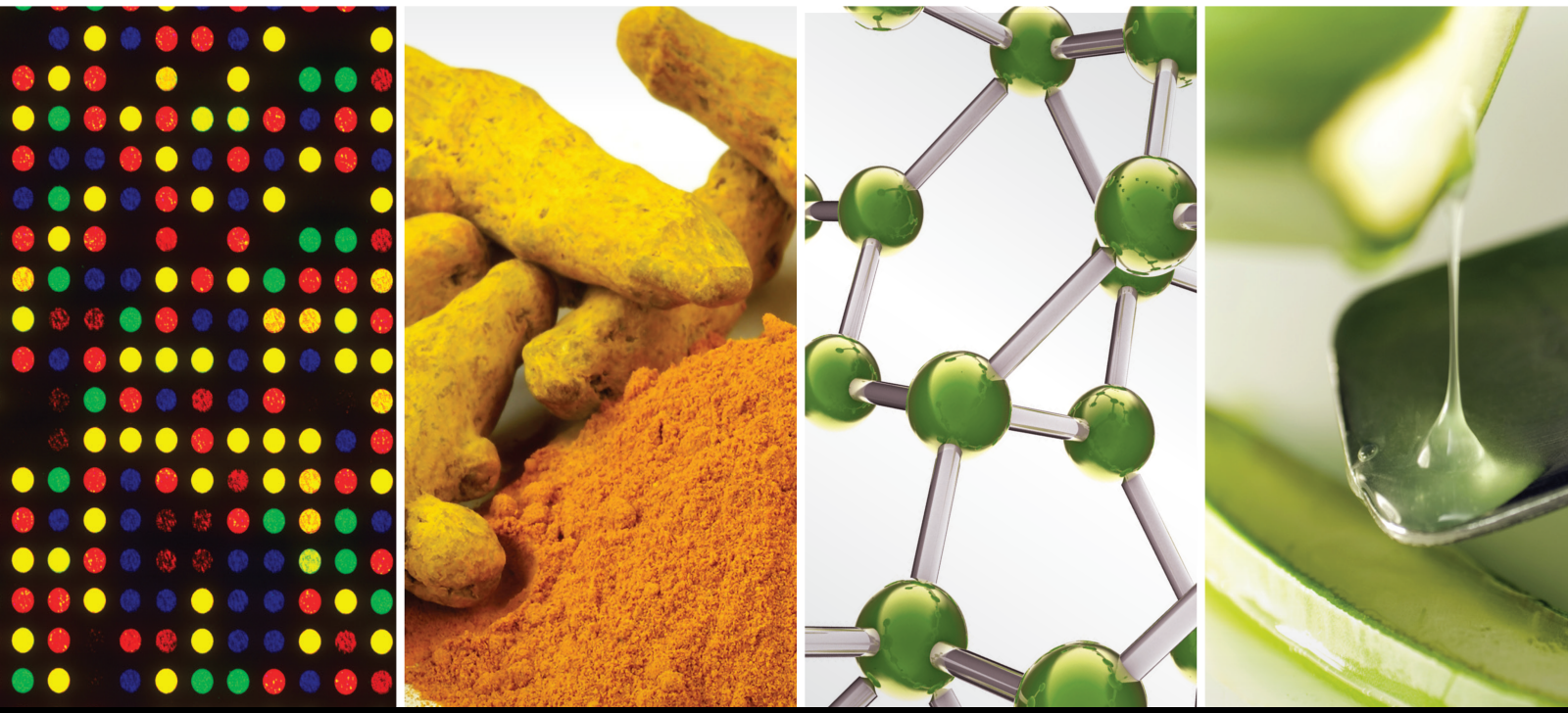
Evidence-Based Complementary and Alternative Medicine

Complementary and Alternative Medicine in Female Reproductive Endocrine Diseases

Lead Guest Editor: Yuehui Zhang
Guest Editors: Xiaoke Wu, Linus R. Shao, Ernest H. Y. Ng, Chi Chiu Wang, and Yong Wang



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Research Article

Effect of Garlic (*Allium sativum*) Supplementation on Premenstrual Disorders: A Randomized, Double-Blind, Placebo-Controlled Trial

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Background. Premenstrual disorders involve physical, behavioral, and mood variations that affect women of childbearing age and interfere with family relationships, household responsibilities, professional duties, and social activities. *Objectives*. Considering the side effects of conventional medications, their use is not recommended except in severe cases of premenstrual disorders. Nowadays, there is a tendency to use traditional and complementary medicine that offers various treatments. The purpose of the current study was to investigate the impacts of garlic as a herbal medicine on the severity of premenstrual symptoms. *Methods*. This study was a double-blind, randomized, controlled trial. After identification of participants with moderate-to-severe PMS through the premenstrual symptoms screening tools questionnaire (PSST), they were randomly assigned to placebo ($n = 64$) or garlic ($n = 65$) groups. Each participant received one tablet daily for three consecutive cycles and logged the severity of their symptoms in the PSST questionnaire during the intervention period. *Results*. There was no significant difference between the two groups in the baseline level of premenstrual symptoms before the intervention. After treatment with garlic for three consecutive cycles, the total score of the severity of premenstrual symptoms significantly ($P < 0.001$) reduced from 34.09 ± 7.31 to 11.21 ± 7.17. In the placebo group, this score changed from 33.35 ± 7.96 to 24.28 ± 7.22. The difference between mean changes in the two groups was 13.78, with a 95% Confidence Interval (CI) of 11.23–16.33. No serious side effects were observed in either group. *Conclusion*. Our findings highlight the potential effect of garlic in reducing the severity of premenstrual symptoms; therefore, the use of garlic can be considered as an alternative therapy in the prevention and treatment of premenstrual disorders.

1. Introduction

Premenstrual disorder (PMD) involves affective, behavioral, and somatic symptoms which occur monthly during the luteal phase of the menstrual cycle and subside after the onset of menstruation [1, 2]. The International Society for the Study of Premenstrual Disorders (ISPMD) newly issued diagnostic standards for PMD present both premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) as one disorder called PMD. Overall, 20 to 30% of women experience clinically significant PMS symptoms and 3–8% experience symptoms meeting PMDD criteria delineated by the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V) [3]. PMDD can disrupt daily activities at home, in the workplace, and during social interactions, and the symptoms can range from moderate to severe in intensity [4–6]. Daily prospective charting of two menstrual cycles to verify the timing of the symptoms precisely is considered essential by all published standards. An asymptomatic week early in the follicular phase is needed for a definitive diagnosis of PMD and to distinguish psychiatric disorders from it [2, 7].

However, the exact etiology of PMDD is still unknown; it seems that instabilities in gonadal sex steroids, predominantly

progesterone, play a role in the pathogenesis of PMDD [8–10]. The metabolites of progesterone with neuroactive properties, including allopregnanolone (3a-hydroxy-5a-pregnan-20-one) and pregnanolone (3a-hydroxy-5b-pregnane-20-one), are positive modulators of gamma-aminobutyric acid (GABA), which is the primary inhibitory neurotransmitter in the brain. Altered functional sensitivity of the GABA receptor and decreased serotonin activity in women with PMD have been reported [11–13]. Consumption of high-calorie diets, sugar, and fat has been identified as crucial risk factor for PMS [14]. On the other hand, enough intake of vitamin D and calcium [15, 16], magnesium, vitamin B1, and vitamin B6 [16–19] might also be beneficial.

Non-pharmacological interventions, such as aerobic exercise, decreased caffeine intake, and increased calcium and carbohydrate intake (before menstruation), are useful for alleviating PMS, but do not improve PMDD symptoms [20, 21]. Some medications, including fluoxetine, sertraline, paroxetine, and oral contraceptives, have been approved by Food and Drug Administration (FDA) in severe PMD [22, 23]. Finally, surgical menopause can be used as the last option to suppress hormones in PMDD [1].

Nowadays, because of the side effects of pharmacological interventions, people are becoming more inclined to use complementary and alternative medicine (CAM) for PMD [24]. Persian medicine as a modality of CAM introduces suitable approaches; for example, Ibn-e-Sina (Avicenna) in his book "The Canon of Medicine" suggested any changes in the menstrual pattern in terms of quantity, quality, and the onset of menstruation could lead to premenstrual disorders. Symptoms potentially subside with regular, adequate, and moderate menstruation [25]. For this, exercise, diet, herbal remedies, and wet cupping are recommended [26].

Among the different modalities of CAM, herbal medicine is the most popular method [27]. Some herbs such as vitex agnus castus (VAC) or chasteberry and *Hypericum perforatum* (St. John's wort) can be effective in the control of PMD symptoms, but it is essential to perform these treatments after gathering sufficient evidence [28–30]. Garlic (*Allium sativum*) is an herb with immunoregulatory effects [31] and reduces anxiety and depression behaviors in diabetic rats, possibly by reducing brain oxidative stress [32]. Recent animal studies have found evidence of the effects of garlic on reducing cognitive and behavioral symptoms through interference with neurotransmitters [33–35]. According to Persian medicine references, garlic is suggested as one of the herbal medicines that can be effective in PMS through lowering blood viscosity and menstruation regulation.

Although research has investigated the effect of garlic on other female disorders such as dysmenorrhea and PCOS, there is a paucity of studies assessed the effects of garlic on PMS. Therefore, the present study aims to explore the efficacy of garlic on young women with PMS.

2. Materials and Methods

2.1. Study Design and Setting. The current study was a single-center with a double-blind, randomized parallel-controlled trial design conducted in the Nasibeh dormitory of Tehran between April 2018 and November 2018. The study protocol was presented to and approved by the Research Ethics Committee of Tehran University of Medical Sciences (TUMS) (no. IR.TUMS.VCR.REC.1396.4670) and was then registered at the Iranian Registry of Clinical Trials (IRCT) Center (IRCT20180311039038N1; 03/25/2018). A consent form was taken before initiation of trial from all eligible participants. Participants were aware of the purpose, procedure, advantages, and disadvantages of study and their legal right to withdraw at any stage of study. Moreover, the CONSORT checklist as the guideline for reporting this study was used (see Appendix S1).

2.2. Participants. The research team presented a summary of study objectives and protocol to the students and asked them whether they would like to participate in the study or not. A total of 790 students living in the dormitory voluntarily filled out the PMS diagnosis questionnaire for two consecutive cycles. Five hundred forty-eight of them were identified to have a provisional diagnosis of PMS. They were then asked to fill out the Iranian version of the PSST questionnaire to rate their PMS severity. Individuals meeting the following criteria were considered as having PMDD or a very severe case of PMS: (A) Answered "severe" to at least 1 of the 4 first questions. (B) Answered "moderate" or "severe" to at least 4 of the 14 first questions. (C) Answered "severe" to at least 1 of the 5 last questions. Those meeting the following criteria were considered having moderate-to-severe symptoms: (A) Answered "moderate" or "severe" to at least 1 of the 4 first questions. (B) Answered "moderate" or "severe" to at least 4 of the 14 first questions. (C) Answered "moderate" or "severe" to at least 1 of the 5 last questions. Other affected individuals were considered as having mild PMS. Those participants with moderate-to-severe symptoms were then invited by the research team for the interview to select participants meeting the inclusion/exclusion criteria of the study.

The inclusion criteria were as follows: women aged 15–49 years, with regular menstrual cycles of 25–34 days, and with moderate-to-severe PMS according to the PSST questionnaire. The exclusion criteria were being pregnant, considering or trying for pregnancy during study, lactating, getting married during the period of study (as might cause unforeseen stress), diagnosed or having symptoms of any other physical illness such as thyroid disease or anemia, being treated for any mental illness, using other medications such as hormonal or herbal medicines, consuming alcohol, tobacco, or illicit substances, having medication allergy, and being drug intolerant such as having an incidence of severe side effects. Based on the inclusion and exclusion criteria, eligible participants amongst female students living in the same dormitory were identified.

2.3. Sample Size Estimation. G*power (version 3.1.9) [36], statistical power and sample size calculator software, was used to compute the required sample size. To achieve a moderate effect size ($d = 0.5$) with static power of 0.8 at a significant level of 0.05, a total of 64 patients were needed in each group.

2.4. Randomization. The randomization process was done using a custom-made computer program that randomly assigned participants to one of the two groups with 1 : 1 allocation ratio. All the study participants and the research personnel involved in running the experiment were blind to the subjects' group allocation during the study.

2.5. Intervention. A single dose of 400 mg tablet per day was taken by all participants for three consecutive cycles. The intervention group received 400 mg Allium-S tablets, while the control group received placebo tablets. Both tablets were manufactured and provided by Dineh Pharmaceutical Company (Qazvin, Iran). The Allium-S tablets were made from dried garlic powder containing 1.1 mg of allicin, its active ingredient, in one 400 mg garlic tablet. The pharmaceutical company made the placebo tablets from starch powder and placed them next to garlic tablets for a month to acquire garlic odor. Placebo and garlic tablets had the same appearance in odor, shape, texture, color, and size. The tablets were encoded by the pharmaceutical company, and the encryption keys were sent to the research team by mail after the completion of the intervention. This allowed both participants and investigators to be blinded to the type of medication during the study.

2.6. Study Procedures. On the first day of their first cycle (i.e., cycle 1), participants started taking the daily tablets for three consecutive cycles (i.e., stopped it at the start of cycle 4). After beginning the intervention, participants completed the PSST questionnaire at the beginning of the first cycle according to the last pre-intervention cycle (cycle 0) and then for three consecutive cycles (cycles 1, 2, and 3) early in the subsequent cycle. During the study, a research team member was present at the dormitory and verified the use of tablets and their possible complications and checked with the participants about their compliance with treatment.

2.7. Measures. Two questionnaires were used in this study: one to identify/diagnose individuals suffering from PMS and the other to rate the severity of PMS. The first questionnaire (i.e., the PMS diagnostic tool) is a self-assessment questionnaire based on the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) of the American Psychiatric Association [37]. It is a questionnaire with a prospective daily rating of symptoms consisting of 11 questions related to (1) depressed mood or dysphoria, (2) anxiety or tension, (3) affective lability, (4) irritability, (5) decreased interest in usual activities, (6) Difficulties in concentrating, (7) marked lack of energy, (8) marked change in appetite, overeating, or food cravings, (9) hypersomnia or insomnia, (10) feeling overwhelmed, and (11) other physical symptoms (such as breast tenderness and bloating). The first four questions are related to affective symptoms. To have a positive PMS diagnosis, multiple criteria need to be met for at least two consecutive symptomatic menstrual cycles. First, it requires a positive response to at least 5 questions, one of them related to the affective symptoms. Then, three criteria must be met: (A) symptoms must occur during the week before menses and remit a few days after onset of menses; (B) symptoms must interfere with work, school, relationships with family, social life, and household responsibilities; and (C) symptoms must not merely be an exacerbation of another disorder. In summary, for at least two consecutive symptomatic menstrual cycles, a person having 5 positive responses and meeting the criteria A, B, and C is identified as the person with PMS.

As the former questionnaire does not measure the severity of PMS, the Iranian version of the premenstrual symptoms screening tools (PSST) questionnaire was used for this purpose [38]. This retrospective self-administrated questionnaire asks participants to rate the listed symptoms/experience if those start before menstruation and stop within the first few days of bleeding. The 4-point Likert-type scale of "not at all," "mild," "moderate," or "severe" was used to rate each question scored from 0 to 3, respectively. The questionnaire consists of 19 questions organized in two sections; the first section assesses the individual's symptoms and impairment during PMS using 14 questions related to physical, behavioral, and mood symptoms. The listed symptoms include depressed mood (hopelessness), anxiety (tension), tearfulness (increased sensitivity to rejection), anger (irritability), decreased interest in work activities, decreased interest in home activities, decreased interest in social activities, difficulty concentrating, fatigue (lack of energy), overeating (food craving), insomnia, hypersomnia, feeling overwhelmed or out of control, and physical symptoms (headaches, muscle aches, chest pain, and flatulence).

The second section consists of 5 questions related to the effects of these symptoms on the quality of life. Women were asked "How your symptoms, as listed above, interfered with any of the following five areas: work efficiency, educational activities, social life, relationships with family, and household responsibilities?" Participants filled out this questionnaire at the beginning of their menstruation, according to symptoms in the previous cycle. The premenstrual disorders criteria listed in this questionnaire are consistent with those of DSM-IV and DSM-V for PMDD [39]. The Iranian version of the PSST was the primary outcome measure of this study. It was also used in identifying eligible participants (i.e., those with moderate-to-severe PMS [40]).

2.8. Statistical Analysis. In this study, all analysis was performed by the intent-to-treat (ITT) principle. The intervention and control groups were compared on demographic variables. Qualitative variables were expressed in percentage (%) and analyzed by chi-square test, while quantitative data were represented as mean ± SD and analyzed by independent samples t-test. The severity of symptoms was compared between the two groups at the pre-intervention cycle (cycle 0) and the three consecutive cycles after intervention (cycles 1, 2, and 3). The repeated measures ANOVA test was used to compare mean changes within and between two groups in pre-post intervention periods. The Bonferroni post hoc test was used for multiple comparisons. Moreover, a Chi-square

test was used to compare potential side effects between groups. Statistical analysis was performed using SPSS 18 software and the statistical significance level was set at the level of $P < 0.05$.

3. Results

3.1. Study Participants. 21 out of 150 eligible patients declined to participate or did not meet included criteria. From 129 students taking part in the study, 65 women were randomized to the garlic group and 64 to the placebo group. During the investigation, seven persons did not complete the study, whose dropout rates were 3 (4.61%) in garlic and 4 (6.25%) in placebo group (Figure 1). All the 122 participants who completed the study took all their tablets during 3 cycles as instructed.

3.2. Demographics. There were no significant differences between the two groups regarding the parameters mentioned in Table 1 (age, body mass index (BMI), duration of the cycle, duration of menstruation, marital status, educational field, and the province of residence), and they were eligible for parallel comparison.

3.3. Study Outcomes. As shown in Table 2, there was no significant difference in the parameters of premenstrual symptoms scores before the intervention between the two groups. However, there was a significant difference ($P < 0.001$) after the intervention in the mean changes between the two groups. At the end of the intervention in the garlic group, 59 people (90.76%) were in the mild or disease-free class. That number was 23 (35.93%) in the placebo group.

After intervention for three consecutive cycles, the total score of premenstrual symptoms in the two groups significantly reduced; in the garlic group from 34.09 ± 7.31 to 11.21 ± 7.17 (mean changes: 22.88, 95% CI 20.72–25.03; $P < 0.001$); in the placebo group from 33.35 ± 7.96 to 24.28 ± 7.22 (mean changes: 9.07, 95% CI 7.68–10.46; $P < 0.001$). The difference between the mean changes in each of the three cycles after the intervention was significant ($P < 0.001$). The mean difference was 6.05, 95% CI 3.26–8.84 at cycle 1; 9.85, 95% CI 7.17–12.53 at cycle 2; and 13.07, 95% CI 10.55–15.57 at the end of the intervention. The results of this analysis showed that the main effect of treatment on reducing the mean total score is significant; this reduction was significantly greater in the treatment group than in the placebo group (P-value <0.001; $F(1, 127) = 35.121$; partial eta squared = 0.217). Also, the results of Bonferroni post hoc test showed a significant difference between each pair of replicates in both groups (Table 3).

3.4. Clinical Complications and Adverse Effects. Nine adverse effects were observed over the intervention. The difference between the garlic and placebo in the frequency of some adverse effects was significant (Table 4). Patients in the garlic group experienced symptoms like acne, itching, and flushing more than placebo, while spotting and bloating occurred more in the control group. There was no considerable difference in other symptoms between groups.

4. Discussion

The results of this study highlight the effects of garlic on reducing premenstrual symptoms. Participants in the study had a moderate-to-severe degree of PMS symptoms before the intervention and following three consecutive menstrual cycle interventions, a significant decrease in mean symptom scores was noted in the garlic group compared with placebo.

Although the pathophysiology of premenstrual disorders is not yet fully understood, the behavioral symptoms are thought to be due to the altered stimulatory impact of progesterone and estradiol on dopamine levels in the brain [41]. Evidence suggests that serotonin is involved in the pathophysiology of PMS, particularly in the prevalence of mood and behavioral symptoms [12, 42]. Clinical evidence has verified that premenstrual symptoms are significantly attenuated through serotonergic neurotransmission enhancer drugs (e.g., serotonin reuptake inhibitors) [42, 43].

Despite their proven efficacy, side effects of SSRIs include fatigue, low mood, sleep disturbances, nausea, headache, reduced libido, and difficulty achieving orgasm. Some of these symptoms may lead to treatment discontinuation [44]. Using unopposed estrogen may increase the risk of endometrial cancer and providing endometrial protection with progestogen may induce premenstrual symptoms [45]. Besides, there is a risk of thromboembolic effects in drospirenone-containing OCs [46]. GnRH agonist is usually reserved for severe cases and when treatment has been resistant to the use of SSRIs. The resulting hypoestrogenic state leads to adverse effects such as vaginitis, osteoporosis, and vasomotor symptoms [47]. Risks of bilateral salpingo-oophorectomy, including the need for postsurgical estrogen replacement, should be considered [48]. Data concerning the therapeutic effects of VAC are promising; however, they are still controversial [49]. So, synthetic drugs are not administered to treat premenstrual disorders because of the side effects except in severe cases.

Based on the findings of this study, garlic can be practical for the treatment of premenstrual symptoms. Globally, the beneficial effects of garlic in the prevention and treatment of diseases have been proven in previous studies. Immunoregulation and modulation of secretion of cytokines by *Allium sativum* may be the mechanism of action of many of its therapeutic effects (antidiabetic, antihypertensive, and hypolipidemic) for metabolic syndrome [31, 50, 51]. Compelling evidence supports the ability of aged garlic extract (AGE) to protect against oxidant-induced diseases, that is, reduced risk of cardiovascular disease, stroke, cancer, and aging, including oxidant-mediated brain cell damage in neurodegenerative disorders, especially Alzheimer's disease (AD) [52–54]. The findings of several studies suggest that garlic intake may lead to inhibition of β-amyloid protein (Aβ) aggregation in the human brain [55–58].

The use of a diet supplement containing garlic and black sesame in ovariectomized rats has shown significant

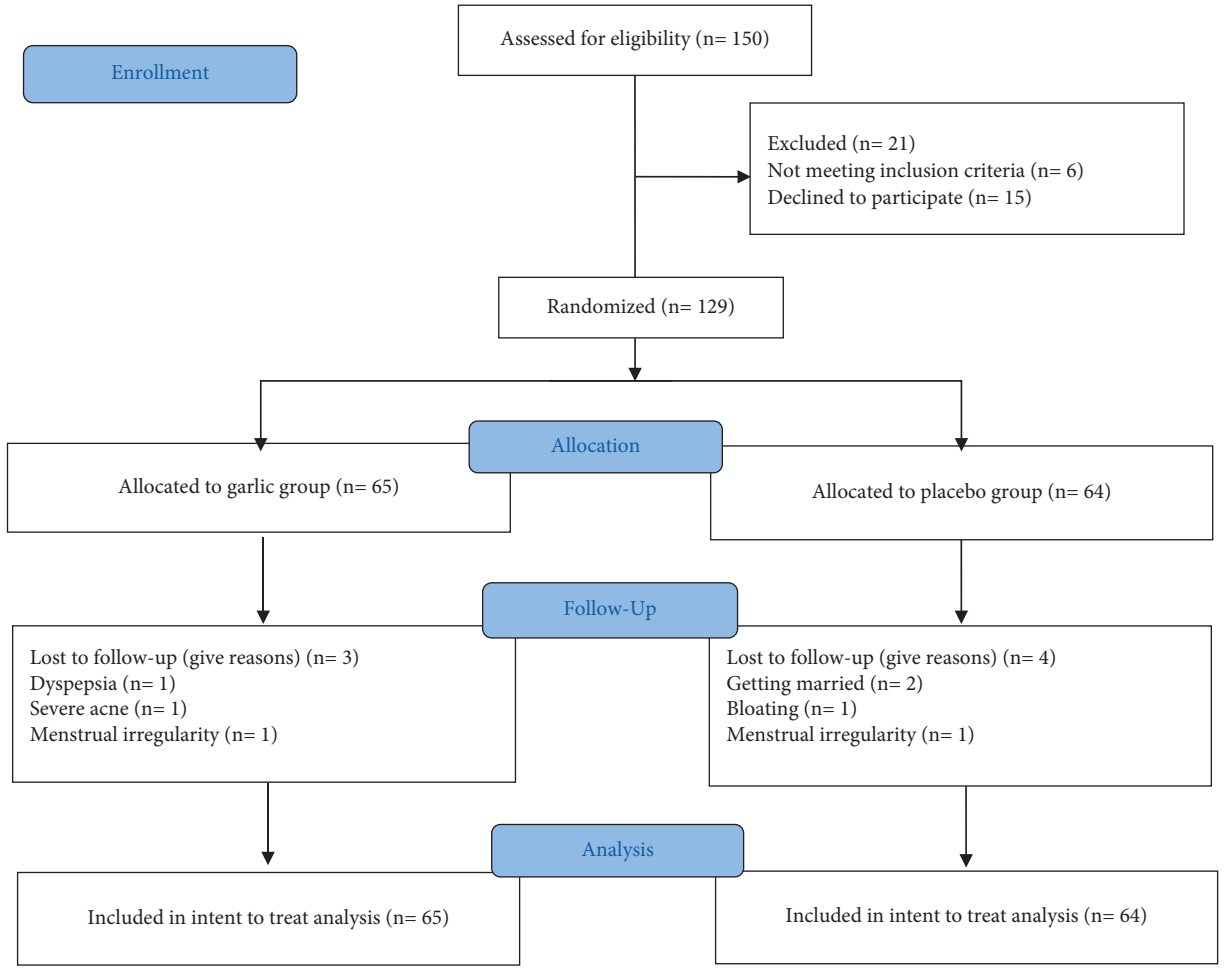

Figure 1: Flow diagram of the study.

Table 1: Demographic data of the participants in the two groups of the study.

Parameter	Garlic group ($n = 65$)	Placebo group ($n = 64$)	P-value
Age (year); mean ± SD	21.5 ± 1.4	20.9 ± 1.3	0.170
Weight (kg); mean ± SD	58.5 ± 7.9	58.6 ± 8.1	0.934
Height (cm); mean ± SD	164.1 ± 4.5	162.9 ± 5.0	0.168
BMI (kg/m^2); mean ± SD	21.7 ± 2.8	22.0 ± 2.6	0.483
Duration of menstruation (day); mean ± SD	6.2 ± 1.1	6.4 ± 1.0	0.329
Duration of cycle (day); mean ± SD	29.6 ± 2.1	30.1 ± 1.8	0.205
Marital status			
Single; number (%)	54 (83.1%)	44 (69.0%)	0.057
Married; number (%)	11 (16.9%)	20 (31.0%)	
Educational field			
Physical education; number (%)	6 (9.2%)	2 (3.1%)	0.151
Nonphysical education; number (%)	59 (90.8%)	62 (96.9%)	
Province; (total: 31 provinces)			
Number (%)	21 (68%)	20 (65%)	0.160

Resulted from independent t-test for quantitative and chi-square test for categorical variables.

antidepressant-like activity [35]. Garlic also reduces anxiety and depression behaviors in diabetic rats, possibly by reducing brain oxidative stress [32]. AGE may improve memory by affecting cholinergic, glutamatergic, and GABAergic systems concerning cognitive impairment in Aβ-induced rats [34, 59]. Evidence for the antidepressant-like activity of garlic extract in mice has been found through inhibition of monoaminoxidase-A (MAO-A) and monoaminoxidase-B (MAO-B) and involvement of adrenergic, dopaminergic, serotoninergic, and GABAergic systems [33]. Evidence also shows that administration of garlic in rats with increased brain serotonin (5-hydroxytryptamine) levels

Table 2: Premenstrual symptoms scores in placebo and garlic groups before (cycle 0) and after intervention (cycles 1, 2, and 3).

Symptoms		Groups		Mean difference between groups (95% CI)	P–value
		Placebo ($n = 64$) mean ± SD	Garlic ($n = 65$) mean ± SD		
Mood symptoms	Cycle 0	7.43 ± 2.18	7.56 ± 2.09	−0.13 (−0.08–0.61)	0.727
	Cycle 1	6.28 ± 2.21	4.95 ± 1.92	1.32 (0.60–2.04)	<0.001
	Cycle 2	5.76 ± 2.02	3.67 ± 2.03	2.08 (1.37–2.79)	<0.001
	Cycle 3	5.18 ± 1.85	2.33 ± 1.92	2.84 (2.18–3.50)	<0.001
Behavioral symptoms	Cycle 0	13.29 ± 4.38	13.46 ± 3.87	−0.16 (−1.60–1.27)	0.822
	Cycle 1	11.73 ± 3.82	9.53 ± 4.16	2.19 (0.80–3.58)	0.002
	Cycle 2	10.50 ± 3.48	7.24 ± 4.04	3.25 (1.93–4.57)	<0.001
	Cycle 3	9.57 ± 3.27	5.07 ± 3.58	4.50 (3.30–5.69)	<0.001
Physical symptoms	Cycle 0	5.64 ± 2.52	5.50 ± 3.01	0.13 (−0.83–1.10)	0.787
	Cycle 1	5.10 ± 2.74	3.81 ± 2.24	1.29 (0.43–2.16)	0.004
	Cycle 2	4.54 ± 2.63	2.72 ± 2.24	1.82 (0.97–2.67)	<0.001
	Cycle 3	4.06 ± 2.43	1.93 ± 1.86	2.12 (1.36–2.88)	<0.001
Interfering symptoms	Cycle 0	6.98 ± 2.34	7.55 ± 2.03	−0.56 (−1.33–0.19)	0.143
	Cycle 1	6.26 ± 2.53	5.03 ± 2.41	1.23 (0.37–2.09)	0.005
	Cycle 2	5.87 ± 2.32	3.18 ± 2.05	2.69 (1.92–3.45)	<0.001
	Cycle 3	5.43 ± 2.18	1.86 ± 1.77	3.57 (2.88–4.26)	<0.001
Total symptoms	Cycle 0	33.35 ± 7.96	34.09 ± 7.31	−0.74 (−3.99–1.93)	0.587
	Cycle 1	29.39 ± 8.33	23.33 ± 7.67	6.05 (3.26–8.84)	<0.001
	Cycle 2	26.68 ± 7.58	16.83 ± 7.78	9.85 (7.17–12.53)	<0.001
	Cycle 3	24.28 ± 7.22	11.21 ± 7.17	13.07 (10.55–15.57)	<0.001

Resulted from repeated measures ANOVA test based on comparing the mean difference between two groups.

Table 3: Mean difference changes of scores of the PMS symptoms in the two groups before and after the intervention.

Parameters	Mean difference (95% CI) within placebo group ($n = 64$)	Mean difference (95% CI) within garlic group ($n = 65$)	Mean difference changes (95% CI) between groups	P-value	Partial eta squared	F (1,127)
Mood symptoms	2.25 (1.77–2.72)	5.23 (4.56–5.89)	2.98 (2.16–3.79)	<0.001	0.177	27.30
Behavioral symptoms	3.71 (2.79–4.64)	8.38 (7.47–9.29)	4.66 (3.38–5.95)	<0.001	0.120	17.28
Physical symptoms	1.57 (1.06–2.08)	3.56 (2.90–4.23)	1.99 (1.16–2.82)	<0.001	0.086	11.96
Interfering symptoms	1.54 (1.08–2.01)	5.69 (5.07–6.31)	4.14 (3.37–4.91)	<0.001	0.173	26.49
Total symptoms	9.07 (7.68–10.46)	22.88 (20.72–25.03)	13.78 (11.23–16.33)	<0.001	0.217	35.121

Resulted from repeated measures ANOVA test and Bonferroni post hoc test.

Table 4: Clinical complications and adverse effects were reported as number per group.

Adverse effects	Garlic ($n = 65$)	Placebo ($n = 64$)	P-value
Acne	0	7	0.007
Flushing	1	9	0.009
Itching	0	6	0.013
Bloating	11	0	<0.001
Spotting	5	0	0.022
Dyspepsia	1	5	0.098
Nausea	2	8	0.051
Dizziness	8	8	0.974
Hypermenorrhea	0	1	0.319

Resulted from chi-square test.

improves cognitive performance [60]. Collectively, these beneficial effects of garlic on improving cognitive and mood symptoms confirm that part of the impact of garlic in our study may be through boosted levels of serotonin and dopamine in the brain.

Besides the beneficial effects of garlic, it is usually accompanied by some mild side effects. In this study, participants in the garlic group announced some mild complaints, such as itching, flushing, and acne. Some studies showed that garlic could induce allergic reactions and irritant dermatitis [61]. Also, heartburn, gastrointestinal irritation, and nausea can be potential side effects of garlic consumption [62].

In addition to the randomized, placebo-controlled, and double-blind design, this study has several strengths. The participants received daily treatment for three consecutive cycles, while in most studies related to premenstrual symptoms, the intervention has been only in the luteal phase. This study had a suitable distribution and participants were present from 26 of the 31 provinces of the country. We could not find any investigation on the effect of garlic on PMS to compare with our study results. In this study, garlic effectively reduced PMS symptoms with no side effects. The main effect of comparing the two types of intervention was significant and large. However, as with some other PMS treatment studies, the placebo effect was significant [63]. It is likely that participants hoped for disease treatment and the expectation of relief of premenstrual symptom severity could reduce their mental stress and alleviate symptoms.

There are some limitations to this study. The voluntary basis of this study opens it to a systematic difference between persons who choose to participate. The study also has the limitation of all self-assessment questionnaires including social desirability bias. The generalizability of the results might be a potential problem as it has been done in only one dormitory. Also, the short duration of study highlights a need for further studies with a longer follow-up period to assess the long-lasting effect of the garlic. Further studies could be done in multiple centers and offer better generalizability by removing some of the mentioned limitations. Future studies are needed to evaluate the effects of garlic on the neurotransmitters involved in premenstrual symptoms.

5. Conclusion

Our findings revealed benefits of garlic for treating premenstrual symptoms without severe side effects. Since many women nowadays tend to use herbal remedies to prevent and treat premenstrual symptoms because of the side effects of synthetic drugs, garlic can be considered as a complementary medicine to improve premenstrual symptoms.

Abbreviations

AGE: Aged garlic extract
AD: Alzheimer's disease
Aβ: β-Amyloid protein
BMI: Body mass index
CAM: Complementary and alternative medicine
CBT: Cognitive-behavior therapy
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th edition
DSM-V: Diagnostic and Statistical Manual of Mental Disorders, 5th edition
GABA: Gamma-aminobutyric acid
GnRH: Gonadotropin-releasing hormone
IRCT: Iranian Registry of Clinical Trials
ISPMD: International Society for the Study of Premenstrual Disorders
MAO-A: Monoaminoxidase-A
MAO-B: Monoaminoxidase-B
OCs: Oral contraceptives
PSST: Premenstrual symptoms screening tools
PMD: Premenstrual disorder
PMDD: Premenstrual dysphoric disorder
PMS: Premenstrual syndrome
SSRIs: Selective serotonin reuptake inhibitors
TUMS: Tehran University of Medical Sciences
US FDA: United States Food and Drug Administration
VAC: Vitex agnus castus.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

There are no conflicts of interest regarding the publication of this paper. Dineh Company was not involved in any part of this study from development to manuscript writing.

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Supplementary Materials

CONSORT 2010 checklist of information to include when reporting a randomized trial. (*Supplementary Materials*)

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Review Article

Effect of Acupuncture on Polycystic Ovary Syndrome in Animal Models: A Systematic Review

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Background. Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age. As a widely used complementary and alternative therapy, acupuncture is increasingly used to treat PCOS. However, the effect of acupuncture in treating PCOS is uncertain, and the mechanisms are unclear. This systematic review aims to determine the efficacy of acupuncture on PCOS in animal preclinical models. *Methods*. Experimental animal studies of acupuncture in PCOS animal models were searched in PubMed, Web of Science, China National Knowledge Infrastructure, and the Chinese Science and Technology Periodical Database from inception to December 2020. The risk of bias was assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool. *Results*. A total of 358 studies were screened based on the title and abstract, and 31 studies were included. A total of 722 animals were involved, and all studies used either Wistar rats or SD rats. Twenty-six studies used electroacupuncture, 9 studies used manual acupuncture, and 5 of them employed both electroacupuncture and manual acupuncture. A total of 22 acupoints were involved; 7 studies followed the modern acupuncture pattern, and the rest followed classic acupuncture theory. *Conclusions*. The present review summarizes the current evidence of the effects of acupuncture on PCOS in animal models. Unfortunately, we could not draw a definite conclusion due to the methodological weakness of the included studies and the high heterogeneity. Well-designed studies are needed in the future to fill this gap.

1. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder among females of reproductive age. The worldwide prevalence of PCOS is 8–13% in women and 6% in adolescent girls [1–3]. Its clinical manifestations are diverse and characterized by irregular menstruation, amenorrhea, androgen excess, chronic anovulation, and infertility. Fifty percent of women with PCOS have insulin resistance (IR) [4], which is associated with an increased risk of metabolic syndrome, type 2 diabetes, and cardiovascular diseases [5]. The pathogenesis of PCOS is not fully understood. In recent years, an increasing number of studies have found that disorders of the hypothalamus-pituitary-ovarian (HPO) axis, abnormal adrenocortical function, and enhanced sympathetic nerve activity are all involved in PCOS development [6–8].

As the prevalence of PCOS increases, studies of effective treatment regimens are particularly important. Acupuncture is an important part of traditional Chinese medicine that has been applied for thousands of years. Some clinical studies found that acupuncture is beneficial for the regulation of hormone levels and ovulatory dysfunction in patients with PCOS [9–11]. Several systematic reviews have been conducted on the efficacy and safety of acupuncture for women with PCOS, but due to the high risk of bias and heterogeneity, the levels of evidence are low [12]. There is an insufficient amount of research evidence to support the clinical efficacy of acupuncture treatment for women with PCOS. Preclinical animal experiments are the link between basic

research and clinical trials. Several animal experimental studies indicate that acupuncture influences PCOS-like symptoms in rats via multiple mechanisms [13, 14]. Peng et al. [13] found that acupuncture can improve insulin resistance by activating the AMPK pathway in PCOS-like symptoms. Xu et al. [14] indicated that acupuncture adjusts hormone levels by regulating ovarian local factors in PCOS rats.

Until now, no systematic review has been published to summarize the effects of acupuncture in PCOS animal models. This systematic review of animal experiments is an efficient means of enhancing the value of animal experiments, which reduces the risk of the translation of animal experiments to the clinic. Therefore, this systematic review aimed to evaluate the currently available evidence of acupuncture in PCOS animal models and provide valuable directions to inform clinical practice.

2. Materials and Methods

2.1. Protocol and Registration. The protocol of this study followed the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines (Additional file 1) [15] and is adapted from the structure provided in the Systematic Review Protocol for Animal Intervention Studies [16]. This study was registered at OSF (Registration DOI: 10.17605/OSF.IO/FNM37).

2.2. Eligibility Criteria

2.2.1. Types of Studies Included. This systematic review included both randomised and nonrandomised controlled studies. There was no restriction on language or date. We included studies published in peer-reviewed journals only [17].

2.2.2. Types of Animal Models. All animal models of PCOS were included regardless of the species or size of the animal.

2.2.3. Types of Intervention and Comparators. Both traditional acupuncture and contemporary acupuncture (in which needles were not inserted in classical meridian points) were included. Hand stimulation, electrical stimulation, or warming needles with moxibustion were included. Acupuncture without needling was excluded, such as acupressure, acupoint injection, tap pricking, and cupping. The comparison group included PCOS animals induced by the same method as the intervention group but without undergoing the intervention [17].

2.2.4. Types of Outcome Measures. The following outcome measures were used [17]:

(1) Primary outcome: homeostatic model assessment-insulin resistance (HOMA-IR: (fasting insulin (μU/mL) × fasting glucose (mmol/L))/22.5).

(2) Secondary outcomes: testosterone (T), LH (luteinizing hormone), LH/follicle-stimulating hormone (FSH) ratio, fasting blood sample (FBG), fasting insulin (FINS), and body weight (BW).

2.3. Search Strategy. PubMed, Web of Science, China National Knowledge Infrastructure (CNKI), and the Chinese Science and Technology Periodical Database (VIP) were searched from inception to December 20, 2020. The main terms "Polycystic ovary syndrome", "Acupuncture", and "Animal Experimentation", indexed in the MeSH system, were combined [17].

2.4. Study Selection. Two independent reviewers (JY and LJZ) screened titles and abstracts for eligibility. Disagreements between reviewers were resolved by a third review (YL). Full texts were obtained and evaluated by the same reviewers using a predesigned form.

2.5. Data Extraction. Two reviewers (JY and LJZ) extracted data independently, and any controversy was resolved by discussion. The following information was recorded using a predesigned form: study design, characteristics of the included animals, characteristics of the animal model, details of the intervention, and outcome measures.

2.6. Risk of Bias Assessment. Two reviewers (YL and JJG) assessed the risk of bias using SYRCLE's tool for assessing risk of bias (SYRCLE ROB) [18].

2.7. Data Synthesis. We performed a meta-analysis using a random-effects model with Review Manager (RevMan) 5.3. Treatment effects were summarized as the standard mean difference (SMD) with a 95% confidence interval (CI). The SMD is an evaluation of the combined effect sizes, and P values below 0.05 were considered statistically significant. The presence of heterogeneity was evaluated by I^2 and chi-square statistical analyses. Funnel plots were performed to evaluate publication bias if there were more than ten studies included [17].

3. Result

3.1. Study Selection. A total of 384 potentially relevant studies were identified from the abovementioned four databases. After removing duplicates, 358 records remained for title and abstract screening. A total of 303 studies were excluded due to at least one of the following reasons: (1) not an animal study, (2) case report or review, or (3) not related to acupuncture. Finally, 55 studies remained after the initial reading. After full-text reading of the remaining studies, 24 studies were excluded, and 31 studies were included (Figure 1).

3.2. Study Characteristics. A total of 31 studies were included [13, 14, 19–46]. Thirteen studies were published in English, and 18 were published in Chinese. A total of 722 animals (9 studies with Wistar rats and 22 studies with SD

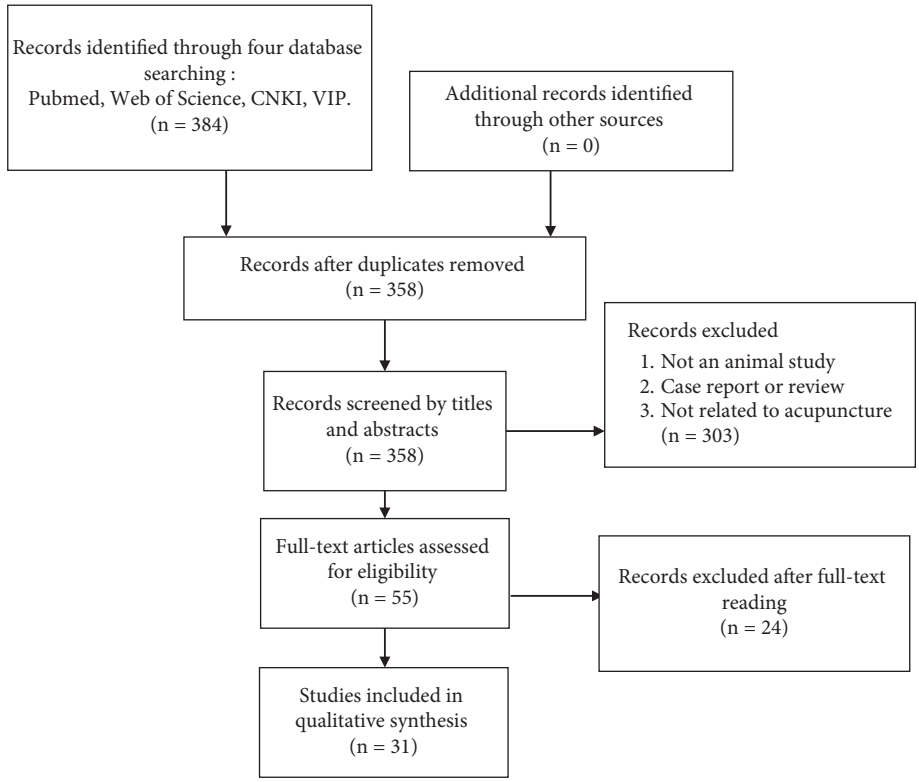

Figure 1: Flow diagram of the study selection process for this systematic review and meta-analysis.

rats) were involved, and the details of the animal models are presented in Table 1. Eleven studies induced a PCOS model by letrozole, 8 studies used dihydrotestosterone (DHT), 6 studies used dehydroepiandrosterone (DHEA), 4 studies used testosterone propionate (TP), 1 study used estradiol valerate (EV), and 1 study used prasterone sulfate. Thirty included studies randomly assigned animals to the acupuncture and control groups, and only 1 study did not report randomisation details. Twenty-six studies used electroacupuncture (EA), 9 studies used manual acupuncture (MA), and 5 of them employed two acupuncture groups, including both EA and MA. Among all the included studies, a total of 22 acupoints were involved. The frequencies of acupuncture points from high to low were as follows: SP6, 20 times; CV4 (RN4), 17 times; CV3 (RN3), 10 times; ST36, 7 times; EX-CA1, 7 times; ST29, 4 times; RN12, 3 times; ST25, 3 times; LI10, 2 times; BL23, 2 times; SP9, 1 time; ST40, 1 time; RN6, 1 time; PC6, 1 time; EX-B3, 1 time; CV12, 1 time; BL18, 1 time; BL20, 1 time; ST27, 1 time; ST28, 1 time; LR3, 1 time; and Hou Hui, 1 time. Seven studies followed the modern acupuncture pattern, in which the basic principle was two needles inserted bilaterally in the abdominal muscles, with two needles placed in each soleus and gastrocnemius hindlimb muscle in somatic segments corresponding to ovarian innervations [18, 19, 23, 24, 26, 31, 36]. The electrical frequencies used were all 2 Hz except for one study that did not mention the EA frequency, and the intensity was 0.6–3 mA. One study reported intervention with a single session; the others lasted from 2 weeks to 8 weeks.

3.3. Risk of Bias within Studies. The results of the risk of bias of the included studies are summarized in Table 2. SYRCLE ROB included domains for selection bias (sequence generation, baseline characteristics, and allocation concealment), performance bias (random housing and blinding), detection bias (random outcome assessment and blinding), attrition bias, reporting bias, and other biases.

3.4. Effectiveness. The meta-analysis was not performed since the heterogeneity was significantly high. Although the heterogeneity analysis was carried out based on sensitivity analysis, the detailed reasons for the potential heterogeneity were not very certain.

3.5. Quality of Evidence Assessment (Table 3). The GRADE recommendations for HOMA-IR, T, FINS, and BW were very low, and those for LH, LH/FSH, and FBG were low. The certainty was downgraded for the following reasons: I values that exceeded 75%; different animal modelling methods; differences in the treatment cycle and dosage; physiological and pathological differences between rodents and humans; small sample size; and the 95% CI included the value of one.

4. Discussion

4.1. Summary of Evidence. To the best of our knowledge, this is the first systematic review summarizing the current evidence of the effects of acupuncture on PCOS in animal experiments. This finding suggests that acupuncture may

Table 1: Characteristics of included studies.

Study ID	Species (Na/Nc)	Randomised	Weight (g)	Model	Acupuncture (acupoints)	Course of treatment	Stimulation parameters	The main outcomes	Result
Stener-Victorin (2003) [18]	SD rats (8/8)	Not reported	195–210	EV	Bilateral in the mm biceps femoris and erector spinae, in somatic segments corresponding to the innervation of the ovaries	Every second or third day, 25 min, 12 times	EA (2 Hz), 1.5 mA	Endothelin-1	$P < 0.05$
Mannerås (2008) [19]	Wistar rats (11/12)	Y	Not reported	DHT	ST29, SP6, in somatic segments that correspond to the innervation of the ovaries	15 min in week 1, 20 min in weeks 2–3, and 25 min, thereafter, 4–5 weeks	EA (2 Hz), 0.8–1.3 mA	BW	NS
Peng (2008) [20]	Wistar rats (11/12)	Y	250 ± 20	TP	ST36, ST40, ST25, SP6	30 min a day, 14 days	EA (2 Hz)	BW FINS	$P < 0.01$ $P < 0.05$
Zhang (2008) [21]	SD rats (14/13)		Not reported	DHT	RN3, RN4, SP6, EX-CA1	15 min, once a day, 6 weeks	MA	T	$P < 0.01$
Zhang (2009)_1 [22]	SD rats (NR)		Not reported	DHT	RN3, RN4, SP6, EX-CA1	15 min, once a day, 6 weeks	MA	T	$P < 0.01$
Zhang (2009)_2 [23]	SD rats (11/12)	Y	Not reported	DHEA	CV4, CV3, SP6, EX-CA1	15 min a day, 5 days	MA	T	$P < 0.05$
Johansson (2010) [23]	Wistar rats (11/12)	Y	299 ± 6; 287 ± 5	DHT	Two needles bilaterally in the abdominal muscles, and two needles were placed in each soleus and gastrocnemius hindlimb muscle, in somatic segments corresponding to ovarian innervations	15 min in week 1, 20 min in weeks 2 and 3, and 25 min thereafter, 5 days/ week, 4–5 weeks	EA (2 Hz), 0.8–1.4 mA	WB Glucose infusion rate	NS $P < 0.001$
Feng (2012) [24]	Wistar rats (8/8/8)	Y	Not reported	DHT	Bilaterally in the rectus abdominis and triceps surae muscles at points in somatic segments corresponding to the innervation of the ovaries	15 min in wk 1, 20 min wks 2–3, and 25 min thereafter, 20–25 treatments	EA (2 Hz), 0.8–1.4 mA; MA	T LH	NS NS
Li (2012) [25]	SD rats (9/10)	Y	50 ± 10	DHEA	BL23, RN6, PC6, ST36, SP6, EX-CA1	30 minutes a day, 6 estrous cycles	EA	T LH	Not reported
Johansson (2013) [26]	Wistar rats (10/10/10)	Y	252.8 ± 12.9; 256.4 ± 15.8; 252.3 ± 23.7	DHT	Two needles inserted in the rectus abdominis, and one in triceps surae muscles bilaterally	15 min in week 1, 20 min in weeks 2 and 3, and 25 min thereafter, 5 days/ week, 4–5 weeks	EA (2 Hz); MA	BW	$P = 0.29$ $P = 0.70$
Sun (2013) [27]	SD rats (10/10)	Y	200 ± 20	Letrozole	CV4, CV3	20 min a day, 14 consecutive days	EA (2 Hz), 2 mA	T	$P < 0.01$
Benrick (2014) [28]	Wistar rats (12/12)	Y	Not reported	DHT	ST27, ST28, ST29, SP6, SP9	45 min, single session	EA (2 Hz), 0.8–1.2 mA; MA	Glucose infusion rate	$P < 0.01$ $P < 0.001$
Lai (2014) [29]	SD rats (8/8)	Y	Not reported	TP + high fat diet	RN12, CV4, ST25	30 minutes, 3 times a week, 6 weeks	EA (2 Hz)	HOMA-IR FINS	$P < 0.05$ $P < 0.05$

Table 1: Continued.

Study ID	Species (Na/Nc)	Randomised	Weight (g)	Model	Acupuncture (acupoints)	Course of treatment	Stimulation parameters	The main outcomes	Result
Chen (2015) [30]	SD rats (10/10)	Y	Y	Prasterone Sulfate + high fat diet	EX-B3, SP6	20 minutes, 5 times a week, 8 weeks	EA (2 Hz), 1.5 mA	T HOMA-IR	$P < 0.05$ $P < 0.05$
Maliqueo (2015) [31]	Wistar rats (10/10)	Y	Not reported	Letrozole	Two needles bilaterally in the abdominal muscles, and two needles were placed in each soleus and gastrocnemius hindlimb muscle, in somatic segments (Th 10- L2) corresponding to ovarian innervations	15 min in week 1, 20 min in weeks 2 and 3, and 25 min thereafter, 5 days/week, 5–6 weeks	EA (2 Hz), 0.6–1.4 mA; MA	T	$P < 0.05$
Zheng (2015) [32]	SD rats (10/10)	Y	45–50	TP + high fat diet	(1) CV12, CV4, SP6; (2) ST36, Hou Hui	20 minutes, 5 times a week, 5 weeks, 2 sets of acupoints alternatively	EA (2 Hz)	T FINS HOMA-IR	$P < 0.05$ $P < 0.01$ $P < 0.01$
Lai (2016) [33]	SD rats (10/10)	Y	Not reported	DHEA + high fat diet	RN4, RN12, ST25	30 minutes, 3 times a week, 5 weeks	EA (2 Hz), 1 mA	HOMA-IR FINS	$P < 0.05$ $P < 0.05$
Li (2016) [34]	SD rats (7/7)	Y	Not reported	DHEA	RN4, SP6, EX-CA1, RN3	15 min in week 1, 20 min in weeks 2 and 3, and 25 min thereafter, 5 days/week, 5 weeks	EA (2 Hz)	HOMA-IR	$P < 0.05$
Lin (2016) [35]	SD rats (6/6)	Y	Not reported	Letrozole + high fat diet	(1) RN4, SP6; (2) BL18, BL20, BL23	15 minutes, 20 days	MA	T HOMA-IR	$P < 0.01$ $P < 0.05$
Maliqueo (2017) [36]	Wistar rats (9/8)	Y	Not reported	Letrozole	Two needles bilaterally in the abdominal muscles, and two needles were placed in each soleus and gastrocnemius hindlimb muscles, in somatic segments (Th 10- L2) corresponding to ovarian innervations	15 min in week 1, 20 min in weeks 2 and 3, and 25 min thereafter, 5 days/week, 5–6 weeks	EA (2 Hz), 0.6–1.4 mA; MA	FINS	NS
Meng (2018) [37]	SD rats (8/8)	Y	Not reported	TP + high fat diet	SP6, RN4, RN12, LI10, ST36	30 min, 5 times a week, 5 weeks	EA (2 Hz)	WB	$P < 0.01$
Xu (2018) [38]	SD rats (10/10)	Y	160 ± 20	Letrozole	ST36, SP6, CV4	20 min, 14 consecutive days	EA (2 Hz), 1–3 mA	WB T	$P < 0.05$ $P < 0.01$
Shi (2019) [39]	SD rats (10/10)	Y	180 ± 20	Letrozole	CV3, CV4	20 min, 14 consecutive days	EA (2 Hz), 2 mA	T	$P < 0.05$
Xu (2019) [40]	SD rats (10/10)	Y	160 ± 20	Letrozole	ST36, SP6, CV4	20 min, 14 consecutive days	EA (2 Hz)	LH LH/FSH	$P < 0.01$ $P < 0.01$
Yu (2019) [41]	SD rats (10/10)	Y	180–200	Letrozole	LI 10, ST36, SP6, RN4	20 minutes, 27 days	EA (2 Hz)	T	$P < 0.01$
Zhou (2019) [42]	SD rats (10/10)	Y	Not reported	Letrozole	CV3, CV4	20 minutes, 14 days	EA (2 Hz), 2 mA	T	$P < 0.01$

Table 1: Continued.

Study ID	Species (Na/Nc)	Randomised	Weight (g)	Model	Acupuncture (acupoints)	Course of treatment	Stimulation parameters	The main outcomes	Result
Xu (2020) [14]	SD rats (10/10)	Y	160 ± 20	Letrozole	CV3 and the point 5 mm next to CV3 at the same horizontal axis	20 minutes, 14 days	EA (2 Hz), 2 mA	WB T LH AMH	$P < 0.05$ $P < 0.01$ $P < 0.01$ $P < 0.01$
Huang (2020) [43]	SD rats (7/7)	Y	130–170	Letrozole	SP6, LR 3	20 minutes, 14 days	EA (2 Hz), 2 mA	T LH AMH	$P < 0.05$ $P < 0.05$ $P < 0.05$
Kuang (2020) [44]	SD rats (8/8)	Y	50 ± 5	DHEA	RN3, RN4, EX-CA1, SP6	15 min in week 1, 20 min in weeks 2 and 3, and 25 min thereafter, 5 days/week, 5 weeks	EA (2 Hz)	WB	$P < 0.05$
Peng (2020) [13]	SD rats (6/6)	Not reported	Not reported	DHEA	ST29, SP6	15 min in week 1, 20 min in weeks 2 and 3, and 25 min thereafter, 5 days/week, 5 weeks	EA (2 Hz), 0.8–1.3 mA;	T LH LH/FSH	$P < 0.001$ $P < 0.001$ $P < 0.001$
Tong (2020) [45]	Wistar rats (11/14)	Y	Not reported	DHT	ST29, SP6	30 min a day, 5 days a week, 4 weeks	EA (2 Hz), 2 mA	Weight	$P < 0.001$

Na = number in acupuncture group; Nc = number in control group; Ev = estradiol valerate; DHT = dihydrotestosterone; BW = body weight; NS = not significant; FINS = fast insulin; TP = testosterone propionate; DHEA = dehydroepiandrosterone; DHT = Dihydrotestosterone; AMH = anti-Mullerian hormone.

Table 2: SYRCLE risk of bias tool for included studies.

Study ID	1	2	3	4	5	6	7	8	9	10	Total
Stener-Victorin (2003) [18]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Mannerås (2008) [19]	U	Y	U	Y	U	U	U	U	Y	Y	4Y6U
Peng (2008) [20]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Zhang (2008) [21]	U	U	U	U	U	U	U	Y	Y	Y	3Y7U
Zhang (2009)_1 [22]	U	U	U	U	U	U	U	Y	Y	Y	3Y7U
Zhang (2009)_2 [23]	U	U	U	U	U	U	U	Y	Y	Y	3Y7U
Johansson (2010) [23]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Feng (2012) [24]	U	U	U	Y	U	U	U	Y	Y	Y	4Y6U
Li (2012) [25]	U	U	U	Y	U	U	U	Y	Y	Y	4Y6U
Johansson (2013) [26]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Sun (2013) [27]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Benrick (2014) [28]	U	U	U	U	U	U	U	U	Y	Y	2Y8U
Lai (2014) [29]	Y	Y	U	Y	U	U	U	Y	Y	Y	6Y4U
Chen (2015) [30]	Y	Y	U	Y	U	U	U	Y	Y	Y	6Y4U
Maliqueo (2015) [31]	U	U	U	Y	U	U	U	Y	Y	Y	4Y6U
Zheng (2015) [32]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Lai (2016) [33]	Y	U	U	Y	U	U	U	Y	Y	Y	5Y5U
Li (2016) [34]	U	U	U	U	U	U	U	Y	Y	Y	3Y7U
Lin (2016) [35]	U	U	U	U	U	U	U	Y	Y	Y	3Y7U
Maliqueo (2017) [36]	U	U	U	Y	U	U	U	Y	Y	Y	4Y6U
Meng (2018) [37]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Xu (2018) [38]	Y	Y	U	Y	U	U	U	Y	Y	Y	6Y4U
Shi (2019) [39]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Xu (2019) [40]	Y	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Yu (2019) [41]	Y	U	U	Y	U	U	U	Y	Y	Y	5Y5U
Zhou (2019) [42]	U	U	U	Y	U	U	U	Y	Y	Y	4Y6U
Xu (2020) [14]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U
Huang (2020) [43]	U	U	U	Y	U	U	U	Y	Y	Y	4Y6U
Kuang (2020) [44]	U	U	U	Y	U	U	U	Y	Y	Y	4Y6U
Peng (2020) [13]	U	U	U	Y	U	U	U	Y	Y	Y	4Y6U
Tong (2020) [45]	U	Y	U	Y	U	U	U	Y	Y	Y	5Y5U

Y = yes; N = no; U = unclear. 1: sequence generation; 2: baseline characteristics; 3: allocation concealment; 4: random housing; 5: blinding; 6: random outcome assessment; 7: blinding; 8: attrition bias; 9: reporting bias, and 10: other biases.

play a potential role in restoring reproductive endocrine function in PCOS-like animal models.

The mechanisms of acupuncture in PCOS animal models are still unclear. It is well known that elevated sympathetic activity contributes to the development and maintenance of PCOS [47, 48]; thus, the sympathetic nervous system may offer a novel therapeutic target in treating PCOS. Normal ovulation requires three components, namely, an intact central hypothalamic-pituitary-ovarian axis, synchronized feedback signals, and normal local responses within the ovary [49]. The evidence suggests that low-frequency EA could lower sympathetic activity, and the effects may be mediated by modulation of NGF expression of sympathetic outflow to the ovaries in PCOS-like rats [50]. Zhang et al. reported that the regulation of EA in reproductive function in PCOS-like rats may not be accomplished by the hypothalamic-pituitary-ovarian axis [22, 23], while Maliqueo et al. reported that low-frequency EA significantly affects the pituitary-ovarian axis by normalizing LH secretion [32]. This contradiction is likely due to different PCOS-like rat models and the different forms of acupuncture employed and it needs to be elucidated with further experiments. It has now been demonstrated that the effect of low-frequency EA on ovarian function is mediated as a reflex response via the ovarian sympathetic nerves, and the response is controlled via supraspinal pathways [51]. It has been newly demonstrated that ovarian innervation likely plays an important role in folliculogenesis, and EA might restore PCOS pathophysiology by regulating ovarian innervation, at least partially mediated through the superior ovarian nerve. The effect of EA is based on the integrity of the nervous system [46].

Other hypotheses have also been reported. EA stimulates the development and maturation of eggs in PCOS-like rats by increasing the level of stem cell factor and reducing the level of TNF-α responsible for follicular fluid [26]. EA could increase P450arom and decrease P450c17α as well as the expression levels of their mRNA in ovarian tissues in PCOS-like rats. These effects may thereby change the local ovarian environment of excessive androgen and improve the reproductive, endocrine, and metabolic disorders associated with PCOS [28]. Adiponectin reduces androstenedione synthesis in human theca cells [52]. EA stimulated the ovarian adiponectin system in rats with letrozole-induced PCOS, and the effect does not seem to be mediated by modulation of sympathetic activity [32]. Oxidative stress is now recognized to play a central role in the pathophysiology of PCOS [53]. Zheng et al. reported that EA decreases serum malondialdehyde levels and increases superoxide dismutase levels, hence improving the oxidative stress status of PCOS-like rats [33]. EA not only regulates abnormal glucose and lipid metabolism in PCOS-like rats but also increases glucose transporter 1 and glucose transporter 4 expression in ovarian tissue, which may alleviate insulin resistance [35]. EA increases angiogenesis in the antral follicles of PCOS-like rats, which favours follicle maturation, and ovulation is suggested as being one of the mechanisms involved in the effects of EA on PCOS [54]. It has also been demonstrated that EA regulates androgen receptor and Connexin 43 (which plays an important role in the process of oocyte meiosis and follicular selection) in PCOS-like rats; however, further studies are needed to clarify whether this is one of the mechanisms involved in the effects of EA on PCOS [14]. It has recently been recognized that autophagy is involved in the occurrence and development of PCOS [55]. Huang et al. demonstrated that EA inhibits autophagy in ovarian tissue through the PI3K/AKT pathway [44]. Sterol regulatory element binding protein-1 (SREBP1) is a key gene in lipid metabolism regulation. Peng et al. suggested that EA regulates SREBP1 expression, thereby improving insulin resistance, mitochondrial dysfunction, and oxidative stress in PCOS-like animals [13]. These studies provide novel insights into the mechanisms of EA in PCOS; however, further studies are needed to confirm the findings.

EA and MA are both widely used in clinical practice; interestingly, studies on PCOS animal models demonstrate that the mechanism of their action is not identical. Feng et al. demonstrated that EA regulates neuroendocrine and reproductive functions through the endogenous opioid receptor system and manual stimulation by regulating steroid

Table 3: Quality of evidence assessment.

No. of studies	Study design	Certainty assessment					No. of patients		Effect			
		Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Acupuncture	Control	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
HOMA-IR												
6	Randomised trials	Not serious	Serious[a]	Serious[b]	Serious[c,d]	None	51	51	—	SMD 1.28 lower (2.77 lower to 0.22 higher)	☐x̂x̂x̂ Very low	Critical
T												
8	Randomised trials	Not serious	Serious[a]	Serious[b]	Serious[c]	None	87	84	—	SMD 2.18 lower (3.42 lower to 0.94 lower)	☐x̂x̂x̂ Very low	Critical
LH												
9	Randomised trials	Not serious	Not serious	Serious[b]	Serious[c]	None	94	91	—	SMD 0.71 lower (1.27 lower to 0.15 lower)	☐☐x̂x̂ Low	Important
LH/FSH												
7	Randomised trials	Not serious	Not serious	Serious[b]	Serious[c,d]	None	68	65	—	SMD 0.59 lower (1.29 lower to 0.12 higher)	☐☐x̂x̂ Low	Important
FBG												
6	Randomised trials	Not serious	Not serious	Serious[b]	Serious[c,d]	None	54	57	—	SMD 0.02 lower (0.8 lower to 0.75 higher)	☐☐x̂x̂ Low	Important
FINS												
7	Randomised trials	Not serious	Serious[a]	Serious[b]	Serious[c,d]	None	62	65	—	SMD 1.46 lower (3.39 lower to 0.48 higher)	☐x̂x̂x̂ Very low	Important
BW												
4	Randomised trials	Not serious	Serious a	Serious[b]	Serious[c,d]	None	44	48	—	SMD 1.67 lower (4.04 lower to 0.7 higher)	☐x̂x̂x̂ Very low	Important

GRADE: Grading of Recommendations, Assessment, Development and Evaluation; CI: confidence interval; SMD: standardized mean difference. [a]I values exceed 75%. [b]Different animal modelling methods; differences in treatment cycle and dosage; physiological and pathological differences between rodents and humans. [c]Small sample size. [d]95% CI included one.

hormone receptors [25]. Johansson et al. reported that MA has a greater effect on glucose tolerance than EA [27]. EA reduces the weight of the subcutaneous fat depot, increases the weight of the soleus muscle, and affects the expression of genes and proteins related to the insulin signaling pathway in the soleus skeletal muscle, while MA improves systemic glucose tolerance and affects gene expression in mesenteric adipose tissue [27]. Benrick et al. further reported that EA has stronger effects on glucose uptake than MA and that it induces more pronounced changes in molecular pathways and improves insulin sensitivity more rapidly, and both EA and MA are equally effective during the poststimulation period [29]. The underlying mechanism of the different actions remains to be elucidated [29].

Different acupoint protocols are employed in studies, and several studies have investigated the different actions. Xu et al. demonstrated that there was no significant difference in body weight when stimulating ST36, SP6, and CV4 separately or in combination. Electrostimulation with ST36 or CV4 alone significantly decreased the T level compared with stimulation with SP6 or their combination. Expression of androgen receptor decreased significantly in the SP6 and CV4 stimulation groups [39]. ST36 significantly improves hormone levels and the expression of receptors in ovarian tissue, but it does not reduce the number of growing follicles. CV4 can regulate follicular development and hormone levels but has no obvious effect on the expression of gonadotropin receptors. The combination group had no evident advantage compared with the single acupoint group [41]. It suggests that the effect of EA at multiple points may not be better than that of a certain empiric point. Whether there is an antagonistic effect on the therapeutic effect of different acupoints is worthy of further study.

4.2. Limitations. The positive findings of acupuncture in PCOS animals should be interpreted with great caution since there were several limitations in the present systematic review. First, the substantial heterogeneity should be taken into consideration. Although we performed sensitivity analysis and subgroup analysis, the reason for the generated high heterogeneity was not identified. The difference between acupuncture protocols and different methods used to induce the PCOS model might contribute to the high heterogeneity. Second, the result of the risk of bias assessed by SYRCLE ROB indicated the methodological weakness of the included studies. A majority of the included studies did not report details of sequence generation, baseline characteristics, allocation concealment, randomizing, blinding, or random outcome assessment, which impaired the power of the evidence generated from the present systematic review.

The main implications for further research are as follows: first, the design of the experiment should follow SYRCLE to minimize the risk of bias; second, the report of acupuncture on PCOS animals should follow the Standards for Reporting Interventions in Controlled Trials of Acupuncture (STRICTA) to prompt better quality reporting on acupuncture interventions and help the interpretation and analysis and enable research replications.

5. Conclusions

The main strengths of this study are that we systematically reviewed acupuncture experiments in PCOS animal models and performed a meta-analysis, which indicated that acupuncture might have the potential to restore hormone levels. Unfortunately, we could not draw a definite conclusion due to the methodological weakness of the included studies and the high heterogeneity. Well-designed studies are needed in the future to fill this gap.

Data Availability

The datasets supporting the conclusions of this article are included within the paper.

Disclosure

The funders do not have any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. This study was registered on OSF (Registration DOI: 10.17605/OSF.IO/FNM37), and the protocol has been submitted as a preprint: "Effect of Acupuncture on Polycystic Ovary Syndrome in Animal Models: Study Protocol for a Systematic Review and Meta-Analysis/Systematic Reviews/2021" [17] (DOI: 10.21203/rs.3.rs-144670/v1).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yan Li, Lijia Zhang, Jinjin Gao, and Jun Yan contributed equally to this work and should be regarded as joint first authors. Yan Li, Jinjin Gao, and Songjiang Liu conceived and designed the study. Lijia Zhang, Jun Yan, Xingu Li, Xiting He, Zhengyi Cui, Junfei Zhao, Fengyi Liu, Xiaowai Liu, Yongfei Liu, Yan Li, Jinjin Gao, and Wan Ren collected the previous related literature and performed the data extraction. Xue Feng performed data analysis. Yan Li, Jinjin Gao, Xiting He, and Hong Jin drafted the manuscript. Lijia Zhang, Jun Yan, and Songjiang Liu were responsible for the revision of the manuscript. All authors approved the final version of the manuscript and approved it for publication.

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Research Article

Body Mass Index Showed No Impact on the Outcome of In Vitro Fertilization in Progestin-Primed Ovarian Stimulation Protocol

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Purpose. To assess whether body mass index (BMI) affects the outcome of in vitro fertilization (IVF) in progestin-primed ovarian stimulation (PPOS) protocol. *Methods*. A retrospective study was conducted in the Reproductive Medicine Center, Renmin Hospital of Wuhan University, from June 2016 to June 2017. 636 infertile women who received PPOS protocol in IVF treatment were divided into three groups according to BMI. The data of basic characteristics, embryological outcomes, and cycle characteristics of controlled ovarian stimulation of different groups were collected and studied. *Result(s)*. There was no significant difference in almost all the basic characteristics, embryological outcomes of controlled ovarian stimulation, and cycle characteristics of controlled ovarian stimulation among the three groups. There was a tendency that the duration of infertility was decreased with the increase of patients' weight, although there was no significant difference ($P = 0.051$). However, overweight patients had a higher fertilization rate than normal weight patients and underweight patients (70.3 vs. 67.7 vs. 66.8, $P = 0.008$), but two-pronuclei (2PN) fertilization rate and cleavage rate showed no significant difference among the three groups. *Conclusion(s)*. BMI showed no impact on the outcome of the ovarian stimulation outcome in PPOS protocol. PPOS protocol may benefit overweight patients, for it attains the same effect with normal patients and requires no increase in gonadotropin (Gn) dose and Gn duration.

1. Introduction

Progestin-primed ovarian stimulation (PPOS) protocol is a new ovarian stimulation regimen based on a freeze-all strategy that uses progestin as an alternative to a gonadotropin-releasing hormone (GnRH) analog for suppressing a premature luteinizing hormone (LH) surge during the follicular phase [1]. PPOS can effectively prevent the activation and transmission phases of 17β-estradiol (E2) induced LH surges and thus serves as an alternative to conventional treatment with GnRH analogs [2]. This new regimen of ovarian stimulation has been proved to availably prevent a premature LH surge and does not compromise oocyte competence in cycles followed by embryo cryopreservation. It has been widely used in patients receiving in vitro fertilization (IVF) since 2016 and showed good IVF outcomes [3, 4]. Indeed, a PPOS protocol may be considered more "user friendly" for the patients in view of its advantage of fewer injections than a conventional protocol [5]. In this study, we showed the efficacy of PPOS protocol in various patients and validated its safety in embryonic morphology. Patients can benefit from this protocol for its effectiveness in

preventing early LH surges and getting satisfactory pregnancy outcomes under much more simple and economic administrations.

Previous studies revealed that various factors may affect and predict clinical outcomes of IVF including age, weight, basal serum follicle-stimulating hormone (FSH) concentration, the number of antral follicles, the newly detected anti-Mullerian hormone (AMH), and so on [6, 7]. According to these researches mentioned above, patients' weight has shown a profound impact on ovarian responses, and it may be related to individual variations. For instance, overweight and underweight patients may suffer a high cycle cancellation rate, poor ovarian response, and a low clinical pregnancy rate and live birth rate after IVF treatments [8–12].

Body mass index (BMI) is usually used as an important factor to calculate the dosage of gonadotropins during the controlled ovarian stimulation (COH). The available evidence about the effects of BMI on the outcome of assisted reproductive technology (ART) is conflicting [13]. Most studies agree that the increase in BMI is related to the increase amounts of gonadotropins used in the process of COH [14], but others found that there is no significant difference in the gonadotropin doses between different BMI groups [15]. Carrell et al. reported that BMI is inversely related to intrafollicular human chorionic gonadotropin (hCG) concentrations, embryo quality, and IVF outcome [16], while some other studies found that BMI has no effect on the final IVF pregnancy outcomes [9, 15, 17]. However, the studies mentioned above were all focused on the traditional COH protocols, such as GnRH agonist (GnRH-a) and GnRH antagonist (GnRH-an) protocols, and there are few studies about PPOS protocol until now.

In conclusion, the impact of BMI on the outcome of PPOS has not yet been evaluated. Therefore, a retrospective study was performed to preliminarily investigate the influence of BMI on PPOS outcome and to provide data for future clinical practice in COH.

2. Patients and Methods

2.1. Study Population. This retrospective study involved 636 infertile women who received PPOS protocol (details as follows) during IVF or intracytoplasmic sperm injection (ICSI) treatment in the Reproductive Medicine Center, Renmin Hospital of Wuhan University, from June 2016 to June 2017. The participants recruited in this study were women who were between 23 and 48 years old, and patients who had chromosomal abnormalities were excluded. According to a previous study about the Chinese BMI and its related risk factors [18], patients were divided into three subgroups according to BMI: $BMI < 18.5\,Kg/m^2$ was assigned to underweight group, $18.5 \leq BMI < 24\,Kg/m^2$ was assigned to normal weight group, and $BMI \geq 24\,Kg/m^2$ was assigned to overweight group.

This study was approved by the ethical committee of Renmin Hospital of Wuhan University, and informed written consent was obtained from all patients (Ethics Number: WDRY2016-K017).

2.2. Controlled Ovarian Stimulation. At the beginning of this process, patients were administrated 10 mg/d medroxyprogesterone acetate (MPA, Beijing ZhongXin Pharmaceutical, China), and they received a daily injection of 150 to 300 IU human menopausal gonadotrophin (hMG, Livzon Pharmaceutical Group Co., China) from menstrual cycle day 2 or 3 on the day of hCG administration. For the purpose of height measurement and clinical requirement, transvaginal ultrasound and serum E2 concentrations were used to monitor the IVF cycle every 2 to 3 days. After testing of the index of E2 concentrations and ovarian responses, the dose of HMG was adjusted according to these indexes. Final oocyte maturation was triggered by 10000 IU hCG (Livzon Pharmaceutical Group Co., China) when three or more dominant follicles reached 18 mm in diameter. Oocyte retrieval was performed 36 hours later under the guidance of transvaginal ultrasound, and all follicles greater than 10 mm in diameter were retrieved.

2.3. IVF Procedures and Embryo Freezing. The procedures of IVF/ICSI, embryo culture, and embryo freezing have previously been described by the colleagues in the same department [19]. Fertilization results were assessed 18 h after IVF/ICSI for the appearance of two distinct pronuclei and two polar bodies. 48 h (day 2) and 72 h (day 3) after oocyte retrieval, the embryo morphology was observed and graded. The grading criteria for the embryos were as follows: grade I (equal size of blastomeres, free of fragmentation), grade II (unequal size of blastomere, fragmentations <20%), grade III (unequal size of blastomere, fragmentations 20%–50%), and grade IV (unequal size of blastomere, fragmentations >50%). Finally, on the 3rd day, all high-quality embryos were cryopreserved, and patients were advised to transfer thawed embryos three months later. The definition of 3 top quality of embryos on day 3 is grade 1–2 embryos at 72 h after oocyte retrieval.

2.4. Data Collection. The basic characteristics such as the age (years), duration of infertility (years), previous pregnancy (%), previous IVF failures, cause of infertility (%) (including tubal factor, endometriosis, dysfunction of ovulation, male factor, and combined factor), antral follicle counts, basal FSH concentration (mIU/mL), basal LH concentration (mIU/mL), and basal E2 concentration (pg/ml) were recorded. The cycle characteristics of controlled ovarian stimulation such as the Gn dose (IU), Gn duration (days), >10 mm follicles on HCG day (*n*), >14 mm follicles on HCG day (*n*), E2 concentration on HCG day (pg/ml), endometrium thickness on hCG day (mm), oocytes retrieved (*n*), fertilized eggs (*n*), cleaved embryos (*n*), fertilization rate (%), cleavage rate (%), 2PN fertilization rate (%), 2PN cleavage rate (%), cancellation rate of the cycle (%), and top quality of embryos of day 3 (*n*) were calculated, respectively.

2.5. Hormone Measurement. The serum basal levels of FSH, LH, and E2 on the 2nd day of the menstrual cycle and E2 on HCG day were also detected. Hormone levels were measured by the chemiluminescence method (Simens, ADVIA Centaur System, USA).

2.6. Statistical Analysis. Continuous data were presented as mean ± standard deviation (SD) and tested using the ANOVA test. Noncontinuous data were presented as a percentage (%) and tested using the chi-Square test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the IBM SPSS Statistic 23 (IBM, Armonk, NY, USA).

3. Results

3.1. Patients' Characteristics. There was no significant difference in almost all the basic characteristics between the three groups, including age, previous pregnancy rate, antral follicle counts, basal hormone concentration, and the causes of infertility. There was a tendency that the duration of infertility was decreased by the patients' weight, although there was no significant difference ($P = 0.051$) (Table 1).

3.2. Ovarian Stimulation and Embryologic Characteristics. During the ovulation stimulation processes, not only the gonadotropins (Gn) dose and Gn duration but also the follicles with diameter greater than 10 mm or 14 mm, E2 concentration, and endometrium thickness on HCG day showed no statistically significant differences among different weight groups. There was no significance in the number of oocyte retrieved and top quality of embryos on day 3. However, overweight patients had a higher fertilization rate than normal weight patients and underweight patients (70.3 vs. 67.7 vs. 66.8, $P = 0.008$), but two-pronuclei (2PN) fertilization rate, cleavage rate, and the cancellation rate of the cycle showed no significant difference among the three groups (Table 2).

4. Discussion

With the utilization of GnRH-a and GnRH-an protocols in controlled ovarian stimulation, the outcome of clinical ovarian stimulation treatment has been obviously promoted. However, there are still some patients suffering from cycle cancelled due to early surges of LH and lack of oocytes, especially for the patients who have a poor ovarian reserve. Kuang et al. firstly reported that using HMG and letrozole in luteal phase can effectively reduce the early surge of LH and can have an optimal pregnancy in the following frozen embryo transfer (FET) treatment for patients who have normal ovarian reserve [4]. Furthermore, they applied this method, which was termed as PPOS protocol later on, artificially creating a high level of progesterone in follicular phase by oral progesterone feeding, and acquired great outcomes in ovarian stimulation and pregnancy [1, 20].

The critical point of PPOS protocol is the continuous high level of progesterone, which could block the positive feedback of the estrogen and can effectively restrain the emergence of LH surges. PPOS protocol has been verified functional in patients with polycystic ovarian syndrome (PCOS) [21] or poor ovarian reserve [22] for its success in the reduction of LH surges. Kuang et al. reported that PPOS protocol in combination with embryo cryopreservation as an ovarian stimulation regimen was as effective as GnRHa long protocol during COH under different endocrine mechanisms [23]. In view of the amount of the benefits for the patients and the fast developments in FET technology, which ensured the security and interests of patients, such as the reduction in administration time and the relief of patients' economic burden, PPOS protocol has become an optimal protocol in patients undergoing IVF treatments. It has been a preferred solution in many Chinese IVF centers for patients with poor ovarian reserve nowadays. As all embryos should be frozen in this protocol, it is also used in these high responders to prevent ovarian hyperstimulation syndrome (OHSS) in our center.

Clinical observations over the effects of BMI on the ovarian stimulation and pregnancy outcome in other protocols are still controversial. In some studies, scholars came to the conclusion that BMI had no effect on the final IVF pregnancy outcome [9, 17], while the others observed oppositely [8, 10] that increasing BMI did not adversely affect the outcome of IVF in nonobese endometriosis patients [15]. Nevertheless, it was acknowledged that overweight patients require a high dose of Gn [9, 17], a long duration of treatment period [9, 10], a lower estradiol concentration peak [8, 9], even a reduction in oocyte retrieved [17], and an increase in cycle cancellation [10, 17]; despite lack of evidence due to the small number of patients, underweight patients tend to have less embryos portable as some studies mentioned [12]. According to the above conclusion, high-weight patients and low-weight patients have their own advantages and disadvantages which may cause BMI to have no impact on the outcome of in vitro fertilization in progestin-primed ovarian stimulation protocol. Furthermore, our results showed that BMI affects neither the Gn dose and duration nor the oocyte retrieved and top quality of embryos on day 3, which may reveal that BMI may not affect the ovarian stimulation outcome in PPOS protocol.

What is interesting is that the current study showed that the fertilization rate in the overweight group was higher than that of the normal and underweight patients, which pointed out that obesity may affect the fertilization process, while 2PN fertilization rate and cleavage rate showed no significant difference among the three groups. A probable explanation for this elevation could be the higher percentage of PCOS patients in the overweight group, who were detected to have a higher rate of abnormal fertilization rate, which was in agreement with a previous study reported by Beydoun et al. [24]. Usually, obesity may be responsible for the Gn resistance during ovarian stimulation according to the previous research [25], possibly due to the overstimulation of ovarian steroidogenesis and decrease of sex hormone-binding globulin blood concentrations mediated by insulin [21]. But there was no significant difference among different weight groups in terms of Gn dose and Gn duration in our study, which means that the high progestin levels in PPOS protocol may partially alleviate the endocrine disorder, and PPOS protocol has potential benefits for overweight patients.

Few studies have shown statistical differences between underweight patients and overweight patients. Some research studies indicated that underweight patients got less

Table 1: Basic characteristics and hormonal profile of different weight patients.

Characteristic	Normal weight ($n = 418$)	Overweight ($n = 173$)	Underweight ($n = 45$)	P
Age (years)	36.2 ± 5.1	36.0 ± 5.8	35.7 ± 5.3	0.801
Duration of infertility (years)	6.3 ± 5.1	5.6 ± 5.0	7.7 ± 6.5	0.051
Previous pregnancy (%)	65.1	68.8	62.2	0.595
Previous IVF failures	0.9 ± 1.0	0.9 ± 1.0	1.1 ± 1.0	0.366
Cause of infertility (%)				
Tubal factor	244 (58.3)	97 (56.1)	29 (64.4)	
Endometriosis	20 (4.8)	17 (9.8)	3 (6.7)	
Dysfunction of ovulation	60 (14.4)	32 (18.5)	5 (11.1)	0.145
Male factor	35 (8.4)	9 (5.2)	1 (2.2)	
Combined factor	59 (14.1)	18 (10.4)	7 (15.6)	
Antral follicle counts	7.9 ± 4.6	7.3 ± 3.7	8.0 ± 4.8	0.249
Basal FSH concentration (mIU/mL)	9.6 ± 4.7	9.6 ± 4.7	9.5 ± 4.4	0.993
Basal LH concentration (mIU/mL)	3.9 ± 2.1	3.8 ± 2.0	3.8 ± 1.9	0.967
Basal E2 concentration (pg/ml)	53.1 ± 43.9	55.8 ± 52.5	55.8 ± 44.9	0.785

Table 2: The cycle characteristics of controlled ovarian stimulation of different weight patients.

Characteristic	Normal weight ($n = 418$)	Overweight ($n = 173$)	Underweight ($n = 45$)	P
Gn dose (IU)	2458 ± 897	2451 ± 810	2695 ± 871	0.207
Gn duration (days)	9.8 ± 2.9	9.8 ± 2.8	9.9 ± 3.2	0.991
>10 mm follicles on HCG day (n)	6.6 ± 5.1	6.9 ± 4.8	7.2 ± 4.1	0.573
>14 mm follicles on HCG day (n)	3.9 ± 2.8	4.0 ± 2.5	4.0 ± 2.2	0.947
E2 concentration on HCG day (pg/ml)	1452 ± 1026	1611 ± 1031	1409 ± 946	0.196
Endometrium thickness on hCG day (mm)	7.8 ± 1.8	7.7 ± 1.8	8.3 ± 2.4	0.139
Oocytes retrieved (n)	4.3 ± 3.5	4.6 ± 3.3	4.6 ± 2.6	0.622
Fertilized eggs (n)	2.9 ± 2.7	3.2 ± 2.8	3.0 ± 2.0	0.430
Cleaved embryos (n)	2.8 ± 2.6	3.1 ± 2.7	2.9 ± 2.0	0.436
Fertilization rate (%)	67.7	70.3	66.8	0.008
Cleavage rate (%)	96.8	97.0	95.6	0.724
2PN fertilization rate (%)	57.7	58.7	54.6	0.567
2PN cleavage rate (%)	83.8	82.5	80.3	0.517
Cancellation rate of the cycle (%)	21.8	17.3	20.0	0.477
Top quality of embryos of day 3 (n)	1.7 ± 1.7	1.8 ± 1.9	1.7 ± 1.4	0.932

embryos [12] as slim patients may face an obstacle of oocyte maturation inhibition. This study found that the underweight patients did not show a poorer ovarian response or a worse embryos outcome compared to normal weight or overweight group, which shows that the PPOS protocol may also have potential benefits for underweight patients.

Since all the embryos were frozen in this protocol and patients should perform the embryo transfer at least one month later, some patients may take much longer time, and only part of these patients have completed the FET process, so the FET pregnancy outcome was not included in the current study, which made it hard to draw a conclusion about whether PPOS has affected the final pregnancy outcome. Moreover, it has been recognized that obesity increased the risk of pregnancy complications, even in natural pregnancy [26]; in the PPOS protocol, the FET outcome may not be the best indicators of the effect of BMI. The FET outcome will be studied in our following studies.

In conclusion, BMI showed no impact on the outcomes of the controlled ovarian stimulation in PPOS protocol. This finding has important implications for overweight patients, as it attains the same effect with normal patients and requires no increase in Gn dose and Gn duration. However, our studies were limited by the sample size and difficulties in reviewing all final pregnancy outcomes. Therefore, further prospective studies with larger sample size and tracking of further results of FET outcomes of PPOS patients should be performed.

Data Availability

All of the original data in this study are from the Reproductive Medicine Management System of the Reproductive Center of Renmin Hospital of Wuhan University.

Disclosure

The abstract of this article has been included in the 2019 IFFS (International Federation of Fertility Societies) Shanghai World Congress.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Zhe Yang and Xuehan Zhao contributed equally to this study.

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Research Article

Erzhi Tiangui Granules Improve In Vitro Fertilization Outcomes in Infertile Women with Advanced Age

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Background. The fertility of females with advanced age declines with aging. Therefore, for medical and social reasons, it is important to establish mechanisms to protect and improve the fertility of such populations. With widespread use of traditional Chinese medicine (TCM) in in vitro fertilization (IVF), studies have evaluated their impact on improving the fertility of females with advanced age. In this study, we performed proteomic analysis of follicular fluid to reveal mechanisms of the Erzhi Tiangui (EZTG) granule (Chinese herbs for replenishing vital essence to tonify the kidney) in improving the outcomes of IVF in infertile women with advanced age. *Methods*. This was a randomized, double-blind, and placebo-controlled trial in which 100 patients with advanced age were divided into the EZTG group and the placebo group by the random number table plus envelope method. Both groups were subjected to controlled ovarian stimulation with a GnRH antagonist regimen. Differences between the two groups were evaluated, including the TCM syndrome score after treatment, gonadotrophin (Gn) days and Gn doses, the number of retrieved oocytes, 2 pronucleus (PN) fertilization, 2PN cleavage, and high-quality embryos. Differentially expressed proteins were identified using the LC-MS/MS method, and their functions were determined through bioinformatics analyses. *Results*. The number of high-quality embryos in the placebo group was significantly lower than that in the EZTG group (2.88 ± 1.85 vs. 4.13 ± 2.83, $p = 0.011$). Eleven differentially expressed proteins were identified between the two groups. Four proteins were highly expressed, whereas seven were suppressed in the control group, compared to the EZTG group. The overall trend suggested that the apoptotic effect in the follicular fluid of the EZTG group was downregulated. *Conclusion*. Treatment with the EZTG granule can improve embryo quality in IVF of advanced age females with both kidney Qi and Yin deficiency syndromes. The mechanism is attributed to downregulation of apoptotic-effector protein expressions in the follicular fluid. This trial is registered with ChiCTR1900025139.

1. Introduction

The threshold for female reproductive aging is ≥35 years [1–3]. Due to increasing late marriages and childbearing (especially since October 2015), the proportion of pregnancy among women with advanced age has further increased, specifically with the "two-child policy" in China. Since fertility decreases with age [4, 5], older women with reduced or lost fertility often seek help from assisted reproductive technologies (ART). There are several adjuvant treatment strategies for infertile advanced age women undergoing IVF, including coenzyme Q10 [6], dehydroepiandrosterone (DHEA) [7], growth hormone (GH) [8], and recombinant luteinizing hormone (r-LH) [9]. However, it has not been determined whether these drugs can improve pregnancy outcomes in advanced age women, especially older women with decreased ovarian reserves.

Kidney-tonifying TCM plays a universal role in delaying human life aging and prolonging the human reproductive period [10–12]. By evaluating the efficacy and mechanism of kidney-tonifying TCM in ART superovulation, we found that kidney-tonifying can improve oocyte quality through a variety

of pathways and improve normal fertilization rates, cleavage rates, and pregnancy rates of in vitro fertilization-embryo transfer in patients with kidney deficiency syndrome [13].

Studies involving TCM are based on two mechanisms: "holistic concept" and "syndrome differentiation and treatment." The decline in female fertility involves multisystem, multiway, and multilink changes of the body, including proteomics. Proteomics, as a component of system biology, reflects the holistic concept of TCM and shows the advantages and roles of TCM in promoting fertility in women with advanced age. Therefore, we performed proteomic analysis of the follicular fluid to establish the mechanisms of Erzhi Tiangui (EZTG) granule (a TCM for replenishing vital essence to tonify the kidney) in improving IVF outcomes in infertile women with advanced age. Our findings provide a scientific, effective, and systematic theory and elucidate on the method of TCM for protecting the fertility of such populations.

2. Materials and Methods

2.1. Patient Enrollment and Grouping. This study is registered in the Chinese Clinical Trial Registry (ChiCTR1900025139). The 100 infertile patients were recruited from the Reproductive and Genetic Center of Integrated Traditional and Western Medicine, the Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China (PRC) from June 2015 to December 2016. Eligible participants were infertile patients undergoing IVF/intracytoplasmic sperm injection (ICSI) procedures without contraindications for adverse IVF/ICSI treatment outcomes.

Participant enrollment was performed by staff not involved in the randomization process. A computer-based random number generator was used for grouping, according to the random number allocation sequence. Women with advanced age were allocated into two groups based on computer-assisted block randomization. Sequence generation and assignment of participants to the experimental and control groups were made by a study staff member who was not involved in intervention delivery, data collection, or data analysis. Both medications (Erzhi Tiangui formula and placebo) were prepared, so that they were identical in shape, taste, and smell. The experimental group ($n = 50$) was orally administered with Erzhi Tiangui, while the control group ($n = 50$) was administered with a placebo.

2.2. Criteria for Syndrome Differentiation for Qi and Yin Kidney Deficiencies

(i) Primary symptoms are as follows: (i) light menstrual color and thin texture, (ii) fatigue, (iii) lumbosacral soreness, (iv) light red tongue with dental marks and a thin white or less coating, and (v) both chi pulses exhibit a deep thready pulse or a deep thready and rapid pulse.

(ii) Secondary syndromes are as follows: (i) dizziness and tinnitus, (ii) dry mouth and throat, (iii) dry vagina, and (iv) lower leg ache or talalgia.

The kidney Qi and Yin deficiency syndrome was diagnosed only by the presence of all primary syndromes with one or two secondary syndromes.

2.3. Inclusion and Exclusion Criteria. Married women aged between 35 and 44 years old, receiving autologous oocytes and who had kidney Qi and Yin deficiency syndromes were enrolled in the study. Patients diagnosed with premature ovarian insufficiency and who had body mass index (BMI) of $\geq 30\,kg/m^2$, endometriosis, polycystic ovarian syndrome, severe malformation of reproductive organs, major operation history, and had used hormonal drugs within 3 months before the study were excluded. A couple with karyotyping abnormalities was also excluded.

2.4. Preparation of the Erzhi Tiangui Granule and the Placebo. The Drug Manufacturing Unit of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine produced the EZTG granule. The EZTG was packaged as 3 g/bag, batch number 01-FZ032-03. The daily dose is equivalent to 15 g of *Ligustrum lucidum* (Nv Zhen Zi), 15 g of *Lotus japonicus* (Han Lian Cao), 15 g of the fruit of Chinese wolfberry (Gou Qi Zi), 15 g of *Cuscuta chinensis* (Tu Si Zi), 15 g of Radix Rehmanniae Preparata (Shu Di Huang), 12 g of *Angelica sinensis* (Dang Gui), 12 g of *Paeonia lactiflora* (Bai Shao), 12 g of *Ligusticum wallichii* (Chuan Xiong), 12 g of *Rhizoma cyperi* (Xiang Fu), and 9 g of Radix Glycyrrhizae Preparata (Zhi Gan Cao). The placebo granule, which was mainly composed of dextrin, was made in a similar color and shape to EZTG. Placebo granules were packaged as 3 g/bag, with the same package of the EZTG, batch number 01-FZ032-03-1. The EZTG or placebo was orally administered after being dissolved in water, 3 g each time, 2 times a day.

2.5. In Vitro Fertilization and Sample Collection. After the 2nd to 3rd day of menstruation and 150–300 units of exogenous gonadotropin controlled ovarian stimulation, the EZTG group was administered with Erzhi Tiangui granules, while placebo granules were administered to the placebo group for 11–14 days. After vaginal ultrasound confirmation of the follicle diameter of between 18 and 20 mm, participants were administered with a single dose of 10000 units of HCG (human chorionic gonadotropin) as a "trigger." Then, 36 h after HCG injection, oocyte retrieval and extraction of ovarian granulosa cells were conducted under transvaginal ultrasound.

The follicular fluid was obtained after oocyte retrieval, and the presence of a cumulus complex was confirmed by inverted microscopy. After centrifugation for 10 min at 3000 rpm to separate red blood cells, leucocytes, and follicle cells, the supernatant was recovered in Eppendorf (EP) tubes, labeled and refrigerated at −80°C for further examination.

2.6. Sample Preparation. Briefly, XX of protein was supplemented with 50 mM ammonium bicarbonate to YY, and DTT was added to a final concentration of 10 mM for 60 min

at 37°C. Iodoacetamide was added to the solution to a final concentration of 50 mM for 30 min at room temperatures in the dark. The solution was transferred to the ultrafiltration tube and centrifuged for 10 min at 14,000 rpm. Pellets were rinsed twice using 50 mM ammonium bicarbonate (containing 0.8% SDC). Trypsin was added after which samples were enzymatically hydrolyzed under incubation at 37°C for 12–16 h. Then, the enzymatic hydrolysates were rinsed using 50 mM ammonium bicarbonate and merged, followed by TFA acidification shaking, centrifugation to remove SDC, and desalting with a C18 desalting column. Desalted samples were lyophilized in a freeze-dryer and redissolved in 0.1% FA for mass spectrometry detection.

2.7. Liquid Chromatography-Mass Spectrometry (LC-MS/MS) Analysis. Desalting of 100 mg lyophilized TMT-labeled peptide pools was performed on a 100 mg C18 solid-phase extraction column Sep-Pak (Waters, Wilmslow, UK) and further fractionated using either reverse-phase chromatography combined with elution at a high pH, isoelectric focusing on an Agilent 3100 OFFGEL fractionator (Agilent, Santa Clara, CA, USA) or HILIC chromatography. Each time, 18–24 fractions were collected and analyzed using a nanoflow LC-MS/MS. Nanoflow LC-MS/MS was performed on an 1100 series capillary LC system (Agilent) coupled with an LTQ-Orbitrap mass spectrometer (Thermo Scientific) operating in the positive mode and equipped with a nanoarray source. Peptide mixtures were trapped in a ReproSil C18 reverse-phase column (column dimensions: 1.5 cm × 100 μm, packed in-house; Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) at a flow rate of 8 μL/min. Peptide separation was performed on the ReproSil C18 reverse-phase column (column dimensions: 15 cm × 50 μm, packed in-house; Dr. Maisch GmbH) using a linear gradient from 0 to 80% B (A = 0.1% formic acid; B = 80% (v/v) acetonitrile, 0.1% formic acid) in 70 min and at a constant flow rate of 200 nL/min using a splitter. Column eluent was directly sprayed into the ESI source of the mass spectrometer. Mass spectra were acquired in a continuum mode and peptide fragmentation performed in a data-dependent mode.

2.8. Database Retrieval and MaxQuant Analysis. Data retrieval from databases was performed using the Proteome Discoverer software (Version PD1.4, Thermo Scientific, city, USA). Protein data were retrieved from the UniProt database (owner, city, country), with the maximum deviation of parent ion molecular weight not exceeding 10 ppm and the maximum deviation of daughter ion molecular weight not exceeding 0.02 Da. Peptide false discovery rate (FDR) was set to <1%. For each group, the experiment was repeated thrice using the MaxQuant software (Version 1.4.0.8, owner(s), city, country). We used "uniprot_Proteomes-Human.fasta" to search the original files. After configuring database files, 12 samples of original files were imported into the analysis software. Corresponding label-free quantification parameters were set and imported into the database for retrieval. The retrieved results were screened using the strict criteria (peptide FDR ≤1% and protein FDR ≤1%). For proteins to be used for quantification, they were to have ≥2 characteristic polypeptides.

2.9. Statistical Analysis. Statistical analysis was performed using the SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant. Quantitative data for each group were expressed as mean ± SD. The data were not statistically described using the median and quartile deviation. Comparison of means between two groups was performed using Student's t-test. Proportions were compared using the chi-square test.

3. Results

3.1. Patient Enrollment. After controlled ovarian stimulation, 3 patients canceled the IVF cycles due to failure to obtain oocytes, including two in the EZTG group and one in the placebo group. The remaining 97 women completed the follow-up without major protocol violations, and all were included in outcome analyses (Figure 1). Compared to the placebo group, the EZTG group did not exhibit statistical differences in all characteristics except for the number of high-quality embryos, which was high (2.88 ± 1.85 vs. 4.13 ± 2.83, $p = 0.011$) (Table 1).

3.2. Quantitative Analysis of Proteins. The LFQ values from MaxQuant analysis were used to characterize protein abundance. The LFQ value of the total protein for each sample was corrected. Differential proteins between EZTG and control groups were screened using a protein abundance ratio of >1.67 and <0.6. A small number of proteins that were relatively specific in the follicular fluid and played a localized role was reduced to >1.1 and <0.9. After comparisons, 11 differentially expressed proteins between the placebo and EZTG groups were identified. Four proteins were highly expressed, whereas seven were suppressed in the placebo group (Table 2).

3.3. Biological Function Analysis of Differentially Expressed Proteins. The differentially expressed proteins were found to be involved in physiological processes, such as lipid metabolism, immunity, cell differentiation, proliferation, and apoptosis. Some of these proteins may be involved in several different biological function processes (Table 3).

3.4. Safety Observation in Clinical Trials. Patients in the EZTG group and the placebo group had no adverse reactions during the medication period.

4. Discussion

Acceleration of apoptotic effects in elderly women results in a decline in egg and embryo quality. Abnormal growth and development of follicles and accelerated apoptosis of granulosa cells lead to follicular atresia and are the

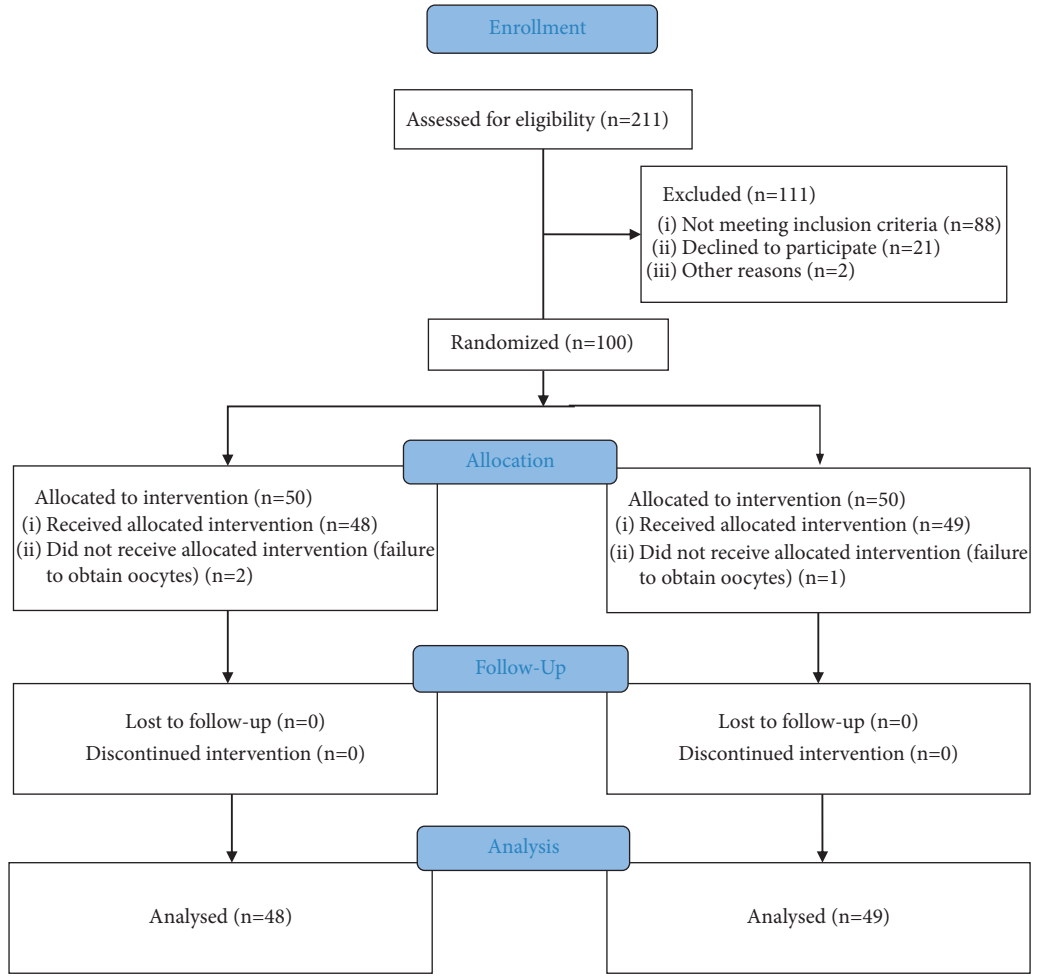

Figure 1: CONSORT 2010 flow diagram.

Table 1: Characteristics of study participants.

Parameter	EZTG group	Placebo group	*P* value
Patients	48	49	—
Age (years)	38.92 ± 4.43	37.61 ± 4.22	0.139
BMI (kg/m^2)	25.42 ± 5.73	24.05 ± 4.33	0.187
AMH (ng/ml)	1.75 ± 0.52	1.90 ± 0.41	0.118
Baseline day 3 FSH (mIU/ml)	10.53 ± 1.72	10.00 ± 2.16	0.185
Baseline day 3 E_2 (pg/ml)	49.70 ± 11.83	53.50 ± 11.20	0.108
E_2 on HCG day (pg/ml)	4258.75 ± 1135.11	4509.83 ± 1249.35	0.303
P on HCG day (ng/ml)	1.29 ± 0.26	1.39 ± 0.33	0.101
TCM clinical syndrome score	13.24 ± 2.17	14.60 ± 3.52	0.155
Gn days (d)	9.26 ± 2.28	9.78 ± 2.79	0.318
Gn doses (U)	3450.75 ± 1237.55	3625.85 ± 1803.25	0.579
Retrieved oocytes (*n*)	8.13 ± 3.22	7.85 ± 2.55	0.636
No. of 2PN fertilization	6.91 ± 2.87	5.87 ± 2.66	0.067
No. of 2PN cleavage	6.11 ± 2.13	5.39 ± 2.74	0.152
No. of good quality embryos	4.13 ± 2.83	2.88 ± 1.85	**0.011**
Transferred embryos per cycle	1.75 ± 0.53	1.80 ± 0.50	0.634
Cumulative pregnancy rate	32.4% (36/111)	28.7% (27/94)	0.566

AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; E_2, estradiol; P, progesterone; TCM, traditional Chinese medicine; Gn, gonadotropin. $P < 0.05$ indicates a statistically significant difference between the 2 groups of data. The value in bold indicates that the number of high-quality embryos was significantly increased in the EZTG group compared with the placebo group.

Table 2: Identification of differentially expressed proteins in follicular fluids of the EZTG group and placebo group.

Protein IDs	Protein names	Gene names	Abundance ratio	Up/down
P02753	Retinol-binding protein 4	RBP4	1.86	Up
P08637	Low affinity immunoglobulin gamma Fc region receptor III-A	FCGR3A	1.69	Up
P24592	Insulin-like growth factor binding protein-6	IGFBP-6	1.17	Up
P17936	Insulin-like growth factor binding protein-3	IGFBP-3	1.13	Up
P01033	Metalloproteinase inhibitor-1	TIMP-1	0.87	Down
Q15582	Transforming growth factor beta-induced protein ig-h3	TGFBI	0.82	Down
P19438-5	Isoform 5 of tumor necrosis factor receptor superfamily member 1A	TNFRSF1A	0.71	Down
P35858	Insulin-like growth factor binding protein complex acid-labile subunit	IGFALS	0.7	Down
Q12789-3	Isoform 2 of the general transcription factor 3C polypeptide 1	GTF3C1	0.63	Down
Q8NEF3	Coiled-coil domain-containing protein 112	CCDC112	0.6	Down
Q07864	DNA polymerase epsilon catalytic subunit A	POLE	0.56	Down

Abundance ratio, EZTG group/placebo group.

Table 3: Biological function analysis for differentially expressed proteins between EZTG and placebo groups.

Biological function	Identified differential proteins
Lipid metabolism	Retinol-binding, protein 4 (RBP4)
Immunization	Low affinity immunoglobulin gamma Fc region receptor III-A (FCGR3A)
Cell differentiation, proliferation and apoptosis	Retinol-binding protein 4 (RBP4), metalloproteinase inhibitor-1 (TIMP-1), insulin-like growth factor binding protein-3 (IGFBP-3), isoform 5 of the tumor necrosis factor receptor superfamily member 1A (TNFRSF1A), insulin-like growth factor binding protein-6 (IGFBP-6), insulin-like growth factor binding protein complex acid-labile subunit (IGFALS), DNA polymerase epsilon catalytic subunit A (POLE), isoform 2 of general transcription factor 3C polypeptide 1 (GTF3C1), transforming growth factor beta-induced protein ig-h3 (TGFBI), and coiled-coil domain-containing protein 112 (CCDC112)

fundamental causes of the decline in ovarian reserve functions and fertility in elderly women [14].

Many of the differential proteins found in this study are involved in apoptotic and proliferative processes. They include matrix metalloproteinases (MMPs), which are crucial in apoptosis, and metalloproteinase inhibitor-1 (TIMP-1), which binds MMPs to suppress their biological activities. In addition, TIMP-1 directly binds cell surface receptors to promote the proliferation of fibroblasts, epithelial cells, smooth muscle cells, and lymphocytes [15, 16]. Anti-apoptotic effects of TIMP-1 are associated with its direct inhibition of caspase 3 activity [17].

Insulin-like growth factor binding protein-3 (IGFBP-3) and IGFBP-6 compete with IGF receptors to bind IGF, thereby blocking IGF receptor formation and inhibiting IGF activity. Moreover, IGFBP-3 has an independent role in promoting apoptosis [18]. The insulin-like growth factor binding protein complex acid-labile subunit (IGFALS) binds free IGFs to form heterotrimers, which may significantly prolong the half-life of IGFs and enhance the effects of IGFs in promoting cell proliferation and inhibiting cell apoptosis [19, 20].

Transforming growth factor beta-inducible protein ig-h3 (TGFβ Ip) is important in cell adhesion, migration, proliferation, and differentiation [21]. Retinol-binding protein 4 (RBP4), a new adipocytokine, participates in the pathological process of human insulin resistance and is associated with the lipid metabolism [22]. RBP4 has been shown to inhibit the first polar body excretion of porcine oocytes. The expressions of GDF-9 and BMP-15 in porcine oocytes treated with RBP4 interventions were found to be significantly suppressed, implying that these proteins might be involved in the apoptotic process [23]. Coiled-coil domain protein (CCDC) is an important regulator of the cell division cycle, and downregulation of CCDC can lead to decreased cyclin A expression, which means cell cycle arrest. Flow cytometry revealed that the proportion of cells in the G1 phase increased, whereas the proportion of cells in S phase and G2/M phase decreased after downregulation of CCDC expression [24]. Comparing the changes in follicular fluid protein expressions between the EZTG and the placebo groups, we found that EZTG prescription, a TCM for tonifying kidney and nourishing Yin, inhibited the apoptosis of cells in follicles to a certain extent and promoted cell proliferation, differentiation, and repair.

The pregnancy rate in IVF-ET cycles is mainly determined by the quality of embryos transferred and the receptivity of the endometrium to embryos, especially the former [25–27]. We found that the number of high-quality embryos per oocyte retrieval cycle was significantly increased in the EZTG group when compared to the placebo group. This significant change in clinical outcomes can improve the chances of successful clinical pregnancy.

According to TCM, women naturally have the essence of kidney deficiency after their "Qi Qi" age. During implementation of modern ART, multiple ovarian follicles are induced to simultaneously develop with gonadotropins. As the human body synchronously develops and matures a large number of follicles in a short period, the kidney secretes a large amount of skull and kidney essence to promote

transient exuberance of kidney Qi. This may be the reason as to why syndrome scores of the two groups after treatment were lower than before treatment, with no significant differences between groups. Behind this phenomenon must be the loss of kidney Qi and kidney Yin (kidney essence), which enhances the essence of kidney deficiency in elderly women. The classic saying "Yang transforms Qi and Yin forms." Yin shaping refers to the process by which Yin and Qi dominate the static state, and the invisible substance is formed into a tangible substance in the movement of "calming." The "shaping" process involves follicle's self-development, maturation, and ovulation and increase in follicle volume, follicular fluid, and proliferation of granulosa cells. They are the functional aspects of kidney Yin. The process of follicular development and shaping is inseparable from cell proliferation. Therefore, the function of kidney Yin "shaping" predominates the whole process of follicular development.

Lian, using a kidney invigoration and Yin nourishment technique, formulated the EZTG prescription [28, 29]. The EZTG prescription replenishes vital essence to tonify kidneys, nourishing blood, and regulating Chong. During follicular development, especially the development of multiple follicles, abundant kidney Yin and decayed water from the human body is required. However, kidney Yin and decayed water cannot be autogenously indefinite. This could be amended by the EZTG prescription, which mobilizes human kidney functions, increases kidney Yin and decayed water reserves, and provides the products needed for the development of multiple follicles through targeted kidney tonification and Yin replenishment. This "reproductive kidney" of the ovary can be excreted to promote the maturation and ovulation of multiple follicles. Normal ovulation functions can greatly improve the chances of conception, the so-called "Jing Tiao Zi Si."

In this study, identification of TCM syndromes was carefully performed by experienced clinicians. Due to individual differences among patients, symptomatic severity was different. The overall dose of TCM granules cannot be adjusted. Therefore, the inability of the total dose of EZTG granules to adjust according to symptomatic severity is a limitation of this study. Moreover, we did not determine any side effects associated with the EZTG granules. In conclusion, the EZTG granule had a positive effect on advanced age women with both kidney Qi and Yin deficiency syndromes.

5. Conclusions

The EZTG prescription intervention can lead to differential expression of some apoptosis-related proteins in the granulosa cell follicular fluid. These proteins should be quantitatively analyzed to establish the specific mechanism involved in replenishing vital essence to tonify kidneys during ovarian granulosa cell apoptosis and their role in assisted reproduction.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Highlights. (1) What do we know about this topic? In China, Chinese herbs for replenishing vital essence to tonify the kidney can improve in vitro fertilization outcomes in infertile women with advanced age. However, their specific roles and internal mechanisms have not been clearly evaluated. (2) How does our research contribute to this field? From the perspective of follicular fluid proteomics, we established the mechanism involved in replenishing vital essence to tonify the kidneys in order to improve in vitro fertilization outcomes in infertile women with advanced age. (3) What are the implications of our research in theory, practice, or policy? Chinese herbs for replenishing vital essence to tonify the kidney should be popularized and applied to benefit infertile women with advanced age.

Ethical Approval

The Ethics Committee of Reproductive Medicine of the Affiliated Hospital of Shandong University of TCM approved this study (AF/SC-08/01.0).

Consent

Participants submitted written informed consents before participation.

Disclosure

Jinlong Sun and Jing-Yan Song are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jinlong Sun and Yao Dong conceived and designed the study. Qiong Guo drafted and critically revised the manuscript for important intellectual content. Yao Dong sought the ethical approval. Jinlong Sun and Shan Xiang coordinated the study and involved in participant recruitment. All authors contributed to manuscript development and approved the final manuscript.

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Research Article

Network Pharmacology Approach for Predicting Targets of Zishen Yutai Pills on Premature Ovarian Insufficiency

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Background and Purpose. Premature ovarian insufficiency (POI) is a serious reproductive disease in females that is characterized by menstrual and ovulation disorders and infertility. The clinical efficacy of complementary and alternative medicine (CAM) has been reported in POI, including compound Chinese medicine. Zishen Yutai Pills (ZSYTP), a well-known patented Chinese medicine, has been widely used for treating POI; however, the pharmacological mechanism and molecular targets of ZSYTP remain unknown. Here, we systematically elucidated the pharmacological mechanism of ZSYTP on POI using a network pharmacology approach and further validated our findings with molecular docking. *Methods*. A comprehensive strategy based on several Chinese herb databases and chemical compound databases was established to screen active compounds of ZSYTP and predict target genes. For network pharmacological analysis, network construction and gene enrichment analysis were conducted and further verified by molecular docking. *Results*. A total of 476 target genes of ZSYTP were obtained from 205 active compounds. 13 herbs of ZSYTP overlapped on 8 active compounds based on the compound-target-disease network (C-T network). 20 biological processes and 9 pathways were strongly connected to the targets of ZSYTP in treating POI, including negative regulation of gene expression, mRNA metabolic process, hypoxia-inducible factor 1 (HIF-1) signaling pathway, and gluconeogenesis. Finally, molecular docking was visualized. *Conclusion*. Intriguingly, the signal pathways and biological processes uncovered in this study implicate inflamm-aging and glucose metabolism as potential pathological mechanisms of POI. The therapeutic effect of ZSYTP could be mediated by regulating glucose metabolism and HIF-1 signal pathway. Collectively, this study sheds light on the therapeutic potential of ZSYTP on POI.

1. Introduction

Premature ovarian insufficiency (POI) is a common reproductive endocrine disorder in females of childbearing age. POI is characterized by amenorrhea before the age of 40, with high gonadotropin and low estrogen [1]. Endocrine dysfunction can cause atrophy of the ovary, hot flashes, depression, and insomnia, all of which are symptoms of perimenopausal women [2]. Owing to the low levels of estrogen, women with POI may suffer from chronic complications, including osteoporosis and cardiovascular disease. As ovarian functional decline affects ovulation, the pregnancy and live birth rates dramatically decrease [3]. POI not only leads to reproductive disorders but also shortens a patient's life span, which has a negative impact on the quality of life [4, 5]. The conventional treatment for POI is hormone replacement therapy (HRT), which relieves perimenopausal symptoms, but is unable to improve ovulation function. Therefore, patients with POI are in urgent need of a more effective treatment.

Complementary and alternative medicine (CAM) refers to a group of diverse medical and healthcare systems, practices, and products that are not generally considered a part of conventional medicine [6] and

include compound Chinese medicine compounds. Previous experimental studies and clinical experiences have suggested that compound Chinese medicine have significant clinical efficacy [7]. ZSYTP is a well-known compound Chinese medicine that is widely used for treating reproductive disorders [8, 9]. ZSYTP can effectively relieve the clinical symptoms caused by low estrogen through lowering serum follicle-stimulating hormone (FSH) levels and elevating serum estrogen levels [10, 11]. Compared with HRT treatment, ZSYTP can induce follicle development and stimulate ovulation [12]. Toxicological experiments have been conducted to prove no perinatal toxicity and observed adverse effects on livers, suggesting the pharmaceutical safety of ZSYTP [13–16]. Thus, ZSYTP might be a novel therapeutic strategy for POI. However, its pharmacological mechanism and molecular targets are unclear yet.

Network pharmacology is an approach to drug design that improves clinical efficacy and understands side effects and toxicity [17]. It enables effective prediction of a complex interplay among TCM components and targets through integrated network analysis [18]. In this study, we conducted a comprehensive network pharmacology approach to predict the potential pharmacological targets of ZSYTP on POI by molecular docking and network analysis. The workflow of this study on the pharmacological mechanism of ZSYTP on POI based on network pharmacology is presented in Figure 1.

2. Methods and Materials

2.1. Data Preparation

2.1.1. Screening for Chemical Compounds of Herbs in ZSYTP. Chemical compounds from all 15 herbs in ZSYTP were obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP; http://lsp.nwu.edu.cn/tcmsp.php, updated in March 2014) [19], TCM Database@Taiwan (http://tcm.cmu.edu.tw) [20], and BATMAN-TCM (http://bionet.ncpsb.org/batman-tcm/) [21]. The TCMSP is a unique pharmacology platform designed for Chinese herbs, the TCM Database@Taiwan is one of the most comprehensive TCM databases in the world, and BATMAN-TCM is a bioinformatics analysis tool for analyzing the active compounds of Chinese herbs.

The screening filters used in this research to maximize drug discovery included oral bioavailability (OB) $\geq 30\%$ [22] and drug likeness (DL) ≥ 0.18 [23]. Active compounds were selected when both these criteria were met in accordance with absorption, distribution, metabolism, and excretion (ADME) consideration to ensure higher efficiency of the active compounds selected.

OB is the percentage value that measures the fractional extent of an orally administered drug that reaches the systemic circulation after ADME. A higher OB value indicates that a lesser amount of drug must reach the intended pharmacological effect, thereby reducing the risk of drug toxicity and potential side effects. A chemical compound with a low OB value indicates a poor drug effect and a higher intersubject variability. Thus, the OB value is one of the most commonly used pharmacokinetic properties in drug screening.

DL is a network pharmacological concept that indicates the similarity or likeness of the compound in question compared with known compounds. The concept originates from Lipinski's Rule of Five, which is used to estimate the possibility of obtaining a pharmacologically active compound, thereby reducing failure rates and increasing efficacy in pharmacological studies [24].

2.1.2. Screening Target Genes of Each Herb in ZSYTP. The canonical SMILES and IUPAC International Chemical Identifier (InChI) of each of the chemical compounds were collated from PubChem Database (http://pubchem.ncbi.nlm.nih.gov/) [25] to ensure the uniqueness of the molecules in the database.

We input all molecular information of the chemical compounds of ZSYTP into the following: (1) STITCH (http://stitch.embl.de) [26], a database of known and predicted interactions between chemicals and proteins; (2) Swiss Target Prediction (http://www.swisstargetprediction.ch/index.php) [27], an online tool that predicts the most probable protein targets of molecules; (3) PubChem Database (http://pubchem.ncbi.nlm.nih.gov/), an online chemistry database providing drug-target identification; and (4) DrugBank (https://go.drugbank.com) [28], an online database containing information on known drugs and their corresponding target genes.

The canonical SMILES of each compound were input into the "chemical structure(s)" search engine of STITCH, and "Homo sapiens" was selected as the organism. Similarly, the canonical SMILES of each active compound were input into the search engine of Swiss Target Prediction; "Homo sapiens" was selected as the species, and target genes with a probability of >70% [27] were included.

Both the canonical SMILES and InChI key of the compounds were searched through the PubChem database, and target genes were subsequently obtained from the "Biological Test Results" panel, only including target genes with known bioactive outcomes.

The InChI key of each chemical compound was searched at DrugBank and consequent target genes were obtained.

2.1.3. POI Targets Database Building. With reference to the POI guideline published by the *European Society of Human Reproduction and Embryology* (ESHRE) in 2015 [2], it was recommended that the term "premature ovarian insufficiency" be used for standard terminology. The guideline recognized a lack in proper clinical diagnostic definition for the condition and provided the diagnosis of POI with two conditions: (1) oligo/amenorrhea for at least four months and (2) an elevated FSH level >25 IU/L on two occasions more than two weeks apart. This study aims to investigate the pharmacological mechanism of ZSYTP on POI, therefore omitting keywords such as "premature menopause" and "premature ovarian failure" in our search for disease targets. "Premature ovarian insufficiency" was, therefore, the keyword used in the search engines listed as follows.

POI targets were collected through searches using databases such as Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org) [29], NCBI Gene Database (https://www.ncbi.nlm.nih.gov/gene) [30], Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/) [31], and MalaCards (https://www.malacards.org) [32].

2.2. Network Construction Method. The network construction was built as follows: (1) ZSYTP chemical compounds-potential target network (C-T network), (2) PPI network of POI disease targets, (3) PPI network of ZSYTP target genes, and (4) PPI network of interaction between POI disease targets and ZSYTP target genes.

The network analysis software Cytoscape (https://cytoscape.org, version 3.8.2) [33] was used to visualize the networks. The nodes in the C-T network represent targets, compounds, pathways, while edges represent interactions.

The C-T network was constructed with Cytoscape software, and the network analyzer tool was used to evaluate the network of chemical compounds and their corresponding gene targets. PPI networks were visualized using BisoGenet plugin for the Cytoscape software.

2.3. Gene Ontology and KEGG Enrichment Analysis. Cytoscape ClueGO [34] application was used for gene ontology (GO) enrichment analysis for biological process, molecular function, cellular components, and KEGG. A p-value <0.05 was set as statistically significant.

2.4. Verification of Molecular Docking. Molecular docking simulations were used to verify the binding of the target and the corresponding compound. 8 active compounds of the highest degree and 5 potential targets of the highest degree were obtained from the C-T network (Figure 2). Data on the construction of macromolecular protein target receptors were acquired via the RCSB PDB database (PDB, http://www.rcsb.org/) [35], and data on small molecule compounds were retrieved via the PubChem Database [25] and TCMSP [19]. The expulsion of water and ligand from macromolecular protein was performed by PyMol 2.4 [36], and format conversion was performed using Open Babel software. Molecular docking simulations of the macromolecular protein targets and the corresponding compounds were performed by AutoDockTool 1.5.6 and AutoDock 4.2.6 software [37]. The results were visualized by Pymol 2.4.

3. Results

3.1. Screened Chemical Compounds of Herbs in ZSYTP. A total of 1364 chemical compounds were found in the process, 76 from *Rehmanniae Radix Praeparata* (SDH), 188 from *Lycii Fructus* (GQZ), 29 from *Cuscutae Semen* (TSZ), 174 from *Morindae Officinalis Radix* (BJT), 134 from *Codonopsis Radix* (DS), 55 from *Atractylodes macrocephala Koidz* (BZ), 30 from *Dipsaci Radix* (XD), 119 from *Eucommiae Cortex* (DZ), 165 from *Amomum aurantiacum* (SR), 190 from *Panax Ginseng* (RS), 46 from *Herba Taxilli* (SJS), 135 from *Folium Artemisiae Argyi* (AY), 17 from *Fallopia multiflora* (HSW), 4 from *Colla Corii Asini* (EJ), and 2 from *Cornu Cervi Degelatinatum* (LJS).

In accordance with the ADME thresholds of OB ≥ 30% and DL ≥ 0.18, 187 chemical compounds were obtained as follows: 2 from SDH, 45 from GQZ, 11 from TSZ, 20 from BJT, 21 from DS, 9 from BZ, 6 from XD, 28 from DZ, 10 from SR, 22 from RS, 2 from SJS, 9 from AY, and 2 from HSW. EJ and LJS were not found in any of the TCM databases. However, a further 18 compounds with lower OB or DL values were consolidated as they hold extensive pharmacological activities: 10 from HSW, 4 from EJ, and 2 from LJS. The final list of 205 active compounds with their parameters and sources are provided in Supplementary Table 1.

3.2. Active Target Gene Prediction of ZSYTP. Target gene prediction of the ZSYTP active compounds based on molecular similarity was conducted by entering each unique molecular data into STITCH, PubChem, Swiss Target Prediction, and DrugBank. 481 target genes were obtained from ZSYTP active compounds upon eliminating duplication. The target genes of each herb of ZSYTP are listed in Supplementary Table 2.

3.3. POI Targets. The keyword "premature ovarian insufficiency" was used in the search through OMIM, TTD, NCBI Gene Database, and MalaCards as a POI disease target. OMIM and TTD yielded no results, and MalaCards provided potential disease targets including premature ovarian failure (POF), which were not included in this study. A total of 119 disease targets were obtained (Supplementary Table 3).

3.4. Constructed ZSYTP Compound-Potential Target Network (C-T Network). Cytoscape software was used to map out a network illustrating the relationship between each herb, its corresponding active compounds and targets, and the C-T network. The nodes depict herbs, active compounds, and target genes, while the edges indicate the correlation between them (Supplementary Files 4-5). The constructed C-T network of 48 active compounds contained 538 nodes and 1418 edges (Figure 2). Compounds and target genes of the highest degrees were noted for molecular docking verification (Table 1; Supplementary File 6B). The median degree of the 48 active compounds in the network was 9.5 (number of related targets), suggesting that the most active compounds influence multiple targets. Specifically, quercetin, kaempferol, and luteolin acted on 384, 255, and 105 targets, respectively (Supplementary File 6A), indicating that they could be crucial in the therapeutic potential of ZSYTP on POI. 13 herbs of ZSYTP overlapped on 8 active compounds; they are indicated in Table 2 with their corresponding degree.

3.5. Core Network Analysis. PPI networks for both potential drug and disease targets were constructed with Cytoscape version 3.8.2 plug-in BisoGenet. Of note, 3938 nodes and

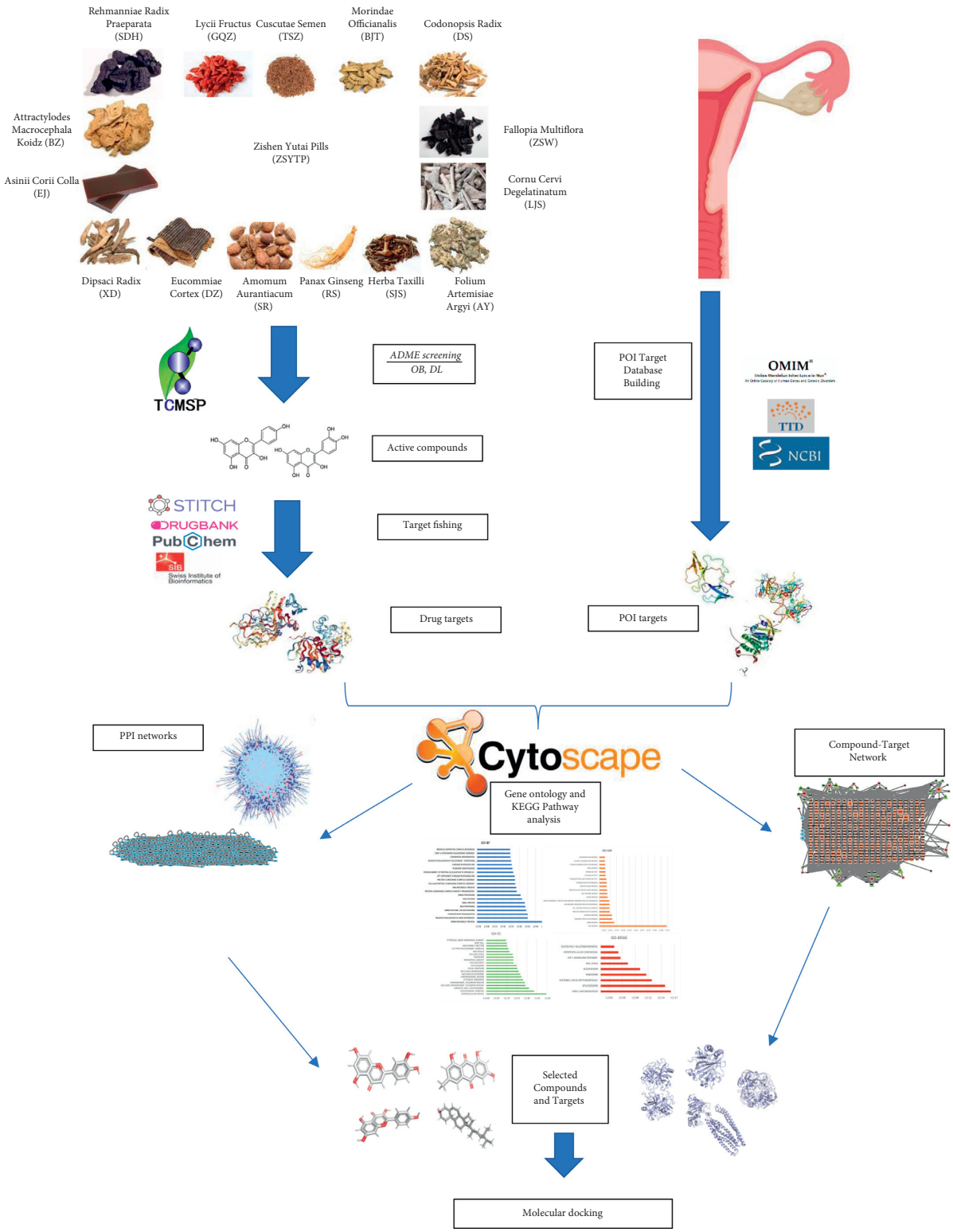


Figure 1: Workflow of the present study.

99,199 edges were established in the PPI network for disease targets, while 7834 nodes and 182,596 edges were found in the PPI network for ZSYTP drug targets.

Topological feature analysis was subsequently performed by intersecting both PPI networks based on "betweenness (BC)," "closeness (CC)," and "eigenvector (EC)," deriving a total of 8511 nodes and 190,297 edges (Figure 3(a)). Based on a previous study by Zhou et al. [16], targets were selected with parameters above twice the median value. The first selection criteria were set as degree >48, and 2352 nodes and 96,928 edges were derived (Figure 3(b)). The 2352 targets were further screened with the second selection criteria of degree, DC > 81,

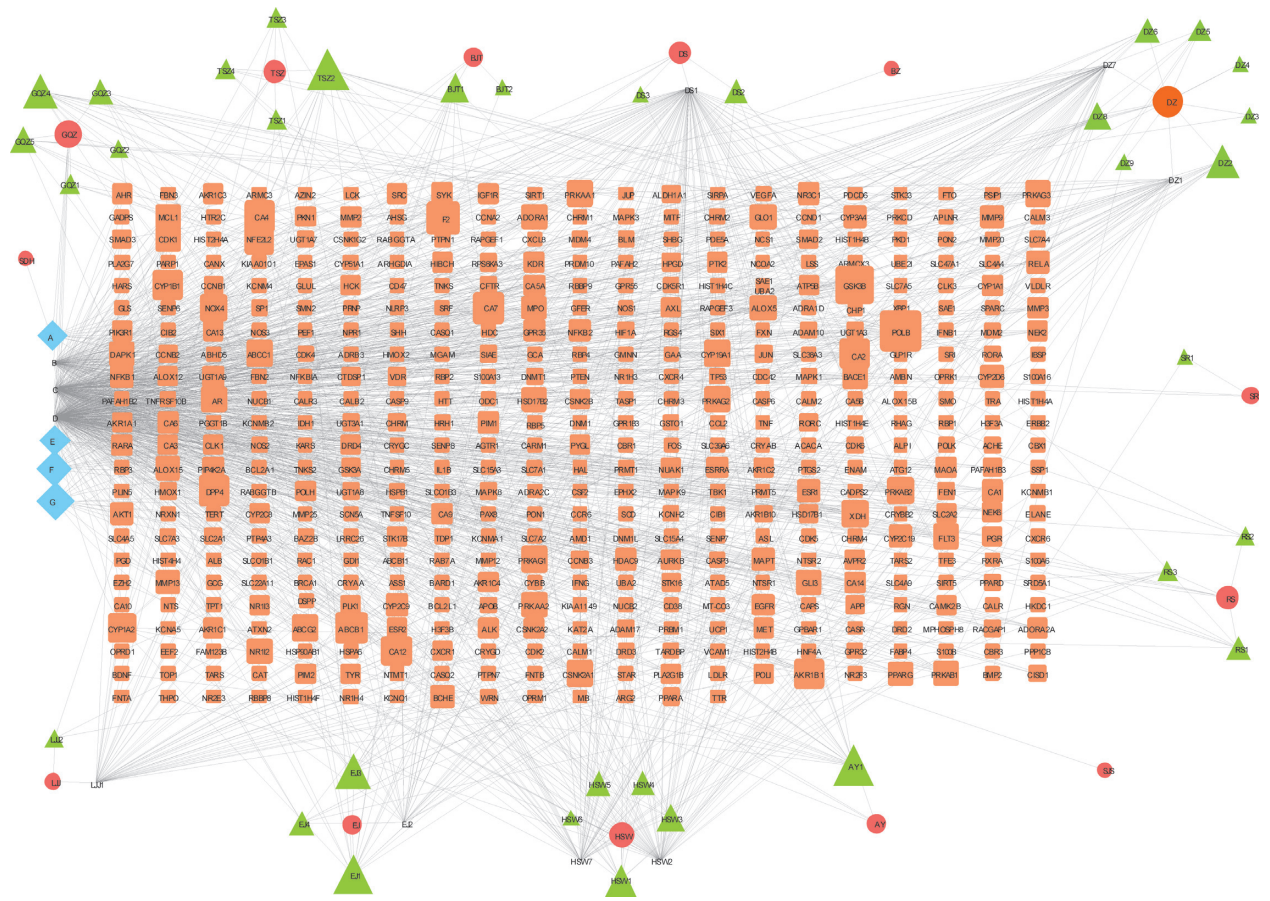


Figure 2: Compound-potential target network. Each red ellipse represents a herb in ZSYTP, and the green triangles represent active compounds. The orange rectangles represent the potential targets, and the blue diamonds represent common active compounds. The size of each node is proportional to the target degree in the network.

Table 1: List of top ten active compounds of ZSYTP based on degree (number of related targets).

Abbreviation	Active compound	Degree	Herbs involved
D	Quercetin	384	GQZ, TSZ, DZ, AY, SJS
C	Kaempferol	255	TSZ, DZ, RS
DS1	Luteolin	104	DS
B	Beta-sitosterol	97	GQZ, TSZ, DZ, AY, RS, BJT, SR, HSW, BZ
HSW2	Emodin	58	HSW
DZ7	Helenalin	48	DZ
HSW7	Rhein	47	HSW
LJJ1	Calcium phosphate	44	LJJ
DZ1	Mairin	29	DZ
EJ2	Histidine	28	EJ

Table 2: List of overlapping active compounds of ZSYTP.

Active compound	Degree	Herbs involved
Quercetin	384	GQZ, TSZ, DZ, AY, SJS
Kaempferol	255	TSZ, DZ, DS
Beta-sitosterol	97	TSZ, BJT, BZ, SR, RS, AY, HSW
Mandenol	18	GQZ, AY
Glycitein	16	BJT, DS, RS
Diop	12	GQZ, DS
Stigmasterol	12	GQZ, SR, RS, DS, AY
CLR	5	GQZ, TSZ

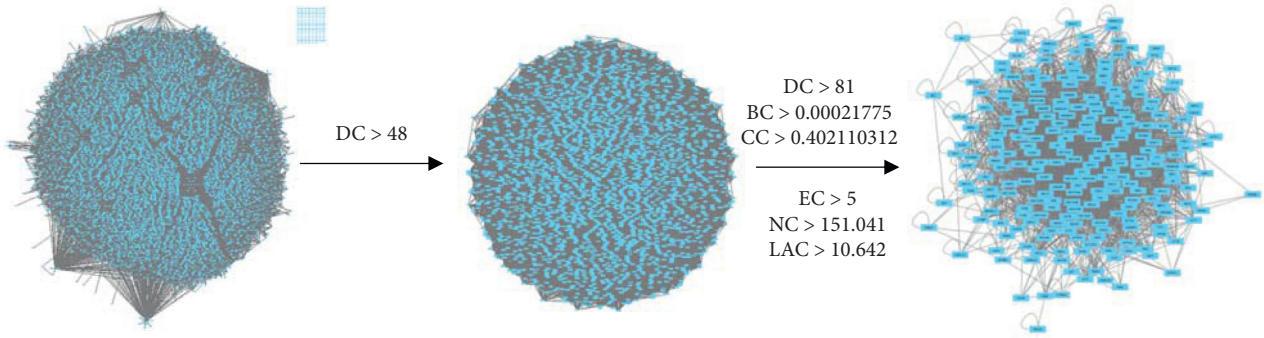


Figure 3: Topological screening process of PPI network.

BC > 0.00021775, CC > 0.402110312, EC > 5, neighborhood connectivity (NC) > 151.041, and node (LAC) > 10.642. A final PPI network of 234 nodes and 2,794 edges was identified (Figure 3(c); Supplementary File 7).

3.6. Gene Ontology and KEGG Analysis

3.6.1. Gene Ontology Biological Process (GO-BP). The enrichment analysis was completed with the Cytoscape ClueGO plugin for visualization. 221 biological processes were retrieved; the p-value was set at $>1.0\times10^{-21}$, and 20 processes were selected for further analysis. The full list of enriched GO-BP terms is presented in Supplementary Table S8A.

The 20 biological processes selected were mainly involved in the negative regulation of gene expression (15.46%), telomere maintenance (10.57%), viral process (8.76%), regulation of protein localization of chromosome, telomeric region (5.41%), mRNA metabolic process (5.41%), and establishment of protein localization to organelles (5.15%). Percentage values were calculated based on the number of processes in a group out of the 221 chart records retrieved.

For negative regulation of the gene expression group, biological processes, such as negative regulation of nucleobase-containing compound metabolic process, negative regulation of RNA metabolic process, negative regulation of macromolecule biosynthetic process, negative regulation of biosynthetic process, negative regulation of nucleic acid templated transcription, and negative regulation of gene expression (epigenetic), were identified. For the mRNA metabolic process group, biological processes, such as RNA processing, mRNA metabolic process, RNA splicing, protein-containing complex subunit organization, and mRNA catabolic process, were identified.

The other biological processes selected for further analysis were as follows: chromosome organization, cellular protein-containing complex assembly, ATP-dependent chromatin remodeling, telomere maintenance, CENP-A containing nucleosome assembly, posttranscriptional regulation of gene expression, protein localization to organelles, SRP-dependent cotranslational protein targeting to membrane, cellular response to DNA damage stimulus, chromatin assembly and disassembly, DNA repair, and double-strand break repair.

The selected processes of GO-BP were visualized (Figure 4(a)) and ranked according to their corresponding p values.

3.6.2. Gene Ontology Molecular Function (GO-MF). Gene ontology analysis of MF identified 45 functions. The results indicated that the active target genes of ZSYTP that act on POI have functions, including transcription factor binding (15.56%), rRNA-binding (8.89%), helicase activity (8.89%), and nucleosome binding (6.67%). 20 molecular functions with p values $>1.0\times10^{-7}$ were selected and visualized; the results are shown in Figure 4(b) with corresponding p values. The list of enriched GO-MF terms is presented in Supplementary Table S8B.

For the transcription factor binding group, the identified functions included transcription factor binding, DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, and transcription coactivator activity.

For the group of rRNA binding, the identified functions included RNA binding, mRNA binding, rRNA binding, and double stranded RNA binding.

Other identified functions included cadherin binding, protein domain-specific binding, cell adhesion molecule binding, kinase binding, nucleosome binding, ubiquitin-like protein binding, protein kinase binding, hormone receptor binding, helicase activity, ATPase activity, steroid hormone receptor binding, and chromatin DNA binding.

3.6.3. Gene Ontology Cellular Component (GO-CC). Gene ontology analysis of CC identified 82 components, including spliceosomal complex (18.07%), cytosolic ribosomes (10.84%), nuclear chromosome, telomeric region (10.84%), and SWI/SNF superfamily-type complex (7.23%). Twenty cellular components with p values $>1.0\times10^{-10}$ were selected for further analysis. The full list of enriched GO-BP terms is presented in Supplementary Table S8C.

GO-CC analysis identified the following cellular components as enriched, extracellular vesicle, spliceosome

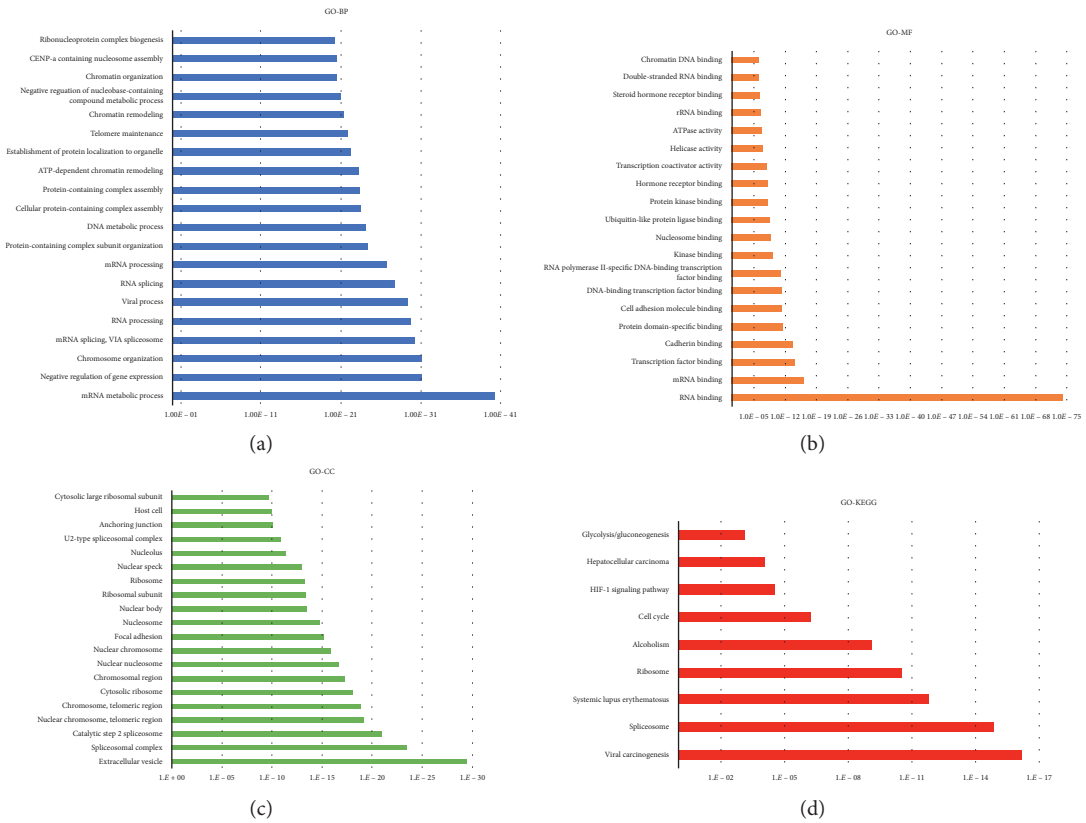


Figure 4: The GO function and KEGG pathway enrichments. (a) Enriched BP functions of active target genes; (b) enriched MF functions of active target genes; (c) enriched CC functions of active target genes; (d) KEGG pathway enrichments. BP: biological process; MF: molecular function; CC: cellular component.

complex, catalytic step 2 spliceosome, nuclear chromosome (telomeric region), chromosome (telomeric region), cytosolic ribosome, chromosomal region, nuclear nucleosome, focal adhesion, nucleosome, nuclear body, ribosomal subunit, ribosome, nuclear speck, nucleolus, U2-type spliceosomal complex, anchoring junction, host cell, and cytosolic large ribosomal subunit.

The 20 selected components of GO-CC analysis are visualized in Figure 4(c) with their corresponding p values.

3.6.4. KEGG Pathway Analysis. The results of KEGG pathway analysis highlighted nine pathways, in which target genes of ZSYTP acting on POI are enriched significantly; 33.33% of the enriched target genes act on viral carcinogenesis and 22.22% on the hypoxia-inducible factor 1 (HIF-1) signaling pathway; hepatocellular carcinoma, cell cycle, spliceosome, and ribosome pathways were equally divided at 11.11%.

All 9 pathways highlighted by KEGG analysis were presented (Figure 4(d)) and ranked according to their corresponding p values (Supplementary Table 8D).

Cytoscape ClueGO plug-in was utilized to better visualize the KEGG pathway as shown in Figure 5.

3.7. Molecular Docking Visualization. The lowest binding energy of the molecular docking of potential targets and their designated compounds are presented in Table 3. The simulations of the molecular docking of F2-beta-sitosterol, CA4-emodin, CA7-beta-sitosterol, and ABCB1-beta-sitosterol are shown in Figures 6–9, respectively.

4. Discussion

ZSYTP is a Traditional Chinese Medicine (TCM) prescription derived from the clinical experience of Professor Luo Yuankai, a nationally acclaimed TCM scholar of Guangzhou University of Chinese Medicine. Luo received a Class 1 award from China's National Health Commission for his contributions with the ZSYTP.

ZSYTP was originally derived as treatment to prevent recurrent and early pregnancy loss. In accordance with the concept of TCM that states that different diseases can be treated with the same therapeutic principle, and on the basis of the TCM theory that kidneys are closely linked with reproduction, it is postulated that ZSYTP could also be used clinically for irregular periods and infertility.

It has been found that ZSYTP can improve ovarian function in patients with POI [11–14]; as a result, we sought to explore the possible etiologies of POI and the pharmacological mechanisms of ZSYTP on POI.

The nine pathways highlighted by KEGG analysis were studied extensively for further corroboration with existing literature that would provide insights and possible hypotheses of the pharmacological mechanisms of ZSYTP in

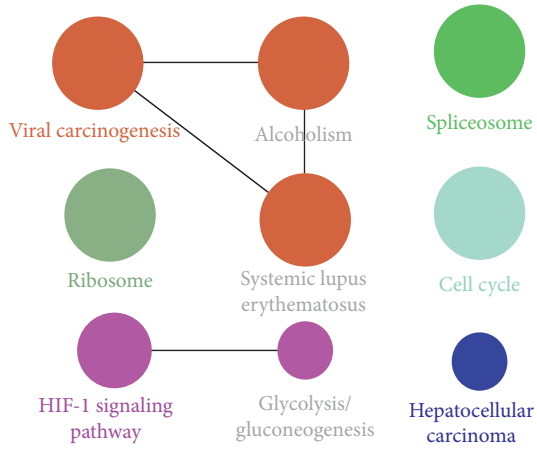


Figure 5: KEGG pathway enrichment analysis visualized by ClueGO.

Table 3: Lowest binding energy of compounds-target molecular docking (kcal/mol).

Target	Degree value	β-Sitosterol	Emodin	Kaempferol	Luteolin	Quercetin
POLB	20	−4.86	−3.98	−3.5	−3.54	−3.22
GSK3B	18	−4.31	−1.53	−2.5	−2.37	−1.86
F2	14	−6.67	−4.62	−4.82	−4.48	−2.97
ABCB1	13	−5.3	−3.4	−2.17	−2.64	−1.73
CA7	13	−5.94	−4.63	−4.27	−4.16	−4.25
CA4	13	−3.7	−5.34	−2.39	−3.55	−1.91
CA12	12	−4.0	−4.4	−3.01	−2.35	−2.83
CA2	12	−5.08	−3.96	−3.22	−3.02	−2.7

the context of POI. In particular, the HIF-1 signaling pathway was highlighted by KEGG analysis due to its multiple roles in ovarian function.

An overview of the C-T network combined with GO and KEGG analysis led us to postulate that the potential mechanism of ZSYTP on POI is likely connected to the anti-inflammatory and antioxidant properties of ZSYTP, as well as participating in glucose metabolism. GO and KEGG analysis also highlighted a change in cell and gene expression as a possible pathological mechanism of POI.

4.1. Inflamm-Aging and POI. A term first coined by Franceschi et al. [38] in 2000, "inflamm-aging" refers to the body undergoing a chronic and progressive inflammatory state [39–41] through the process of aging.

Chronic inflammation has been closely linked with oxidative stress [42], cytokines, and DNA damage. Oxidative stress refers to the imbalance of reactive oxygen species (ROS) and antioxidants in the body. ROS help to fight pathogens while being kept in check by antioxidants, an imbalance between the two leads to ROS damage of proteins, DNA, and fatty tissue. The oxidative stress levels and neutrophil-to-lymphocyte ratio were found to be elevated in subjects with POI, indicating a state of inflammation in these patients [43].

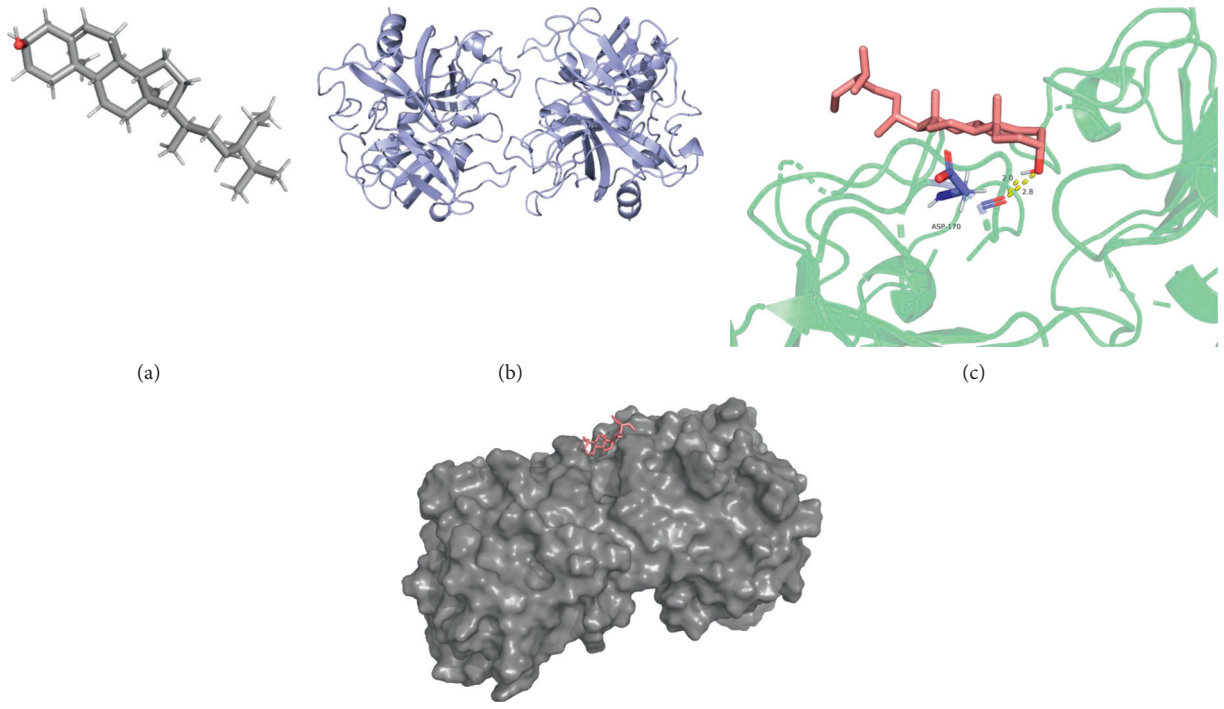
In reference to the C-T network, active compounds, such as quercetin, kaempferol, luteolin, beta-sitosterol, and emodin, were found to be key hub compounds of ZSYTP, in descending degree, respectively.

Quercetin, kaempferol, and luteolin are natural flavonoids that demonstrate anti-inflammatory, antioxidant, anticarcinogenic properties and gene expression-modulating potential [44]. A recent study indicated that quercetin could protect ovarian function in female albino mice with cyclophosphamide-induced premature ovarian failure. Primordial follicles and serum anti-Müllerian hormone (AMH) were increased, while the number of atretic follicles was decreased under quercetin treatment, suggesting a protective effect of quercetin on ovarian function in cyclophosphamide-induced POF [45].

The C-T network also highlighted NFE2L2 and NOX4 as key targets of ZSYTP. NFE2L2 encodes a transcription factor that regulates genes containing antioxidant response element (ARE) in their promoters; these genes encode proteins involved in the production of free radicals. NOX4 encodes a family of enzymes that catalyzes the reduction of molecular oxygen to various ROS.

It is, therefore, possible that the potential anti-inflammatory and antioxidant effect of ZSYTP could be achieved by the combined actions of quercetin, kaempferol, and luteolin via key gene targets; together, these actions could reduce ovarian inflammation and possibly slow ovarian degradation in POI.

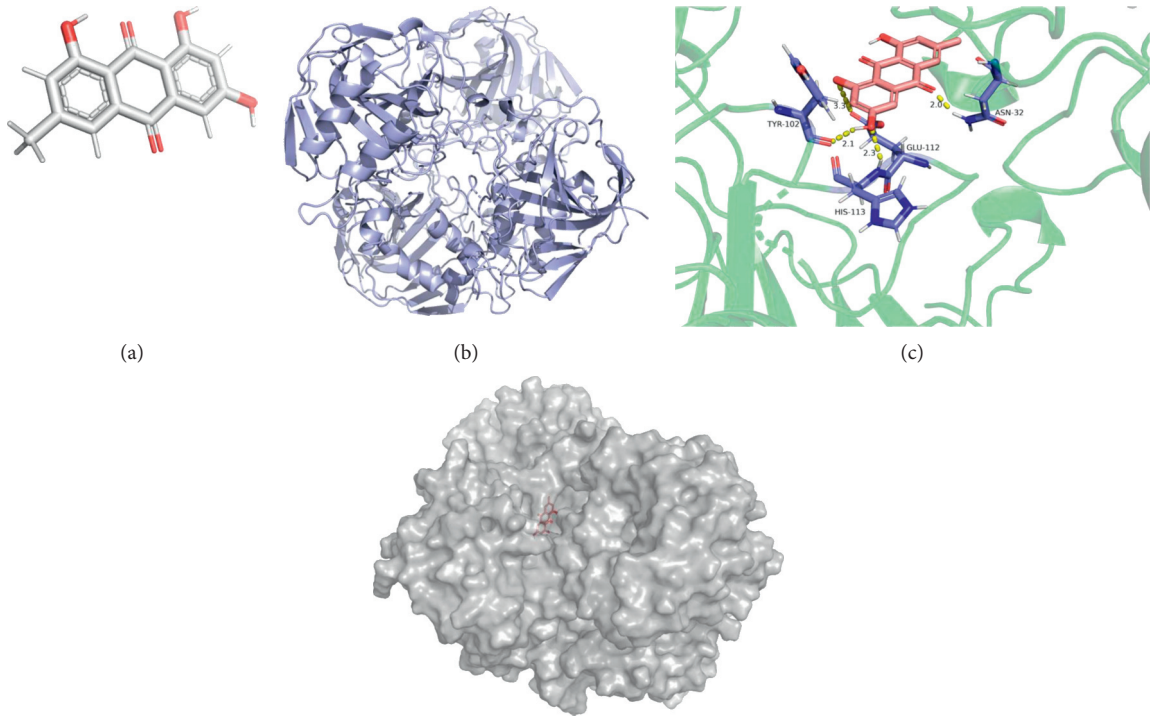
4.2. Glucose Metabolism in POI. POI describes a hypoestrogenic state in women that is associated with metabolic changes [46]. As estrogen optimizes insulin activity, multiple studies have aimed to understand glucose metabolism and



(a) (b) (c)

(d)

Figure 6: F2 beta-sitosterol molecular docking. 3D structures of (a) beta-sitosterol and (b) F2; (c) molecular docking simulation; (d) display protein surface of molecular docking simulation.



(a) (b) (c)

(d)

Figure 7: CA4 emodin molecular docking. 3D structures of (a) emodin and (b) CA4; (c) molecular docking simulation; (d) display protein surface of molecular docking simulation.

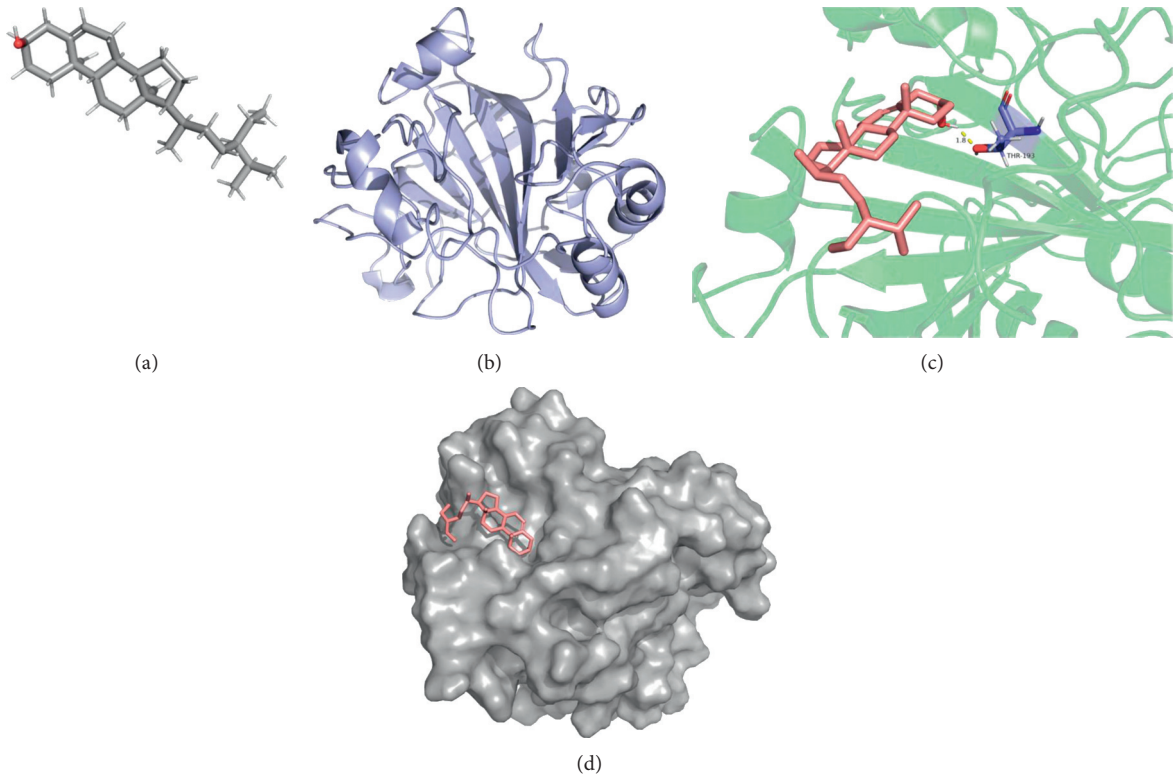


Figure 8: CA7 beta-sitosterol molecular docking. 3D structures of (a) beta-sitosterol and (b) CA7; (c) molecular docking simulation; (d) display protein surface of molecular docking simulation.

insulin resistance levels in patients with POI [47]. A meta-analysis was conducted to investigate the association of POI with type 2 diabetes (T2DM) [48], the results of which demonstrated that women with POI presented with a higher risk of T2DM compared with women of normal menopausal age (45–55 years) [49–51].

KEGG analysis implicated the HIF-1 signaling pathway and glycolysis as being associated with the underlying therapeutic mechanisms of ZSYTP in POI. HIF-1 is a transcription factor of two subunits, HIF-1a and HIF-1b [52]. The activity of HIF-1 is mainly determined by HIF-1a, which is regulated by hypoxia and hyperglycemia. Many studies have aimed to elucidate the relationship between HIF-1a gene expression in cells and hyperglycemia. Although the molecular mechanism of this relationship is still unclear, researchers have come to an agreement that hyperglycemia is directly linked to a compromised HIF-1a expression level.

Inhibition of HIF-1a expression was found to have triggered atresia in large follicles of mice with polycystic ovary syndrome (PCOS) and, therefore, prevented ovulation [53]. One study showed that HIF-1a signaling is inhibited in PCOS rat models [54], and after clinical PCOS treatment of dimethyldiguanide, PCOS symptoms were improved by rescuing this pathway, increasing HIF-1a gene expression in the process. Another study exposed human primary granulosa cells of subjects with PCOS to mitochondrial and glycolysis inhibitors and compared mitochondrial activity and glycometabolism with controls [55]. It was found that HIF-1a gene expression decreased, while ROS levels increased upon inhibition, and the researchers concluded that glycolysis and high HIF-1a expression in human primary granulosa cells are required for oocyte competence of PCOS. Therefore, it can be surmised that decreased levels of HIF-1a gene expression have a negative effect on ovarian follicle development; these hypothesized pathways are summarized in Figure 10.

Hyperglycemia augments oxidative stress and contributes to the overproduction of ROS [56], which, in turn, downregulates HIF-1a levels [57] via multiple possible mechanisms [58]. The significance of identifying the potential pathological mechanism of HIF-1 signaling pathway and glycolysis in POI could guide future pharmacological research to focus on possible therapeutic approaches.

In ZSYTP, active compounds that act on glucose metabolism include quercetin, kaempferol, emodin [59], luteolin, and chrysophanol; among them, quercetin is also involved in the inhibition of intestinal glucose absorption, insulin secretion, and insulin-sensitizing activities. This is substantiated by reports that quercetin intake results in a significant decrease in insulin resistance in PCOS cases [60–62].

It is possible that the active compounds of ZSYTP collectively act on regulating glucose metabolism and HIF-1 expression, therefore improving ovarian follicle development, slowing the rate of ovarian degradation in POI.

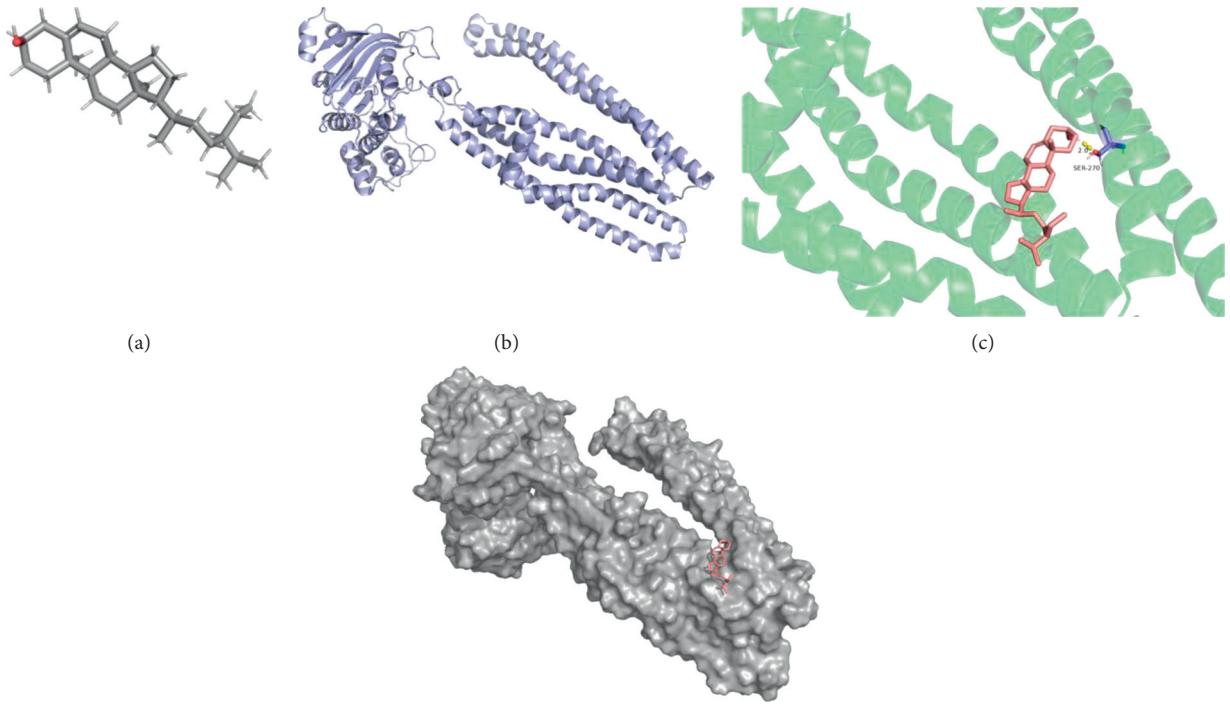


Figure 9: ABCB1 beta-sitosterol molecular docking. 3D structures of (a) beta-sitosterol and (b) ABCB1; (c) molecular docking simulation; (d) display protein surface of molecular docking simulation.

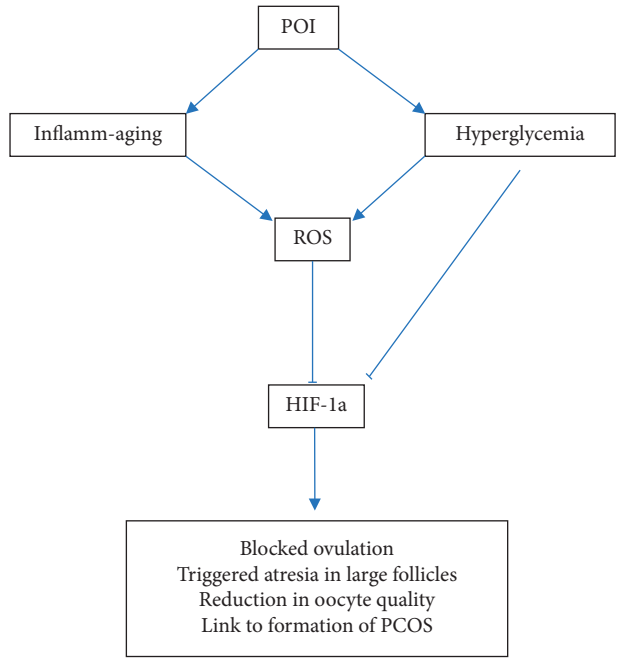


Figure 10: Possible etiology of POI leading to repressed HIF-1a protein levels.

4.3. Change in Gene and Cell Expression. Subsequent GO and KEGG analysis aided in the elucidation of another possible pharmacological mechanism of ZSYTP on POI. The enriched GO term of BP with the smallest *p*-value was "mRNA metabolic process," which refers to the process of carrying messages transcribed from DNA to the protein assembly at ribosomes. Moreover, "negative regulation of gene expression" was the group of biological processes highlighted in the GO-BP analysis, implying abnormal regulation of gene expression in POI.

The most enriched GO-CC was "extracellular vesicles (EVs)." Recent studies have suggested the ability of EVs to transfer functional RNA from cell to cell [63, 64] and their involvement on immune responses. Indeed, a previous study reported that HIV coreceptors could be transferred between cells with EVs, increasing the body's susceptibility to infection [65, 66]. EVs have been a topic of interest in the treatment for POI. Several studies indicated that EVs derived from human umbilical cord mesenchymal stem cells (HUCMSCs) can restore ovarian function of chemically induced POI [67].

The most significant enriched GO-MF was "RNA binding," which further implies the importance of RNA in the mechanism of ZSYTP on POI.

KEGG analysis indicated "viral carcinogenesis" as the most enriched process. The mechanisms of viral carcinogenesis include direct transformation through the expression of viral genes and indirect transformational activities in cells. These activities increase translation of modified proteins with altered cell function, which corroborate with the GO-BP analysis of multiple negative regulation of gene expression processes identified.

Multiple studies have shown decreased protein expression in POI [68–72], signifying the significance of a downregulated gene as a possible cause for POI.

4.4. Molecular Docking. According to previous research, we considered the binding activity of molecular docking simulations to be practical when the binding energy was < −1.2 kcal/mol (−5.0 kJ/mol) and dynamite when the binding energy was < −5.0 kcal/mol. In our study, all of the binding energies were < −1.2 kcal/mol, and 12.5% of binding energies were < −5.0 kcal/mol. Furthermore, it was found that beta-sitosterol could stably dock to the F2 protein structure, while the H-bond plays a critical role at residue ASP-170. These active compounds may provide a foundation for treating POI, and the therapeutic action could be performed by correlative pathways in ZSYTP.

5. Conclusion

POI remains a debilitating disease for women with no fixed treatment protocol currently. ZSYTP has previously been shown to have a clinical effect on POI and warrants further research [11–14]. Our study mapped out the active compounds and corresponding gene targets of ZSYTP and further explored the pharmacological mechanisms underlying the effects of ZSYTP on POI using the method of network pharmacology.

Our results provide future research directions for the therapeutic use of ZSYTP in POI into three aspects: (1) the anti-inflammatory and antioxidant effect, (2) regulation of glucose metabolism, and (3) negative regulation on mRNA metabolic process. Our results also indicated that quercetin and kaempferol, as the two major active compounds found in ZSYTP, have potential pharmacological effects on POI. Future studies should aim to validate the effect of quercetin and kaempferol on POI to elucidate the underlying mechanism. In addition, future studies should also ascertain the status of glucose metabolism in patients with POI.

In conclusion, it is possible that the therapeutic potential of ZSYTP on POI is a multipathway effect, and therefore, more research is warranted to fully elucidate this relationship. The results of this study brought focus to potential pharmacological mechanisms of ZSYTP and its effect on POI. Multiple hypotheses of the pathological mechanism of POI were thereby formulated. Here, we provide a preliminary platform showcasing a comprehensive study of a TCM formula for POI, establishing a protocol enhancing TCM drug discovery to be more systematic and efficient.

Data Availability

The data used in this study are available from the corresponding author upon request.

Disclosure

Xinyi Chai and Yihui Feng are the co-first authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Xinyi Chai and Yihui Feng contributed equally to this work. Xinyi Chai, Yihui Feng, and Ying Zhao designed the study. Xinyi Chai, Yihui Feng, and Yingying Chen drafted the manuscript. Xinyi Chai, Yingying Chen, and Yan Ning are responsible for data analysis and interpretation. Yihui Feng and Ying Zhao supervised the study and conducted the revision of the manuscript. All authors have read and approved the final version of this paper.

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Supplementary Materials

Supplementary Table 1: active compounds of each herb in ZSYTP. Supplementary Table 2: corresponding targets of active compounds: (A) two target genes of SDH; (B) 138 target genes of GQZ; (C) 202 target genes of TSZ; (D) 25 target genes of BJT; (E) 121 target genes of DS; (F) 11 target genes of BZ; (G) 283 target genes of DZ; (H) 13 target genes of SR; (J) 112 target genes of RS; (K) 76 target genes of SJS; (L) 115 target genes of AY; (M) 149 target genes of HSW; (N) 68 target genes of EJ; (P) 46 target genes of LJJ. Supplementary Table 3: POI targets. Supplementary Table 4: compounds and targets attributes of C-T network. Supplementary Table 5: data of compound-target network. Supplementary Table 6: analysed C-T network: (A) active compounds of C-T network and corresponding degrees;

(B) targets of C-T network and corresponding degrees. Supplementary Table 7: ZSYTP core PPI network. Supplementary Table 8: gene ontology and KEGG analysis: (A) GO-BP analysis; (B) GO-MF analysis; (C) GO-CC analysis; (D) KEGG pathway enrichment analysis. (*Supplementary Materials*)

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Research Article

Regulation of Mild Moxibustion on Uterine Vascular and Prostaglandin Contents in Primary Dysmenorrhea Rat Model

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Objective. Primary dysmenorrhea (PD) is a common and high incidence disease in gynecology, which seriously affects the quality of life in young women. Our previous study found that mild moxibustion could treat abdominal pain of PD patients, but the mechanism is still unclear. Therefore, this study aims to partly investigate the treatment mechanism of moxibustion for PD, especially on uterine microcirculation. *Methods*. Forty 3-month-old Sprague Dawley female rats were randomly divided into four groups, including group A (saline control group, $n = 10$), group B (control plus moxibustion group, $n = 10$), group C (PD model group, $n = 10$), group D (PD. model plus moxibustion group, $n = 10$). The PD rat model was established by injecting estradiol benzoate and oxytocin. Mild moxibustion on Sanyinjiao (SP6) and Guanyuan (CV4) acupoints was once a day, 20 minutes per time, for 10 consecutive days. A vaginal smear was used to test the estrous cycle of rats. Uterine microvascular thickness was observed by stereomicroscope. And we detected the content of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) in uterine tissue by enzyme-linked immunosorbent assay. *Results*. Mild moxibustion can enlarge the microvessels, improve the microcirculation disturbance, and relieve the swelling of the uterus in PD rats. During the mild moxibustion intervention, the contents of $PGF_{2\alpha}$ and PGE_2 in uterus issues were synchronous increases or decreases and the changes of PGE_2 were more obvious, but the changes of uterine microvasculature and morphology caused by the decrease of $PGF_{2\alpha}$ were greater than PGE_2. *Conclusion*. Mild moxibustion at SP6 and CV4 acupoints may relax uterine microvascular obstacle by reducing the content of $PGF_{2\alpha}$ in uterine tissue, improve the microcirculation disorder, and then alleviate the PD rat's uterine swelling.

1. Introduction

Primary dysmenorrhea (PD) is defined as pain occurring with menses in the absence of pelvic pathology [1, 2]. Patients may suffer lower backache associated with painful menstruation, a condition more pronounced in women whose uterus is in the retroflexed position [2, 3]. The pain typically lasts for 8–72 h and is most severe during the first or second day of menstruation [3, 4]. PD is always accompanied by symptoms such as nausea, vomiting, diarrhea, fatigue, and insomnia. There is considerable variation in the prevalence of dysmenorrhea, depending on the definition used: 45–72 percent of all women and 43–93 percent of adolescent girls experience the condition [2]. Women with PD have lower quality of life (QOL) score because of recurrent abdominal pain, general health condition, and physical and social dysfunctions [5–7]. At present, it is treated with nonsteroidal anti-inflammatory drugs (NSAIDs) or oral contraceptive pills (OCPs), both of which work by reducing myometrial activity. However, these treatments are accompanied by renal and gastrointestinal side effects [3, 4]. Our goal of treatment is to provide adequate pain relief and reduction of symptoms with the least adverse effects [8].

Moxibustion is a traditional method of burning moxa sticks (usually made from herbal preparations containing

Artemisia vulgaris) near an acupoint to cause a warm and painless sensation [9]. Moxibustion showed desirable merits in managing menstrual pain [10–16], given their treatment effects and economic costs. However, the mechanisms of action of moxibustion therapy are still largely unknown [17]. The factors are likely to be concluded as follows: temperature, infrared radiation, smoke, odor, and the type of moxa [18]. Many clinical and experimental studies have shown that moxibustion stimulation applied to the SP6 or CV4 acupoints (Sanyinjiao and Guanyuan moxibustion point) performed well in the treatment of pain induced of primary dysmenorrhea [10, 19–23]. SP6 and CV4 points are common compatibility schemes for the treatment of PD [24, 25]. The supporting degree of acupoint selection and compatibility is as high as 53.33%. Abaraogu et al. [26] found that pressing the SP6 acupoint can effectively relieve PD pain and reduce anxiety. However, the curative mechanisms of moxibustion remain poorly understood, which limits the application of moxibustion on a larger scale.

The pathogenesis of PD is still unclear, including abnormal uterine contraction, endocrine factors, nerve and neurotransmitter, and mental factors [27]. Uterine microcirculation disturbance is one of the main pathogenesis of dysmenorrhea [28]. The pathophysiology of primary dysmenorrhea is likely a result of the cyclooxygenase pathway producing increased prostanoids, particularly prostaglandins (PGs) [27]. Prostaglandin content plays an important role in the pathogenesis of PD. Further literature studies have found that excessive secretion of prostaglandins in the uterus was considered to be one of the main causes of dysmenorrhea pain [1, 29, 30]. The content of PGs in the endometrium of dysmenorrheal patients is higher than healthy women, and $PGF_{2\alpha}/PGE_2$ is significantly greater as well. Fajrin et al. found that the greater the pain intensity score, the higher the levels of $PGF_{2\alpha}$ [31]. The increased production or unbalanced levels of PGs (PGF_{2a} and PGE_2) create pain due to increased uterine contractility, decreased uterine blood flow, and increased sensitivity of peripheral nerves [32]. The uterine cavity pressure increases and the uterus wall blood flow reduces, so the microcirculation gets disordered and finally causes dysmenorrhea [33, 34]. Li et al.'s study [35] has shown that immediate acupuncture of SP6 may improve the uterine microcirculation disturbance through the nerve reflex pathway to the uterus, affect the synthesis and release of prostaglandins in the endometrium, and realize the analgesic effect. A previous study [36] has also shown that acupoint catgut embedding SP6 and CV4 can regulate the level of PGF_{2a} to treat PD.

At present, the research about moxibustion on PD in traditional Chinese medicine focuses on the regulation of endocrine hormones, immune function, nerve-related factors, improvement of uterine microcirculation, and other aspects. Our previous study demonstrated that moxibustion at the SP6 and CV4 acupoints can regulate the high resistance and low flow state of uterine microcirculation in PD patients to relieve pain [20]. It is not clear whether moxibustion can improve the microcirculation and shape of the uterus to relieve pain by regulating the balance of $PGF_{2\alpha}/PGE_2$. Hence, this study aimed to investigate the effect and mechanism of moxibustion on SP6 and CV4 acupoints on the release of $PGF_{2\alpha}/PGE_2$ and the regulation of uterine microcirculation.

2. Materials and Methods

2.1. Ethical Statement. Animal care and experimental procedures used in the current study were filed and supervised by the animal experimental center of Chengdu University of traditional Chinese medicine (ethics no. 2019-13). This study was carried out adhering to guidelines provided by the National Institutes of Health for the Care and Use of Laboratory Animals and all efforts were made to minimize the suffering of animals.

2.2. Experimental Animals. Adult female Sprague Dawley (SD) rats weighing 240 ± 20 g were purchased from the Chengdu Dashuo experimental animal Co. (medical laboratory animal certificate number: SCXK (Chengdu) 2015-030). All the rats were kept in the Experimental Animal Center of Chengdu University of Traditional Chinese Medicine. The animal room was under controlled conditions (temperature, humidity, and a 12-hour light-dark cycle).

2.3. Construction of PD Rats. Forty female SD rats were fed adaptively for 7 days, and a vaginal smear was used to screen the estrous cycles of rats for 7 days. During the experiment, the typical four cycles of vaginal smear were observed (Figure 1). Rats with regular estrous cycles were selected for this study. According to the random number table, they were divided into four groups, including group A (control group), group B (control plus moxibustion group), group C (PD model group), group D (PD model plus moxibustion group). The rats of groups C and D were injected with estradiol benzoate subcutaneously and intraperitoneal oxytocin to establish the PD model [37]. The rats were in the estrus period and began to inject estradiol benzoate. The rats were injected subcutaneously with estradiol benzoate injection for ten days (0.5 mg/day on the 1^{st} and 10^{th} days, 0.2 mg/day on the 2^{nd}–9^{th} days), and 2 u per rat intraperitoneal injection of oxytocin 1 hr after the last injection of estradiol benzoate. Group A and group B rats were injected subcutaneously with saline of the same volume as estradiol benzoate on the 1^{st}–10^{th} days. One hour after the last injection, they were injected with the same volume saline of oxytocin. Group A and group B were injected subcutaneously with the same volume of saline as estradiol benzoate on the 1^{st}–10^{th} days. One hour after subcutaneous injection of saline on the 10^{th} day, the rats were injected intraperitoneally with the same volume of 2 u saline as oxytocin [33, 35]. A flowchart of experiment development is shown in Figure 2.

2.4. Evaluation of PD Model. After the completion of the model building, observed the writhing times of every rat within 20 minutes. The model rats showed twisting responses (abdominal contraction, concave, trunk, and hind

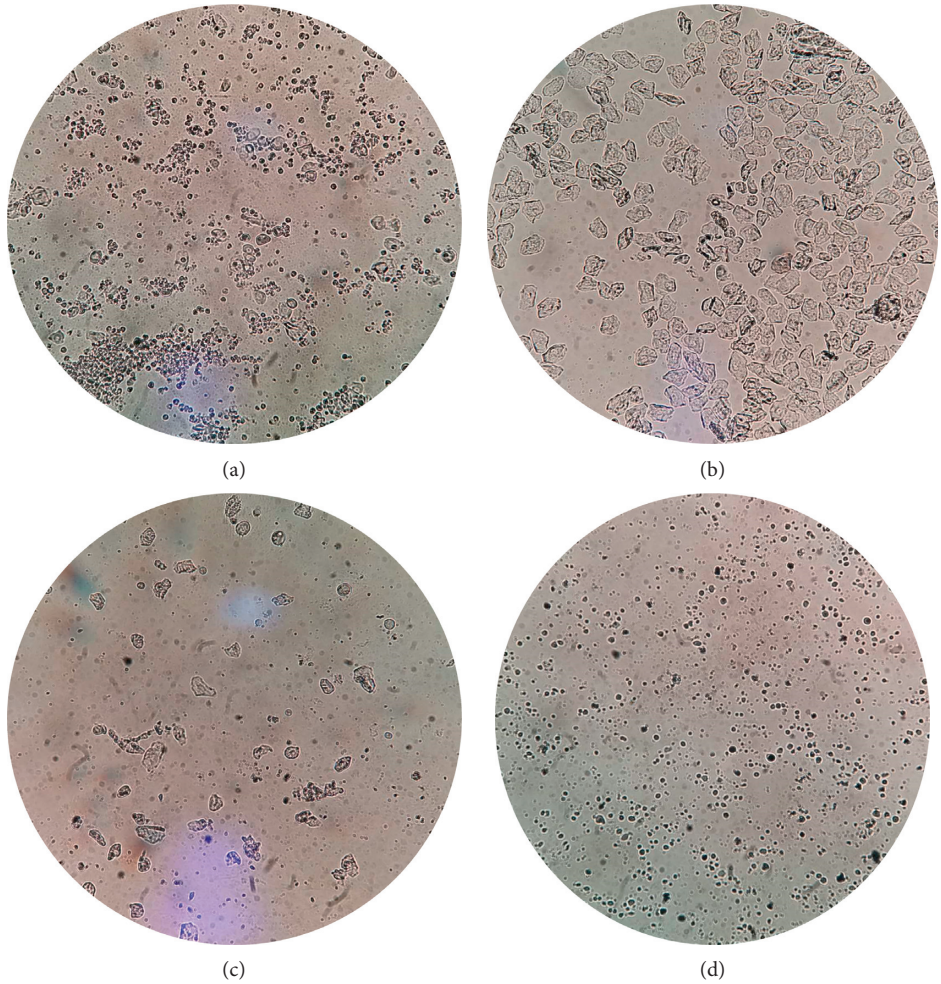

Figure 1: The regular vaginal smear of the typical rat estrus cycle. The cells of the different estrous cycles in rats were as follows: the small and round nucleated epithelial cells were the main cells in the prophase of estrus. In estrus, irregular squamous epithelial cells predominate. White blood cells, nucleated epithelial cells, and keratinized epithelial cells can be seen in the later stage of estrus, but the total number is small. During the estrus, most of them were white cells, and a few of them were nuclear epithelial cells and keratinized epithelial cells. (a) Propstrum. (b) Estrus. (c) Postestrus. (d) Diestrus.

limbs extension, one limb rotation, uterine contraction), indicating that the PD rats model was successfully prepared.

2.5. Treatment of Moxibustion. Groups A and C were put on the self-made rat clothes and fixed on the frame to expose their abdomen and leg and received no treatment (Figures 3(a) and 3(b)). Groups B and D received moxibustion on "SP6" and "CV4" from the 1st to 10th days after model establishment. First, we put on the self-made rat clothes and fixed them on the frame to expose their abdomen and leg. The locations of SP6 and CV4 acupoints were shown in Figure 3(a). Shave the hair of acupoints by 1×1 cm, mark the acupoint position with a marking pen, and light the moxa stick and placed it 2-3 cm away from the skin of the rat. Then, make moxibustion of the above two acupoints gently, and replace the moxa stick when it was close to burnout, once a day, each time for 20 min [38], for 10 days [12] (choose the right SP6 for 1–5 d and the opposite side for 6–10 d). All manipulations were performed between 8:00 a.m. and 12:00 a.m. every day to minimize the influence of circadian rhythms.

2.6. Observed the Number and Shape of Microvasculature under the Microscope. After the rats were anesthetized with 0.2 ml/100 g of 1.5% pentobarbital sodium, we made an opening about 2-3 cm long at about 0.5 cm outside the midline of the abdomen and then pulled out one side of the uterus and its ligament. We selected the middle section of the uterus and put it under the stereomicroscope. We observed the color and number of uterus microvasculature under the stereomicroscope (40X) and took pictures. At last, we calculated the microvascular diameters (μm) of pictures by Image Proplus Software.

2.7. Measurement of Uterine Thickness In Vitro. After the observation of uterine microvasculature under the stereomicroscope, the rats were further killed by spinal dislocation. The uterus was quickly and completely removed (in a "V"

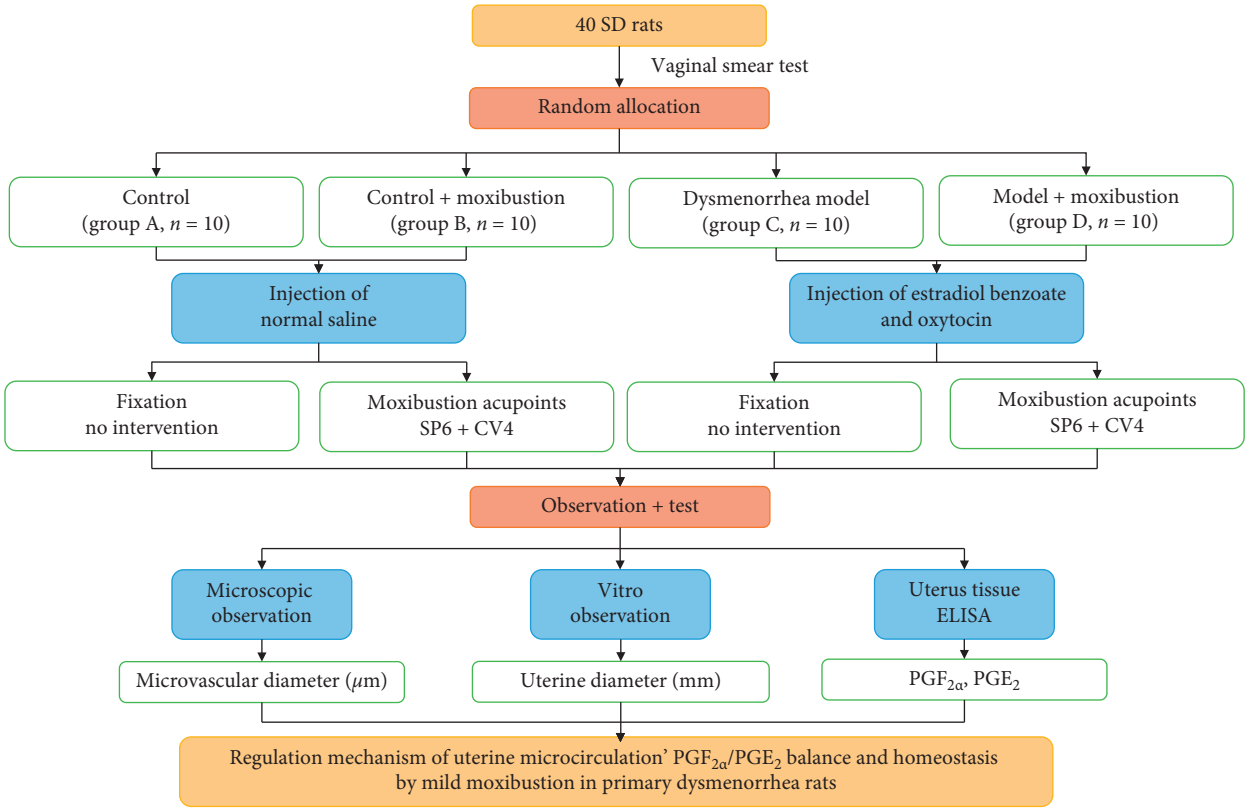

Figure 2: Flowchart of experiment development. SP6 = Sanyinjiao; CV4 = Guanyuan.

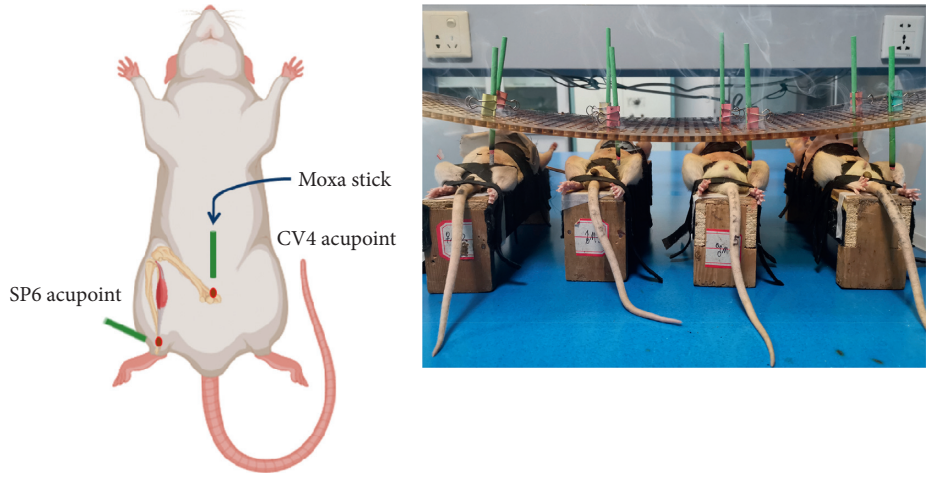

Figure 3: (a, b) The location of SP6 and CV4 acupoints on the body of SD rats. SP6 is located 10 mm above the tip of the inner ankle of the hind limb, and CV4 is located about 25 mm below the umbilicus.

shape). The uterine tissue was placed in a culture dish (the culture dish was placed above the ice plate), and the connective tissue and fat attached to the uterine wall were carefully removed (attention should be paid to not retaining the ovaries at the distal end of the bilateral uterus). In the process of stripping, it is necessary to avoid puncturing the uterine body due to instrument operation and ensure the liquid filling in the uterine body. The thickness (mm) of the isolated uterus was measured at the midpoint between the distal end of the uterus and the uterine horn. After the measurement, the uterus was stored in Eppendorf (EP) tube and transferred to liquid nitrogen. All uteruses were stored at −80°C.

2.8. Detection of the Content of $PGF_{2\alpha}$ and PGE_2 in Tissue by Enzyme-Linked Immunosorbent Assay. We took 100 mg of frozen uterine tissue from the ipsilateral segment and

ground it with a 9-fold homogenate medium. Then, the grinding liquid was centrifuged at 3000–4000 r for 10 min. The supernatant was taken and prepared into 10% tissue homogenate, which was placed in a refrigerator at 4°C for testing. Diluent and standard liquid were prepared according to the instructions. The samples were incubated at room temperature for 1.5 hours and then washed. Antibodies, enzymes, signal enhancers, and enzymes were successively added for incubation and washing. Finally, after adding the substrate color development and termination solution, the absorbance values of each hole were detected at 450 nm. The standard curve was drawn with the absorbance value of the standard hole and its corresponding concentration, and the contents of PGF_{2a} and PGE_2 in the uterine tissues of the rat samples were calculated. The contents of PGE_{2a} and PGF_2 were determined by enzyme-linked immunosorbent assay (ELISA) and operated strictly according to the kit instructions (the detection kit brand is Elabscience). Figure 4 shows the schematic diagram of the whole experimental process of moxibustion intervention on healthy and PD rats.

2.9. Statistical Analysis. GraphPad Prism 8.0 software was used for statistical analysis. The normal data were expressed as mean ± standard deviation (SEM) and the nonnormal distribution data as median (lower quartile, upper quartile). The data of each group conform to a normal distribution. Two-way ANOVA was used for the comparison among the groups, and the Turkey test was used for the multiple comparison after the event. $P < 0.05$ was considered as the difference was significant.

3. Results

3.1. Difference of Writhing Times after Molding. Because groups A and B did not use estradiol benzoate and oxytocin to induce pain, there was no writhing reaction within 20 minutes. After modeling, groups C and D showed a significant writhing response (Table 1). For groups C and D, the writhing latency was shortened. The results showed that the model of estradiol benzoate combined with oxytocin was successful, and the baseline of groups C and D was comparable.

3.2. Effect of Moxibustion on the General State of Rats. The results showed that moxibustion at the SP6 and CV4 acupoints for ten consecutive days can regulate the general state of rats. The hair color of rats in group A was glossy and they moved freely. In group C, the hair color was haggard. And they depilated with obvious grasping stress. In group D, the overall state of rats was better than that of group C, and the hair color was glossy and active.

3.3. Effect of Moxibustion on the Thickness of Uterus In Vitro (mm). A previous study by Dmitrovic et al. found that the uterus of dysmenorrhea women was larger than that of healthy women, especially in the transverse, anterior, and posterior diameters [39]. We wanted to know the relationship between uterine morphology, uterine microvasculature, and uterine prostaglandin. Therefore, we dissected the uterine tissue and measured its thickness. The two main effects (independent variables) described below refer to the body state of rats (primary dysmenorrhea model rats/healthy rats) and moxibustion or not.

In terms of the thickness of the isolated uterus, the two main effects were statistically significant ($P < 0.0001$, $P < 0.0001$), and there was a significant interaction between the two main effects ($P < 0.0001$). The effect of moxibustion on the thickness of the isolated uterus varies with the body state (A in Figure 5(a)).

Compared with group A, there was no statistical difference between group A and group B ($P > 0.05$), but the diameter of the uterus in vitro has a trend of increased in group B. In group C, the diameter of the uterus in vitro has increased significantly ($P < 0.0001$), and in group D, the diameter of the uterus in vitro increased ($P < 0.001$). Compared with group B, the diameter of the uterus in vitro in group C has increased significantly ($P < 0.0001$). The diameter of the uterus in vitro has a trend of increase in group D ($P > 0.05$), the difference was not statistically significant. Compared with group C, the results showed that moxibustion at the SP6 and CV4 acupoints for ten consecutive days significantly decreased the diameter of the uterus in vitro ($P < 0.0001$) (B and C in Figure 5(a)).

3.4. Effect of Moxibustion on Uterine Microvascular Diameter (μm). Microcirculation includes arterioles, posterior arterioles, true capillaries, and blood capillary. Uterine microvasculature is an important part of microcirculation. Some studies found that there are obvious microcirculation disorders in PD rats, such as the uneven thickness of uterine microvasculature and capillaries, the contraction of diameter [35], and the common fracture of capillaries, even the contraction to the smooth muscle layer of the uterus [40]. Acupuncture at CV4 and SP6 acupoints can improve microcirculation disorders. The related factors affecting uterine microcirculation include temperature and body fluid regulation. Therefore, this part of the study mainly observed the influence of moxibustion on SP6 and CV4 acupoints through observing uterine microvasculature in vivo.

In terms of microscopic microvascular, the main effects of the two factors were statistically significant ($P < 0.0001$, $P < 0.0001$), and there was no interaction between the two main effects ($P > 0.05$). The effect of moxibustion on uterine microvasculature is not different from the body state of rats (A in Figure 5(b)).

Compared with group A, the diameter of uterine microvasculature in group B tended to be coarser ($P > 0.05$). The microvascular diameter in group C decreased significantly ($P < 0.0001$), and that in group D decreased ($P < 0.05$). Compared with group B, the microvascular diameter of groups C and D decreased significantly ($P < 0.0001$), and the difference was statistically significant. Compared with group C, the microvascular diameter in

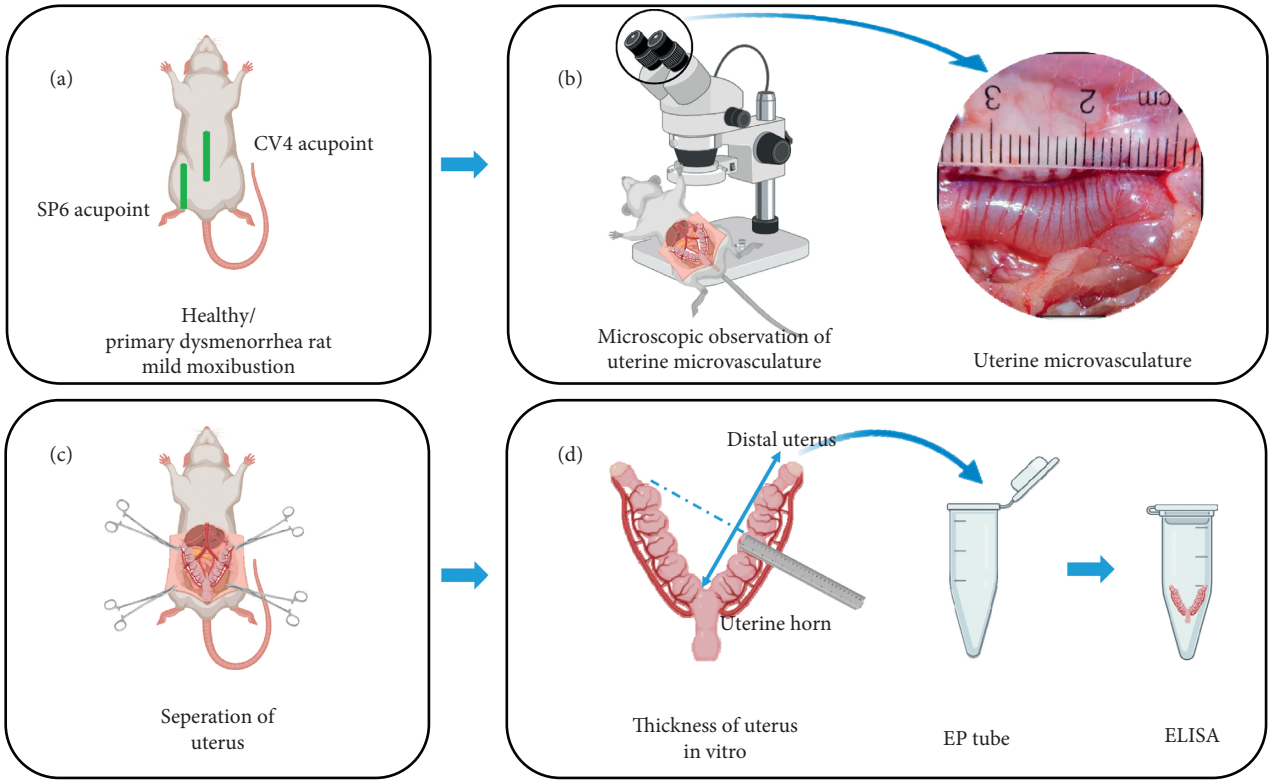

Figure 4: The schematic diagram of the whole experimental process of moxibustion intervention on healthy and PD rats. (A) The moxibustion on health and PD rats. (B) Microscopical observation of uterine microvasculature in rats. (C) The separation of the uterus. (D) Measurement of uterine thickness in vitro and detection $PGF_{2\alpha}$ and PGE_2 of uterus tissue by ELISA.

Table 1: Writhing latency of rats after modeling.

Groups	Numbers	Writhing latency (min)	Writhing times
C	10	8.50 ± 6.45	11.30 ± 9.84
D	10	10.00 ± 5.98	8.30 ± 5.43

Data are expressed as the mean ± SEM.

group D was significantly thicker ($P < 0.0001$), and the difference was statistically significant (B and C in Figure 5(b)).

3.5. The Effect of Moxibustion Applied to the SP6 and CV4 Acupoints on the PGs ($PGF_{2\alpha}$ and PGE_2) Levels. In terms of PGF_{2a}, the main effect of the two independent variables was not statistically significant ($P > 0.05$), but the interaction effect was statistically significant ($P < 0.05$). The effect of moxibustion on PGF_{2a} in uterine tissue varies with different body states (A in Figure 6(a)). Compared with group A, the $PGF_{2\alpha}$ in groups B and D increased ($P > 0.05$). The $PGF_{2\alpha}$ in group C increased ($P < 0.05$), and the difference was statistically significant. Compared with group B, $PGF_{2\alpha}$ in group C increased ($P > 0.05$), while $PGF_{2\alpha}$ in group D decreased ($P > 0.05$). Compared with group C, $PGF_{2\alpha}$ in group D decreased ($P > 0.05$) (B in Figure 6(a)).

In terms of PGE_2, the main effect of the two independent variables was not statistically significant ($P > 0.05$), but there was a significant interaction effect between the two variables ($P < 0.0001$). The effect of moxibustion on PGE_2 in uterine tissue varies with different body states (A in Figure 6(b)). Compared with

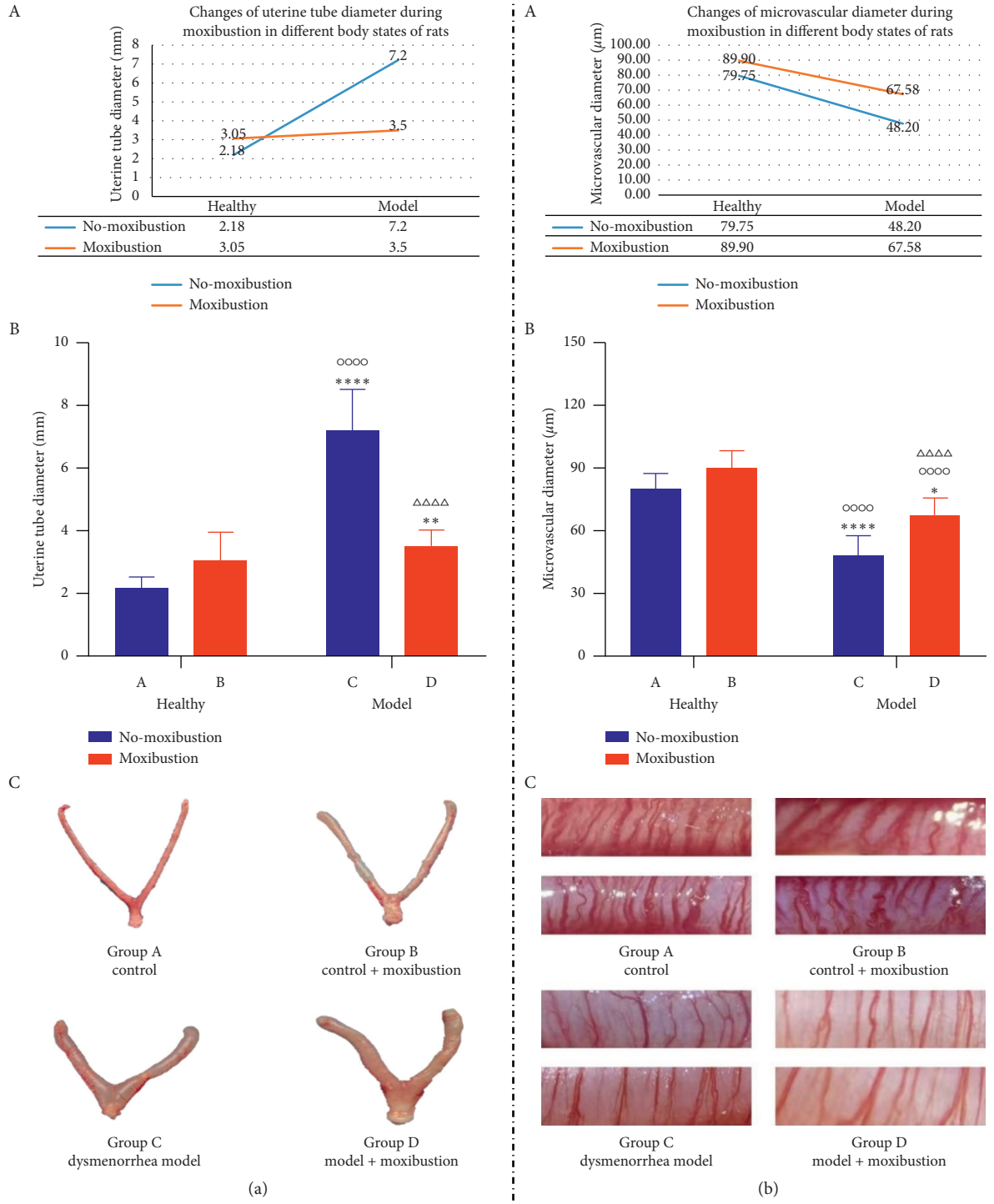

Figure 5: In (a), changes of uterine tube diameter during moxibustion in different body states of rats (A), thickness of uterus in vitro (mm) (B), and four groups of the typical uterus in vitro (C) are shown. In (b), changes of microvascular diameter during moxibustion in different body states of rats (A), effects of moxibustion on uterine microvascular diameter under microscope (B), and microscopic view of uterine microvascular (C) are shown. Data are expressed as the upper quartile and the lower quartile. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ versus group A. $^{oooo}P < 0.0001$ versus group B. $^{\Delta\Delta\Delta\Delta}P < 0.0001$ versus group C.

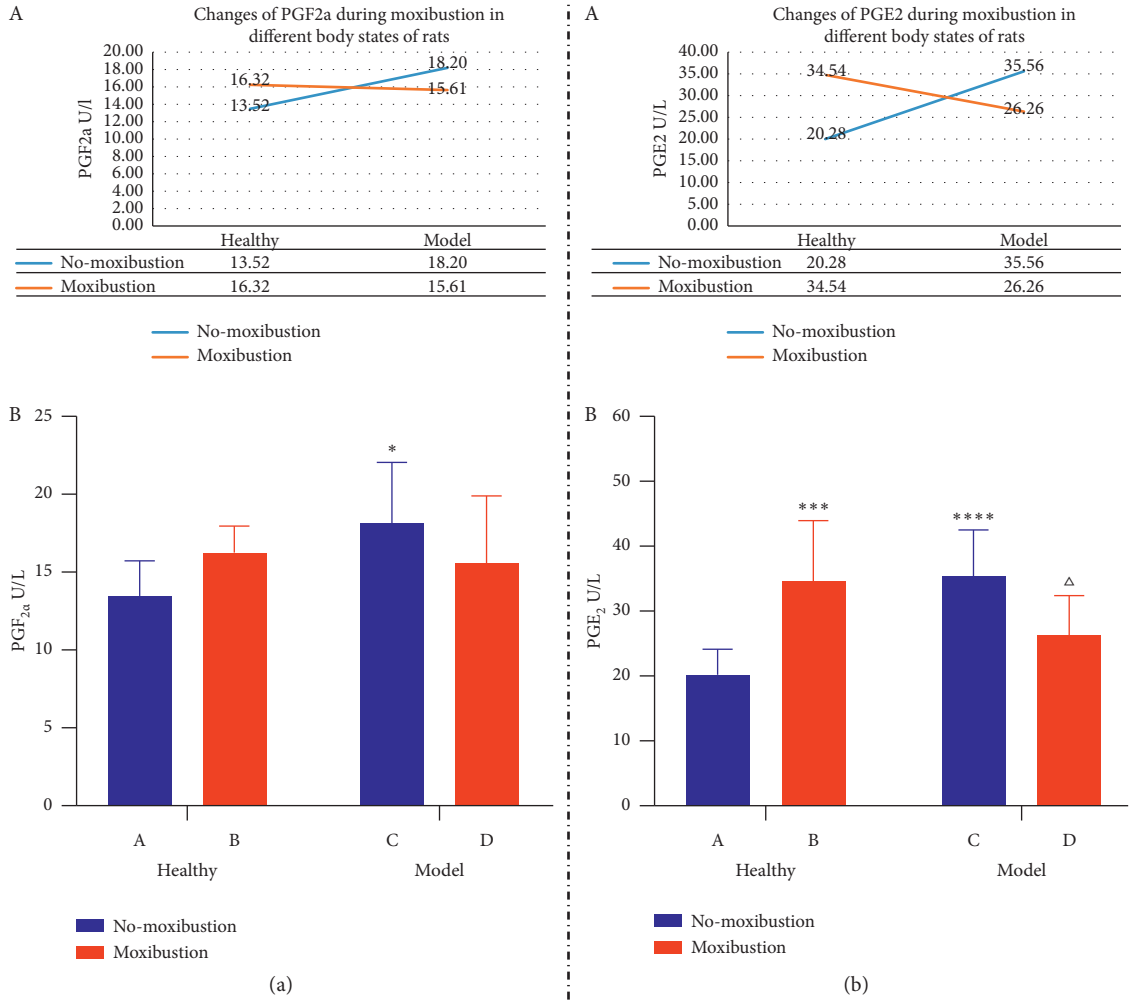

Figure 6: In (a), changes of PGF_{2a} during moxibustion in different body states of rats (A) and effects of moxibustion on the content of PGF_{2a} in uterine tissue (B) are shown. In (b), changes of PGE_2 during moxibustion in different body states of rats (A) and effects of moxibustion on the content of PGE_2 in uterine tissue (B) are shown. PGF_{2a} data are expressed as the mean ± SEM. $^{*}P < 0.05$ versus group A. PGE_2 data are expressed as the upper quartile and the lower quartile. $^{***}P < 0.001$, $^{****}P < 0.0001$ versus group A. $^{\Delta}P < 0.05$ versus group C.

Table 2: Index changes and interaction effects of moxibustion in different body states of rats.

Observation index	Source of difference	SS	MS	F	P value
The thickness of uterus in vitro (mm)	Row factor	74.80	74.80	102.23	$P < 0.0001$
	Column factor	20.02	20.02	27.36	$P < 0.0001$
	Interaction	52.21	52.21	71.36	$P < 0.0001$
Microvascular diameter (μm)	Row factor	7253.87	7253.87	99.53	$P < 0.0001$
	Column factor	2180.05	2180.05	29.91	$P < 0.0001$
	Interaction	212.89	212.89	2.92	$P > 0.05$
PGF_{2a} (U/L)	Row factor	39.34	39.34	3.89	$P > 0.05$
	Column factor	0.11	0.11	0.01	$P > 0.05$
	Interaction	72.42	72.42	7.16	$P > 0.05$
PGE_2 (U/L)	Row factor	122.49	122.49	2.72	$P > 0.05$
	Column factor	61.46	61.46	1.36	$P > 0.05$
	Interaction	1387.89	1387.89	30.80	$P < 0.05$

Row factor: different body states of rats (healthy/primary dysmenorrhea model); column factor: moxibustion or not; SS: sum of squares between groups; MS: mean square.

Table 3: Summary of the effects of moxibustion on the regulation of uterine microvascular in healthy and PD rats.

Intergroup comparison	The thickness of uterus in vitro (mm)	Microvasculature diameter (μm)	$PGF_{2\alpha}$ (U/L)	PGE_2 (U/L)
A-B	Thickening trend $P > 0.05$	Thickening trend $P > 0.05$	Rising trend $P > 0.05$	Rising $P < 0.001$
A-C	Obvious thickening $P < 0.0001$	Obvious thinning $P < 0.0001$	Rising $P < 0.05$	Rising $P < 0.0001$
C-D	Thinning $P < 0.0001$	Obvious thickening $P < 0.0001$	Downward trend $P > 0.05$	Downward $P < 0.05$
A-D	Thickening $P < 0.001$	Thinning $P < 0.05$	Rising trend $P > 0.05$	Rising trend $P > 0.05$
B-D	Thickening trend $P > 0.05$	Obvious thinning $P < 0.0001$	Downward trend $P > 0.05$	Downward trend $P > 0.05$

A = control group; B = control plus moxibustion group; C = PD model group; D = PD model plus moxibustion group.

group A, the PGE_2 in groups B and C increased ($P < 0.001$, $P < 0.0001$), and the difference was statistically significant. The PGE_2 in group D increased ($P > 0.05$). Compared with group B, PGE_2 in group C increased ($P > 0.05$), while PGE_2 in group D decreased ($P > 0.05$). Compared with group C, PGE_2 in group D decreased ($P < 0.05$) (B in Figure 6(b)).

4. Conclusion

According to Tables 2 and 3 and the above results, the following conclusions can be further deduced.

(1) After estradiol benzoate and oxytocin made the model, the uterine microvessels of rats became thinner, the isolated uterus became swollen and thicker, and the contents of $PGF_{2\alpha}$ and PGE_2 increased.

(2) Moxibustion can thicken the uterine microvessels of PD rats, thin the isolated uterus, and reduce the contents of PGF_{2a} and PGE_2.

(3) Moxibustion can thicken the uterine microvessels of healthy rats, thicken the isolated uterus, and increase the contents of PGF_{2a} and PGE_2.

(4) Moxibustion has different regulatory effects on rats in different body states.

① The regulating effect of moxibustion on the microvessels of PD rats is greater than that of healthy rats.

② Moxibustion can regulate the prostaglandin index in two ways. Moxibustion decreased the contents of PGF_{2a} and PGE_2 in PD rats and increased the contents of PGF_{2a} and PGE_2 in healthy rats.

(5) Combined with the changes of microvascular effect in rats, although the content of PGE_2 changed more obviously, the contractile effect of PGF_{2a} was dominant.

(6) In conclusion, moxibustion at SP6 and CV4 can reduce dysmenorrhea in PD rats, and its mechanism may be to reduce the content of $PGF_{2\alpha}$ and PGE_2 in uterine tissue, relax uterine microvessels, improve microcirculation disorder, so as to reduce uterine swelling, and achieve the purpose of treating dysmenorrhea.

5. Discussion

The literature research shows that $PGF_{2\alpha}$ mainly plays a contractile role in the uterus, and PGE_2 plays a diastolic role in the uterus. Dysmenorrhea is characterized by fluctuation and intermittence, with the most intense pain at the first 24–36 hours of menstruation, which is consistent with the maximum time of prostaglandin release into menstrual fluid [41]. The occurrence of PD pain is related to the production of large amounts of PGs in the uterus [1, 42–45], and the intensity of pain is directly proportional to the content of $PGF_{2\alpha}$ [31, 46]. The most basic structure and function unit of the uterine microcirculation system is the microcirculation between uterine microvasculature and capillary. $PGF_{2\alpha}$ can constrict the uterine microvascular [47], resulting in the decrease of the local blood flow of the uterus, the disturbance of the microcirculation of the uterus, the abnormal tissue fluid exchange caused by the long-term tissue ischemia and hypoxia, the morphological changes of the uterus, and finally a series of symptoms of PD.

This study found that the content of $PGF_{2\alpha}$ and PGE_2 in uterine tissue increased in the intervention of moxibustion on healthy rats, and the microvasculature became coarser. After models were made by estradiol benzoate combined with oxytocin, the two kinds of prostaglandins in the uterus of PD rats increased, the content of PGE_2 was higher than that of $PGF_{2\alpha}$, and the microvasculature was thinner, suggesting that the contraction effect of $PGF_{2\alpha}$ was stronger than the relaxation effect of PGE_2. Moxibustion intervention in PD rats, the two prostaglandins in uterine tissue were reduced, the content of PGE_2 was significantly lower than $PGF_{2\alpha}$, and the microvessels were significantly thicker, suggesting that the contraction effect of $PGF_{2\alpha}$ was more obvious than the relaxation effect of PGE_2. To sum up, $PGF_{2\alpha}$ and PGE_2 in the uterus of rats showed a homotropic change, and the change of PGE_2 was more obvious, but PGF_{2a} played a leading role in contraction.

Combined with the literature research and this study (Table 1), it is speculated that the content of $PGF_{2\alpha}$ and the contraction of uterine microvasculature may cause local ischemia and anoxia, microcirculation disturbance, and the change of uterine tissue morphology and thickening. Moxibustion can reduce the content of $PGF_{2\alpha}$ and the relaxation of uterine microvasculature in PD rats, which can lead to the relief of local ischemia and hypoxia, the

improvement of microcirculation disturbance, and the reduction of swelling of the uterus in vitro. To sum up, moxibustion of SP6 and CV4 points may relax the uterine microvasculature by reducing the content of $PGF_{2\alpha}$ in the uterine tissue, to improve the microcirculation of the uterus, then alleviate the degree of uterine swelling, and finally achieve the effect of relieving dysmenorrhea.

We regarded prostaglandins as the main entry point, combined with PGF_{2a}/PGE_2 and uterine microcirculation to explore the possible mechanism of moxibustion treat PDs. Many clinical and experimental studies have shown that moxibustion stimulation applied to the SP6 or CV4 acupoints (Sanyinjiao and Guanyuan moxibustion point) performed well in the treatment of pain induced of primary dysmenorrhea [10, 19–23]. SP6 and CV4 points are common compatibility schemes for the treatment of PD [24, 25]. SP6 can relieve the pain of PD immediately [44, 48, 49], especially for PD patients with cold and dampness stagnation pattern [50]. Moxibustion may regulate the content of prostaglandin in the uterus of PD through its light effect, heat effect, moxa fume, drug composition, and so forth. Further, improve the microcirculation of the uterus, finally, relieve the shape of the uterus, and achieve the effect of relieving dysmenorrhea [51].

In the whole process of the experiment, animal selection, model making, intervention, and efficacy evaluation were strictly controlled. Moxibustion significantly improved uterine microvascular and isolated uterine morphology, while the regulation of prostaglandin only showed a trend change. Combined with the particularity of moxibustion as a supplementary alternative therapy, the whole experimental process was reviewed and analyzed. The reasons considered may be related to the lack of moxibustion quantity and treatment course. Further research directions include but are not limited to the following. 1. In this study, moxibustion intervention on the prostaglandin of PD rats shows a trend change, which may be related to the course of treatment, cycle, and insufficient amount of moxibustion. In the further experiment, different control groups are set up to study the quantity effect relationship of moxibustion intervention on PD. 2. This experiment speculates that the therapeutic effect of moxibustion on PD rats is greater than that of healthy rats. We can set up the matching experiment to study the index change before and after the intervention and further clarify the specific effect difference of moxibustion on healthy and PD rats. 3. Moxibustion regulates the contraction of uterine microvascular by prostaglandin, causes the change of uterine morphology, and relieves the pain process. It is worth further exploring whether there is a new target in the information transduction process between prostaglandin and uterus. 4. There may be differences in the effects of different cycles of moxibustion on PD, which can be studied by incorporating rats in different emotional periods to determine the best intervention time of moxibustion for PD.

In conclusion, moxibustion of SP6 and CV4 acupoints may relax the uterine microvasculature by reducing the content of $PGF_{2\alpha}$ in the uterine tissue, to improve the microcirculation of the uterus, then alleviate the degree of PD rat's uterine swelling, and finally, achieve the effect of relieving dysmenorrhea. It will be further studied whether moxibustion can relieve dysmenorrhea symptoms through light effect, heat effect, or drug composition of moxa fume.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xuemei Li, Sha Guo, and Zhaoheng Chen drafted the manuscript. Xuemei Li, Sha Guo, Zhaoheng Chen, and Kuiyu Ren completed the animal experiment. Hong Zhang, Shuguang Yu, and Sha Yang designed the experiment scheme. Sha Yang revised the manuscript. All authors have read and approved the publication of the final manuscript. Xuemei Li, Sha Guo, and Zhaoheng Chen are co-first authors and contributed equally to this paper.

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Review Article

Complementary and Alternative Medicine for Dysmenorrhea Caused by Endometriosis: A Review of Utilization and Mechanism

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Endometriosis (EM) is a common and benign estrogen-dependent gynecological disorder among women of reproductive age, and secondary dysmenorrhea is one of the more severe symptoms. However, the mechanism behind the development of dysmenorrhea is poorly understood, and there is a lack of effective methods for diagnosing and treating EM dysmenorrhea. In this regard, complementary and alternative medicine (CAM) has recently come into widespread use due to its limited adverse reactions and high efficiency. This review updates the progress of CAM in the treatment of EM dysmenorrhea and seeks to identify the therapeutic efficacy as well as the mechanisms behind these effects based on the available clinical and experimental studies. According to the literature, CAM therapy for EM dysmenorrhea, including herbs (herbal prescriptions, extracts, and patents), acupuncture, and Chinese herbal medicine enema (CHM enema), is effective for relieving dysmenorrhea with fewer unpleasant side effects when compared to hormonal and surgical treatments. In addition, we discuss and analyze the existing gaps in the literature. We hope to provide some instructive suggestions for clinical treatment and experimental research in the future.

1. Introduction

Endometriosis (EM) is defined as endometrial tissue, including glands and stroma, which grows abnormally in locations outside the uterus. EM is the second most common benign female genital disease after uterine myoma [1]. About 15% of women of reproductive age suffer from EM [2], but the actual incidence is higher because this figure is only for symptomatic cases [3]. EM is characterized by chronic pelvic pain, secondary dysmenorrhea, dyspareunia, infertility, abnormal uterine bleeding, and so forth. As one of the more severe symptoms, secondary dysmenorrhea refers to pain or cramps before or during menstruation. In 78.7% of women with EM, dysmenorrhea is the symptom that led to their diagnosis and severely reduces their quality of life [4]. The pathophysiology of EM dysmenorrhea may include immune factors [5], excessive production of prostaglandin causing ischemia [6], activation of mechanoreceptors [7], increased neovascularization [8], neurological factors [9, 10], and so forth (Figure 1).

Treatment for EM dysmenorrhea can be medical and/or surgical. For mild cases, nonsteroidal anti-inflammatory drugs play an important role in alleviating menstrual pain. However, for cases who are unresponsive to these drugs, they can be treated by hormone replacement therapy, including progestogenics, gestrinone, danazol (androgen derivatives), and gonadotropin-releasing hormone agonists to alleviate pain [11]. Surgical methods are also needed for those patients who cannot accept oral medicines. About 18% of women with dysmenorrhea are unresponsive to nonsteroidal anti-inflammatory drugs [12], and women receiving hormone replacement therapy are at increased risk for some cancers and cardiovascular diseases. Moreover, EM has a high recurrence rate. Thus, there is an urgent need to develop better strategies for treating EM.

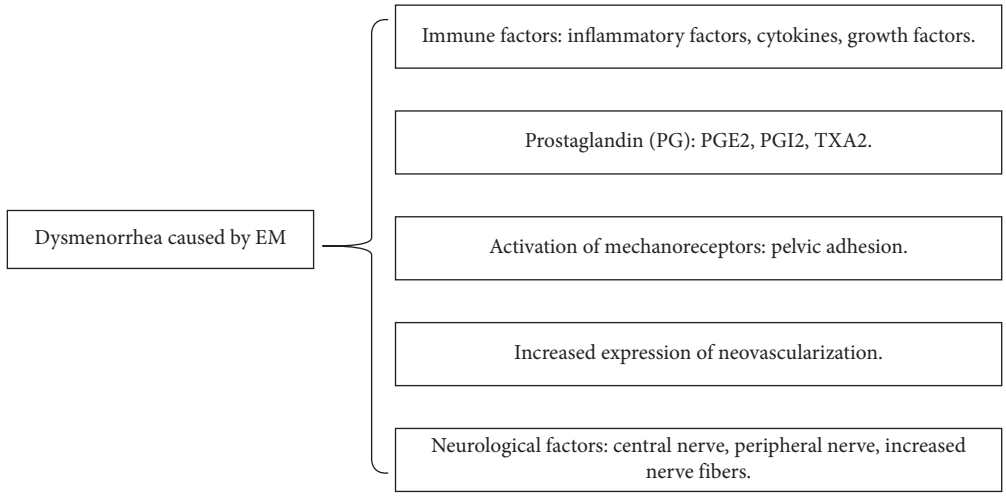

Figure 1: Risk factors for EM dysmenorrhea.

Complementary and alternative medicine (CAM) refers to various health care and treatment systems that are independent of Western medicine, such as traditional Chinese medicine (TCM), qi gong, and homeopathy [13]. The acknowledged advantages of CAM are that they are natural, convenient, and affordable, and thus, CAM has gained favor with many nationalities and peoples [14]. CAM is an effective strategy to alleviate the pain associated with many diseases, and both academic researchers and governmental agencies have started to incorporate CAM recommendations into chronic pain management strategies [15]. Therefore, this review updates the progress of CAM in the treatment of EM dysmenorrhea. According to the available literature, CAM methods for EM dysmenorrhea mainly include herbal products, acupuncture and moxibustion, and CHM enema (Table 1).

2. Search Strategy and Selection Criteria

The literature search was performed using the key words "Endometriosis," "Dysmenorrhea," "Menstrual Pain," "Traditional Chinese Medicine," "Complementary and alternative medicine," and "Herbal Extracts" in PubMed, CNKI, VIP, Web of Science, Embase, and Wanfang from the date of establishment of the database to 2021. References in the identified studies were also searched to identify relevant literature. A total of 1,060 articles were identified in the search, and 116 articles were deemed potentially relevant. Among them, there are 51 articles on Chinese products, 30 articles on acupuncture and moxibustion, and 15 articles on CHM enema.

3. Herbal Products

Herbal products mainly include compound preparations (consisting of two or more herbs) in which all of the medicines are boiled together in water and then processed into herbal extracts, pills, or capsules (patents). Tables 2 and 3 list the most commonly used herbs for treating EM dysmenorrhea. In China, compound preparations are generally more effective than other treatment methods. Researchers have suggested that the potential mechanism of herbal products on EM dysmenorrhea is through (1) reducing the viscosity of whole blood, improving pelvic microcirculation, and adjusting the expression level of related factors [16, 17], (2) inhibiting the expression of inflammatory factors such as interleukin-1 (IL-1) and interleukin-6 (IL-6) [18], (3) reducing the expression of prostaglandin E2 (PGE2), prostaglandin f 2α (PGF2α), and nerve growth factor (NGF) in patients with EM dysmenorrhea [19], and (4) inhibiting uterine smooth muscle activity and relieving uterine smooth muscle spasm [20].

3.1. Herbal Decoction Therapies. There are several common decoctions used to treat EM dysmenorrhea in China, including Shaofu Zhuyu decoction (SZD), Wengjing decoction (WJD), Xuefu Zhuyu decoction (XZD), and Danggui Sini decoction (DSD). Many clinical and experimental studies have confirmed that Chinese herbal decoctions play an important role in the treatment of EM dysmenorrhea.

3.1.1. SZD. SZD comes from QR Wang's *Correction of Errors in Medical Works*, which was written in the Qing dynasty and is still widely used in the clinic. In the theory of TCM, SZD is mainly used in EM dysmenorrhea due to cold blood stasis. SZD can reduce the expression of tumor necrosis factor (TNF-α), IL-6, and interleukin-8 (IL-8); the mRNA expression of extracellular regulated protein kinases (ERK), vascular endothelial growth factor (VEGF), and matrix metalloprotein-9 (MMP-9); and the protein expression of nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), and MAPK-ERK kinase (MEK), thus influencing the MAPK/ERK signaling pathway as a way to treat EM [21]. SZD can inhibit proliferation in the eutopic endometrium by inducing apoptosis in eutopic endometrial stromal cells in a rat model of EM [22]. A randomized controlled trial (RCT) showed that SZD could relieve dysmenorrhea more effectively than ibuprofen capsules. The total effective rates were 90% and 70%, respectively, after 3

Table 1: General view of all therapeutic approaches.

Therapeutic approaches	Specifications	Efficacy	Precautions	Refs.
Herbal products	Appropriate TCM prescriptions are proposed according to TCM doctors' judgment.	Alleviating dysmenorrhea.	Allergy to drugs or contraindications.	[16–66]
Acupuncture and moxibustion	Use the appropriate acupoints or moxa-moxibustion therapy in light of the disease status of the patient. Most acupuncture treatments are 30 min (needling and auricular point), while moxibustion treatments last 40–50 min.	Alleviating dysmenorrhea.	Be careful of fainting conditions.	[67–96]
CHM enema	Ask patients to take the left lateral decubitus position. Put the boiled TCM herbal liquid into a 20 mL syringe, and wait for the temperature to reach 38–40°C. With a disposable catheter connection, slowly push the TCM herbal liquid into the rectum. Tell patients to relax and to retain the TCM herbal liquid for at least 2 hours.	Alleviating dysmenorrhea.	Use caution in patients with intestinal lesions.	[97–111]

Table 2: Herbal mixture for dysmenorrhea caused by EM treatment in the literature.

Herbal mixture sample/case number (n)	Ingredients	Control sample number (n)	Total clinical effect rate	Model used	Therapeutic effects and actions	Refs.
Shaofu Zhuyu decoction (SZD); $n = 20$	Xiao Hui Xiang, Gan Jiang, Yuan Hu, Mo Yao, Dang Gui, Chuan Xiong, Guan Gui, Mu Dan Gen, Pu Huang, Wu Ling Zhi	Ibuprofen; $n = 20$	T: 90.00% versus 70.00%	Human study	Alleviating dysmenorrhea. CA-125↓, PEG2↓	[23]
Wengjing decoction (WJD); $n = 48$	Wu Zhu Yu, Mai Dong, Dang Gui, Mu Dan Pi, Chuan Xiong, Ren Sheng, Gui Zhi, E Jiao, Bai Shao, Sheng Jiang, Ban Xia, Gan Cao	Mifepristone; $n = 48$	T: 93.75% versus 79.17%	Human study	Alleviating dysmenorrhea. CD4+↓, CD4+/CD8+↓, IL-4↓, IL-10↓	[26]
Xuefu Zhuyu decoction (XZD); $n = 60$	Dang Gui, Sheng Di Huang, Tao Ren, Hong Hua, Zhi Qiao, Chi Shao, Chai Hu , Gan Cao, Jie Geng, Chuan Xiong, Niu Xi	Mifepristone; $n = 60$	T: 90.0% versus 73.3%	Human study	Alleviating dysmenorrhea. Reducing VAS scores.	[30]
Danggui Sini decoction (DSD); $n = 37$	Dang Gui, Gui Zhi, Shao Yao, Xi Xin, Tong Cao, Zhi Gan Cao, Da Zao	Progesterone; $n = 37$	D: 89.19% versus 67.57%	Human study	Alleviating dysmenorrhea.	[34]
Danggui Shaoyao Powder (DSP); $n = 40$	Dang Gui, Shao Yao, Chuan Xiong, Fu Ling, Ze Xie, Bai Zhu	Progesterone; $n = 40$	T: 97.5% versus 82.5%	Human study	Alleviating dysmenorrhea. Reducing VAS scores. PEG2↓, P↓	[35]

Note: T (Total effect rate) = number of effective cases/total number of cases; where effective case refers to the patients or animal models whose signs and symptoms were improved after treatment. D: dysmenorrhea alleviation rate.

months of treatment in a study group of 20 patients [23]. SZD can reduce cell proliferation, increase apoptosis, and inhibit angiogenesis and hypoxia inducible factor-1α (HIF-1α) expression, and SZD seems to be helpful in preventing the recurrence of EM after surgery [24].

3.1.2. WJD. WJD has been widely used to treat various disorders in China since the Han dynasty. WJD has an inhibitory effect on the growth of ectopic endometrium in a mouse model of EM and reduces the levels of inflammatory factors and improves the inflammatory response [25]. In the clinic, the results have been similar to those in animal trials. Compared with mifepristone, WJD could significantly lower serum $CD4^+$, $CD4^+/CD8^+$, IL-4, and IL-10 levels to relieve pain [26]. In addition, Tang and Wu [27] selected 60 patients who were randomly divided equally into the treatment group (WJD) and control group (ibuprofen sustained-release capsules) for 3 months. The total effective rate of the treatment group was 90.0%, which was significantly higher than 76.7% in the control group, and the visual analogue scale (VAS) scores of the two groups were significantly reduced. WJD appears to be effective in treating EM dysmenorrhea, and thus should be considered for clinical applications.

3.1.3. XZD. XZD is also from Wang's *Correction of Errors in Medical Works*. In TCM, most doctors agree that EM dysmenorrhea's pathogenesis is blood stasis, so XZD or SZD is widely applied [28]. XZD acts on multiple EM targets and participates in regulating multiple signaling pathways

Table 3: Chinese traditional patent medicines for treating dysmenorrhea caused by EM.

Chinese traditional patent; sample number (n)	Ingredients	Control; sample number (n)	Total clinical effect rate	Model used	Therapeutic effects and actions	Refs.
Guizhi Fuling capsules (GFC); $n = 93$	Gui Zhi, Fu Ling, Mu Dan Pi, Tao Ren, Bai Shao	Gestrinone; $n = 94$		Human study	CA-125↓, CA-199↓	[52]
ELeng capsules (ELC); $n = 25$	San Leng, E Zhu, Dan Shen, Yu Jin, Bie Jia, Chi Shao, Ji Nei Jin, Zhe Bei Mu	Nemestran; $n = 25$	D: 84.00% -48% vs. 80%-36%	Human study	Alleviate dysmenorrhea CA-125↓, PRL↓	[57]
Dan Bie capsules (DBC)	Dang Shen, San Qi, San Leng, Tao Ren, Dang Gui, Bie Jia, Hai Zao, Du Zhong, Bai Zhu, Ban Zhi Lian, Gui Zhi	GFC		SD rat model	PGF2α↓, PGE2↓	[58, 59]
Sanjie analgesic capsules (SAC); $n = 112$	Long Xue Jue, San Qi, Zhe Bei Mu, Yi Yi Ren	Danazol; $n = 46$	T: 92.9% vs. 77.5%	Human study	Alleviate dysmenorrhea Shrink endometrioic lesion Recurrence rate↓	[63]
Dane Fukang paste (DFP); $n = 75$	Dan Shen, E Zhu, Chai Hu, San Qi, Chi Shao, Dang Gui, San Leng, Xiang Fu, Yuan Hu, Gan Cao	Gestrinone; $n = 75$	T: 95.45% vs. 81.82%	Human study	Alleviate dysmenorrhea	[66]

Note: T (Total effect rate) = number of effective cases/total number of cases; effective case refers to the patients or animal models whose signs and symptoms were improved after treatment. D: dysmenorrhea alleviation rate.

through its influence on TNF and estrogen [29]. Fu and Xie [30] treated 94 patients with EM dysmenorrhea with XZD or mifepristone, and the total effective rates were 89.4% and 78.7%, respectively. XZD can also reduce the VAS score of patients with EM and can relieve pain [31].

3.1.4. DSD. DSD contains angelica, cinnamon, and asuram and is a common decoction for treating EM dysmenorrhea. According to modern pharmacological research [32], angelica promotes the proliferation of hematopoietic progenitor cells related to antithrombotic and antiplatelet aggregation. Cinnamon contains coumarins, organic acids, volatile components, and other chemical components that exhibit antiviral, antiallergic, anti-infective, diuretic, antipyretic, and analgesic properties. Asarum has anti-infection, vasodilator, heart strengthening, antipyretic, and analgesic effects. Liu [33] applied DSD combined with different doses of mifepristone to evaluate the postoperative curative effect on EM and found that 5 mg of mifepristone combined with DSD could reduce serum levels of CA-125 and sex hormones and ked to a lower recurrence rate compared to 10 mg mifepristone combined with DSD. Li et al. [34] treated EM dysmenorrhea patients with DSD and reported an overall efficiency of 89.19%.

3.1.5. Other Herbal Decoction Therapies. Some reports of treating EM with Danggui Shaoyao Powder (DSP) showed that it could reduce serum progesterone levels, effectively regulate serum PGE2 and PGF2α concentrations, and significantly alleviate pain in EM dysmenorrhea patients [35, 36]. In addition, Bushen Zhuyu decoction (BSZYD) is 94.12% effective in treating EM, and IL-6, IL-8, and TNF-α levels are significantly reduced after treatment, indicating that BSZYD can improve EM dysmenorrhea and the pelvic microcirculation of patients with dysmenorrhea, regulate the expression level of related factors, and relieve pain [37]. Chai and Wang [38] treated EM dysmenorrhea patients with Gexia Zhuyu decoction (GZD), and it was proposed that GZD can reduce serum CA-125 levels, plasma viscosity, and the erythrocyte sedimentation rate. A rat trial showed that Qingre Huayu decoction could reduce the mRNA expression of NF-κB, VEGF, and cyclooxygenase-2 (COX-2), thus inhibiting inflammation and angiogenesis [39]. Various herbal mixtures are used, but only the most commonly used are discussed in this section.

3.2. Herbal Extract Therapies. Several herbal extracts are commonly used to treat EM dysmenorrhea in China, including rhizoma zedoariae water decoction, triterpenoid saponin, and emodin. All of these may affect cell proliferation, apoptosis, angiogenesis, immunity, and the inflammatory microenvironment through multiple pathways, thus playing an important role in EM dysmenorrhea.

3.2.1. Rhizoma Zedoariae Water Decoction. Rhizoma zedoariae water decoction is from the dried tuber of *Curcuma phaeocaulis valeton* (the traditional method is to add water and then boil to concentrate to 2 g/mL), and this can inhibit the growth of ectopic endometrium in a rat model of EM [40]. The mechanism is related to the downregulation of JAK2, STAT3 phosphorylation, and protein overexpression and to the reduction of JAK2 and STAT3 levels in ectopic endometrial tissues. STAT3 signaling has been shown to stimulate cell proliferation, inhibit apoptosis, promote angiogenesis, mediate immune evasion, and participate in tumorigenesis and development, and the interaction

between JAK and STAT3 promotes these cellular responses [41]. Similarly, studies in rats also demonstrated that curcumenol, the main active ingredient in zedoary, has antibacterial, anti-inflammatory, antioxidation, antitumor, antiplatelet aggregation, and antithrombotic activities and showed that curcumenol significantly reduces the levels of human macrophage chemoattractant protein-1 (MCP-1), migration inhibition factor (MIF), TNF-α, IL-1β, and IL-6 in the peritoneal fluid in the rat model of EM and has obvious inhibitory effects on inflammatory reactions in the abdominal microenvironment [42].

3.2.2. Triterpenoid Saponin. Triterpenoid saponin types are more widely distributed in nature than steroidal saponins and are predominantly found in Leguminosae, Araliaceae, Polygalaceae, and Cucurbitaceae such as ginseng, cohosh, Panax, and licorice. According to some rat trials [43, 44], the mechanism of action of triterpenoid saponins may be related to the inhibition of NF-κB activity, leading to increased IFN-γ cytokine secretion and decreased expression of cytokines such as IL-6 and TNF-α, thus inhibiting the formation and development of lesions. These cytokines, which are immune factors, play important roles in EM occurrence and development.

3.2.3. Emodin. As the main component of many Chinese herbal medicines, emodin is a cymbidium compound with many biological activities such as immunosuppressive, antibacterial, anti-inflammatory, and antitumor properties [45]. By regulating the integrin-linked kinase (ILK) pathway, which in turn regulates cell survival, proliferation, and invasion by regulating various signal transduction pathways, emodin induces the development of endometrial stromal cells' MET, thereby inhibiting their migration and invasion [46, 47].

3.2.4. Other Herbal Extracts. Other herbal extracts also play particular roles in treating EM, and the mechanism of five Chinese medicine monomers on uterine smooth muscle was reported, including *Atractylodes macrocephala*, rhein, alizarin, turmeric, and corydalis [48]. Their functions are mainly through H1 receptors and L-type calcium channels, and the H1 receptor functions in prostaglandin (PG) formation and release. To sum up, herbal extracts play a vital role in treating EM dysmenorrhea and should be investigated further.

3.3. Herbal Patent Medicine Therapies. Herbal patent medicines are based on Chinese medicine theory using CHM as raw materials. These are processed into various dosage forms and provide new Chinese medicine forms for patients who find Chinese medicine decoctions to be inconvenient to take. Chinese patent medicines are easy to carry and taste good, including Guizhi Fuling capsules (GFC), ELeng capsules (ELC), Dan Bie capsules (DBC), Sanjie Analgesic capsules (SAC), and Dane Fukang paste (DFP).

3.3.1. GFC. GFC has been shown to be effective in treating EM dysmenorrhea. The results of modern pharmacological studies show that the active ingredients in GFC have anti-inflammatory, analgesic, and immune-regulating effects [49–51]. Clinical studies have shown that GFC can effectively reduce the levels of serum CA-125 and CA-199 in patients with EM dysmenorrhea and at the same time can effectively improve the TCM syndromes of patients, including menstrual cycle and menstrual volume [52]. A clinical study conducted by Tao and Yu showed that GFC could lower the levels of MEK-2, ERK-5, p-ERK, VEGF, T-cad, and VE-cad, demonstrating that GFC can significantly inhibit the cell proliferation and differentiation of EM by inhibiting the activity of MEK and ERK proteins and blocking the signal transduction pathways between cells, thereby inhibiting the abnormal proliferation of endometrial cells [53].

3.3.2. ELC. ELC can significantly inhibit EM tissue development in rats. Its mechanism of action may be related to inhibition of VEGF and its receptor, which are proangiogenic factors, with the strongest action and highest specificity for inhibiting angiogenesis in ectopic lesions [54]. A retrospective study found that ELC application before surgery can improve the pelvic microenvironment and make it more conducive to surgery. The mechanism behind this is likely through inhibition of the expression of soluble intercellular molecule-1 (sICAM-1), which is a member of the immunoglobulin superfamily of adhesion molecules and functions in damage repair, inflammation, immune response, and tumor metastasis. In addition, ELC can block the adhesion of ectopic lesions. Simultaneously, reduction of sICAM-1 reduces the promotion of vascular proliferation and adhesion formation [55]. Interestingly, Xu et al. [56] showed that the level of sICAM-1 in patients treated with ELC and mifepristone was higher than that in the control group treated with mifepristone alone, and the specific mechanism behind this effect needs further study. Huang et al. [57] performed an RCT using ELC with EM patients, and the total effective rate of dysmenorrhea was decreased from 84% to 48%.

3.3.3. DBC. DBC can reduce the concentration of PGF2α and PGE2 in EM patient's serum, which can relax the small blood vessels, reduce pelvic stasis, improve the pelvic microcirculation, and relieve pain according to a rat model [58, 59]. DBC has many functions such as anti-inflammatory and analgesic effects and improving blood rheology and microcirculation to treat EM dysmenorrhea [60].

3.3.4. SAC. The active ingredients in SAC have significant biological activities such as anti-inflammatory, analgesic, and hormone-like effects. They can act on inflammation, cell invasion, metastasis, coagulation, and smooth muscle contraction, thereby improving endometrial blood vessel function, improving the blood perfusion of the pelvis and uterus, inhibiting smooth muscle contraction, and regulating the immune inflammatory response [61, 62]. SAC has been shown to treat EM more effectively than danazol with a total effective rate of 92.9% vs. 77.5% [63].

3.3.5. DFP. Clinical studies have shown that DFP promotes blood circulation, removes blood stasis, soothes the liver, regulates qi, regulates menstruation, and relieves pain [64]. Ye and Wu [65] divided 150 patients with EM into an observation group and a control group with 75 cases each according to a random number table. The control group was treated with gestrinone, and the patients in the observation group were treated with DFP. The total effective rate in the observation group was 95.45%, which was significantly higher than the effective rate of 81.82% in the control group. Studies have shown that DFP may treat EM dysmenorrhea by regulating the body's immunity and inhibiting the proliferation, migration, and invasion of endometrial cells [66].

4. Acupuncture and Moxibustion Treatment

Acupuncture has been practiced for more than 3000 years in China, and it spread throughout Europe and America from the sixteenth to the nineteenth centuries. The history of acupuncture research was initiated in the eighteenth century and has developed rapidly since then [67]. Acupuncture has attracted increasing attention as a safe and easy-to-perform treatment [68]. Acupuncture has evolved from its original methods to include moxibustion, acupoint catgut implantation therapy, electroacupuncture, auricular acupoint treatment, and acupuncture combined with other therapies [69]. All of these can effectively relieve the symptoms of dysmenorrhea caused by EM (Tables 4 and 5). Based on a large number of clinical and animal experiments, the different mechanisms of acupuncture treatment for dysmenorrhea include relaxation of the meridians and promotion of blood circulation, modulation of immunity, activation of various neurotransmitters, reduction of VEGF, and regulation of abnormal prostaglandins, β-endorphin, dynorphin, electrolytes, and substance P levels in the body [70, 71]. Therefore, it plays a significant role in treating diseases.

4.1. Acupuncture Treatment. Acupuncture can relieve pain in the central and peripheral regions by activating various neurotransmitters or modulators, including serotonin, norepinephrine, and adenosine [72]. Xu et al. [73] carried out a systematic review and meta-analysis to determine the effects of acupuncture on treating EM-related pain. Patients in the intervention group were treated with acupuncture, and patients in the control group were treated with sham acupuncture, TCM, or western medicine. The results showed that the total effective rate of the intervention group reached 95%, and acupuncture had obvious advantages in relieving pain, reducing CA-125 concentration, and improving clinical symptoms. Shen and Lu [74] treated 50 EM patients with acupuncture or mifepristone and showed that acupuncture significantly reduced the extent of dysmenorrhea and reduced serum CA-125 levels. Xiao et al. [75] analyzed the relevant literature on modern acupuncture and moxibustion treatments for EM (Table 6).

4.2. Moxibustion Treatment. Moxibustion is the traditional method of burning the dried leaves of the mugwort plant (*Artemisia vulgaris*) to stimulate acupuncture points [76]. Correspondingly, moxibustion's effects are associated with properties from burning the dried herb, including the thermal stimulation. Chen et al. [77] sought evidence to confirm moxibustion's effect. Fifty-four EM patients were randomly divided into the moxibustion treatment group and the ibuprofen sustained-release capsule control group. The VAS scores and the days of dysmenorrhea were decreased in the treatment group and were less than those in the control group ($P < 0.05$).

4.3. Acupuncture Combined with Moxibustion Treatment. Acupuncture combined with moxibustion is a common practice in TCM and can effectively stimulate the regulatory function of meridians and collaterals, thus improving local blood stasis [78]. Mu [79] treated 42 patients with acupuncture combined with moxibustion. The acupuncture treatment was performed in the Zhongwan (CV12), Xiaguan (ST7), Qihai (RN6), Zhongji (CV3), Guanyuan (CV4), Qixue (KI13), Shuidao (S28), Taixi (K13), and Zigong (EX-CA1) acupoints. The acupuncture needles were retained for 30 min after de qi, and moxibustion was performed with ai zhu after acupuncture, which was effective in 29 patients.

Warming needle moxibustion, which combines acupuncture and moxibustion, involves wrapping moxa on the needle handle (or fixing a suitable length of moxa stick onto the needle handle) and igniting it during the needle retention process [80]. The needle body transfers the heat into the acupoint to treat disease by warming the meridians and promoting qi and blood circulation. It has a wide range of indications and is often used in the treatment of pain. Pan [81] treated 35 EM patients with existing dysmenorrhea with warming needle moxibustion at the following acupoints: Zusanli (ST36), Siman (KI14), Sanyinjiao (SP5), Qihai (RN6), Shuidao (RN9), Tianshu (ST25), and Zhongwan (RN12). After 3 months of treatment, the pain score had decreased significantly compared to the Western medicine group.

4.4. Acupuncture Combined with TCM. The combination of acupuncture with Chinese medicine is an important means of treating diseases. Acupuncture combined with Chinese medicine can reduce VEGF, a highly specific provascular endothelial cell growth factor, and this in turn promotes increased vascular permeability, extracellular matrix degeneration, and the migration, proliferation, and angiogenesis of vascular endothelial cells in a rat model of EM [82]. Another experiment in a rat model of EM showed that the combination of acupuncture and Chinese medicine could increase the levels of 6-keto-PGF1α and decrease the levels of thromboxane B2 (TXB2), thus regulating the imbalance of the two, which may be the mechanism through which acupuncture combined with Chinese medicine relieves the pain caused by EM [83]. Cui and Yang [84] performed a clinical study in which acupuncture was given one week prior to menstruation (acupoints: Guanyuan

Table 4: The specifications and efficacy of acupuncture methods.

Therapeutic approach	Specifications	Efficacy	Refs.
Acupuncture	Puncturing a needle into the patient's body at a certain angle.	Relieving pain, reducing serum CA-125, improving clinical symptoms such as irregular menstruation.	[72]
Moxibustion	Burning the dried leaves of mugwort (*Artemisia vulgaris*) to stimulate acupuncture points.	Relieving pain and improving pelvic microcirculation by thermal stimulation.	[76]
Warming acupuncture	Maintaining the position of the needle, twisting the moxa mass around the needle handle to heat it, and transferring the heat into the acupoints through the needle.	Relieving pain, warming meridians, and promoting qi and blood circulation.	[80]
Acupoint catgut embedding	Implanting absorbable catgut into acupoints.	Similar to acupuncture's efficacy, but the stimulation of acupoints is continuous for a few days.	[85]
Electroacupuncture	Addition of electric current to strengthen the stimulating effect of acupuncture on acupoints.	Alleviating inflammatory and neuropathic pain and improving blood circulation.	[87]
Auricular points	Stimulating acupoints distributed on the auricle.	Controlling pain, regulating immunity, and so forth.	[93]

Table 5: Acupuncture for dysmenorrhea.

Treatment; sample number (n)	Control; sample number (n)	Total clinical effect rate	Model used	Therapeutic effects and actions	Refs.
Acupuncture; $n = 25$	Mifepristone; $n = 25$	T: 92.0% vs. 52.0%	Human study	Pain score↓, CA-125↓, recurrence rate↓	[74]
Moxibustion; $n = 27$	Ibuprofen; $n = 27$		Human study	VAS score↓, the days of dysmenorrhea↓	[77]
Acupoint catgut implantation therapy; $n = 36$	Acupuncture; $n = 36$	T: 96.97% vs. 90.63%	Human study	PGF2α↓, VAS score↓	[86]
Electroacupuncture; $n = 36$	Mifepristone; $n = 36$	T: 94.4% vs. 91.7%	Human study	Pain score↓, CA-125↓, recurrence rate↓	[90]
Auricular acupuncture; $n = 37$	Herbal decoction; $n = 30$	T: 91.9% vs. 60.0%	Human study	β-EP↑, dysmenorrhea score↓	[96]

Note: T (total effect rate) = number of effective cases/total number of cases; effective case refers to the patients or animal models whose signs and symptoms were improved after treatment.

Table 6: Main acupoints for the treatment of endometriosis in reports.

The main acupoint	English name	N	Percentage (%)
Guanyuan	RN4	117	14.11
Sanyinjiao	SP6	84	10.13
Qihai	RN6	75	9.05
Zhongji	RN3	73	8.81
Zigong	EX-CA1	54	6.51

(CV4), Qihai (RN6), Zhongji (CV3), Zigong (EX-CA1), Sanyinjiao (SP5), Diji (SP8), and NeiYiJian)) and found that the clinical efficacy of acupuncture combined with Chinese medicine (86.67%) was higher than that of mifepristone (60.00%), and the uterine artery hemodynamics and EM-related serological parameters were reduced.

4.5. Acupoint Catgut Implantation Therapy. Acupoint thread-embedding therapy is based on acupuncture and moxibustion therapy. Absorbable protein filaments are implanted into acupoints to maintain the continuous stimulation of acupoints for a long time, which allows for the easy dissipation of local congestion [85]. Cong et al. [86] applied the catgut-embedding method at the Xuehai (SP10), Sanyinjiao (SP5), Diji (SP8), Zigong (EX-CA1), and Guanyuan (CV4) acupoints once every 2 weeks six consecutive times, resulting in tonifying the kidneys, warming the meridians, removing blood stasis, and clearing collaterals, with a total effective rate of 96.97%.

4.6. Electroacupuncture Treatment. Electroacupuncture is a modified form of acupuncture that uses electrical stimulation and is a widely used TCM therapy [87]. Direct stimulation can be carried out on the transtendon point through electric conduction, and the current can reach areas that acupuncture needles cannot reach, which can effectively improve blood circulation. Electroacupuncture activates the nervous system differently in healthy patients compared to

those in pain, and it alleviates both sensory and effective inflammatory pain and inhibits inflammatory and neuropathic pain more effectively at 2–10 Hz than at 100 Hz. Electroacupuncture blocks pain by activating various bioactive chemicals through peripheral, spinal, and supraspinal mechanisms [88, 89]. Zhang and Li [90] treated patients with electroacupuncture (acupoints: Qihai (RN6), Guanyuan (CV4), Zhongji (CV3), Zigong (EX-CA1), Diji (SP8), Sanyinjiao (SP5), Hegu (LI4), Taichong (LR3)). After de qi, a G6805-I electronic pulse generator was attached to needles at the bilateral Zigong (EX-CA 1), Guanyuan (CV 4), and Zhongji (CV 3) acupoints, and a continuous wave was generated at a frequency of 70 Hz and an intensity of 3 mA. The control group was treated with mifepristone. The curative group's total effective rate was 94.4%, and the recurrence rate within a 1 year was low.

4.7. Acupuncture Combined with Acupoint Sticking Treatment. Acupoint sticking is the combination of acupoints and drugs, which can dredge channels and collaterals and promote blood circulation by stimulating the effects of drugs on the body's corresponding acupoints in order to achieve the treatment goal [91]. Chen et al. [92] treated 73 EM dysmenorrhea patients who were divided into the observation group (36 cases) with acupuncture combined with acupoint sticking (acupoints: Zhongji (CV3), Guanyuan (CV4), Zigong (EX-CA1), etc.) and the control group (37 cases) treated with Jiawei Mojie tablet. The long-term total effective rates for the treatment group and the control group were 97.1% and 69.4%, respectively, and the treatment group had good long-term effects and stable conditions.

4.8. Auricular Acupoint Treatment. Auricular acupuncture involves the stimulation of auricular points on the ear. According to TCM theory, there is a natural mode of essence in the human body [93], and a study showed that auricular points are effective in controlling pain and regulating immunity through multiple mechanisms [94]. Auricular acupuncture is easy and convenient, has good analgesic effects, and is long lasting [95]. Xiang et al. [96] found that the total effective rate of ear acupuncture in EM patients with mild-to-moderate dysmenorrhea was 91.9%.

Although doctors' understandings of dysmenorrhea caused by EM are different based on years of clinical practice, starting from basic theories of TCM four diagnostic parameters, syndrome differentiation, and individualized treatment plans can be made, which rely on the patient's, physical condition, ages, and flexible use of TCM. Acupuncture, patching, moxibustion, acupoint embedding, etc. are used as pain relief methods and have achieved good clinical results. TCM has unique advantages in treating EM; therefore, we should pay more attention to performing more research in order to provide more evidence-based treatments for this disease.

5. CHM Enema and Related Therapies

As an important part of the external treatment in TCM, CHM enema has been widely used in the clinic. CHM enema delivers TCM decoctions into the rectum from the anus so that the medicine can be retained in the intestine and the intestinal mucosa can absorb the drug to achieve the purpose of treating and preventing the disease [97]. The advantage of this therapy is that it can effectively avoid the "first elimination" effect of the liver and avoid the digestive effect of the gastrointestinal tract that can destroy the drug before it reaches the pelvic cavity and can alleviate the pain caused by EM [98]. CHM enema is often combined with oral Chinese medicine, microwave physiotherapy, and patch therapy in the treatment of EM dysmenorrhea, as listed in Table 7.

5.1. CHM Enema. The mechanism of CHM enema to treat EM dysmenorrhea may be by inhibiting the activation of NF-κB in EMT cells, reducing the expression and secretion of regulated on activation in normal T-cell expressed and secreted (RANTES), thereby reducing inflammation and pain [99]. Yu and Li [100] treated patients suffering from EM dysmenorrhea with CHM enema (drugs: danshen 15 g, chishao 15 g, mudanpi 15 g, wulingzhi 15 g, yanhusuo 15 g, zaojiaoci 15 g, danggui 15 g, ezhu 15 g, muxiang 10 g, rougui 10 g, chenpi 10 g, and quanxie 3 g) or oral Sanjie analgesic capsules. After treatment, the serum CA-125 and VEGF levels decreased significantly compared with the control group, indicating a clear clinical effect of enema treatment on EM dysmenorrhea. Tian [101] treated 94 patients with EM-related pain who were randomized into two treatment groups with CHM enema (drugs: danshen 20 g, sanleng 15 g, ezhu 15 g, zaojiaoci 10 g, yimucao 15 g, and yanhusuo 20 g) or mifepristone. The efficiency rates were 89.4% and 78.7% for the treatment and control groups, respectively. In general, it appears that CHM enema can achieve twice the result with half the effort.

5.2. CHM Enema Combined with Oral CHM. In most Chinese hospitals, CHM enemas combined with oral CHM are the most commonly utilized treatments for dysmenorrhea caused by EM. Wu and Li [102] selected 86 patients with EM and divided them into control and observation groups using a random number table. The control group was given oral gestrinone capsules, and the observation group was given Jiawei Xuefu Zhuyu decoction both orally and as an enema. After 6 months of treatment, the observation group's total clinical effective rate was 95.35%, while that of the control group was 86.05%. The total clinical effective rate the observation group was significantly higher than that of control group. The difference in estradiol (E2), prolactin (PRL), and progesterone (P) hormone levels was statistically significant ($P < 0.05$). Lou [103] selected 92 patients with EM and randomly divided them into observation and control groups. The observation group was given oral CHM combined with CHM enema, while the control group was given

Table 7: CHM enema for dysmenorrhea caused by EM.

Treatment; sample number (n)	Control; sample number (n)	Total clinical effect rate	Model used	Therapeutic effects and actions	Refs.
Proprietary CHM enema decoction; $n = 30$	Oral Sanjie analgesic capsules; $n = 30$		Human study	CA-125↓, VEGF↓	[100]
CHM enema; $n = 47$	Mifepristone; $n = 36$	T: 89.4% vs. 78.7%	Human study	Pain score↓, recurrence rate↓	[101]
CHM enema, Dihuang Jisheng decoction; $n = 46$	Mifepristone; $n = 46$	T: 91.30% vs. 73.91%	Human study	E2↓, FSH↓, LH↓	[103]
CHM ointments; $n = 36$	Danazol; $n = 36$	T: 94.40% vs. 77.80%	Human study		[107]
CHM enema combined with patch therapy; $n = 32$	Ibuprofen sustained-release capsules; $n = 32$		Human study	VAS score↓	[110]

Note: T (total effect rate) = number of effective cases/total number of cases; effective case refers to the patients or animal models whose signs and symptoms were improved after treatment.

mifepristone. Comparing the two groups of patients after treatment, the total effective rate in the Chinese medicine combined enema group was 91.30%, which was significantly greater than the effective rate of 73.91% in the control group.

5.3. CHM Enema Combined with Microwave Physiotherapy. Microwave physiotherapy has the advantages of simple, safe operation, and low side effects in treating gynecological diseases [104]. Microwaves are a kind of high-frequency electromagnetic wave with strong penetrability. The principle in using microwave's thermal effects is to expand local blood vessels, promote blood circulation, and improve local nutrition [105]. Microwave therapy uses the magnetocaloric effect of microwaves on the tissues to stimulate different areas around the rectal wall. The microwave treatment shrinks and softens the endometrial sac, accelerates blood circulation around the lesion, improves capillary permeability, and relieves smooth muscle spasms and thereby improves the patient's clinical symptoms [106]. Tang et al. [107] randomly divided 72 patients with EM into the observation and control groups. The observation group received microwave therapy with TCM ointments, while the control group was given danazol orally for 3 months. The total effective rate of TCM ointments combined with microwave treatment on dysmenorrhea was 94.40% compared to 77.80% for the controls.

5.4. CHM Enema Combined with Patch Therapy. The mechanism of patch therapy is induction and conduction through the meridian, and the drug is absorbed through the skin. This method allows the drug to remain on the local skin surface for a long time, stimulating the skin receptors and reaching the disease through the skin so that the drug is supplied continuously [108]. Chinese herbal enema combined with patch therapy can alleviate the clinical symptoms and reduce the level of IL-8 in EM patients. The mechanism of action in relieving pelvic inflammation may be by improving the local microenvironment of the uterine cavity and increasing pelvic blood circulation [109].

Wan et al. [110] randomly divided 64 patients with secondary dysmenorrhea into an observation group receiving CHM enema combined with patch therapy and a control receiving oral ibuprofen sustained-release capsules. The relief rate of dysmenorrhea in the treated group was significantly higher than that of ibuprofen control group ($P < 0.05$). Deng [111] used CHM retention enema and external application of Chinese medicine compared to CHM retention enema alone. The results showed that the total effective rate of CHM retention enema combined with external application of Chinese medicine was 93.48%, while for the control group it was 63.04%. Therefore, CHM enema combined with external application has good therapeutic effects for treating EM and has high safety for reducing the focus of disease, alleviating dysmenorrhea, and regulating the ovarian axis.

6. Other CAM Therapies

There are some other CAM therapies for dysmenorrhea caused by EM, including Chinese herbal soaking method, copper Bian stone scraping therapy, and foot massage [112]. The TCM dip stain method is to wet the affected area with gauze full of medicinal juice so that the medicinal juice can pass through the Xuanfu and enter the hair follicle, thereby promoting blood circulation, dredging collaterals, and relieving pain. Jiang et al. [113] selected 80 patients with EM and randomly divided them into observation and control groups. The observation group was treated with the TCM dip stain method, and the control group was treated with Shaofu Zhuyu granules. The dysmenorrhea score and total effective treatment rate in the observation group were significantly higher than those in the control group ($P < 0.05$). Traditional Gua Sha therapy has the functions of dredging meridians, promoting qi, and increasing blood circulation. A clinical study reported that using Zusanyin meridian curettage therapy to treat dysmenorrhea is effective [114], and copper Bian stone scraping therapy compared with traditional Gua Sha therapy has greater ability to regulate qi and blood circulation and has stronger local penetration [115]. Cong et al. [116] randomly divided 56 patients with EM dysmenorrhea into observation and control groups. The control group was treated with Guixiang Wenjing Zhitong capsules orally, and the observation group was treated with Cuban scraping therapy. After three courses of treatment, the observation group's total effective rate was 96.43%, which

was significantly higher than the control group's 78.57%. There is still a lack of studies into the detailed mechanisms of foot massage. We will continue to focus on future avenues of research in this field.

7. Conclusions

There are increasing CAM therapies for secondary dysmenorrhea caused by EM, including herbs, acupuncture, and CHM enema. CAM therapies have been widely utilized because their curative effect is well accepted. These therapies can relieve pain, reduce the recurrence rate, and improve quality of life; however, they cannot fully eradicate the endometriotic lesions. In summary, the active principle of CAM therapies has a strong scientific foundation, and researchers have shown increased interest in this area of medical treatment. Standardizations of effective CAM therapies are still needed in order to increase the benefits of these alternative medical interventions for patients with EM dysmenorrhea throughout the world. In the future, larger samples and RCTs are needed to confirm the efficacy and safety of CAM for the treatment of EM dysmenorrhea and to provide new approaches for the management of EM dysmenorrhea.

Data Availability

No data were used to support the findings of this study.

Disclosure

Ying Guo and Fang-Yuan Liu should be considered co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Ying Guo and Fang-Yuan Liu contributed equally to this work.

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Research Article

Efficacy and Safety of the Two Ayurveda Drug Regimens in Uterine Fibroids: A Randomized Single-Blind Clinical Trial

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This study aims to assess the efficacy and safety of two Ayurveda drug regimens for the treatment of uterine fibroids (UF) in a randomized single-blind clinical trial. 120 participants with UF (volume $\geq 2\,cm^3$) were randomly allocated at a 1 : 1 : 1 ratio to 2 experimental groups and the control group. The 12-week intervention period was followed by 12-week follow-up. The primary efficacy endpoint was the change of the largest UF volume. The secondary efficacy endpoints were assessed by the pictorial bleeding assessment score (PBAC), UF symptoms, and quality of life score. The safety endpoints were changed in hepatic and renal safety parameters and patients experiencing adverse effects. Significant decrease was observed in the volume of UF in the arm II but not in arm I, while a significant increase was observed in the volume of the largest UF in the control group at 12^{th} week. The PBAC score remained stable in all groups. Further mean value of the intervention arms symptom severity subscale (SSS) was significantly reduced compared to the control arm. Health-related quality of life (HRQL) value improved in 12^{th} week of both experimental arms compared to baseline. Control arm HRQL value was reduced compared to baseline. The volume of the largest UF and both SSS and HRQL values remained stable within the follow-up period in the tested arms. The findings of this study demonstrated the safety and efficacy of selected two Ayurveda drug regimens in reducing the volume of UF and related symptoms and improving quality of life.

1. Introduction

Uterine fibroids are the most common reproductive tract tumor in the child-bearing age of women [1, 2]. Women with fibroids create considerable personal and social costs including diminished quality of life, disruption of usual activities and roles, lost work time associated with symptoms, and substantial healthcare expenditures [3]. Eventhough there are limited medical therapies, surgical and other invasive interventions still dominate in fibroid treatment. Many women are opposed to having a hysterectomy due to the undesirable comorbidities such as inpatient hospitalization, prolonged fever, transfusion, scarring, relatively long recovery time to presurgical levels of activities, and elimination of future pregnancies [4].

In last few decades, pharmacologic agents are used to provide relief for fibroid patients with mild symptoms, including combined oral contraceptives, progesterone (by oral, injection, or intrauterine device), and nonsteroidal anti-inflammatory drugs, antifibrinolytics, gonadotropin-releasing hormone agonists, selective estrogen or progesterone receptor modulator progestin, danazol, and aromatase inhibitors. Some of these medications are also useful in reducing tumor growth [5]. But still, there is no any gold stranded treatment option being developed for this common condition. In this context, various complementary and alternative medicine treatments have been administered for the uterine fibroids amelioration process including the Sri Lankan Ayurveda which is the most popular complementary medicine in Sri Lanka. But,

further research studies are still an immense requirement for these treatment modalities.

Sri Lanka Ayurveda is a mixture of Ayurveda and indigenous medicine. Sri Lanka developed its own Ayurveda system based on a series of prescriptions handed down from generation to generation over a period of 3,000 years. Uterine fibroids have been treated by Ayurveda medicine regimens in Sri Lanka. It was noticed that a considerable number of patients search Ayurveda treatment for this condition to prevent surgery and preserve fertility [6]. The treatment regimens selected for this study were freely available in Sri Lanka and extensively used in the treatment of uterine fibroids without any adverse reactions and proven effectiveness clinically [7]. However, the effectiveness and safety of selected two treatment regimens in uterine fibroid patients have not been explored by the scientific clinical trials. Therefore, this research was designed as a randomized clinical trial which has been considered as a gold standard from the clinical research paradigm. The study aimed to compare the efficacy and safety of selected two Ayurveda drug regimens in the treatment of uterine fibroids.

2. Methodology

2.1. Study Design and Oversight. This study was a randomized single-blind clinical trial conducted in gynecology clinic of National Ayurveda Teaching Hospital, Borella, Sri Lanka during years 2018–2020. The ethics approval for this clinical trial was obtained from the Research Approval Committee of the Faculty of Graduate Studies in the University of Colombo and the Ethics Review Committee of Institute of Indigenous Medicine in University of Colombo, Sri Lanka. The trial was registered in ISRCTN registry (trial number: ISRCTN16108738). The study was conducted adhering to good clinical practice guidelines. Written informed consent was obtained from each participant before conducting the trial. The participants were given sufficient time to ask questions and decide whether they wish to participate in this study or not.

2.2. Study Population. Sample size was calculated with the main outcome parameter as reduction in the uterine fibroid size (each arm, 30 + loss to follow-up 10) [8]. A total 120 women aged between 18 and 50 years with at least one fibroid $\geq 2\ cm^3$ in volume as assessed by ultrasonography were included for the study. The main exclusion criteria were menorrhagia (score ≥ 100 by PBAC), serum hemoglobin level less than 11 g/dL, having used any steroid hormonal therapy for a minimum of 03 months prior, pregnancy, lactating females, menopausal women, presence of condition other than fibroids, contributing to dysmenorrhea or pressure symptoms, and presence of any severe medical or psychological condition that, in the opinion of the investigator, would compromise the patient's safe participation.

2.3. Randomization and Intervention. Participants were randomly allocated at a 1 : 1 : 1 ratio to either the two experimental groups or to the control group (40 per each arm) by block randomization generated using an online program. The two experimental groups (I and II) received two Ayurveda drug regimens (Table 1), and the control group was kept as observation without treatment. Selected 11 Ayurveda products purchased from Sri Lanka Ayurvedic Drugs Corporation were included in Sri Lankan Ayurveda Pharmacopoeia [9, 10].

The duration of intervention was 12 weeks followed by a 12-week follow-up period. All the cases were advised to visit clinic once in two weeks for monitoring. The drugs for the next two weeks were issued for the experimental group. The duration of interventions, drugs, and treatment procedures are given in Table 1.

2.4. Efficacy Assessment. The primary efficacy endpoint was the change of the largest uterine fibroid volume at the end of the treatment. The secondary efficacy endpoints were assessed by the pictorial bleeding assessment score scale, the uterine fibroid symptoms, and the quality of life scale.

Fibroid volume was measured with the help of the GE Voluson P6 ultrasound machine 4C-RS convex probe or RICS-9A endocavitary probe by an expert ultrasonographer, who was blind to this trial. Volume of the largest fibroid was measured by transvaginal ultrasound scan at the screening, the posttreatment (12^{th} week), and at the follow-up (24^{th} week) by applying the prolate ellipsoid method (formula $V = 0.5233\ (D1 \times D2 \times D3)$ [11].

2.5. Assessment of Uterine Bleeding. Cases of menorrhagia were excluded from this study. Since the selected drug regimens were not tested for menstrual behaviors. Patients were advised to record their bleeding pattern by the pictorial bleeding assessment chart (PBAC) at the screening and after the treatment.

2.6. Assessment on Symptom Severity and Quality of Life (QOL). Symptom severity and their impact on health-related quality of life (HRQL) were assessed by a Uterine Fibroid Symptom and Health-Related Quality of Life Questionnaire (UFS-QOL) already translated by a scientific method and validated in a small population at the same study center [12]. The questionnaire consists of an 8-item symptom severity scale and 29 HRQL questions, which comprise 06 subscales including concern, activities, energy/mood, control and self-consciousness.

2.7. Safety Analysis. Each patient underwent the hematological investigations (full blood count (FBC), aspartate aminotransferase (ALT), alanine aminotransferase (AST), serum creatinine, glomerular filtration rate (GFR), and urine full report (UFR)) before and after the treatment. The vital signs were measured and recorded in a patient diary at each visit. The safety endpoints were considered as the number and proportion of patients withdrawing from treatment early for safety reasons, changes in hepatic and renal safety parameters, and the number and proportion of patients experiencing adverse effects.

Table 1: Procedure of intervention.

Period	No.	Arm I	Arm II	Arm III
02 weeks	1	Panchamoolilaghudrakshadi decoction, 30 ml, bd		No intervention (control arm)
	2	Chandraprabha Vati, 2 pills (500 mg × 2), bd		
	3	Manibadra Choorna, 05 g powder, at night		
03rd–12th week	4	Thiplagugul decoction, 30 ml, bd	Punarnavashtaka decoction, 30 ml, bd	
	5	Panchatiktagritaguggul, 2 pills (500 mg × 2), bd	Kanchanaragugulu, 2 pills (500 mg × 2), bd	
	6	Krishna Jeeraka Choorna 5 g powder, bd	Satapushpa Choorna 5 g powder, bd	
	7	Sharshapadi oil—external application on lower abdomen for 07 days after each menstruation	Nirgundyadi oil—external application on lower abdomen for 07 days after each menstruation	
13th–24th week		No intervention (follow-up)		

2.8. Statistical Analysis. Statistical analysis was performed using the SPSS statistical package program (ver. 22.0), and the level of significance was established at $\alpha = 0.05$. Descriptive analysis for categorical variables was expressed as numbers and proportion. The continuous data were assessed for normalcy using QQ plots and the Kolmogorov–Smirnov test. Continuous data were described using mean and standard deviation for normally distributed variables, while skewed data were described using IQR. Between-group comparisons were carried out by analysis of variance (ANOVA) for normal data, while for nonnormal data, the Kruskal–Wallis test was used. For before and after data comparisons, the paired *t*-test and the related samples Wilcoxon sign rank test were used for normally distributed and nonnormal distrusted data, respectively. Similarly, for categorical variables, the chi-square test and Mac Nemar chi-square test were used, respectively. Intention to treat analysis was performed for all efficacy outcomes and safety outcomes.

3. Results

3.1. Consort Flow Diagram of Group's Enrollment, Allocation, Follow-Up, and Analysis. 150 participants who were referred to the Gynecology Unit of National Ayurveda Teaching Hospital were assessed for eligibility, and possible patients who fulfilled the inclusion criteria were enrolled to this study (Figure 1). 22 cases did not met with the inclusion criteria and 8 cases declined considering the study perspective after discussing the information provided by the investigator. Excluded cases were continued with the regular clinical management. Included 120 cases were randomly divided into three arms. The total study period of the randomized clinical trial was completed by 102 women: 35 (87.5%) cases from arm I, 37 (92.5%) from arm II, and 30 cases (75%) from control (arm III). Within the study period with follow-up of 12 weeks, 04 cases left without completing the programme in arm I due to not regularly attending the clinics and 01 case left the trial due to menorrhagia. In arm II, 02 cases left without completing the trial due to not regularly attending the clinics and 01 case underwent myomectomy due to no improvement of the symptoms. All the cases recruited to arms I and II completed the follow-up period (12^{th}–24^{th} week). In control (arm III), all the cases visited the regular clinics in first 12 weeks, but the follow-up period (12^{th}–24^{th} week) was not completed due to their planned myomectomy.

3.2. Baseline Characteristics of the Study Sample. Baseline characteristics of the 102 participants who completed the study and who entered to three arms were very similar characters such as most women were in their late thirties or early forties married stage, employed, and Buddhist. The *P* value shows a nonsignificant difference ($P > 0.05$) between the arms (Table 2).

3.3. Efficacy Outcomes of Three Arms. As given in Table 3, there was no significant reduction in the volume of the fibroids in the arm I (MD: 13.81, $P = 0.312$) and significant reduction in arm II (MD: 0.38, $P = 0.001$), and there was a significant increase in the size of fibroids in the control group (Arm III MD: −45.43, $P = 0.001$). Mean difference of fibroid volume in two experimental arms from 12^{th} week to 24^{th} week (follow up) was nonsignificantly increased in mean volume by 0.22 cm^3 in Arm I and 3.03 cm^3 in Arm II.

The difference of the PBAC score between groups at 12^{th} week was nonsignificant. The symptom severity mean value differences from baseline to 12^{th} week of all the arms were significant ($P < 0.05$). The experimental arms mean values were not changed at the follow-up compared to after treatment. HRQL subscale items mean values of experimental arms were increased in 12^{th} week compared to the baseline ($P < 0.05$). There was no significant difference shown in HRQL total score in 24^{th} week when comparing with the posttreatment values.

3.4. Safety Outcomes of Three Arms. The mean values of serum AST, ALT, and serum creatinine at baseline and the 12^{th} week differences were not significant in all the groups. Important parameters of serum creatinine, GFR assessment, FBC, and UFR at baseline and the 12^{th} week are given table. The urobilinogen, bile pigments, WBC, platelet count, and hematocrit mean values remained stable in the study period.

4. Discussion

This was the first study assessing the 6-month clinical outcome of selected 02 Ayurveda drug regimens for the management UF. In general, guidelines for methodologies on research and evaluation of traditional medicine also recommend the use of both herbal medicine and traditional

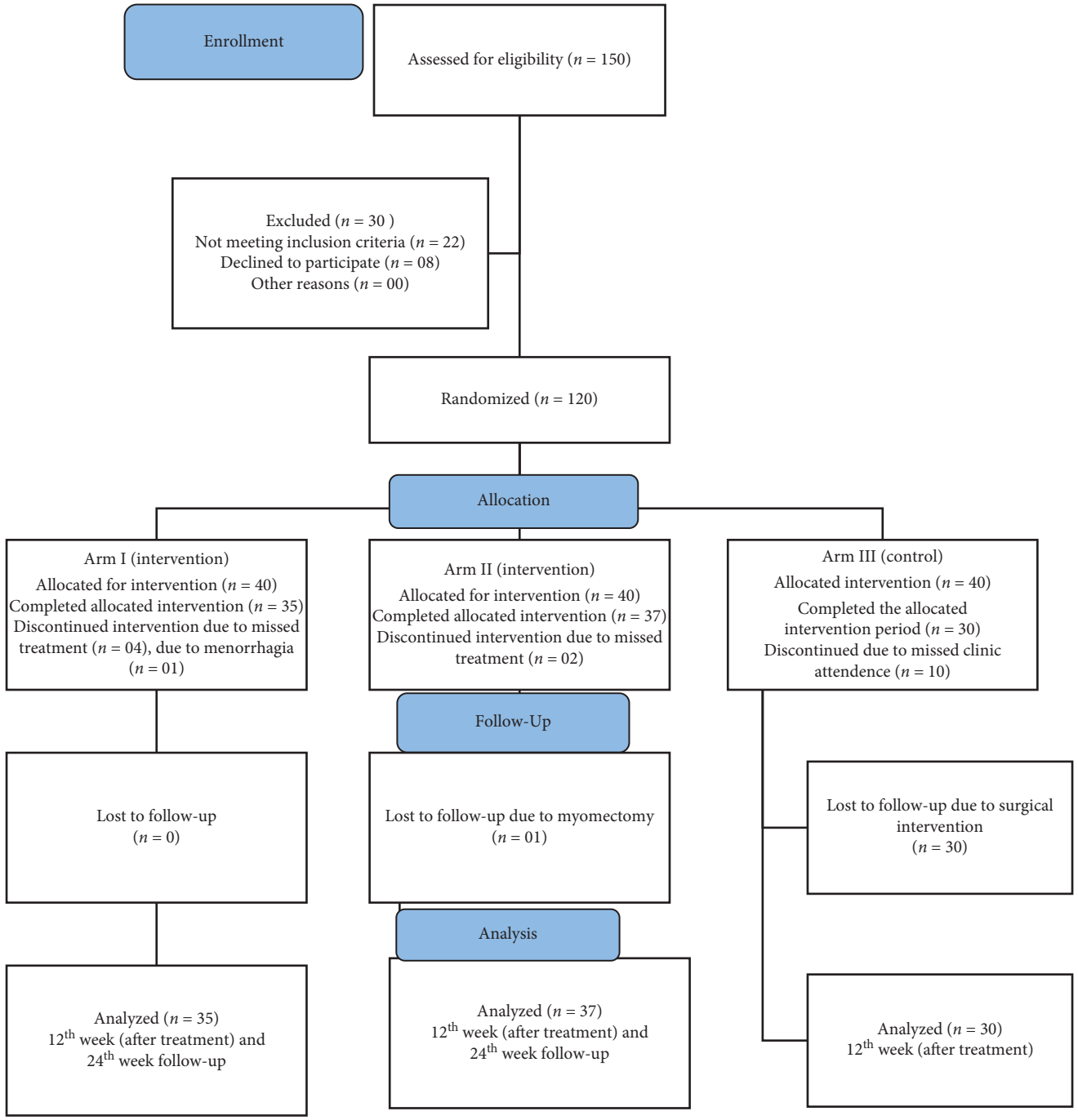

Figure 1: Consort diagram of the study.

procedures-based therapies together with the treatment. Because they believe that the successful treatment is often the consequence of both types of treatment acting synergistically [13].

This randomized single-blind clinical trial was completed with 102 participants with UF (Figure 1). The mean age range between 37 and 39 was reported in this study following the normal age range reported by the previous studies [14]. The clinical trial participants were also reflecting the religious diversity of Sri Lanka. Furthermore, occupation and education showed the normal distribution pattern of urban women of Sri Lanka. The mean BMI of arms I, II, and III was 23.61 (3.39), 23.40 (4.11), and 23.29 (4.27), respectively. Comparison between three arms on Hb%, mean PBAC score, the volume of the largest fibroid, and QOL score also showed nonsignificant ($P > 0.05$) variation. Demographic data were not significantly variable in the three arms (Table 2). Therefore, a comparable study can be made in this research' findings where most of such other studies were unable to perform [15].

The results indicated a reduction of the fibroid volume at 12^{th} week in both experimental groups (arm I mean difference:

Table 2: Baseline characteristics of the study sample.

Characteristics	Arm I ($n = 35$)	Arm II ($n = 37$)	Arm III ($n = 30$)	P value**
Age (y), mean (SD)	38.66 (6.54)	37.11 (5.82)	39.59 (6.33)	0.258
Marital status, n (%)				
Married	26 (74.3)	27 (71.1)	22 (75.9)	0.708
Single	07 (20.0)	10 (26.3)	7 (24.1)	
Widowed	02 (5.7)	01 (2.6)	0 (0.0)	
Occupation, n (%)				
Work outside the home	26 (74.3)	26 (68.4)	21 (72.4)	0.851
Homemaker	09 (25.7)	12 (31.6)	08 (27.6)	
BMI (kg/m^2), mean (SD)	23.61 (3.39)	23.40 (4.11)	23.29 (4.27)	
Hemoglobin (%), g/dL	11.83 (0.80)	12.20 (1.06)	12.12 (0.81)	0.185
PBAC score, mean (SD)	54.17 (31.31)	50.86 (25.81)	62.23 (27.71)	0.262

Arm I and Arm II, experimental groups; Arm III, control group; BMI, body mass index; PBAC, pictorial bleeding assessment chart. **Significant level of $P < 0.05$ by ANOVA.

Table 3: Fibroid volume change in 12th week and 24th week of three arms.

Fibroid volume (cm^3)	Arm I	Arm II	Arm III	P value**
Baseline	$n = 35$	$n = 37$	$n = 30$	
Baseline (mean ± SD)	82.03 (173.08)	86.67 (161.20)	66.63 (151.14)	0.525
12th week (after treatment)	$n = 35$	$n = 37$	$n = 30$	
12th week (mean ± SD)	68.22 (142.11)	86.29 (237.19)	112.06 (240.52)	
Mean difference from baseline to 12th week (MD)	13.81	0.38	−45.43	0.322
P value*	0.312	0.001	0.001	
Follow-up period				
24th week (follow-up)	$n = 35$	$n = 37$	$n = 00$	
24th week (mean ± SD)	68.45 (137.19)	89.32 (221.58)	—	
Mean difference from 12th week to 24th week (MD)	−0.22	−3.03	—	0.813$^{\#}$
P value*	0.145	0.780	—	

Arm I and Arm II, experimental groups; Arm III, control group; SD, standard deviation; MD, mean difference. **Significant level of $P < 0.05$, based on the related samples Wilcoxon sign rank test (before and after within group). **Significant level of $P < 0.05$, based on the Kruskal–Wallis value test (between group). #Between Arm I and Arm II.

13.81 cm^3; arm II mean difference: 0.38 cm^3) which was significant in arm II ($P > 0.05$) (Table 3). In the control arm, the mean fibroid volume significantly increased ($P > 0.05$). These findings were in agreement with the results reported in previous case series and case studies conducted on uterine fibroid volume reduction with several Ayurveda drug regimens [16–20]. The mean UF volume measurements of both intervention arms in the follow-up period did not report a significant change from 12th week ($P > 0.05$). When comparing both the arms, arm II showed a slight increase of the volume than arm I at the end of 24th week of the follow up. Post-treatment fibroid regrowth reported by some of the medical management includes asoprisnil, [21] and stable UF volume in 3 months follow-up after three months treatment with ulipristal acetate (UPA) [22]. But still, no published data are available from Ayurveda drug regimens to compare the results. In the follow-up period (12th–24th week), results of the arms I and II could not be compared with the control (arm III). In the follow-up period (12th week to 24th week), comparing the effects of two Ayurveda treatment regimens (arms I and II) indicated that the UF volume was not significantly changed even after cessation of the treatment (Table 3).

Research studies have had to define their own criteria for treatment success, and the PBAC is one [23]. It is commonly understood that the PBAC score of 100 or more is heavy menstrual bleeding, 99–02 considered normal bleeding, and PBAC score less than 02 is amenorrhea. In this study, the menstruation pattern was not changed in either group after the treatment; furthermore, 07 cases reported menorrhagia in arm I and 04 cases in arm II which might get some effects by the treatment (Table 4). But these reported changes were not taken seriously as they were controlled without any added intervention or discontinuation of the study intervention. Furthermore, all the cases remained normal hemoglobin levels throughout the period which supported the augment made. Anyhow, some relations of menstrual bleeding with the treatment may be proved by a large-scale clinical study. Cases were not reported with amenorrhea by this study where most of the medical managements, UPA [24], GnRH, agonist selective progesterone receptor modulators (SPRMs) of the disease were faced.

Studies explored that, with the increasing availability of noninvasive therapies to hysterectomy, it will be important to assess symptom reduction of uterine fibroids of patients who choose these treatment options [25]. The patients with symptomatic uterine fibroids treated by Ayurveda drug regimen experienced significant alleviation of fibroid-related symptoms (by SSS) and increased in HR-QOL. Baseline mean values of SSS remained nonsignificant between three arms ($P = 0.615$). This was a moderate level of severity when

Table 4: The secondary efficacy endpoints changes in the 12th week and 24th week.

Variable	Arm I ($n = 35$)	Arm II ($n = 37$)	Arm III ($n = 30$)	P value
PBAC score MD (% change)	1.02 (44.31)	−10.97 (−33.73)	−2.90 (−14.44)	
PBAC at 12th week				
≥100 (menorrhagia)	01 (02.9)	04 (10.8)	02 (06.7)	
99–02 (normal flow)	34 (97.1)	33 (89.2)	28 (93.3)	0.410*
<02 (amenorrhea)	00 (0.0)	00 (0.0)	00 (0.0)	
UFS-QOL-symptom severity$^{\alpha}$				
Baseline (mean ± SD)	33.21 (15.91)	32.26 (16.52)	30.72 (14.21)	0.815**
12th week (mean ± SD)	25.71 (16.45)	22.63 (12.74)	34.16 (14.77)	
P value*** (baseline to 12th week)	0.001	0.001	0.006	0.006**
24th week mean difference from 12th week (MD ± SD)	0.00 (2.00)	0.00 (0.00)	—	
P value*** (12th–24th week)	1.00	0.05	—	
UFS-QOL-HRQL$^{\alpha\ \alpha}$				
Baseline (mean ± SD)	51.45 (16.98)	58.57 (17.48)	59.13 (15.51)	0.112**
12th week (mean ± SD)	57.73 (17.37)	64.63 (17.63)	55.35 (16.47)	
P value*** (baseline to 12th week)	0.005	0.035	0.010	0.081**
24th week (mean ± SD)	57.53 (17.14)	64.60 (17.14)	—	
P value*** (12th–24th week)	0.160	0.786	—	

Arm I and Arm II, experimental groups; Arm III, control group; PBAC, pictorial blood loss assessment chart; score value ≥ 100, menorrhagia; 99–02, normal flow; <02, amenorrhea; HRQL, health-related quality of life; UFS-QOL, Uterine Fibroid Symptom and Health-Related Quality of Life Questionnaire; SD, standard deviation; MD, mean difference. *Significant level of $P < 0.05$, based on the chi-square test (between groups). **Significant level of $P < 0.05$, based on ANOVA (between groups). ***Significant level of $P < 0.05$, based on the paired t-test (before and after within groups). $^{\alpha}$Score range from 0 to 100, a higher score indicates greater symptom severity; $^{\alpha\ \alpha}$score range from 0 to 100, higher scores indicate better HRQL.

the highest score indicates greater symptom severity. This could be a specific factor of following Ayurveda treatment by the study population as they can bear up the disease-related difficulties up to some extent till the treatment initiates it action. In posttreatment, assessment mean values of SSS were reduced in experimental arms, while the increased mean value was shown in control. Then, the SSS values of baseline and after treatment were compared by the paired t-test. Both the experimental arms mean values of SSS at 12th week (after treatment) were reduced (symptoms reduced), while in control, SSS was improved ($P < 0.05$). This result proved that there was an effect on Ayurveda treatment on controlling symptoms related to the uterine fibroid. Considerable symptoms at the enrollment and a substantial decrease in mean symptom levels after intervention appear to be a clinical amelioration of the disease. The UFS-QOL has a second scale that measures six dimensions of health-related QOL (HR-QOL). In contrast to the SSS, on this 100-point scale, a lower number indicated improved QOL. These QOL impacts have included fatigue, self-consciousness, weight gain, interference with physical activities, and interference with daily and social activities and effect on relationships with partners and with family and friends, impaired ability to take care of home or children, and missed workdays [26]. Two intervention arms reported a significant improvement in HRQL after treatment compared to baseline. The control arm value was significantly reduced. Previous studies claimed that the clinical efficacy of alternative uterine sparing treatments for UF cannot be fully described by objective measurements, such as changes in UF volume, because symptom alleviation is highly subjective [27]. At this point, the achieved positive clinical outcome by the UFS-QOL can be regarded as a marker for clinical success of the selected drug regimens. Follow-up values of the UFS-QOL (both SSS and HRQL scales) were not changed significantly and persist near to the posttreatment stage (Table 4). The fact that the UFS-QOL score changes follow volume reduction behavior occurred (changed significantly at posttreatment and then static at the follow-up) argues against this being a result of bias or a placebo effect.

Safety biomarkers are important tools in clinical trials as they are measurable indicators of normal biological processes or biological responses to a therapeutic intervention. Eventhough herbal remedies and Ayurveda treatments are believed to be safe, it is highly recommended to carry safety studies in every clinical trial. The results of this study show the efficacy and the safety of two regimens. Serum AST, ALT, creatinine, GFR, and parameters of full blood count and urine full report remained unchanged and within the normal range (Table 5). None of the treated patients had treatment-related serious adverse effects. There were no major adverse effects and clinical implications noted in this conducted trial. There were no deaths, life-threatening events, or unintended second procedures reported. A single participant continued heavy menstruation in the second week after the treatment initiation, requiring switch to bleeding, controlling Ayurveda treatments by which cases were controlled in ten days. She was discontinued from the study. Another complaint of treatment emergent gastritis is at the initial stage, but it was suppressed by proper food habits. Hence, these two herbal treatment approaches may be a safety answer for short-term treatment of uterine fibroids where some studies could not be achieved. Most of other medical interventions were questionable in the safety aspect [28] when treating leiomyoma. Considering treatment approaches of the conducted trial, there were no safety implications raised.

It is clear that both the Ayurveda drug regimens demonstrated efficacy and safety in treating uterine fibroids. The

Table 5: The safety outcomes of three arms.

Variable	Arm I ($n = 35$)	Arm II ($n = 37$)	Arm III ($n = 30$)
AST (IU/L)			
Baseline	20.47 (9.45)	18.38 (6.39)	18.77 (8.15)
12th week	20.13 (7.48)	18.87 (5.43)	18.40 (8.17)
P value	0.78	0.63	0.31
ALT (IU/L)			
Baseline	20.63 (10.30)	20.09 (9.01)	19.86 (11.19)
12th week	20.90 (9.8)	19.38 (9.10)	19.71 (11.12)
P value	0.84	0.54	0.35
Creatinine (mg/dl)			
Baseline	0.68 (0.10)	0.63 (0.10)	0.69 (0.14)
12th week	2.1 (8.67)	2.04 (8.44)	0.69 (0.14)
P value	0.33	0.31	0.20
GFR (mL/min/1.73 m^2)			
Baseline	>90	>90	>90
12th week	>90	>90	>90
Urobilinogen (mg/dL)			
Baseline	Normal	Normal	Normal
12th week	Normal	Normal	Normal
Bile pigments			
Baseline	Nil	Nil	Nil
12th week	Nil	Nil	Nil
WBC** (10^9/L)			
Baseline	28.34 (107.48)	28.47 (121.42)	24.19 (101.11)
12th week	28.64	28.25	26.30
PLT** (10^9/L)			
Before	343 .57 (80.30)	339.41 (89.87)	331.63 (86.73)
12th week	354.71 (69.74)	342.22 (77.77)	339.03 (88.78)
Hematocrit (%)			
Before	34.52 (4.89)	37.48 (5.17)	36.29 (4.34)
12th week	36.65 (9.67)	37.47 (8.63)	36.38 (4.26)

Values are presented as mean ± standard deviation. AST, aspartate transaminase; ALT, alanine aminotransferase; GFR, glomerular filtration rate; TB, total bilirubin; WBC, white blood cells; PLT, platelet. *Based on urine full report. **Based on full blood count. *Significant level of $P < 0.05$ by the paired t-test.

results of this study may provide answers for the women with uterine fibroid who have pain and pressure effects, women who wish to retain the option of childbirth, women who wish to save their uterus, women who are not fit for surgical intervention, and women with infertility can take advantage of this type of treatment.

5. Conclusion

In conclusion, the findings of this study demonstrated the efficacy of selected two Ayurveda drug regimens in fibroid shrinking, reducing fibroid-related symptoms, and improving quality of life. It was confirmed that the two drug regimens were safe for use in uterine fibroid treatment. Furthermore, we suggest that multicentered randomized controlled trials are needed to confirm our findings in future.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Review Article

An Overview of Systematic Reviews of Using Chinese Medicine to Treat Polycystic Ovary Syndrome

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Objective. This review sought to evaluate the strength and validity of the existing evidence for the use of Chinese medicine for the treatment of polycystic ovary syndrome (PCOS). *Methods*. We retrieved systematic evaluations and meta-analyses of randomized controlled trials (RCTs) evaluating Chinese herbal interventions in polycystic ovaries, including the use of decoctions or Chinese patent medicines. The quality of these systematic evaluations was assessed using AMSTAR2 tools, and ovulation rate, pregnancy rate, effective rate, serum hormones (testosterone, luteinizing hormone, and follicle-stimulating hormone), and adverse reactions were recorded. Finally, the reliability of each result was evaluated according to the GRADE system. *Data Sources*. PubMed, Embase, Cochrane Library, China National Knowledge Infrastructure (CNKI), Wanfang Data, CQVIP, and SINOMED databases were searched up to January 1, 2021. *Outcomes*. A total of 18 publications were included, all of which showed that PCOS symptoms were improved with Chinese medicine compared with control groups. However, most of the evaluations did not have good research designs and had issues with the analysis of their results. The reliability of most outcome measures was rated low or very low, and it is presumed that the reliability of the results was low due to the poor quality of the RCTs. *Conclusions*. At present, there is insufficient evidence to suggest that improved efficacy is achieved by the combined use of Chinese and Western medicine compared with Western medicine alone in treating PCOS. Therefore, it is recommended that multicenter, large-sample RCTs adopting standard designs and rigorous methods be carried out in the future while introducing standardized assessment plans for the systematic review of clinical trials so as to improve the quality of the resulting clinical evidence.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder among women of reproductive age, and it is the main cause of anovulatory infertility [1]. Its major manifestations include ovulation disorders, irregular menstrual cycles, high levels of androgens, and depression and other emotional disorders [2, 3]. Meanwhile, patients often develop insulin resistance, obesity, and other metabolic disorders, which puts women with PCOS at a very high risk of developing diabetes [4, 5]. In addition, PCOS patients are prone to serious complications, and their risk of cardiovascular disease is higher than the general population [6], and they tend to suffer from fatty liver, metabolic syndrome, and other diseases [7, 8]. Clinically, PCOS is often managed by controlled ovulation stimulation and androgen suppression. However, while these methods can achieve certain therapeutic effects, they may also produce adverse effects such as vomiting and diarrhea [9]. Therefore, some Chinese herbal medicines are popular because of their low levels of side effects and adverse reactions even after long-term use [10]. These include cinnamon and other medicines that appear to work at the level of sex hormones and that are believed to play a role in the regulation of the menstrual cycle [11, 12]. In addition, Chinese medicine can increase the ovulation rate and improve hormone levels by regulating qi, blood, yin, and yang in all phases of the menstrual cycle [13]. Although several recent studies have systematically reviewed the efficacy of Chinese medicine for the treatment of PCOS, the quality of their methods and conclusions requires further verification. In this study, the methodological quality and the

quality of evidence of existing systematic reviews on Chinese medicine for the treatment of PCOS were evaluated using the AMSTAR2 and GRADE systems in order to provide guidance for planning future studies on this subject.

2. Materials and Methods

2.1. Protocol and Registration. This study provides an overview of systematic evaluations based on existing recommendations and in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [14]. The review was registered in the PROSPERO database (CRD42021242641).

2.2. Inclusion and Exclusion Criteria

2.2.1. Research Types. This study included systematic reviews and meta-analyses of randomized controlled trials (RCTs) written in Chinese or English.

2.2.2. Research Subjects. Articles that enrolled patients with confirmed diagnoses of PCOS were included in this study, and there was no restriction on the age of the patients or the course of the disease.

2.2.3. Interventions. Articles included in this study must have adopted an intervention in which the experimental group was treated with either Chinese medicine alone or in combination with Western medicine, while the control group was treated with Western medicine alone or placebo. There were no restrictions on the type of Chinese or Western medicine used.

2.2.4. Outcome Indicators. This study included articles with the primary indicators of live birth rate, pregnancy rate, ovulation rate, and clinical efficiency and the secondary indicators of adverse effects and serum hormone levels (testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and LH/FSH).

2.2.5. Exclusion Criteria. Exclusion criteria were as follows: (i) articles that were not systematic reviews or meta-analyses, (ii) meta-analyses of protocols and network meta-analyses, (iii) duplicate publications, (iv) trials containing other treatments such as acupuncture, (v) studies with outcome indicators that did not include at least two of the primary indicators listed above, (vi) studies with an inappropriate search strategy or coverage of fewer than two databases, and (vii) studies with erroneous conclusions or data.

2.3. Search Strategy. Systematic reviews and meta-analyses were searched for in the PubMed, Cochrane Library, Embase, CNKI, Wanfang Data, QVIP, and SINOMED databases. The search period was set from the inception of the database to January 1, 2021, with literature only in Chinese or English included. Two examples of the search strategies are shown in Table 1. A manual search of protocol registries and other unpublished sources was also performed as a supplement to avoid missing relevant literature.

2.4. Literature Screening and Data Extraction. Two researchers conducted independent screening of the literature by merging the search results and then removing duplicates using Endnote X9 (Clarivate Analytics, USA), followed by literature screening based on the aforementioned criteria. Once the cross-checks were completed, the two researchers performed the data extraction and quality evaluation separately. Disagreements were resolved after discussion between the two researchers, with the assistance of a third researcher if required.

2.5. Evaluation Methods

2.5.1. Evaluation of the Methodological Quality. The methodological quality of the included studies was evaluated using the AMSTAR2 tool [15]. The quality of the 16 items in the tool was rated individually for each study, with items 2, 4, 7, 9, 11, 13, and 15 being prioritized.

2.5.2. Evaluation of the Quality of the Evidence. The quality of the evidence of the included studies was evaluated using the GRADE evaluation system [16]. The limitation, inconsistency, indirectness, imprecision, and publication bias of each outcome indicator of the systematic reviews were objectively evaluated and assigned a confidence rating.

3. Results

3.1. Literature Screening Process and Results. The initial search yielded 312 relevant articles, and after removing 149 duplicates, a total of 163 articles were screened, including 122 Chinese and 35 English articles. After initial screening and rescreening, 18 articles were eventually included in this study; all of which were written in Chinese. The detailed literature screening process is shown in Figure 1 [14].

3.2. Characteristics of the Included Studies. Of the 18 systematic reviews/meta-analysis included in this review [17–34], 14 were journal articles [17, 19–24, 27, 28, 30–34], and four were theses [18, 25, 26, 29]. None of the studies were registered in the Cochrane Library, PROSPERO, or the like. The basic characteristics of the included studies are listed in Table 2.

3.3. Quality Evaluation of the Included Systematic Reviews

3.3.1. Evaluation of Methodological Quality. The AMSTAR2 assessment showed that five items (1, 4, 5, 8, and 9) were relatively complete and were reported by ≥70% of the articles, while there were five items (2, 3, 7, 10, and 16) that were not reported by any article (0%). The percentages of articles reporting the prioritized items were as

Table 1: Search strategy for databases.

Database	Search	Search strategy
PubMed	#1	(("Polycystic Ovary Syndrome" (MeSH term)) OR (Stein-Leventhal syndrome) OR (PCOS) OR (polycystic ovarian syndrome))
	#2	(("Meta-Analysis" (MeSH term)) OR (Meta-Analysis) OR (Systematic Reviews))
	#3	(("Medicine, Chinese Traditional" (MeSH term)) OR (Drugs, Chinese Herbal) OR (traditional Chinese medicine) OR (Integrative Medicine) OR (integrated Chinese and Western medicine) OR (Cinnamon) OR (Berberine) OR (Resveratrol) OR (Paeoniflorin) OR (Cryptotanshinone))
	#4	#1 AND #2 AND #3
CNKI	#1	SU = polycystic ovaries + polycystic ovary syndrome + polycystic ovarian syndrome + PCOS + "Stein-Leventhal syndrome"
	#2	SU = meta-analysis + systematic review
	#3	SU = Chinese medicine + Chinese herbs + Chinese herbal medicine + proprietary Chinese medicine + Chinese and Western medicine
	#4	#1 AND #2 AND #3

MeSH: medical subject headings, SU: subject.

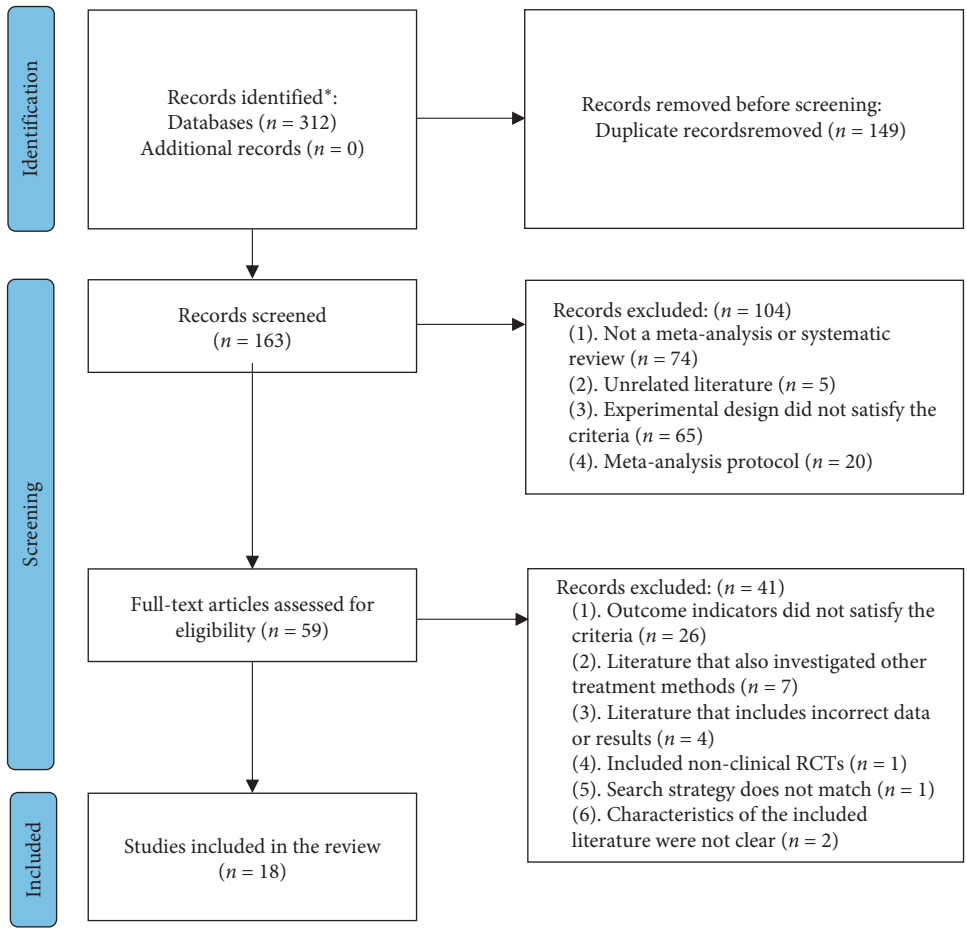

Figure 1: Literature screening process. *PubMed (n = 33), Embase (n = 15), Cochrane Library (n = 1), CNKI (n = 70), Wanfang (n = 79), QVIP (n = 58), and SINOMED (n = 56).

follows: Item 2: 0%, Item 4: 100%, Item 7: 0%, Item 9: 89%, Item 11: 39%, Item 13: 56%, and Item 15: 67%. The overall credibility of the included systematic reviews was very low. Specific evaluation results are listed in Table 3.

3.3.2. Evaluation of the Quality of the Evidence. The quality-of-evidence ratings for the outcome indicators of the included reviews were moderate, low, or very low quality. All quality-of-evidence ratings were downgraded due to research limitations because the methods adopted by these reviews to include RCTs were significantly biased and featured irregular, incorrect, or even semirandomized methods. Moreover, most RCTs did not state the use of a blinded method. In terms of serum hormone levels, significant heterogeneity was observed in the levels of T,

Table 2: Characteristics of the systematic reviews included in this study.

Included systematic reviews	Number of databases searched	Number of studies included	Sample size	Experimental group	Control group	Evaluation tools
Yan Lun et al. 2015 [17]	4	13	1,148	Chinese medicine + Western medicine[#]	Western medicine	Cochrane risk of bias assessment tool
Xiao Chao 2016 [18]	7	12	1,213	Chinese medicine + Western medicine	Western medicine	Jadad
Li Nan et al. 2017 [19]	3	23	NA	Chinese medicine + Western medicine[#]	Western medicine	NA
Lu RuLing et al. 2018 [20]	7	22	1,676	Kidney tonifying herbs + Diane-35	Diane-35	Jadad
Xu LiFang et al. 2018 [21]	6	22	NA	Chinese medicine to tonify the kidneys and invigorate the blood + Western medicine[#]	Western medicine	Jadad
Xu Huayun et al. 2018 [22]	3	14	978	Herbal manual cycle + Western medicine[#]	Western medicine	Jadad
Huang Wenfang et al. 2018 [23]	5	14	1,057	Liver relaxation method + Western medicine	Western medicine	Cochrane risk of bias assessment tool
Liu Ying et al. 2019 [24]	5	11	1,128	Kuntai capsule + Western medicine	Western medicine	Cochrane risk of bias assessment tool
Yuan BoChao 2019 [25]	7	7	634	Chinese herbal remedies to tonify the kidneys and invigorate the blood	Clomiphene	Cochrane risk of bias assessment tool
Ji Lin 2019 [26]	7	34	NA	Chinese medicine to tonify the kidneys and invigorate the blood + Western medicine[#]	Western medicine	Cochrane risk of bias assessment tool
Xie Peng Peng et al. 2019 [27]	7	20	1,484	Plus or minus CangFu Guiphlegm Tang + Western medicine	Western medicine	Jadad
Zhong Yizheng et al. 2019 [28]	7	15	1,259	Compound Xuanju capsules + Western medicine	Western medicine	Jadad
Dong YuFang 2020 [29]	7	43	3,056	Chinese medicine	Western medicine	Cochrane risk of bias assessment tool
Li Nan et al. 2020 [30]	5	13	1,305	Kuntai capsule + letrozole	Letrozole	Jadad
Du Xiu et al. 2020 [31]	6	14	1,100	Compound Xuanju capsules + Western medicine	Western medicine	Cochrane risk of bias assessment tool
Lin BeiBei 2020 [32]	8	26	1,299	Chinese herbs + Western medicine for kidney and liver	Western medicine	Cochrane risk of bias assessment tool
Chen JinMing et al. 2020 [33]	5	7	502	Gueiren pills + Western medicine	Western medicine	Cochrane risk of bias assessment tool
Huang Ting et al. 2020 [34]	8	13	797	Kidney tonifying herbs + clomiphene	Clomiphene	Cochrane risk of bias assessment tool

Note. NA: not reported. [#]The experimental group in the study used both Chinese medicine and a combination of Chinese and Western medicine.

LH, FSH, and LH/FSH, which contributed substantially to the inconsistencies in the results. Furthermore, imprecise conclusions and publication bias arising from the wide 95% confidence intervals and the small number of RCTs in some reviews had a negative impact on the quality of the evidence. Specific GRADE quality-of-evidence ratings are listed in Tables 4–11.

Table 3: AMSTAR2 quality evaluation results (items 1–16).

Included systematic reviews	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Credibility
Yan Lun et al. 2015 [17]	Y	N	N	Y	Y	N	N	PY	N	N	Y	NP	N	Y	Y	N	Very low
Xiao Chao 2016 [18]	Y	N	N	Y	Y	N	N	Y	Y	N	N	Y	Y	N	Y	N	Very low
Li Nan et al. 2017 [19]	Y	N	N	Y	Y	N	N	PY	N	N	N	NP	N	N	NP	N	Very low
Lu RuLing et al. 2018 [20]	Y	N	N	Y	Y	N	N	PY	Y	N	N	N	N	N	Y	N	Very low
Xu LiFang et al. 2018 [21]	Y	N	N	Y	N	N	N	PY	Y	N	N	N	N	Y	Y	N	Very low
Xu Huayun et al. 2018 [22]	Y	N	N	Y	Y	Y	N	PY	Y	N	N	Y	Y	N	Y	N	Very low
Huang Wenfang et al. 2018 [23]	Y	N	N	Y	Y	Y	N	PY	Y	N	Y	Y	Y	Y	Y	N	Very low
Liu Ying et al. 2019 [24]	Y	N	N	Y	Y	N	N	PY	Y	N	Y	NP	NP	Y	N	N	Very low
Yuan BoChao 2019 [25]	Y	N	N	Y	Y	Y	N	PY	Y	N	NP	Y	Y	N	N	N	Very low
Ji Lin 2019 [26]	Y	N	N	Y	Y	Y	N	PY	Y	N	Y	Y	Y	Y	Y	N	Very low
Xie Peng Peng et al. 2019 [27]	Y	N	N	Y	Y	Y	N	PY	Y	N	N	Y	Y	N	Y	N	Very low
ZhongYizheng et al. 2019 [28]	Y	N	N	Y	Y	Y	N	PY	Y	N	Y	Y	Y	Y	Y	N	Very low
Dong YuFang 2020 [29]	Y	N	N	Y	Y	Y	N	PY	Y	N	N	Y	Y	N	Y	N	Very low
Li Nan et al. 2020 [30]	Y	N	N	Y	Y	Y	N	PY	Y	N	Y	NP	N	Y	NP	N	Very low
Du Xiu et al. 2020 [31]	Y	N	N	Y	Y	Y	N	PY	Y	N	N	NP	N	N	Y	N	Very low
Lin BeiBei 2020 [32]	Y	N	N	Y	Y	Y	N	PY	Y	N	N	NP	Y	N	Y	N	Very low
Chen JinMing et al. 2020 [33]	Y	N	N	Y	Y	Y	N	PY	Y	N	Y	NP	N	Y	NP	N	Very low
Huang Ting et al. 2020 [34]	Y	N	N	Y	Y	Y	N	PY	Y	N	N	Y	Y	N	N	N	Very low
Percentage of reports	100	0	0	100	94	67	0	100	89	0	39	50	56	44	67	0	

Note. Item 1: did the research questions and inclusion criteria for the review include the components of PICO? Item 2: did the report of the review contain an explicit statement that the review methods were established prior to conducting the review, and did the report justify any significant deviations from the protocol? Item 3: did the review authors explain their selection of the study designs for inclusion in the review? Item 4: did the review authors use a comprehensive literature search strategy? Item 5: did the review authors perform study selection in duplicate? Item 6: did the review authors perform data extraction in duplicate? Item 7: did the review authors provide a list of excluded studies and justify the exclusions? Item 8: did the review authors describe the included studies in adequate detail? Item 9: did the review authors use a satisfactory technique to assess the risk of bias (RoB) in individual studies that were included in the review? Item 10: did the review authors report on the sources of funding for the studies included in the review? Item 11: if meta-analysis was performed did the review authors use appropriate methods for statistical combination of results? Item 12: if meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis? Item 13: did the review authors account for RoB in individual studies when interpreting/discussing the results of the review? Item 14: did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review? Item 15: if they performed quantitative synthesis, did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review? Item 16: did the review authors report any potential sources of conflict of interest, including any funding they received to conduct the review? *Y* = Yes; PY = Partially yes; *N* = No; NP = no meta-analysis performed.

3.4. Primary Outcome Indicators

3.4.1. Live Birth Rate. None of the included systematic reviews listed live birth rate as an outcome indicator.

3.4.2. Pregnancy Rate. A total of 17 systematic reviews reported on the pregnancy rate. Of these, 10 compared the pregnancy rate after combined treatment with Chinese and Western medicine with that achieved after treatment with Western medicine alone [18, 20, 23, 24, 28, 30–34]; two compared the pregnancy rate achieved after treatment with Chinese medicine alone with that after treatment with Western medicine alone [25, 29], and the remaining five compared the pregnancy rate achieved after combined treatment with Chinese and Western medicine with that after treatment with either Chinese or Western medicine alone [17, 19, 21, 22, 26]. All of the results suggested a higher pregnancy rate in the experimental group than in the control group. In addition, only the subgroup analysis of one review [22] indicated that the combined value of the experimental group after treatment with Chinese medicine alone on the pregnancy rate crossed the line of null effect when compared with that of Western medicine.

3.4.3. Ovulation Rate. A total of 13 systematic reviews reported on the ovulation rate. Of these, nine reviews compared the ovulation rate after combined treatment with Chinese and Western medicine with that of Western medicine alone [18, 23, 24, 27, 28, 30–33]. Of these, only one suggested that the combined value of the experimental group crossed the line of null effect [18], whereas the rest showed a higher ovulation rate in the experimental group. Conversely, one review compared the ovulation rate after treatment with Chinese medicine alone with that of Western medicine alone and showed that not only did the combined value of Chinese medicine alone cross the line of null effect when compared with Western medicine but the center of the diamond also favored the control group [25]. The remaining three studies compared the ovulation rate after the combined treatment with Chinese and Western medicine with that of either Chinese or Western medicine alone [17, 19, 26]. Of these, the subgroup analysis of one article showed no statistical significance between the ovulation rates after treatment with Chinese and Western medicine [26]. Therefore, the existing literature does not support the hypothesis that Chinese medicine is more effective than Western medicine in improving the ovulation rate of patients with PCOS.

Table 4: GRADE quality-of-evidence ratings for pregnancy rate.

Included systematic reviews	Number of studies included	Pregnancy rate effect (95% CI)	GRADE quality of evidence	Relegation factors
Yan Lun et al. 2015 [17]	16	OR = 3.44, 95% CI (2.66, 4.43)	Low	①④
Xiao Chao 2016 [18]	9	RR = 1.91, 95% CI (1.59, 2.29)	Low	①⑤
Li Nan et al. 2017 [19]	12	OR = 2.96, 95% CI (2.35, 3.74)	Very low	①④⑤
Lu RuLing et al. 2018 [20]	8	OR = 3.34, 95% CI (2.23, 5.02)	Very low	①②④⑤
Xu LiFang et al. 2018 [21]	18	OR = 3.83, 95% CI (2.95, 4.96)	Very low	①④⑤
Xu Huayun et al. 2018 [22]	11	RR = 1.70, 95% CI (1.39, 2.09)	Moderate	①
Huang Wenfang et al. 2018 [23]	3	OR = 1.97, 95% CI (1.19, 3.25)	Very low	①④⑤
Liu Ying et al. 2019 [24]	11	RR = 1.71, 95% CI (1.46, 2.01)	Low	①⑤

Note. CI: confidence interval; OR: odds ratio; RR: relative risk; ①: limitation; ②: inconsistency; ③: indirectness; ④: publication bias; ⑤: imprecision.

Table 5: GRADE quality-of-evidence ratings for ovulation rate.

Included systematic reviews	Number of studies included	Ovulation rate effect (95% CI)	GRADE quality of evidence	Relegation factors
Yan Lun et al. 2015 [17]	9	OR = 2.18, 95% CI (1.63, 2.92)	Very low	①④⑤
Xiao Chao 2016 [18]	8	RR = 1.10, 95% CI (0.87, 1.39)	Very low	①②④⑤
Li Nan et al. 2017 [19]	6	OR = 2.70, 95% CI (1.32, 5.45)	Very low	①②④⑤
Huang Wenfang et al. 2018 [23]	6	OR = 2.18, 95% CI (1.77, 2.68)	Low	①⑤
Liu Ying et al. 2019 [24]	8	RR = 1.34, 95% CI (1.23, 1.46)	Low	①⑤
Yuan BoChao 2019 [25]	6	RR = 0.97, 95% CI (0.86, 1.09)	Very low	①②④⑤
Ji Lin 2019 [26]	14	OR = 1.92, 95% CI (1.40, 2.64)	Low	①②
Xie Peng Peng et al. 2019 [27]	10	RR = 1.17, 95% CI (1.02, 1.34)	Low	①④
Yuan BoChao 2019 [25]	5	RR = 1.18, 95% CI (1.03, 1.37)	Low	①④
Li Nan et al. 2020 [30]	4	OR = 3.91, 95% CI (1.95, 7.84)	Very low	①④⑤
Du Xiu et al. 2020 [31]	6	RR = 1.17, 95% CI (1.03, 1.34)	Very low	①②④
Lin BeiBei 2020 [32]	6	RR = 1.31, 95% CI (1.16, 1.48)	Low	①④
Chen JinMing et al. 2020 [33]	3	RR = 1.21, 95% CI (1.07, 1.37)	Very low	①④⑤

Note. CI: confidence interval; OR: odds ratio; RR: relative risk; ①: limitation; ②: inconsistency; ③: indirectness; ④: publication bias; ⑤: imprecision.

3.4.4. Clinical Efficiency. A total of 15 systematic reviews reported on clinical efficiency. Of these, nine compared the clinical efficiency of the combined use of Chinese and Western medicine with that of Western medicine alone [18, 20, 23, 27, 28, 32–34]; one compared the clinical efficiency of Chinese medicine alone with that of Western medicine alone [29], and the remaining five compared the clinical efficiency of the combined use of Chinese and Western medicine with that of either Chinese or Western medicine alone [17, 19, 21, 22, 26]. Although all results showed that the efficiency in the test group was higher than that in the control group, two articles indicated that the combined value of Chinese medicine alone crossed the line of null effect when compared with Western medicine [21, 22].

3.4.5. Testosterone Level. A total of 10 systematic reviews reported on the T level. Of these, 7 compared the T level after the combined treatment with Chinese and Western medicine with that of Western medicine alone [18, 27, 28, 30–33]; 2 compared the T level after treatment with Chinese medicine alone with that of Western medicine alone [25, 29]; and 1 compared the T level after combined treatment with Chinese and Western medicine with that of either Chinese or Western medicine alone [26]. Except for 1 article [26], all studies suggested that the T level of the test group was significantly lower than that of the control group.

3.4.6. Luteinizing Hormone Level. A total of 10 systematic reviews reported on the LH level. Of these, 7 compared the LH level after the combined treatment with Chinese and Western medicine with that of Western medicine alone [18, 20, 27, 28, 30, 32, 33]; 2 compared the LH level after treatment with Chinese medicine alone with that of Western medicine alone [25, 29], and 1 compared the LH level after the combined treatment with Chinese and Western medicine with that of either Chinese or Western medicine alone [26]. All results indicated that the LH level of the experimental group was lower than that of the control group.

3.4.7. Follicle-Stimulating Hormone Level. A total of six systematic reviews reported on the FSH level. Of these, four compared the FSH level after the combined treatment with Chinese and Western medicine with that of Western medicine alone [27, 28, 30, 33]. All but one study indicated a lower FSH level in the test group than in the control group [33]. In contrast, one study compared the FSH level after treatment with Chinese medicine alone with that of Western medicine alone and showed no significant differences [29].

Table 6: GRADE quality-of-evidence ratings for efficiency.

Included systematic reviews	Number of studies included	Efficiency effect (95% CI)	GRADE quality of evidence	Relegation factors
Yan Lun et al. 2015 [17]	14	OR = 5.32, 95% CI (3.82, 7.41)	Low	①④
Xiao Chao 2016 [18]	7	RR = 1.27, 95% CI (1.19, 1.36)	Low	①⑤
Li Nan et al. 2017 [19]	8	OR = 3.90, 95% CI (2.92, 5.20)	Low	①④
Lu RuLing et al. 2018 [20]	11	OR = 4.22, 95% CI (2.86, 6.23)	Very low	①④⑤
Xu LiFang et al. 2018 [21]	18	OR = 2.83, 95% CI (2.06, 3.88)	Very low	①④⑤
Xu Huayun et al. 2018 [22]	13	RR = 1.19, 95% CI (0.87, 1.63)	Low	①⑤
Huang Wenfang et al. 2018 [23]	7	OR = 2.63, 95% CI (1.67, 4.15)	Very low	①④⑤
Ji Lin 2019 [24]	21	OR = 3.38, 95% CI (2.59, 4.41)	Moderate	①
Xie Peng Peng et al 2019 [27]	14	RR = 1.13, 95% CI (1.02, 1.24)	Moderate	①
ZhongYizheng et al. 2019 [28]	10	RR = 1.27, 95% CI (1.13, 1.44)	Moderate	①
Dong YuFang 2020 [29]	31	RR = 1.26, 95% CI (1.20, 1.32)	Moderate	①
Li Nan et al. 2020 [30]	4	OR = 3.42, 95% CI (1.76, 6.64)	Very low	①④⑤
Lin BeiBei 2020 [32]	18	RR = 1.26, 95% CI (1.17, 1.36)	Very low	①②④
Chen JinMing et al. 2020 [33]	6	RR = 1.26, 95% CI (1.15, 1.37)	Low	①④
Huang Ting et al. 2020 [34]	8	RR = 1.25, 95% CI (1.13, 1.37)	Very low	①④⑤

Note. CI: confidence interval; OR: odds ratio; RR: relative risk; ①: limitation; ②: inconsistency; ③: indirectness; ④: publication bias; ⑤: imprecision.

Table 7: GRADE quality-of-evidence ratings for testosterone level.

Included systematic reviews	Number of studies included	Testosterone effect (95% CI)	GRADE quality of evidence	Relegation factors
Xiao Chao 2016 [24]	8	SMD = –0.81, 95% CI (–1.46, –0.16)	Very low	①②④⑤
Yuan BoChao 2019 [25]	5	MD = –1.51, 95% CI (–1.64, –1.37)	Very low	①④⑤
Ji Lin 2019 [26]	24	SMD = –0.64, 95% CI (–0.97, –0.36)	Very low	①②⑤
Xie Peng Peng et al 2019 [27]	13	WMD = –0.93, 95% CI (–1.38, –0.28)	Very low	①②④⑤
ZhongYizheng et al. 2019 [28]	9	SMD = –1.59, 95% CI (–1.76, –1.41)	Very low	①②④
Dong YuFang 2020 [29]	37	SMD = –0.40, 95% CI (–0.65, –0.15)	Very low	①②④
Li Nan et al. 2020 [30]	3	SMD = –0.68, 95% CI (–3.99, 2.62)	Very low	①②④⑤
Du Xiu et al. 2020 [31]	5	RR = –0.53, 95% CI (–0.90, –0.16)	Very low	①②④
Lin BeiBei 2020 [32]	19	SMD = –0.20, 95% CI (–0.55, 0.16)	Very low	①②④⑤
Chen JinMing et al. 2020 [33]	2	MD = 0.95, 95% CI (0.15, 1.75)	Very low	①②④⑤

Note. CI: confidence interval; MD: mean difference; SMD: standardized mean difference; WMD: weighted mean difference; ①: limitation; ②: inconsistency; ③: indirectness; ④: publication bias; ⑤: imprecision.

Another study [26] compared the FSH level after combined treatment with Chinese and Western medicine with that of either Chinese or Western medicine alone and also showed no significant differences.

3.4.8. LH/FSH Level. A total of six systematic reviews reported on the LH/FSH level. Of these, four compared the LH/FSH level after the combined treatment with Chinese and Western medicine with that of Western medicine alone [18, 20, 27, 32]; one compared the LH/FSH level after treatment with Chinese medicine alone with that of Western medicine alone [29]; and one compared the LH/FSH level after the combined treatment with Chinese and Western medicine with that of either Chinese or Western medicine alone [26]. All results suggested that the LH/FSH level was lower in the experimental group than in the control group.

3.4.9. Adverse Reactions. A total of seven systematic reviews reported adverse reactions. Of these, two compared the adverse effects of the combined use of Chinese and Western medicine with the use of Western medicine alone [18, 32]; one compared the adverse effects of Chinese medicine alone with that of Western medicine alone [29]; and four compared the adverse effects of the combined use of Chinese and Western medicine with that of either Chinese or Western medicine alone [17, 19, 21, 26]. Apart from one study [18], all reviews indicated fewer adverse effects in the experimental group than in the control group.

4. Discussion

4.1. Poor Methodological Quality of Systematic Reviews/Meta-Analyses of Using Chinese Medicine for the Treatment of PCOS. As systematic reviews/meta-analyses are an important source of evidence for guiding clinical decision-making in evidence-based medicine, they need to be strictly standardized. The low overall quality of the 18 reviews included in this study suggests that existing systematic reviews/meta-analyses of treatment with Chinese medicine for PCOS need to be improved and rigorously planned according to the PRISMA protocol. Moreover, the included reviews were neither registered nor provided a detailed exclusion list, which might have affected the accuracy of the results.

Table 8: GRADE quality-of-evidence ratings for luteinizing hormone level.

Included systematic reviews	Number of studies included	Luteinizing hormone effect (95% CI)	GRADE quality of evidence	Relegation factors
Xiao Chao 2016 [18]	7	SMD = –1.16, 95% CI (–1.66, –0.66)	Very low	①②④⑤
Lu RuLing et al. 2018 [20]	18	MD = –1.84, 95% CI (–1.98, –1.70)	Very low	①②⑤
Yuan BoChao 2019 [25]	5	MD = –6.72, 95% CI (–7.32, –6.13)	Very low	①④⑤
Ji Lin 2019 [26]	23	SMD = –0.55, 95% CI (–0.74, –0.37)	Low	①④
Xie Peng Peng et al 2019 [27]	13	WMD = –0.95, 95% CI (–1.41, –0.52)	Very low	①②④
ZhongYizheng et al. 2019 [28]	9	SMD = –1.24, 95% CI (–1.39, –1.08)	Very low	①②④
Dong YuFang 2020 [29]	39	SMD = –0.38, 95% CI (–0.59, –0.16)	Very low	①②④
Li Nan et al. 2020 [30]	5	SMD = 1.67, 95% CI (–1.97, –1.37)	Very low	①②④
Lin BeiBei 2020 [32]	17	SMD = –0.78, 95% CI (–1.22, –0.34)	Very low	①②④
Chen JinMing et al. 2020 [33]	2	MD = 7.55, 95% CI (2.05, 13.04)	Very low	①②④⑤

Note. CI: confidence interval; MD: mean difference; SMD: standardized mean difference; WMD: weighted mean difference; ①: limitation; ②: inconsistency; ③: indirectness; ④: publication bias; ⑤: imprecision.

Table 9: GRADE quality-of-evidence ratings for follicle-stimulating hormone level.

Included systematic reviews	Number of studies included	Follicle-stimulating hormone effect (95% CI)	GRADE quality of evidence	Relegation factors
Ji Lin 2019 [26]	19	SMD = 0.12, 95% CI (–0.29, –0.53)	Very low	①②④
Xie Peng Peng et al 2019 [27]	11	WMD = –0.59, 95% CI (–0.98, –0.20)	Very low	①②④
ZhongYizheng et al. 2019 [28]	8	SMD = 0.66, 95% CI (0.51, 0.82)	Low	①④
Dong YuFang 2020 [29]	37	SMD = 0.01, 95% CI (–0.22, 0.25)	Very low	①②④⑤
Li Nan et al. 2020 [30]	5	SMD = –1.67, 95% CI (–3.05, –0.30)	Very low	①②④⑤
Chen JinMing et al. 2020 [33]	2	MD = 0.13, 95% CI (–0.39, 0.66)	Very low	①②④⑤

Note. CI: confidence interval; MD: mean difference; SMD: standardized mean difference; WM: weighted mean difference; ①: limitation; ②: inconsistency; ③: indirectness; ④: publication bias; ⑤: imprecision.

Table 10: GRADE quality-of-evidence ratings for luteinizing hormone/follicle-stimulating hormone level.

Included systematic reviews	Number of studies included	Luteinizing hormone/follicle-stimulating hormone effect (95% CI)	GRADE quality of evidence	Relegation factors
Xiao Chao 2016 [18]	4	MD = –0.81, 95% CI (–1.17, –0.45)	Very low	①②④⑤
Lu RuLing et al. 2018 [20]	12	MD = –0.25, 95% CI (–0.44, –0.06)	Very low	①②⑤
Ji Lin 2019 [26]	11	SMD = –0.45, 95% CI (–0.68, –0.23)	Low	①②
Xie Peng Peng et al. 2019 [27]	3	WMD = –1.04, 95% CI (–1.78, –0.33)	Very low	①②④⑤
Dong YuFang 2020 [29]	22	SMD = –0.39, 95% CI (–0.60, –0.19)	Very low	①②④
Lin BeiBei 2020 [32]	14	MD = –0.37, 95% CI (–0.53, –0.21)	Very low	①②④

Note. CI: confidence interval; MD: mean difference; SMD: standardized mean difference; WMD: weighted mean difference; ①: limitation; ②: inconsistency; ③: indirectness; ④: publication bias; ⑤: imprecision.

Furthermore, failure to declare conflicts of interest makes it difficult to rule out potential conflicts, thereby affecting the objectivity of the review to some extent.

All the data and findings from the included studies suggested that the combined use of Chinese and Western medicine could improve the efficacy of PCOS treatment. However, due to the poor quality of the systematic reviews, the credibility of the results and evidence was compromised. Except for efficiency, which had a quality rating of moderate, all other evidence had a quality rating of low or very low. The analysis showed that ratings were reduced primarily because of methodological limitations and incorrect selection of the included studies. This was reflected mostly in the inadequate blinding, inappropriate randomization, wide 95% confidence intervals, and small sample sizes, which reduced the credibility of the conclusions.

With regard to the very low-quality evidence found in our analysis, an overview of systematic reviews of the use of acupuncture for the treatment of PCOS also generally resulted in low quality of evidence [35]. However, subsequent studies have confirmed that acupuncture does not support the treatment of PCOS [36]. While this does not

Table 11: GRADE quality-of-evidence ratings for adverse effects.

Included systematic reviews	Number of studies included	Adverse effects effect (95% CI)	GRADE quality of evidence	Relegation factors
Yan Lun et al. 2015 [17]	4	OR = 0.19, 95% CI (0.08, 0.46)	Low	①⑤
Xiao Chao 2016 [18]	4	RD = –0.05, 95% CI (–0.13, 0.03)	Very low	①②⑤
Li Nan et al. 2017 [19]	3	OR = 0.07, 95% CI (0.02, 0.23)	Very low	①②④⑤
Xu LiFang et al. 2018 [21]	10	OR = 0.26, 95% CI (0.09, 0.80)	Low	①⑤
Ji Lin 2019 [26]	13	OR = 0.26, 95% CI (0.12, 0.55)	Very low	①②⑤
Dong YuFang 2020 [29]	8	RR = 0.12, 95% CI (0.06, 0.25)	Low	①④
Lin BeiBei 2020 [32]	13	RR = 0.36, 95% CI (0.20, 0.63)	Low	①④

Note. CI: confidence interval; OR: odds ratio; RR: relative risk; ①: limitation; ②: inconsistency; ③: indirectness; ④: publication bias; ⑤: imprecision.

directly imply that low-quality evidence must not be credible, examples suggest that low-quality evidence does contain the possibility of not supporting the treatment.

4.2. Suggestions for Future Systematic Reviews/Meta-Analyses of Chinese Medicine Treatment for PCOS. At present, letrozole and other drugs are considered first-line medications to treat PCOS, but their side effects and adverse reactions have been shown to reduce patient compliance [37]. Traditional Chinese medicine is becoming more and more widely used because of its milder side effects and adverse reactions and individual relevance [38]. Particularly, berberine not only improves symptoms but also reduces the risk of cardiovascular disease [39]. Meanwhile, the mechanism of traditional Chinese medicine in the treatment of PCOS needs to be further explored. Compared with letrozole, which has a standard of quantitative use, the use of traditional Chinese medicine is difficult to quantify. Different doctors may use different traditional Chinese medicines, and this difference may have a negative impact on the efficacy of the treatment. Despite this, Chinese medicine is a good way to treat PCOS.

It is recommended that future systematic reviews/meta-analyses of treatment with Chinese medicine for PCOS prepare a research plan in advance, including a literature exclusion list, and that they use the effect size in a reasonable manner and analyze sources of heterogeneity and biases carefully during the review. To minimize study limitations, correct randomization and appropriate blinding methods should be introduced as inclusion criteria. Moreover, although this study found that the quality rating for the evidence for efficiency was higher than that of other evidence, most articles did not specify how efficiency was evaluated. Therefore, it is suggested to unify the evaluation criteria of effective efficiency and choose more main indicators that can reflect the curative effect [40]. Furthermore, existing evidence does not support the advantage of Chinese medicine over Western medicine in aspects such as the ovulation rate. The fact that the center of the diamond of some studies was biased toward the control group indicated that the real results might even be that Western medicine is more effective. Therefore, it is recommended that subsequent research should carry out multicenter, large-sample RCTs, or factorial tests to verify the efficiency of Chinese medicine, thereby providing more reliable evidence for clinical guidance.

4.3. Study Limitations. This study utilized the AMSTAR2 tool and the GRADE system to review and evaluate the existing evidence for the treatment of PCOS with Chinese medicine. This study has the following limitations. First, only Chinese-language reviews of poor overall quality were included in this study, which may have resulted in biased and inaccurate results. Second, the AMSTAR2 tool and the GRADE system are highly subjective. Even with two evaluators, subjective factors or user error cannot be fully eliminated, and this can introduce biases and errors. Third, subgroup analysis was performed only for studies in which Western medicine was used in combination with Chinese medicine in the experimental groups, and no individual analysis of different types of Chinese medicine was carried out, thus making it difficult to identify the efficiencies of particular Chinese medicines or treatment theories.

5. Conclusions

At present, only low-quality evidence is available to suggest that combined treatment with Chinese and Western medicine is superior to Western medicine alone in improving the pregnancy rate, ovulation rate, serum hormone levels, and adverse effects of patients with PCOS. Future clinical trials and reviews of higher quality are recommended to clarify the efficacy of Chinese medicine and provide more accurate evidence.

Data Availability

The data used to support the finding of this study are stored in the FAIRDOMHub database (https://fairdomhub.org/projects/230) [41].

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Both Linjing Wang and Runyu Liang are the first authors.

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Supplementary Materials

The supplementary materials include the extracted characteristics of each study, the score of each outcome, the AMSTAR2 quality evaluation results, and the GRADE quality-of-evidence ratings. These are the same data uploaded to FAIRDOMhub (https://fairdomhub.org/projects/230). (*Supplementary Materials*)

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Research Article

Follicular Metabolites-Assisted Clinical Evaluation of IVF/ICSI Outcomes

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As infertility became a significant public health problem, assisted reproductive technologies (ARTs) were introduced. However, the fertilization rate of in vitro fertilization (IVF) per cycle varied, and patients needed to repeat IVF or change to intracytoplasmic sperm injection (ICSI). Here, 75 couples suffering from female fallopian tubal blockage (tubal group) and 42 spouses beset by male abnormal sperm status (dysspermia group) were recruited. We comprehensively explored the relationship among couples' clinical factors, follicular metabolites, and IVF/ICSI stepwise outcomes. IVF/ICSI outcomes were affected by follicular metabolites and physical status in both women and men, regardless of which side infertility came from. Particularly, in the tubal group, the energy supporting pathways—glycolysis and pyruvate metabolism—were most essential in follicles, and IVF/ICSI outcomes were also related to sperm parameters. However, in the dysspermia group, in addition to sperm conditions, oocyte quality acted as a compensation for poor sperm quality, for which aminoacyl-tRNA biosynthesis and the related supporting metabolism were critical in the follicular environment, and ultimately played a decisive role in IVF/ICSI outcomes. The respective logistic regression models in combination with selective male sperm parameters, estradiol (E2), follicular alanine, glutamine, glycoprotein, lipid, and acetic acid, were constructed to predict IVF or ICSI outcomes. No matter which sex infertility comes from, factors from both men and women should be considered. The current study provides a feasible option for pre-IVF evaluation, as well as guidance for follow-up clinical intervention to improve IVF/ICSI success rates.

1. Introduction

Data from both the United States [1] and UK [2] governments pointed out that 12–15% of couples suffered infertility in their productive age, which was identified as a significant public health issue. The most common cause of female infertility was fallopian tube problems, affecting over 25% of infertile women. The structural abnormality and/or pelvic inflammation stops spermatozoa from traveling in the fallopian tube and subsequently fertilizing locates [3]. About half of the infertilities involve male factors, either the male factor alone or male-female combined problems, while most of the male infertility is preventable, ameliorable, or treatable [4]. Invasive tools were introduced to assist infertile couples. However, the fertilization rate per cycle varied widely based on assorted issues including disease, ovulation, induction control, oocyte selection, operator's experience, and ART processes. A large proportion of patients need to repeat IVF or switch to ICSI for satisfactory fertilization. This not only brings physical but also financial burdens to patients.

Efforts have been carried out toward improving IVF outcomes. Summarizing overall biological events, metabolites can reflect system dynamics into a series of detectable small molecules with sophisticated modern metabolomic techniques [5]. Hence, besides blood cytology or biochemical indicators, some researchers targeted metabolites from the embryo culture medium (ECM) [6, 7], follicular fluid [8, 9], and female blood [10] for biomarkers to predict IVF outcomes. However, inconsistent findings exist in different studies [2]. It is presumably because metabolic pictures in the productive area were far more complex than those diseases whose status can be considered in single organs. The combination of sperm and oocytes, as well as the following zygotic development, involved both paternal and maternal individuals. Hence, both females' and males' physical and endocrine status, in addition to follicular metabolic profiling, could have nonnegligible impacts on reproductive outcomes.

In the oocyte environment, the follicular fluid includes information on granulosa cell secretion and conduction, as well as metabolites released during oocyte growth [11]. In our previous study of altered follicular metabolic profiling in PCOS patients, we discovered significant effects of common clinical characteristics on follicular metabolites, which potentially influence IVF outcomes [9]. To expand on it, the present study aims to investigate both male and female clinical factors, as well as follicular components that influence IVF outcomes in two different disease groups, infertility with male sperm problems and infertility with female fallopian tube problems. After collecting the follicular metabolite information by ^{1}H NMR, we firstly identified the influence of females' physical and endocrine parameters on follicular metabolites. With this knowledge, we observed the relationships of IVF/ICSI outcomes with couples' clinical parameters and follicular metabolites in two different disease groups. Logistic regression models were constructed to predict IVF results, to assist clinicians to evaluate the success rates of IVF, and to determine whether to conduct IVF or ICSI directly.

2. Experimental Section

2.1. Sample Collection. This study involved 117 couples who underwent IVF/ICSI in Renmin Hospital of Wuhan University, China. Among them, 75 couples suffered from female fallopian tubal blockage (tubal group) without any male problems. The other 42 couples were purely affected by male abnormal sperm status (dysspermia group), consisting of 27 oligozoospermia and 15 azoospermia cases. Sperm status was measured according to WHO-5 standards [12]. No other reproductive or endocrine diseases were found in this sample population. The whole sample cohort included Chinese females from Hubei, China. The study was approved by the Institutional Review Board (IRB) of Renmin Hospital of Wuhan University, China. Written consent has been obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used.

All recruited females were advised to have proper meals for at least seven days before ovulation. Oocytes were stimulated by the GnRH-long protocol. Sex hormone levels were measured, and ultrasonography was performed regularly. Follicles were collected 34–36 hours after injection of human chorionic gonadotropin (HCG). Oocyte retrievals were carried out under the guidance of transvaginal ultrasound. The follicular fluid from the same patient was combined and centrifuged at 12000 rpm for 10 min under 4°C to remove tissue, debris, and granulosa cells. Finally, the supernatant was transported under dry ice and stored at −80°C until analysis. The relative clinical information of the couples was collected.

2.2. Assisted Reproductive Technology (ART) Protocol and Information. This study included only one cycle per woman. After oocyte retrieval, insemination underwent IVF within three hours using gradient-prepared spermatozoa in the fertilization medium. The appearance of two pronuclei (2PN) was observed closely, and intracytoplasmic sperm injection (ICSI) was performed also within 4 hours if necessary. Thirty couples received ICSI directly because they had at least one of the following problems, (1) sperm activity rate (level a + b) $< 1\%$; (2) sperm density $< 1\times10^6$/ml; (3) normal sperm morphology rate <1%; (4) fertilization failure in IVF in the last ART cycle. The oocytes were then transferred to a cleavage medium for further development. The overall fertilization rate (FR) was calculated as the ratio of successfully fertilized oocyte number to the oocyte number that was involved in the insemination. Next, the number of 2PN was counted under a microscope. The ratio of the 2PN number to the fertilized oocyte number was assigned as the effective fertilization rate (2PN-FR). The standard embryo quality evaluation strategy was exerted three days after cleavage [13] as in Table S1. Those embryos belonging to grade I or II and blastomeres numbering between six and eight on day three were defined with top quality. The top-quality embryo rate (TQER) was calculated as the ratio of the top-quality embryo number to the 2PN cleavage number.

2.3. ^{1}H NMR Experiments. Frozen follicular fluid samples were thawed, vortexed, and centrifuged. A volume of 530 μL of each sample was transferred to a 5 mm NMR tube with a coaxial capillary containing 60 μL TSP (0.53 mmol) in D_2O, serving as the chemical shift and quantitative reference. The experiment sequence was randomized and carried out at 25°C on a Bruker DRX500-MHz NMR spectrometer using pulse sequences of CPMG (Carr-Purcell-Meiboom-Gill) with a water presaturation pulse. For each spectrum, 64 transients were collected and 16K data points were acquired using a spectral width of 6000 Hz. An exponential weighting function corresponding to 0.5 Hz line broadening was applied to the free-induced decay followed by Fourier transformation. Phasing and baseline correction was applied in Bruker XwinNMR software version 3.5.

2.4. Data Analysis. All statistical analysis was performed using SPSS Statistics (ver.21). For clinical parameters and IVF/ICSI outcomes, the average and standard deviation

(Ave. ± STD) were provided, and a 95% confidence interval (CI) was used as the statistical threshold. A *T*-test, Mann–Whitney *U* test (for abnormally distributed data), and Chi-square test were used for comparison among groups. Pearson correlation was used to explore the relationship between variables. Since the IVF/ICSI results of FR, 2PN-FR, and TQER came from the step-by-step processes, a non-negligible correlation existed among them. As a result, in the following sections, when observing the parameters that affected individual steps, the respective partial correlation coefficients were calculated with the control of the previous steps. In this way, the impacts from previous steps were removed. Moreover, for correlation calculation, a tightened significant threshold of $p < 0.03$ was chosen as the significant cutoff to minimize interference factors and false-positive results. Multivariate analysis was carried out by logistic regression analysis with a forward stepwise variable selection algorithm, which automatically retained the variables that are most conducive to discrimination. Nighty-five percent CI of model coefficients, as well as the prediction results of Receiver Operating Characteristic (ROC), were provided. Bonferroni adjustments of p values were performed using R software.

3. Results

3.1. Clinical Information. The sample cohort of this study involved 117 couples, including 75 couples in the tubal group and 42 couples in the dysspermia group. The clinical information for both female and male subjects was collected, and the representative demographic information including age, body mass index (BMI), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sperm density is summarized in Table 1 by group. Most of the characteristics were comparable between groups, except the sperm-related parameters (sperm density, sperm motility A and B, level A and level B sperm percentage, total motility, and PR + NP percentage), for which the tubal group was superior to the dysspermia group.

3.2. Follicular Metabolites Detected by ^{1}HNMR. CPMG ^{1}H NMR spectra focused on small molecules were collected for each follicular fluid sample. A total of 67 peak regions were identified with reference to their chemical shift and multiplicities [14]. Detailed information of these 67 peaks was listed in Table S2, including amino acids, glucose, choline, and lipids. The integrals of each peak region were obtained. Subsequent analysis with relevance to FF metabolites was employed on these 67 peak integrals.

3.3. Effects of Clinical Parameters on Follicular Metabolites. Correlation analysis was performed on 67 follicular metabolite peak integrals of 117 female patients and their clinical parameters. The correlation coefficients and corresponding log 10 based p values are visually presented in Figure 1. Those pairs shown in orange to dark red colors were closely related pairs, having a correlation with a significant p value less than 0.01.

Firstly, the women's pulse rate (P. min^{-1}), respiration rate (R. min^{-1}), and systolic blood pressure were the most effective clinical parameters. P. and R. acted reversely on follicular metabolites, including lactate, acetate, proline, glutamine, 3-hydroxybutyric acid, pyruvate, glutamate, β-glucose, tyrosine, and formic acid. The influential trends of BP sys. on follicular metabolites were almost the same as R. Secondly, liver function index—DBIL—and renal function index—BUN—had an influence on isoleucine, β-glucose, tyrosine, and pyruvate, glutamine, and glucose. Thirdly, age performed a positive correlation with lactate and alanine levels. BMI was positively correlated with glycoprotein and glucose. Lastly, there is no doubt that endocrine parameters, E2 and FSH, affected the follicles. FSH had a negative impact on lactate, while E2 had a positive effect on glutamate. Besides, total small follicle number (SFN) in women's ovaries was positively associated with follicular lactate, alanine, and α-glucose (Figure 1).

3.4. IVF/ICSI Outcomes. In the whole sample cohort, 88 couples were treated by IVF, 47 couples underwent ICSI, and 18 spouses participated in both techniques. In Figure 2 and Table S3, the stepwise IVF/ICSI results in the forms of overall fertilization rate (FR), 2PN fertilization rate (2PNFR), and the top-quality embryo rate (TQER) were presented upon the technique used. The overall outcomes of both groups were summarized. There was no significant difference between the groups in each parallel comparison. For example, the IVF FR was similar in the tubal group (62.4 ± 26.9%) to that in the dysspermia group (59.3 ± 26.1%), and ICSI 2PNFR of the tubal group (87.3 ± 22.3%) was comparable with that of the dysspermia group (90.9 ± 15.1%c).

It was not surprising that, because of different insemination techniques, in both the tubal and dysspermia groups, the FR (around 80%) from ICSI treatments was clearly higher than that (~60%) from IVF treatment ($p < 0.01$). Similarly, in the tubal group, 2PNFR of ICSI was elevated ($p < 0.05$). But, in the tubal group, TQER from IVF (67.6 ± 32.2%) exceeded the result from ICSI (50.2 ± 35.1%), with $p < 0.05$. When zoomed in the dysspermia group, this trend of higher embryo top-quality rate of IVF was also found in 15 couples with azoospermia, with the p value of 0.051 at the borderline.

3.5. Factors Affecting IVF/ICSI Outcomes in the Tubal Infertility Group. Correlations between IVF/ICSI stepwise outcomes and couples' available clinical parameters were explored. Firstly, a significant positive correlation was found between FR and male age in both the IVF subset and the whole tubal group. It was not surprising that the IVF FR was positively correlated with sperm quality parameters, including normal sperm morphology and progressive motility (PR). But the ICSI FR was negatively correlated with oocyte retrieved number (ORN) and the total SFN from female ovaries (Figure 3, Table S4). Similarly, ORN appeared to have a negative effect on 2PNFR. According to the number of oocytes retrieved, we further divided the tubal ICSI cohort into two subgroups, one with the oocytes number greater

Table 1: Representative demographic information by group.

Clinical characteristic	Tubal group ($n = 75$)	Dysspermia group ($n = 42$)	Corrected p value (95% CI)
Females			
Age	32.0 ± 5.1	31.1 ± 4.9	NS
BMI (kg/m^2)	22.2 ± 3.3	21.6 ± 2.9	NS
Basal FSH (mIU/ml)	6.2 ± 1.7	6.9 ± 2.4	NS
Basal LH (mIU/ml)	4.1 ± 2.0	4.4 ± 2.2	NS
Estradiol, (E2, pg/ml)	40.6 ± 15.9	48.7 ± 22.1	NS
BP sys. (mmHg)	101.6 ± 3.8	101.9 ± 3.3	NS
BP dia. (mmHg)	65.5 ± 6.3	64.4 ± 5.5	NS
Pulse rate (P., min^{-1})	66.1 ± 7.2	66.3 ± 7.3	NS
Respiration rate (R., min^{-1})	20.2 ± 1.1	20.3 ± 1.1	NS
DBIL (umol/L)	3.6 ± 2.1	3.5 ± 1.9	NS
BUN (mmol/L)	4.2 ± 1.1	4.3 ± 1.1	NS
Fasting blood glucose (mmol/L)	5.0 ± 1.3	4.8 ± 1.2	NS
Creatinine, (Cr, μmol/L)	49.8 ± 8.1	51.8 ± 10.3	NS
SFN	13 ± 6	12 ± 6	NS
ORN	12 ± 7	12 ± 6	NS
Males			
Age	33.5 ± 8.0	33.6 ± 5.4	NS
BMI (kg/m^2)	22.7 ± 6.4	23.4 ± 6.4	NS
BP sys. (mmHg)	108.3 ± 23.6	106.3 ± 24.7	NS
BP dia. (mmHg)	68.0 ± 16.1	66.1 ± 16.2	NS
Sperm density (×10^6/mg)	88.6 ± 52.1	44.0 ± 48.2	<0.01
Sperm motility A (%)	22.9 ± 8.6	12.7 ± 10.3	<0.01
Sperm motility B (%)	37.3 ± 8.5	23.7 ± 15.2	<0.01
Level a sperm (%)[1]	13.6 ± 9.0	3.3 ± 4.0	<0.01
Level b sperm (%)[2]	21.0 ± 11.1	7.5 ± 7.5	<0.01
Total motility (PR + NP, %)	36.4 ± 11.5	14.9 ± 9.3	<0.01

FSH, follicle-stimulating hormone; LH, luteinizing hormone; BP sys., systolic blood pressure; BP dia., diastolic blood pressure; DBIL, direct bilirubin; BUN, blood urea nitrogen; PR, progressive; NP, nonprogressive; SFN, small follicle number in ovaries; ORN, oocyte retrieved number. Level a sperm: fast-moving sperms; Level b sperm: slowly moving sperms.

than 15 ($n = 9$), and the other less than 15 ($n = 12$). The subgroup with more oocytes retrieved number had statistically lower FR ($p < 0.05$). Similarly, the 2PN rate was significantly reduced ($p < 0.05$) when the retrieved oocyte number achieved more than 15. The same negative trend was found in female urinary creatinine.

The embryo quality evaluation step was affected by more sophisticated factors. Again, sperm parameters and male age were influential. However, male age here negatively contributed to TQER, in comparison with its positive effect on FR. Like that in FR, the SFN in female ovaries was again negatively affecting ICSI EQTR. Also, female blood pressure was closely connected to ICSI TQER.

The follicular metabolites also presented a significant correlation with ART outcomes (Table S5). For patients who participated in ICSI, increased histidine and decreased glucose appeared to improve FR. Lactate and alanine seemed beneficial to overall ART FR. Creatinine played a role in the following 2PN transforming step, contributing positively to IVF 2PN-FR. Less glucose appeared profitable on overall ART 2PN-FR. Moreover, the high content of lipids presented helpful to ICSI TQER.

A summary of all factors impacting stepwise IVF/ICSI outcomes is presented in Figures 3 and S1 with more details.

3.6. Logistic Regression Predicting IVF/ICSI Outcomes for the Tubal Infertility Group. In the tubal infertility group, patients who participated in IVF ($n = 68$) were further divided into two portions, one with the IVF FR less than 70% ($n = 39$, entered as "0") and the other with IVF FR above 70% ($n = 29$; entered as "1"). The logistic regression model automatically retained the most discriminant variables with an overall predicted accuracy of 83.8%. ROC space is plotted in Figure 4, with the AUROC (area under ROC) of 0.85 (CI: 0.76~0.95), illustrating a satisfactory discriminative ability. The coefficients of the included variables in the model, as well as their significance, 95% CI, are provided in Table 2. Higher levels of alanine and sperm viability, more homogeneous cytoplasm, and lower levels of E2 were favorable for IVF FR. Among them, follicular alanine was dedicated the most to the IVF FR with its high odds ratio (OR).

Similarly, tubal infertility couples that participated in IVF ($n = 68$) were split into two groups based on their TQER outcomes. Those with TQER lower than 70% were included in one group ($n = 29$, entered as "0"), and those with TQER higher than 70% were placed into another group ($n = 39$, entered as "1"). Follicular glutamine, arrangement of oocytes, radial crown, sperm volume, and viability were recruited in the final model generating 80.9% overall

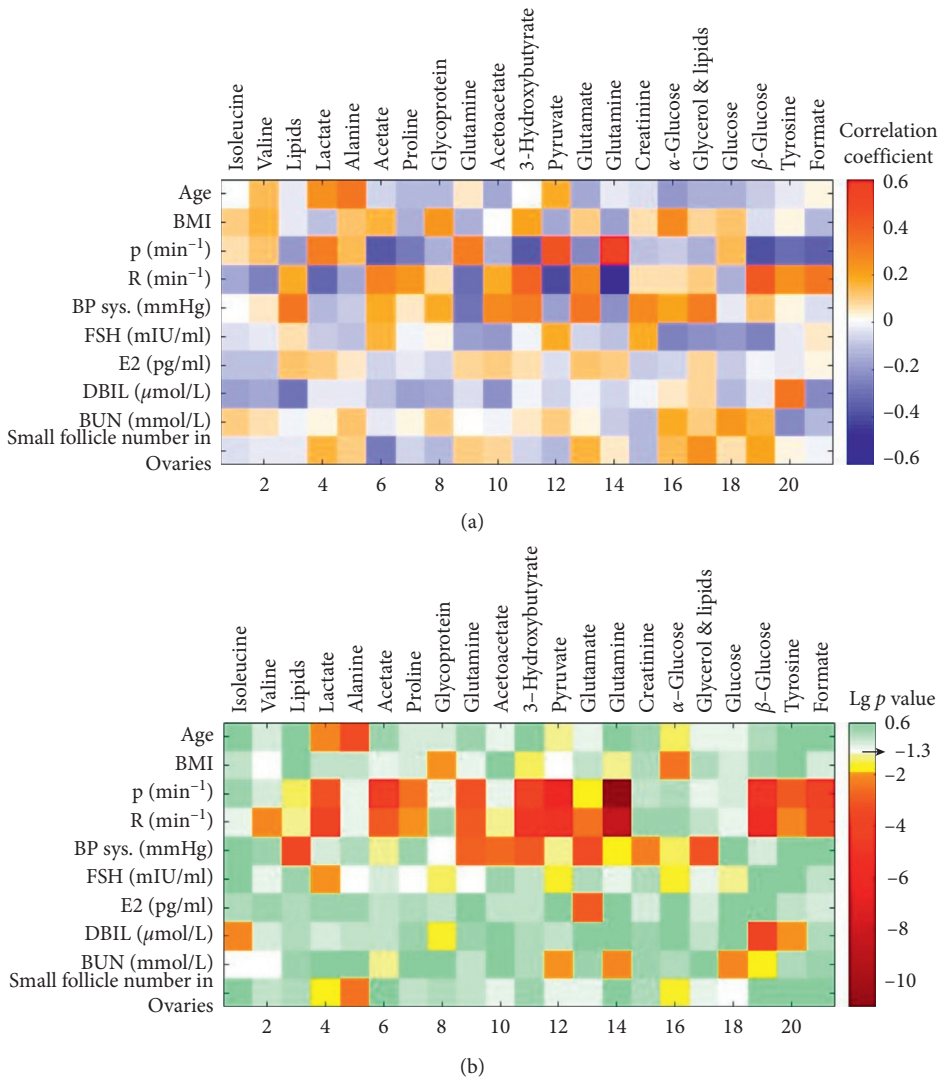

Figure 1: (a) Pearson's correlation coefficients between clinical parameters and NMR detected metabolites. The stained color represents correlation coefficients ranging from −0.6 to 0.6. (b) Pearson's correlation p values of each paired data. The visual color represents log 10 (p value) ranging from −11 to 0.6. The pairs with p value of 0.05 and 0.01 were dyed light yellow and orange, respectively.

predicted accuracy. From the model parameters and sensitivity analysis, the glutamine level represented the greatest impact on IVF TQER. The model AUROC achieved 0.83 (95% CI: 0.73~0.93, Figure 4). However, due to the limited ICSI sample population of this group, we were not able to construct any models for ICSI TQER.

3.7. Factors Affecting IVF/ICSI Outcomes for the Dysspermia Group. From the correlation results, it was not surprising to discover that sperm's overall progressive (PR) and nonprogressive (NP) motility composition was positively affecting IVF FR in the dysspermia group (Figure 3, Table S6). That is because, in IVF, sperm and oocytes are still self-combining, depending on the sperm's activity capacity. On the other hand, this activity was not as important as in the ICSI fertilization step. However, the harmfulness of sperm malformation on ICSI FR was highlighted. Moreover, women's endocrine and physical factors, including FSH, BMI, and systolic and diastolic blood pressure, also affected ICSI FR. A series of metabolites displayed influence on the fertilization step for dysspermia couples (Table S7). It demonstrated that glycoprotein, lysine, isoleucine, creatinine, and 3-hydroxybutyrate had had negative effects on FR in both IVF and ICSI methods. Proline affected IVF FR, while leucine, valine, and glutamine had an influence on ICSI FR.

In the dysspermia group, men's BMI and BP sys. exhibited importance in ICSI and IVF 2PN-FR, respectively. Women's FSH, ORN, and SFN displayed a negative correlation with 2PNFR again. During this step, 3-hydroxybutyrate, lysine, and arginine were negatively connected with both the IVF and ICSI results. The functions of creatinine, lactate, and leucine were more apparent with the IVF 2PN-FR outcome. In addition, males' blood pressure and follicular leucine were closely linked to the embryo's quality.

All factors impacting stepwise IVF/ICSI outcomes were summarized in Figures 3 and S1 with more details.

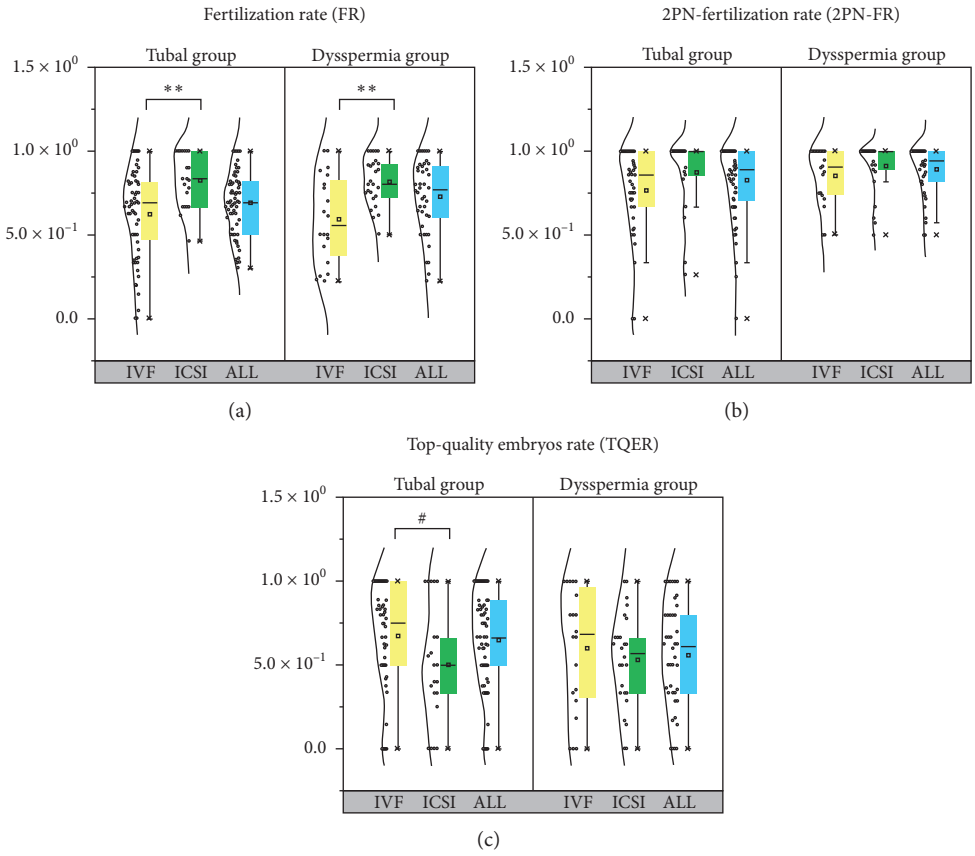

Figure 2: Group box plot for FR, 2PN-FR, and TQER. $^{**}p$ value <0.01; $^{\#}p$ value = 0.053.

3.8. Logistic Regression Predicting IVF/ICSI Outcomes for the Dysspermia Group. In the dysspermia IVF group ($n = 20$), 12 couples obtained satisfactory IVF fertilization rates (FR ≥ 70%, $n = 12$, entered as "1"). The remaining couples received an FR of less than 70% ($n = 8$, entered as "0"). The forward stepwise algorithm only left the follicular glycoprotein in the logistic regression, but with 75% prediction accuracy and an AUROC of 0.84 (CI: 0.67~1.00, Table 3). Half of these 20 couples received more than 70% IVF TQER (TQER≥70%, $n = 10$, entered as "1"). Both follicular lipids and female alanine aminotransferase (ALT) in blood appeared discriminative for IVF TQER, producing a high model prediction accuracy of 90.0% and an AUROC of 0.96 (CI: 0.87~1.00, Figure 4). Among the retained variables, follicular lipids showed greater importance.

The patients who underwent ICSI were further divided into two halves, using a TQER of 60% as the cutoff. Logistic regression retained the only variable, follicular acetic acid, giving the model prediction accuracy of 76.9% and AUROC of 0.81 (CI: 0.64~0.98, Figure 4).

3.9. Importance of Follicular Metabolism in IVF/ICSI Outcomes. The metabolites that played roles in ART outcomes in the tubal and dysspermia groups were submitted to MetPA for pathway investigation. Based on their pathway impact and −log (p) from enrichment analysis, the important metabolisms involved in IVF/ICSI outcomes in the two groups were provided in Table S8 separately. The overall pathway map combining all related metabolites is presented in Figure 5. It mainly included aminoacyl-tRNA biosynthesis, arginine, and proline metabolism, as well as alanine, aspartate, and glutamate metabolism.

4. Discussion

In the present study, we focused on the two most common infertilities caused by the women's problem (fallopian tubal blockage) and the men's problem (sperm defects), respectively. We observed the influence of clinical indicators on follicular fluid metabolites in females and investigated the effects of follicular metabolites and couples' clinical parameters on IVF/ICSI stepwise outcomes. Our aim was to explore a feasible and objective method for IVF FR estimation and TQER evaluation, to assist the clinical staff to decide whether to carry out IVF or switch to ICSI directly. In the meantime, the comprehensive study revealed potential factors that influenced the IVF/ICSI outcomes, which could provide information for follow-up clinical intervention to improve the success rate.

From our data, the IVF TQER overwhelmed ICSI TQER, although ICSI had a much better FR due to its advanced insemination method. It suggested that although ICSI can improve fertilization, zygotic development depends more on the quality of both oocytes and sperm. In the tubal group (representing female infertility), the sperm's normal

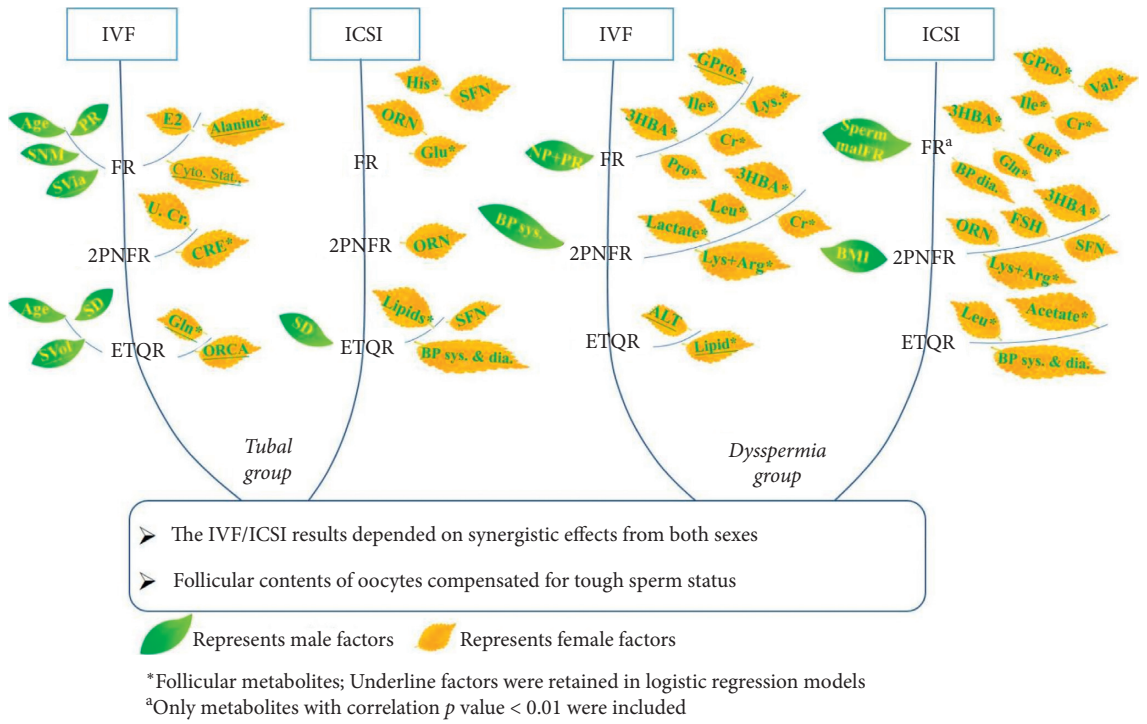

Figure 3: Summary of factors impacting stepwise IVF/ICSI outcomes by group. PR, progressive; NP, nonprogressive; Sperm NM, sperm normal morphology (%); Sperm Via., sperm viability; Sperm Vol., sperm volume; Sperm Den., sperm density; Sperm malFR, Sperm malformation rate; BP sys., systolic blood pressure; BP dia., diastolic blood pressure; FSH, follicle-stimulating hormone; E2, estradiol; Cyto. Stat., cytoplasmic states; U Cr., urinary creatinine; Gln, glutamine; Oocyte arr., oocyte radial crown arrangement (1. compact; 2. slightly dilated; 3. radial); SFN, small follicle number in ovaries; ORN, oocyte retrieved number; ALT, alanine aminotransferase; Ala, alanine; His, Histidine; Glu, glucose; GPro, glycoprotein; Lys, lysine; Ile, Isoleucine; Leu, leucine; Cr, creatinine; CRE, creatine; 3HBA, 3-hydroxybutyric acid; Val, valine; Gln, glutamine; Pro, proline.

morphology and progressive motility exhibited importance only in the IVF fertilization process. Without the concern of sperm activity, the oocytes' parameter elevated more concerns in ICSI FR. However, sperm density was effective in the following TQER of both IVF and ICSI. In the dysspermia group (representing male infertility), both female physical factors and follicular metabolites had significant impacts on both FR and TQER, no matter which intervention approach was taken. It inferred that an index of follicular metabolites representing oocyte quality acted as a repairing role, compensating for the poor sperm quality, just like what it did in older male partners [15].

From our study, it is evident that clinical parameters, as well as their cross-influenced follicular metabolites, were closely associated with IVF/ICSI outcomes. Next, we will focus on these two aspects, respectively.

4.1. Clinical Parameters. BMI was positively associated with follicular glycoprotein in the current study, which was harmful in IVF fertilization of the dysspermia group as shown in the logistic regression model. Meanwhile, male BMI was also negatively associated with ART outcomes. Quite a few studies tried to correlate women's BMI with ART outcomes, but the results were conflicting because of reproductive intricacies [16]. Nevertheless, as the researchers pointed out, the BMI varied, and serum metabolites were reflected in follicular fluid, especially in protein concentration [17]. On the other hand, paternal obesity resulted in reduced reproductive potential, even with normality on conventional semen parameters [18], further supporting our findings.

Age is another concerning issue in the reproduction field. In our sample cohort, male age played a positive role in IVF FR, while it was harmful to high-quality embryo formation. A similar negative effect of male age was discovered in former studies where increased paternal age may induce adverse effects on sperm parameters [19], blastocyst embryo formation [20, 21], and IVF/ICSI success rate [19, 22]. The result could be caused by unexpected DNA fragmentation [23], chromosomal aneuploidies, and epigenetic mutation [22]. In females, age was negatively correlated with follicular glucose but positively associated with lactate, pyruvate, and alanine. This trend was also found in women with advanced age or impaired ovarian reserve, which accounts for the elevated glycolysis in granulosa cells [11]. In addition, we also observed that a negative correlation existed between female age and ORN. It suggested that the ovary stimulation protocol should be not only adjusted to the concern of disease but also according to female age and ovarian reserve capacity.

The ORN and SFN negatively correlated with not only ICSI and FR but also 2PNFR and TQER, respectively. The

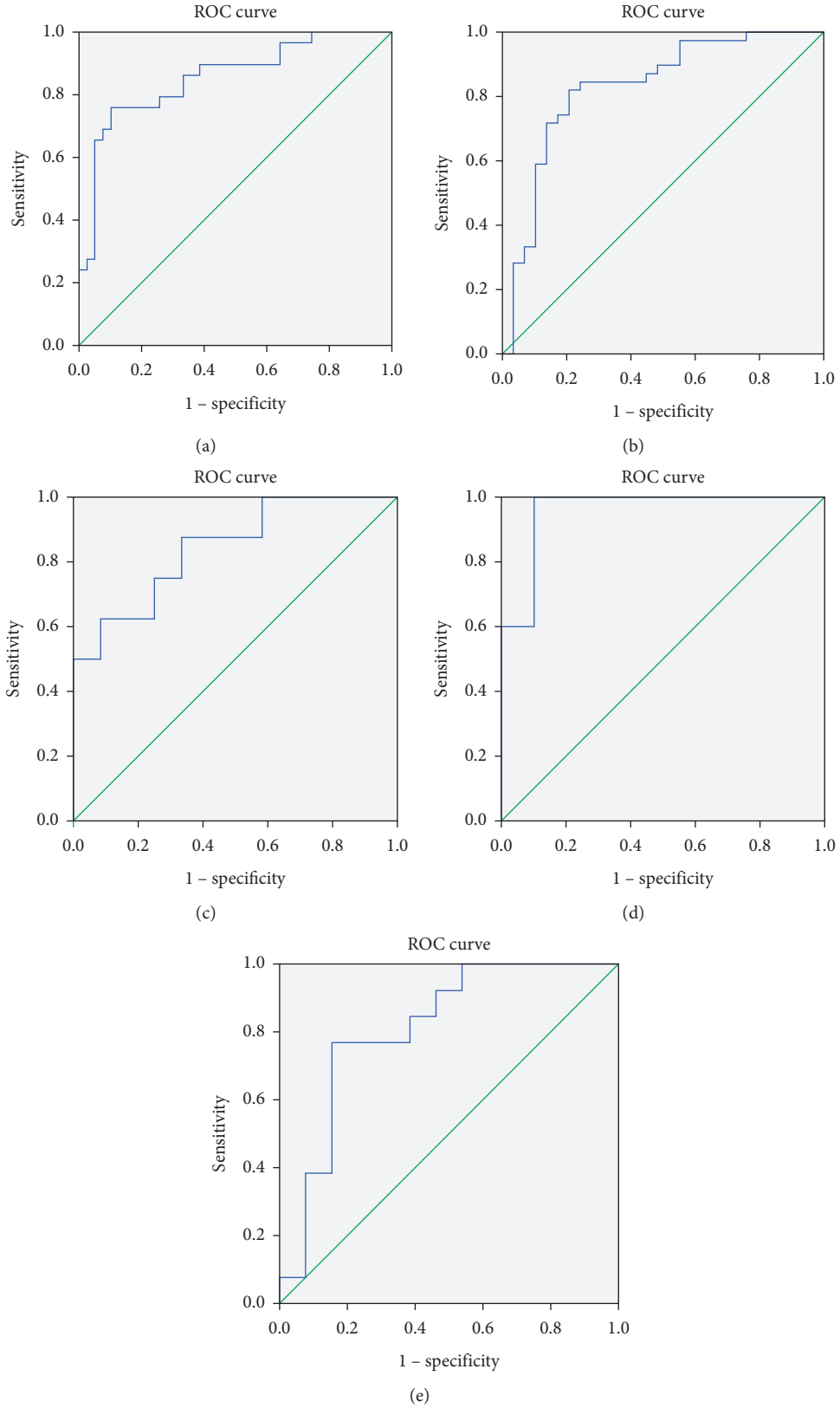

Figure 4: Plotted receiver operating characteristic (ROC) space from logistic regression prediction for (a) tubal group IVF FR, (b) tubal group IVF TQER, (c) dysspermia group IVF FR, (d) dysspermia group IVF TQER, and (e) dysspermia group ICSI TQER results.

Table 2: Logistic regression model for tubal group.

	Coefficient details					Model parameter				
	B	Sig.	Exp (B)	95% CI for exp (B) lower bound	95% CI for exp (B) upper bound	Model -2 log-likelihood	Overall predicted percentage	ROC area	95% CI lower Bound	95% CI upper Bound
IVF fertilization rate for tubal group										
Alanine	$1.92E+02$	0.013	$1.83E+83$	$4.76E+17$	$7.01E+148$					
E2 pg ml^{-1}	$-5.37E-02$	0.016	$9.48E-01$	$9.07E-01$	$9.90E-01$					
Cytoplasmic states (1. granular; 2. homogeneous)	$2.64E+00$	0.001	$1.41E+01$	$2.94E+00$	$6.72E+01$	60.60[a]	83.82	0.85	0.76	0.95
Sperm viability	$1.16E-01$	0.002	$1.12E+00$	$1.04E+00$	$1.21E+00$					
IVF top-quality embryos rate for tubal group										
Glutamine	$2.87E+02$	0.012	$2.72E+124$	$6.56E+27$	$1.13E+221$					
Sperm volume (mL)	$1.22E+00$	0.003	$3.38E+00$	$1.52E+00$	$7.52E+00$					
Sperm viability	$6.98E-02$	0.022	$1.07E+00$	$1.01E+00$	$1.14E+00$	69.92[a]	80.88	0.83	0.73	0.93
Oocyte radial crown arrangement (1. compact; 2. slightly dilated; 3. radial)	$1.62E+00$	0.005	$5.03E+00$	$1.62E+00$	$1.56E+01$					

[a]Estimation terminated at iteration number 6 because parameter estimates changed by less than 0.001.

Table 3: Logistic regression models for the dysspermia group.

	Coefficient details					Model parameter				
	B	Sig.	Exp (B)	95% CI for exp (B) lower Bound	95% CI for exp (B) upper Bound	Model -2 log-likelihood	Overall predicted percentage	ROC area	95% CI lower Bound	95% CI upper Bound
IVF fertilization rate for dysspermia group										
Glycoprotein	$-3.43E+02$	0.035	$8.57E-150$	$2.27E-288$	$3.23E-11$	17.96[a]	75.00	0.84	0.67	1.00
IVF top-quality embryos rate for dysspermia group										
Lipid	$6.00E+02$	0.028	$5.02E+260$	$4.37E+28$		10.97[b]	90.00	0.96	0.87	1.00
ALT	$-4.69E-01$	0.040	$6.26E-01$	$4.00E-01$	$9.80E-01$					
ICSI top-quality embryos rate for dysspermia group										
Acetate	$5.73E+02$	0.025	$8.06E+248$	$3.80E+31$		29.09[c]	76.92	0.81	0.64	0.98

[a]Estimation terminated at iteration number 6 because parameter estimates changed by less than 0.001. [b]Estimation terminated at iteration number 9 because parameter estimates changed by less than 0.001. [c]Estimation terminated at iteration number 5 because parameter estimates changed by less than 0.001.

finding was coincident with a considerable retrospective study ($n = 2\,578$) where transferable embryos declined with ORN [24]. In addition, from our results, as total SFN increased, several follicular metabolites altered, including lactate, alanine, and glucose, which were essential in IVF/ICSI outcomes. It suggested that the increased number of these two factors might result in decreased quality of oocytes. In both the tubal and dysspermia groups, smaller ORN and SFN were expected to achieve a more satisfactory outcome.

Female hormone levels impacted IVF/ICSI outcomes. In the tubal group, without other endocrine diseases, a lower E2 level would result in elevated FR, given by the regression model. Similarly, a lower E2 level was found in the higher clinical pregnancy rate group [25], and the higher E2 exposure group resulted in an increased rate of preterm delivery and a lower birth rate [26]. Besides, from the correlation analysis, increased FSH was favorable for both fertilization and 2PN FR. It was also illustrated that a higher FSH level within an appropriate range was plausible for good embryos' quality [27].

BP sys. and BP dia. represent the cardiovascular regulatory function. In both groups, negative correlations were found repeatedly between BPs and IVF/ICSI stepwise outcomes regardless of gender. On the other hand, we also observed that BP sys. had an impact on a series of follicular metabolites. It has been reported that men with higher BP had a lower quality of semen [28]. There is also great evidence supporting the association between high BP and declined renal function [29]. Some reports indicated that BP data was associated with end-organ health [30]. Furthermore, pulse rate and respiration rate, which reflect cardiac function, revealed their prominent role in multiple follicular metabolite fluctuations.

Female renal and liver function in terms of BUN and DBIL caused fluctuation of follicular composition. It was

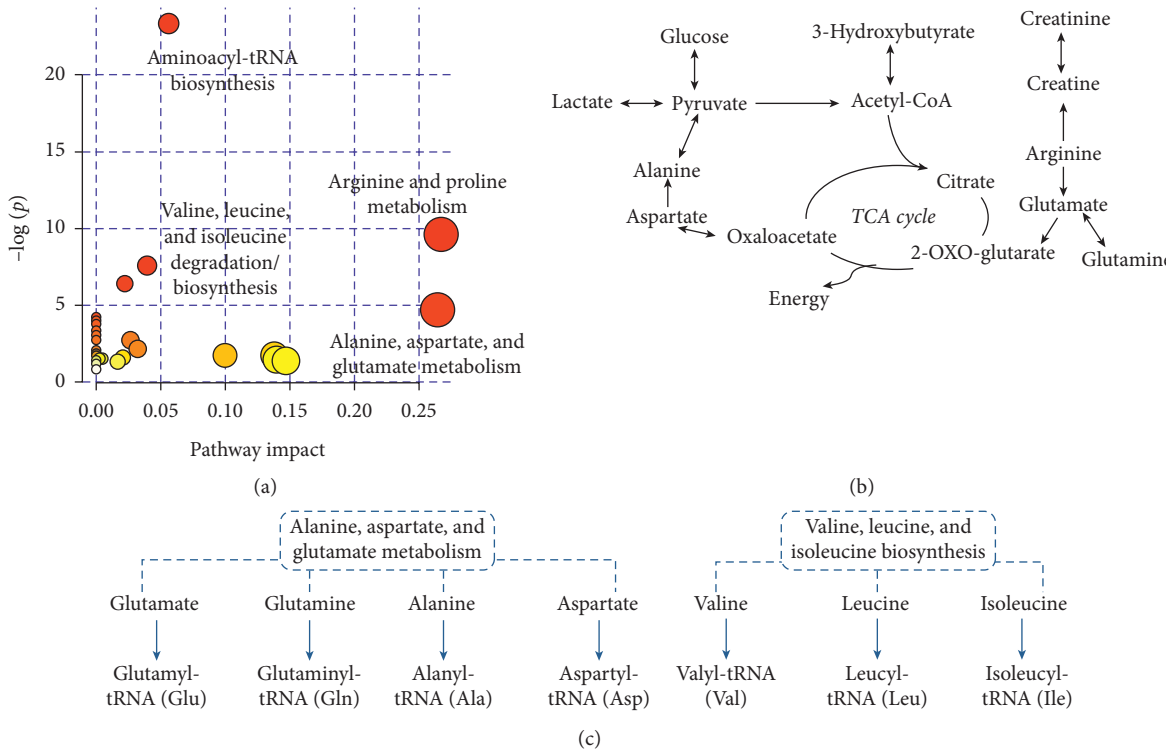

Figure 5: (a) Overview of fluctuated metabolisms in follicular fluid suggested by MetPA. (b) Metabolic pathway diagram showing perturbations in energy production metabolism involved essential follicular metabolites that were related to ART outcomes. (c) Metabolic pathway diagram showing perturbations in aminoacyl-tRNA biosynthesis involved follicular metabolites that were related to ART outcomes.

echoed by female urinary creatinine, another indicator of renal function, which possessed negative correlations with IVF 2PNFR in the tubal group. It was also consistent with follicular creatinine concentration, which correlated negatively with both FR and 2PNFR in the dysspermia group.

4.2. Follicular Metabolites. Follicular metabolite variation was attributed to the combinational effects of all physical factors. A series of metabolites eventually influenced IVF/ICSI outcomes, especially those showing discriminant properties in logistic regression models. In the tubal group, enriched follicular alanine and glutamine were favored for IVF, FR, and TQER, respectively. It is consistent with the finding that alanine supplementation was beneficial for embryonic developmental competence through increasing mRNA expression in pig oocytes [31]. It has been observed that follicular glutamine levels were positively correlated with FR in PCOS patients [32]. In addition, the lack of follicular glutamine resulted in low reproductive performance sows [33]. In the dysspermia group, glycoprotein was reserved as the only predictor in the IVF FR regression model, with a large negative coefficient. The increased glycoprotein could be an indicator of various inflammatory disorders, such as PCOS [32, 34], diabetes, cardiovascular disease, and infection [35]. For IVF TQER, elevated follicular lipid was expected. Although, in the NMR technique, we could not specifically identify the exact lipid, our previous MS data suggested that most of the glycerophospholipids and sphingolipids were lower in PCOS and were expected to increase for better IVF/ICSI outcomes [9]. A positive coefficient of acetic acid in the ICSI TQER model implied the demand of the acetyl group, which could provide the center for carbohydrates and fat metabolism when binding to coenzyme A. The usage of acetic acid could also perform as an alternative energy path just like in T cells [36].

The involved metabolic pathways showed different importance in IVF/ICSI outcomes in different groups. In the tubal group, glycolysis, along with pyruvate metabolism, was a determinant in IVF/ICSI outcomes. Generally, the energy supporting the oocyte to undergo fertilization, cleavage, and proliferation was provided via glycolysis, in which glucose was converted to pyruvate and entered a tricarboxylic acid (TCA) cycle [37]. Consumption of lactate and amino acids was helpful in the process. In the dysspermia group, similar to what we found in our previous study [32], amino acids such as leucine and isoleucine were negatively correlated with IVF/ICSI outcomes. It was consistent with a study in which repeated IVF failure found an elevation of 11 amino acids in follicular fluids [8]. The predominant role of amino acids highlighted aminoacyl-tRNA biosynthesis, in which amino acids were delivered to the ribosome and incorporated into the polypeptide chain for protein synthesis. Investigated in different species, the deficiency of amino acids has been related to chromosome aneuploidy [38], DNA damage [39], etc., and ultimately revealed its relationship

with oocyte quality. The adequate consumption of amino acids in protein synthesis helps oocytes prepare for their high-quality status to get ready for following tough fertilization and development processes because of the sperm defects. On the other hand, the energy production related to arginine and proline metabolism, as well as alanine, aspartate, and glutamate metabolism, was also provoked to provide support.

The most unfortunate thing about our study is that we did not have the opportunity to trace further the molecular biology of sperm other than the ordinary clinical sperm parameters. Combination analysis of paternal DNA fragmentation, chromosomal aneuploidies, and/or epigenetic mutations would be of expectation in future work. On the other hand, the relatively small number of the dysspermia sample group was the major limitation. Moreover, the logistic regression model in the current study pointed out the possibility of using follicular metabolites and couples' physical parameters to predict IVF/ICSI outcomes objectively. More sample cohorts are expected to improve and verify the model for clinical application.

5. Conclusion

The present study explored the relationship among couples' clinical factors, follicular metabolites, and IVF/ICSI stepwise outcomes. Our findings indicated that in the case of fallopian tubal blockage as a cause of female infertility, the factors of both males (sperm volume and viability, etc.) and females (follicular alanine, glutamine, and cytoplasmic status, etc.) need to be evaluated, while in the case of male infertility caused by sperm problems, oocyte quality in terms of the follicular metabolites index (especially glycoprotein, lipids, and acetic acid) as a compensatory role should be considered. In the treatment of infertility, synergistic effects from both sexes cannot be ignored. In addition, ovary stimulation protocols should be adjusted with the concern of both the disease and female age to achieve proper ORN. Despite the limited sample size, the study provided us with a feasible option for pre-IVF/ICSI evaluation from a molecular point of view and constitutes a framework for future intervention improvement by considering both female and male factors.

Abbreviations

Ala: Alanine
ALT: Alanine aminotransferase
Arg: Arginine
ART: Assisted reproductive technology
AUROC: Area under ROC
Ave: Average
BMI: Body mass index
BP sys.: Systolic blood pressure
BP dia.: Diastolic blood pressure
CI: Confidence interval
CPMG: Carr-Purcell-Meiboom-Gill
Cr: Creatinine
CRE: Creatine
Cyto. Stat.: Cytoplasmic states
E2: Estradiol
ECM: Embryo culture medium
FR: Fertilization rate
FSH: Follicle-stimulating hormone
Gln: Glutamine
Glu: Glucose
GPro: Glycoprotein
HCG: Human chorionic gonadotropin
His: Histidine
IRB: Institutional review boards
ICSI: Intracytoplasmic sperm injection
Ile: Isoleucine
IVF: In vitro fertilization
Leu: Leucine
LH: Luteinizing hormone
Lys: Lysine
PR: Progressive
Pro: Proline
NP: Nonprogressive
OCRA: Oocyte radial crown arrangement (1. compact; 2. slightly dilated; 3. radial)
ORN: Oocyte retrieved number
ROC: Receiver operating characteristic
STD: Standard deviation
SFN: Small follicle number
SFN: Small follicle number
Sperm Den.: Sperm density
Sperm malFR: Sperm malformation rate
Sperm NM: Sperm normal morphology (%)
Sperm Via.: Sperm viability
Sperm Vol.: Sperm volume
TQER: Top-quality embryos rate
U. Cr.: Urinary creatinine
Val: Valine
2PN: Two pronuclei
2PNFR: 2PN fertilization rate
3HBA: 3-Hydroxybutyric acid.

Data Availability

The data underlying this article are available upon reasonable request to the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Bing Qu and Yunhe Xiong contributed equally to this work.

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Supplementary Materials

Figure S1: summary of factors impacting stepwise IVF/ICSI outcomes by group. PR, progressive; NP, nonprogressive; Sperm NM, sperm normal morphology (%); Sperm Via., sperm viability; Sperm Vol., sperm volume; Sperm Den., sperm density; Sperm malFR, Sperm malformation rate; BP sys., systolic blood pressure; BP dia., diastolic blood pressure; FSH, follicle-stimulating hormone; E2, estradiol; Cyto. Stat., cytoplasmic states; U. Cr., urinary creatinine; Gln, glutamine; Oocyte arr., oocyte radial crown arrangement (1.compact; 2. slightly dilated; 3. radial); SFN, small follicle number in ovaries; ORN, oocyte retrieved number; ALT, alanine aminotransferase; Ala, alanine; His, Histidine; Glu, glucose; GPro, glycoprotein; Lys, lysine; Ile, Isoleucine; Leu, leucine; Cr, creatinine; CRE, creatine; 3HBA, 3-Hydroxybutyric acid; Val, valine; Gln, glutamine; Pro, proline. Table S1: embryo quality evaluation strategy on day 3. Table S2: integrated 67 metabolite regions. Table S3: stepwise ART outcomes by group. Table S4: correlation coefficients between clinical parameters and IVF/ICSI outcomes in the tubal group. Table S5: correlation coefficients between follicular metabolites and IVF/ICSI outcomes in the tubal group. Table S6: correlation coefficients between clinical parameters and ART outcomes in dysspermia group. Table S7: correlation coefficients between follicular metabolites and ART outcomes in dysspermia group. Table S8: summary of metabolic pathway enrichment analysis. (*Supplementary Materials*)

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Review Article

The Treatment with Complementary and Alternative Traditional Chinese Medicine for Menstrual Disorders with Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is a frequent gynecological female endocrinopathy, characterized by chronic anovulation, hyperandrogenism, and insulin resistance (IR). Menstrual disorders are one of the main clinical manifestations of PCOS. Other symptoms include hirsutism and/acne. At present, the treatment of PCOS with irregular menstruation is mainly based on oral contraceptives, but there are some side effects and adverse reactions. In recent years, more and more attention has been paid to the complementary and alternative medicine (CAM), which has been widely used in clinical practice. Modern Western medicine is called "conventional medicine" or "orthodox medicine," and the complementary and alternative medicine is called "unconventional medicine" or "unorthodox medicine." CAM includes traditional medicine and folk therapy around the world. Around 65–80% of world health management business is classified into traditional medicine by the World Health Organization, which is used as alternative medicine in Western countries. In our country, Chinese medicine, acupuncture, and other therapies are commonly used due to their significant efficacy and higher safety. Therefore, this review aims to summarize and evaluate the mechanisms and the effect of current complementary replacement therapy in the treatment of menstrual disorders caused by PCOS, so as to provide guidance for the following basic and clinical research.

1. Introduction

PCOS is a common gynecologic endocrine disease, characterized by high androgen, persistent anovulation, and polycystic ovary changes. The prevalence of PCOS in women of childbearing age ranges from 6% to 21% [1],; however, the cause of this disease is still unclear [2, 3]. As one of the main clinical symptoms of PCOS, menstrual disorders mainly include premenstrual period, post-menstrual period, menorrhagia, less menstrual flow, and other types, which afflict women of all ages. The study by Rajiwade et al. [4] found that adolescent girls are easily affected by anxiety and other factors due to the rapid transformation of their physical development, which leads to endocrine and metabolic disorders, and finally menstrual abnormalities. In addition, an article written by Rostami et al. [5] also summarized and proposed that women have irregular menstruation due to the dysfunction of the ovario-thyroid axis. The menstrual disorder caused by PCOS are mainly caused by the complex endocrine characteristics of PCOS, high blood androgen level, elevated luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio, and excessive insulin, which will affect ovulation of the ovary and lead to menstrual disorders. At present, the treatment for menstrual disorders caused by PCOS is mainly based on drug therapies. The first-line treatment drugs are oral contraceptives; but, these may have adverse effects on glucose tolerance, fertility, and so on [6–8]. At the same time, experimental have data confirmed that long-term usage of contraceptives has a close relationship with hypertension, venous thrombosis, breast

cancer, and other cancers [9–12]. Therefore, it is particularly important to select a complementary alternative therapy, which has high safety and more selectivity, and few reports of adverse reactions [13].

Nowadays, CAM is getting more and more attention from all over the world, which also plays a great role in the prevention and treatment of diseases. The rapid development of CAM techniques in TCM diagnosis and treatment has provided more options for complementary and alternative treatments. When facing illness, not only the Asian people will seek help from CAM including TCM but also a large portion of Western people will pick it as one of their choices [14]. The paper summarized and evaluated the mechanisms and effects of current TCM complementary and alternative therapies, such as monomers, compounds, acupuncture, auricular acupoints, massage, Qigong, Tai Chi, cognitive behavioral therapy, and healthy lifestyle, in the treatment of menstrual disorders caused by PCOS, so as to provide guidance for subsequent basic and clinical research.

2. The Treatment with Traditional Chinese Medicine in PCOS with Menstrual Disorders

TCM, as a major part of CAM, plays an irreplaceable role in the prevention and treatment of diseases whose existence is irreplaceable and unrepeatable. Recently, with the culture of TCM spreading all over the world, it is being widely known and applied in practice. Moreover, it also exerts its unique advantages in the treatment of PCOS. According to relevant data, a survey of 493 Australian women diagnosed with PCOS found that more than 70% of these women chose to use CAM [15]. Generally speaking, TCM is divided into the monomer of Chinese medicine and compound Chinese medicine. For a long time, TCM has been used to treat gynecological diseases and its application is gradually increasing. The following paragraphs will describe and analyze the treatment of TCM in PCOS with menstrual disorders according to the monomer and compound of Chinese medicine.

2.1. The Monomer of Chinese Medicine

2.1.1. Cryptotanshinone. Cryptotanshinone (CRY) is an effective monomer extracted from the stem root of Salvia miltiorrhiza [16]. TCM believes that *Salvia miltiorrhiza* has the function of a blood-activating, menstruation-regulating, stasis-dispelling analgesic, cooling the blood, eliminating the carbuncle, and acting as an anxiety-reliving tranquilizer. In modern medicine, Salvia miltiorrhiza, regarded as a kind of precious Chinese herbal medicine with the function of protecting heart and brain vessels, is widely used in treating cardiovascular diseases in Asian countries, including coronary heart disease, myocardial infarction, angina pectoris, and atherosclerosis because it is safe [17]. In addition, it can also improve the condition of acne, lupus erythematosus, and melanoma [18–20]. According to the recent studies, specialists have found that Salvia miltiorrhiza also has anticancer activity, which can be used to treat cancers like lung cancer [21–24]. As one of the most important extractions, CRY plays a significant role in the prevention and treatment of diseases like PCOS, which cannot be underestimated. With regard to treating PCOS, CRY can improve the statement of hyperandrogen in PCOS patients through a variety of methods. Clinical trials have confirmed that CRY has a curative effect on PCOS patients. In Shen et al.'s study [25], 100 patients were randomly assigned to tanshinone or placebo group, with 50 patients in each group. Tanshinone or placebo capsules were given orally for 12 weeks, and the main outcome parameters, such as plasma testosterone (T), human chorionic gonadotropin-induced androgen response, IR, reproductive hormone, fasting blood lipid, oral glucose tolerance test, quality of life, and side effects were recorded and compared. The results showed that all indicators were significantly improved after taking CRY compared with the placebo group.

In the animal experiment conducted by Yu et al. [26], PCOS model rats were induced by subcutaneous injection of dehydroepiandrosterone (DHEA), observing the weight and ovarian morphology of rats after the CRY interventions and using the RIA method to detect serum biochemical indexes. The results showed that CRY could significantly recover the estrus of PCOS rats, and the serum biochemical indexes were also improved. It was proved that CRY could rebalance the reproductive and metabolic disorders of PCOS rats by regulating the expression of CYP 17 and AR, so as to restore the menstrual cycle. According to an experiment conducted by Yang et al. [27], PCOS rats model was established by injecting human chorionic gonadotropin and insulin daily for 22 days. Compared with the control group, the weight of PCOS rats, ovarian weight, and LH were significantly increased. It was proved that CRY could protect against PCOS-induced damage of ovarian tissue, possibly through a regulatory pathway involving HMGB1, TLR4, and NF-κB. At the same time, it can also recover the regular menstruation by protecting the functions of the ovary from being hampered. In addition to the above mechanism, the experiment conducted by Ye et al. [28], granulosa cells (PGCs) were isolated from porcine ovary to culture and conduct experiments. The results showed that CRY could regulate androgen synthesis of porcine granulosa cells through ERK/c-fos/CYP 17 pathway. All the basic experiments mentioned above proved that CRY has a therapeutic effect on the hyperandrogenesis in PCOS patients.

2.1.2. Berberine. Berberine (BBR) is an alkaloid separated from Chinese medicinal Coptis chinensis. It is the main active component of Coptis chinensis. TCM holds the thought that Coptis chinensis has functions of reducing body heat, drying dampness, purging fire, and detoxification. Owing to the antibacterial and other functions of BBR, according to the modern pharmacological research, it is widely used in TCM for anti-infection, hypercholesterolemia, type 2 diabetes, and other diseases [29]. At the same time, BBR also plays a significant role in improving metabolism. Besides, BBR can also be used to treat IR in patients with PCOS. Studies have shown that there appeared no such

serious adverse reactions but gastrointestinal reactions [30] compared with the common treatment of Western medicine. According to the related literature, BBR treating women with PCOS menstrual disorder is concerned with the improvement of IR [31–35]. Experiments showed that BBR plays the same role as metformin in improving IR [31, 32], which means BBR can improve obesity by improving insulin level in vivo, thereby regulating menstruation [29]. In the experiment of Orio et al. [33], a total of 100 people, that is, 50 obese PCOS patients were selected as the experimental group, and 50 menopausal healthy women who were matched with age and weight were selected as the control group. BBR was given to the experimental group for six months. The results showed that BBR could significantly increase the menstrual frequency; at the same time, the increasing of the SHBG and total T in PCOS patients also proved that BBR could improve the metabolism and hormone level of PCOS patients [34]. In addition, BBR is superior to metformin in improving IR and safety in PCOS patients. A randomized controlled trial was conducted by An et al. [35] with 150 PCOS infertile women. They were randomly divided into the BBR group, metformin group, and placebo group, with 50 women in each group. This experiment lasted for 12 weeks with results showing that the recovery of ovarian function was reflected by in vitro pregnancy, and indirectly reflected the improvement of irregular menstruation. Apart from that, improvement of BMI, TC, LDL, and other indicators in BBR group was better than that in the metformin group and placebo group. Moreover, the adverse events of gastrointestinal tract in BBR group were less than the metformin group, and the survival rate of in vitro pregnancy was higher than the other two groups. It was concluded that BBR was safer for premenopausal women at the same time giving the least stimulation to ovary. BBR could improve the metabolism of ovarian stimulation and the response to ovarian stimulation. Comparatively speaking, BBR can significantly improve PCOS menstrual disorder [34]; it is also effective in improving IR in PCOS patients and regulating menstrual cycle by improving metabolism.

Zhang et al. [36] showed that BBR could improve glucose transporter 4 (GLUT4) and reduce IR in PCOS rats through dual regulation of PI3K/Akt and MAPK. By measuring the protein levels of GLUT4, PI3K/Akt, and MAPK dual pathway, it can help to restore the HOMA-IR and ISI values to the normal level, and enhance the expression of GLUT4, so as to restore the normal ovarian function and menstrual regularity.

We find that the experiment conducted by Zhang [37] studied the pharmacologic effects of Diane-35, probiotics, and BBR on PCOS patients from the perspective of intestinal microflora. The authors had consulted other experiments showing that BBR could improve IR of PCOS patients during the experiment. In their experiment, they compared three treating methods including Diane-35 (estrogen and progesterone), probiotics, and BBR. They found that Diane-35 and probiotics could improve the reproductive and metabolic functions in the PCOS rats along with restoring the diversity of the gut microbiota leading to the improvement of the reproductive function in PCOS-like rats. As for the BBR administration to PCOS rats, the composition and diversity of intestinal microflora were significantly reduced, showing no improvement in terms of metabolism or reproduction Phenotypic in PCOS, which was contrary to the previous findings.

2.1.3. Cinnamon. As one of the Chinese herbal medicines, cinnamon has the functions of tonifying fire, helping Yang, dispersing cold and relieving pain, activating blood, and unblocking meridians. Moreover, as a common edible spice, it is considered to have anti-PCOS and anti-diabetes characteristics [38]. Cinnamon can prevent IR caused by high fructose diet, and early injection can prevent the development of IR [39]. Many clinical trials have also shown that cinnamon can improve metabolism by improving IR. For example, in the placebo-controlled, double-blind, randomized trial conducted by Kort and Lobo [40], 45 women were randomly selected to receive cinnamon replacement therapy. Among them, 26 women completed the 3-month study and the remaining 17 women completed the 6-month study. The results showed that the menstrual cycle of the patients taking cinnamomum cassia was more frequent than those taking placebo, and the improvement of the menstrual cycle was more obvious in women who took for 6 months, suggesting that cinnamon supplement can improve menstrual cycle. Hajimonfarednejad et al. [41] conducted a randomized, double-blind, placebo-controlled clinical trial on cinnamon powder in 66 patients with PCOS. It was also proved that cinnamomum cassia can significantly reduce fasting insulin and IR in PCOS patients. In the experiment [42] of Wang et al., 15 patients with polycystic bursa were randomly divided into oral cinnamon and placebo group for 8 weeks. After treatment, compared with the baseline insulin sensitivity index of fasting and 2-hour oral glucose tolerance test, IR in cinnamon group was significantly reduced, while that in placebo group was not. It was found that cinnamomum cassia could enhance the effect of insulin by increasing the activity of phosphatidylinositol 3-kinase in insulin signaling pathway.

In the experiment of Qin et al. [38], the rats were divided into oral saline group and cinnamomum cassia group, comparing the glucose infusion rate after insulin 3 mU/kg was injected. With the increase in insulin injection volume proportion, the glucose infusion rate of cinnamomum cassia group was significantly raised. Compared with other groups, such as IR -β and IRS-1 tyrosine phosphorylation level and IRS-1/PI-3 kinase combined with oral cinnamon group, the results showed that cinnamon extraction can improve the effect of insulin by increasing glucose uptake in vivo. Furthermore, it can be concluded that cinnamomum cassia can improve IR and regulate the metabolic level of the body, so as to improve the menstrual disorder of PCOS patients. Referring to the relevant literature, in the basic experiment [43] conducted by Dou et al., 60 female mice were randomly divided into three groups for 20 days, with 20 mice in each group. Blank group: injected sesame oil and methylcellulose; model group: injected DHEA and methylcellulose to

establish PCOS mouse model; experimental group: injected equal amount of DHEA and cinnamon powder mixed with methylcellulose. The three groups were given insulin intraperitoneal injection 20 days later for glucose tolerance test. Body weight was observed at any time during the experiment, and reproductive and metabolic characteristics were evaluated. After testing the levels of T and serum insulin, the results showed that cinnamomum cassia could restore the cell cycle and ovarian morphology of PCOS mice induced by DHEA. In addition, cinnamomum cassia also inhibited the expression of IGF-1 and IGFBP-1 induced by DHEA. It is suggested that cinnamomum cassia can be used as a supplementary treatment to improve IR, restore ovarian function, and improve menstrual disorder in PCOS mice.

However, the placebo-controlled, double-blind, randomized trial conducted by Kort and Lobo [40] reported four nonserious adverse events, including headache, heartburn, menstrual cramps, nausea, and diarrhea. In this paper, [44] speculated that the occurrence of adverse reactions may be related to variable dose.

2.2. Compound Chinese Medicine

2.2.1. Cangfu Daotan Decoction. Cangfu Daotan decoction [45] is a TCM prescription widely used in patients with PCOS of phlegm and dampness type. Its original prescription is Atractylodis Rhizoma, Cyperi Rhizoma, Aurantii Fructus Immaturus, Citri Unshius Pericarpium, Poria Sclerotium, Arisaematis Rhizoma Preparata cum Bovis Fel, and Glycyrrhizae Radix et Rhizoma. It has the effect of removing the phlegm and dampness. There are many randomized controlled clinical trials for the treatment of PCOS patients with Cangfu Daotan decoction. Yang's randomized controlled clinical trial [46] showed that Cangfu Daotan decoction had a significant effect on late menstruation caused by hyperinsulinemia of kidney deficiency and phlegm dampness type; 58 patients who met the diagnostic criteria were randomly divided into two groups, 29 cases in each group. The treatment group was treated with Cangfu Daotan decoction. The prescription consists of Atractylodes lancea, Rhizoma Cyperi, Pericarpium Citri Reticulatae, Pinellia ternata, Poria cocos, dannanxing, Zhishi, jinneijin, silkworm excrement, raw hawthorn, Cuscuta chinensis, epimedium, Cistanche, antler tablets, Morinda officinalis, and the control group was treated with metformin. The total effective rate was 82.7% in the treatment group and 65.5% in the control group. In clinical application, Cangfu Daotan decoction is usually used in combination with conventional Western medicine for the treatment of PCOS. Huang's randomized controlled clinical trial [47] showed that Cangfu Daotan decoction combined with clomiphene has a good long-term effect in treating PCOS amenorrhea of phlegm dampness type. There were 68 cases of PCOS, 35 cases in the treatment group and 33 cases in the control group. The control group was given clomiphene, and the treatment group was treated with Cangfu Daotan decoction on the basis of the control group. The prescription is Atractylodes Rhizoma, Rhizoma Cyperi, Pinellia ternata, Poria cocos, tangerine peel, dannanxing, Fructus aurantii, ginger, and liquorice. The course of treatment in both groups was 6 menstrual cycles. The levels of LH, prolactin (PRL), and T in the two groups were significantly decreased, and the short-term effect of the treatment group was similar to that of the Western medicine control group. However, 3 months after the drug withdrawal, the hormone level of the control group returned to the level before treatment, the treatment group remained at the level after treatment, and the long-term total effective rate of the treatment group was significantly higher than that of the control group. Hua et al.'s randomized controlled clinical trials [48] showed that Cangfu Daotan decoction (Rhizoma Cyperi, Atractylodes lancea, Rhizoma Pinelliae, Acorus tatarinowii, Gleditsia sinensis, Pericarpium Citri Reticulatae, Poria cocos, Xianlingpi, Yam, Astragalus membranaceus, Angelica, and Salvia miltiorrhiza) combined with Dian-35 and metformin can reduce the menstrual volume, infertility, obesity, hairiness, and other TCM Syndromes of PCOS patients, improve the levels of sex hormone and blood lipid, and reduce insulin resistance index (HOMA-IR). Du's clinical trial [49] showed that Cangfu Daotan decoction (Atractylodes macrocephala, Rhizoma Cyperi, Poria cocos, Fructus aurantii, Pericarpium Citri Reticulatae, dannanxing, Rhizoma Pinelliae, Atractylodis Macrocephalae, coix seed, Chinese yam, prepared radix rehmanniae, dodder seed, antler gum, Angelica sinensis, Ligusticum chuanxiong, and Glycyrrhiza uralensis) combined with Diane-35 can improve TCM syndrome, basic sex hormone, menstrual cycle, menstrual volume, and polycystic ovary of PCOS.

Yi et al.'s experiment [50] showed that Cangfu Daotan decoction decreased the serum levels of TCHO, TG, LDL-c, LH, T, IL-1β, IL-6, and TNF-α and increased the levels of HDL-c, follicle-stimulating hormone (FSH), and estradiol (E2) in PCOS rats model. The mechanism of Cangfu Daotan Decoction in the treatment of PCOS may be related to its regulation of lipid metabolism, sex hormone secretion, inflammatory reaction, and induction of the expression of OATP2B1 and OATP3A1 in ovarian and uterine tissues. Xu et al.'s research [51] showed that 111 active components were extracted from 1433 components of Cangfu Daotan decoction, involving 118 protein targets. 736 genes were found to be closely related to PCOS, and 44 of them overlapped with Cangfu Daotan decoction. Pathway enrichment analysis identified the AGE-RAGE signaling pathway in diabetic complications, endocrine resistance, the IL-17 signaling pathway, the PRL signaling pathway, and the HIF-1 signaling pathway. In addition, PI3K-Akt, IR, Toll-like receptor, MAPK, and AGE-RAGE were related to the treatment of PCOS.

2.2.2. Others. Li et al.'s experiments [52] have shown that the long-term efficacy of TCM in the treatment of PCOS menstrual related symptoms is more stable and lasting than that of Western medicine. In the randomized controlled clinical trial of Li et al., 66 patients with PCOS were randomly divided into TCM group and Western medicine groups, with 33 cases in each group. TCM group was treated with modified Zigui Decoction (Prescription: Cornus officinalis, Fructus Ligustri Lucidi, Herba Ecliptae, dodder, radix rehmanniae, Radix

Paeoniae Alba, amethyst, and Xianlingpi) and the Western medicine group was treated with Diane-35. The normal rate of menstrual cycle in the TCM group was 57.58% after one menstrual cycle; 63.64% in the Western medicine group; 45.45% in the TCM group after six menstrual cycles; 21.21% in the Western medicine group; and the TCM group was better than that of the Western medicine group. After one menstrual cycle, the volume of ovaries in both groups decreased significantly; the number of follicles was significantly reduced, and the ratios of LH, T, and LH/FSH were lower than that before treatment. After 6 menstrual cycles, the volume of bilateral ovaries in the TCM group was still significantly reduced compared with before treatment, while the Western medicine group returned to the state before treatment; The number of follicles in the TCM group was still significantly reduced, and the Western medicine group was restored to the state before treatment; the hormone level in the TCM group still decreased significantly, and the Western medicine group returned to the level before treatment. The clinical trials conducted by Lai et al. [53] showed that the TCM with the prescription of Paeonia lactifloraPall, Bupleurum chinenseDC, Citrus reticulatataBlanco, Ligusticum chuanxiongHort, Angelica sinensis (Oliv.) Diels, Glycyrrhiza uralensisFisch, Lycium barbarumL, Cinnamomum cassiaPresl, Carthamus tinctoriusL, Prunus persica (L.) Batsch, Cuscuta chinensisLam, Cyperus rotundusL, Leonurus japonicusHoutt, Citrus aurantiumL can improve the menstrual regularity of PCOS patients, including from amenorrhea to hypomenorrhea or menorrhagia, from hypomenorrhea to menorrhagia, or pregnancy. In addition, the modified ferriman Gallwey score was significantly lower than that before treatment, and hirsutism was improved. The data of liver and kidney function and adverse events were basically normal. This prescription has the function of soothing liver and replenishing qi, tonifying the kidney yang, nourishing blood, and activating blood circulation. The randomized controlled clinical trial of Liu and Mao [54] showed that Danzhi Xiaoyao Pill (Radix Bupleuri, Angelica sinensis, Radix Paeoniae Alba, Atractylodes Macrocephalae, Poria cocos, Glycyrrhiza uralensis, cortex moutan, and Gardenia jasminoides) can improve the ovulation rate of PCOS patients with IR, and has better curative effect in improving symptoms such as irritability, chest tightness, hypochondria, premenstrual chest pain, bitter mouth, dry mouth, less menstruation, menstrual color, abnormal menstruation frequency, pulse, tongue, etc., and has obvious advantages over Western medicine alone. Tao et al.'s randomized controlled clinical trial [55] showed that modified Longdan Xiegan decoction (Gentian grass, Scutellaria baicalensis, Gardenia jasminoides, Alisma orientalis, plantain seed, Angelica sinensis, Shengdi, bupleurum, Cortex Moutan, and Prunella vulgaris) could significantly improve the symptoms of PCOS patients of stagnant fire in Gan channel type, menstrual disorder, acne, and basal body temperature, and the adverse reactions were lower than those in the Western medicine group. Hou et al.'s randomized controlled clinical trial [56] showed that Tiangui decoction (Anemarrhena asphodeloides, Ophiopogon japonicus, Polygonatum sibiricum, Angelica sinensis, Psoralea corylifolia, Rhizoma Polygoni cuspidatum, Verbena officinalis, Xianlingpi, Shengdi, and Taoren) can reduce the high insulin concentration of PCOS patients, induce ovulation, and resume menstrual cycle. The efficacy of Tiangui Capsule, a kind of TCM compound, in the treatment of PCOS was evaluated by a randomized controlled clinical trial by Kuek and compared with metformin and Diane-35 [57]. The effect of Tiangui Capsule on hyperandrogenemia was not as good as Diane-35, but better than metformin. Tiangui capsule is not as effective as metformin in the treatment of hyperinsulinemia, but better than Diane-35. It was concluded that Tiangui capsule can treat PCOS by regulating ovarian function and reducing the insulin level.

Zhao et al.'s experiment [58] showed that the therapeutic effect of Heqi San (*Curculigo orchioides* Gaertn, *Schisandra chinensis* (Turcz.) Baill, *Cynanchum otophyllum* C. K. Schneid, *Citrus medica* L. var. sarcodactylis Swingle, *Crataegus pinnatifida* Bunge, *Rhus chinensis* Mill, *Clinopodium megalanthum* (Diels) C. Y. Wu & Hsuan ex H. W. Li, *Cuscuta chinensis* Lam, *Poncirus trifoliata* (L.) Raf, *Hordeum vulgare* L, *Polygala tenuifolia* Willd, and *Epimedium davidii* Franch) on the rats model of PCOS includes altering serum hormone levels, healing ovary morphological lesions, and improving IR, which is mediated through the PI3K/Akt pathway. Azeemuddin's study [59] evaluated "DXB-2030," a polyherbal combination of Trigonella foenum-graecum, Aloe vera, Sphaeranthus indicus, Nardostachys jatamansi, and Symplocos racemosa extracts in an experimental model of testosterone propionate (TP), which induced PCOS in female rats. Results showed that "DXB-2030" reversed the TP-induced changes by increasing the GLUT4 expression and decreasing the body weight, T levels, AUC of glucose in Oral Glucose Tolerance Test (OGTT), and the cystic follicles of the ovaries. The effect of "DXB-2030" on the reproductive system of women with PCOS may include reversion of estrus cyclicity, reduction in ovary volume and size of the cyst, antiandrogenic effect, decreased T levels, and restoration of the histology of ovarian tissue. Bushen Huatan decoction [60] can improve the serum metabolites in patients with PCOS, and has a certain therapeutic effect on PCOS by reducing inflammatory reaction and oxidative stress. In addition, Wang et al.' s experiment [61] has shown that Siwu decoction may has the effect of promoting follicular development and establishing normal menstrual cycle in patients with PCOS.

The current problem is that the sample size of randomized controlled trials is too small. In addition, most randomized controlled trials use the method of observing the treatment effect for three or six months, which means that the observation time needs to be extended. Lack of placebo control may affect the analysis of drug efficacy. The lack of blinding may affect the predicted treatment outcomes, especially subjective outcomes, such as self-reported quality of life, psychology, and compliance. Therefore, randomized controlled trials (double-blind placebo-controlled) are needed to verify the efficacy of the drug.

For summarizing the randomized clinical trials, please refer to Table 1.

Table 1: The summary of randomized studies of Chinese medicinal treatment on PCOS menstrual disorders.

Study ID	Design	Sample size (human)	Interventions	Treatment duration	Outcomes	Limitation
26	RCT	100	Treatment arm: the tanshinone capsules Control arm: no intervention	12 weeks	Treatment arm: not mentioned Control arm: not mentioned	Small sample size
33	Controlled trial	100	Treatment arm: BBR Control arm: no intervention	6 months	Treatment arm: effective rate, 48.3 % (22 of 50) Control arm: no changes	Not mentioned blindness Not mentioned placebo
35	RCT	150	Treatment arm: BBR Control arm: metformin Placebo: no intervention	3 months	Treatment arm: effective rate, 48.6 % (18 of 37) Control arm: effective rate, 36.8 % (14 of 38) Placebo: effective rate, 20.6 % (7 of 37)	Small sample size
40	RCT	15	Treatment arm: cinnamon Placebo: no intervention	3–6 months	Treatment arm: not mentioned Control arm: not mentioned	Small sample size
41	RCT	66	Treatment arm: cinnamon Placebo: no intervention	12 weeks	Treatment arm: not mentioned Placebo: not mentioned	Small sample size
42	RCT	15	Treatment arm: cinnamon Placebo: no intervention	8 weeks	Treatment arm: not mentioned Placebo: not mentioned	Small sample size
46		58	Treatment arm: Cangfu Daotan decoction Control arm: metformin	6 months	Treatment arm: effective rate, 82.7% (24 of 29) Control arm: effective rate, 65.5% (19 of 29)	Not mentioned blindness Not mentioned placebo
47		68	Treatment arm: clomiphene and Cangfu Daotan decoction Control arm: clomiphene	6 months	Treatment arm: effective rate, 88.6 % (31 of 35) Control arm: effective rate, 84.8 % (28 of 33)	Not mentioned blindness Not mentioned placebo
49		60	Treatment arm: Cangfu daotan decoction and Diane-35 Control arm: no intervention	3 months	Treatment arm: effective rate, 81.6 % (49 of 60) Control arm: not mentioned	Not mentioned blindness Not mentioned placebo Not mentioned control group
52		66	Treatment arm: Zigui decoction Control arm: Diane-35	3 months	Treatment arm: effective rate, 45.45 % (15 of 33) Control arm: effective rate, 21.21 % (7 of 33)	Not mentioned blindness Not mentioned placebo
53		40	Treatment arm: standardized Chinese herbal medicine Control arm: no intervention	6 months	Treatment arm: effective rate, 52.6 % (10 of 19) Control arm: not mentioned	Not mentioned blindness Not mentioned placebo Not mentioned control group
54		60	Treatment arm: metformin, Dian-35 and danzhi xiaoyao pill Control arm: metformin and Dian-35	3 months	Treatment arm: effective rate, 87.5 % (21 of 24) Control arm: effective rate, 58.3 % (14 of 24)	Not mentioned blindness Not mentioned placebo
55		48	Treatment arm: Longdan xiegan decoction Control arm: Diane-35	3 months	Treatment arm: effective rate, 82.6 % (19 of 23) Control arm: effective rate, 78.26 % (18 of 23)	Not mentioned blindness Not mentioned placebo
56		22	Treatment arm: Tiangui decoction Control arm: metformin	3 months	Treatment arm: effective rate, 60 % (6 of 10) Control arm: effective rate, 33.3 % (4 of 12)	Not mentioned blindness Not mentioned placebo

3. Acupuncture Treatment for Menstrual Disorder in PCOS

Acupuncture, as the major part of TCM, the history of can be traced back to more than 3000 years [62,63], is based on the basic theory of TCM, selecting acupoints according to the syndrome differentiation and experience, thus achieving the purpose of treatment of diseases through a variety of manipulation. In recent years, due to the limited efficacy and common side effects of Western medicine in the process of treating PCOS patients [64], acupuncture plays an important role in the treatment of menstrual disorders caused by PCOS [65]. Therefore, acupuncture has been used as an alternative treatment for PCOS, which has gradually become a researching hot spot recently and received extensive attention [29]. As a kind of TCM treatment, acupuncture is divided into acupuncture, moxibustion, acupoint catgut embedding, and other categories. The following paragraphs

will be discussing the acupuncture treatment for menstrual disorder in PCOS patients according to the different categories.

3.1. Acupuncture Treatment for PCOS Menstrual Disorder

3.1.1. Clinical Application of Acupuncture for Menstrual Disorder of PCOS. As one of the complementary and alternative therapies, acupuncture is getting more and more attention in the treatment of PCOS patients with menstrual disorders [66], and there have been a number of reports on the efficacy of acupuncture in treating PCOS. However, there has been no study on the method and quality of standard reports on the effectiveness of acupuncture and moxibustion on PCOS [67–70]. In Zheng et al. 's randomized controlled clinical trial [71], 86 patients were randomly divided into abdominal acupuncture group and metformin group, with 43 patients in each group. The acupuncture group received 30 minutes of treatment twice a week.

After 6-month-treatment, in terms of reducing BMI and increasing menstrual frequency, the abdominal acupuncture group appeared better than the metformin group. Both abdominal acupuncture and metformin can improve the endocrine and metabolic functions of obese PCOS patients. Nevertheless, the advantages of the abdominal acupuncture are more outstanding apparently, which reflected in less side effects, reducing BMI significantly by reducing visceral adipose tissue [72]. In Li et al.'s double-blind, placebo-controlled, multicenter randomized controlled study [73] subsequently, 342 patients with PCOS and IR were randomly divided into three groups: true acupuncture + metformin placebo, sham acupuncture + metformin, and sham acupuncture + metformin placebo, with 114 patients in each group. Results of the oral glucose tolerance test and insulin release test collected 3 months after treatment showed that acupuncture could improve the sensitivity of insulin in PCOS patients. The IR of PCOS is caused by hyperinsulinemia and deficiency of insulin signaling pathway; therefore, high concentration of the insulin reduces the circulating level of SHBG and increases the level of free T, resulting in menstrual disorder [73,74]. Consequently, it is essential to reduce insulin levels. These two clinical trials have proved that acupuncture can improve the insulin sensitivity of PCOS patients, thereby improving hyperinsulinemia and relieving the symptoms of menstrual disorders. It has been found that acupuncture and moxibustion could regulate the reproductive and endocrine function of PCOS patients by affecting various signal pathways and targets of hypothalamus pituitary gonadal axis [75]. In Yu et al.'s randomized, drug-controlled, parallel grouping trial [76], they compared the efficacy and safety of Dong's acupuncture therapy and oral contraceptives on sex hormones in patients with PCOS; 60 women aged 18–45 years with PCOS were randomly divided into the acupuncture group and control group, with 30 patients in each group. In the acupuncture group, Fuke, Huanchao, Tianhuang, renhuang, Guanyuan, and uterus (EX-CA1) were selected, and the participants received acupuncture twice a week. After 12 weeks, compared with the control group, the LH/FSH ratio, LH, and T value in the acupuncture group were significantly reduced, indicating that acupuncture can effectively reduce the LH/FSH ratio, thereby regulating menstrual frequency and treating menstrual disorders caused by PCOS. Other studies have shown that low-frequency electroacupuncture can induce ovulation [77], thus making menstrual frequency return to normal. In addition, repeated electroacupuncture treatment can also reduce the ratio of LH/FSH and improve menstrual frequency [77]. The study found that women with PCOS had higher sympathetic nervous system activity [78]. In a prospective randomized controlled trial conducted by Jedel et al. [79], 84 women were selected and divided into the intervention group and low-frequency electroacupuncture group, with 42 patients in each group. Each participant experienced a 12-week-observation period. The results showed that serum T in the low frequency EA group was significantly lower than that in the intervention group, and menstrual frequency continued to improve. Therefore, low-frequency electrical stimulation reduces high sympathetic activity [80], which may be helpful in the treatment of hyperandrogenemia and oligomenorrhea/amenorrhea. In the clinical trial, Julia Johansson et al. conducted a prospective randomized controlled clinical study; 32 female patients with PCOS were divided into manual group and low frequency electrical stimulation acupuncture group, with 16 patients in each group. The acupuncture points were ST1, CV3, CV6, ST29, SP6, SP9, LI4, GV20, ST2, ST25, ST29, LR3, and PC6 (Figure 1), the ovulation frequency in the acupuncture group was higher than that in the control group, 10–13 weeks later. Normal ovulation frequency makes regular changes in endometrium, which further improves the menstrual cycle of PCOS patients [82]. In recent years, there have been a number of meta-analyses on acupuncture treatment of PCOS menstrual disorders. Although the conclusions of each study are different, they all enrich the specific clinical application of acupuncture as CAM. Among them, a systematic review summarized and evaluated the effective data for the acupuncture treatment of PCOS menstrual disorders, focusing on the menstrual rate, and found that acupuncture was more likely to improve the menstrual rate under low levels of evidence compared with the group which did not receive acupuncture and metformin [83].

Acupuncture and moxibustion in the treatment of menstrual diseases caused by PCOS have been constantly making meaningful explorations, but there are also some controversial parts. For example, some adverse reactions may occur in the process of acupuncture in clinical trials, such as pain, redness, hematoma, and nausea after acupuncture [84,85]. In addition, some researchers have proposed that superficial acupuncture, compared with true acupuncture, cannot be regarded as placebo control, for these control methods are not inert [86,87]. Relevant experiments have shown that both true acupuncture and sham acupuncture can improve the LH/FSH ratio of patients with PCOS, thus affecting menstrual frequency [84]. Several systematic reviews on acupuncture and moxibustion for

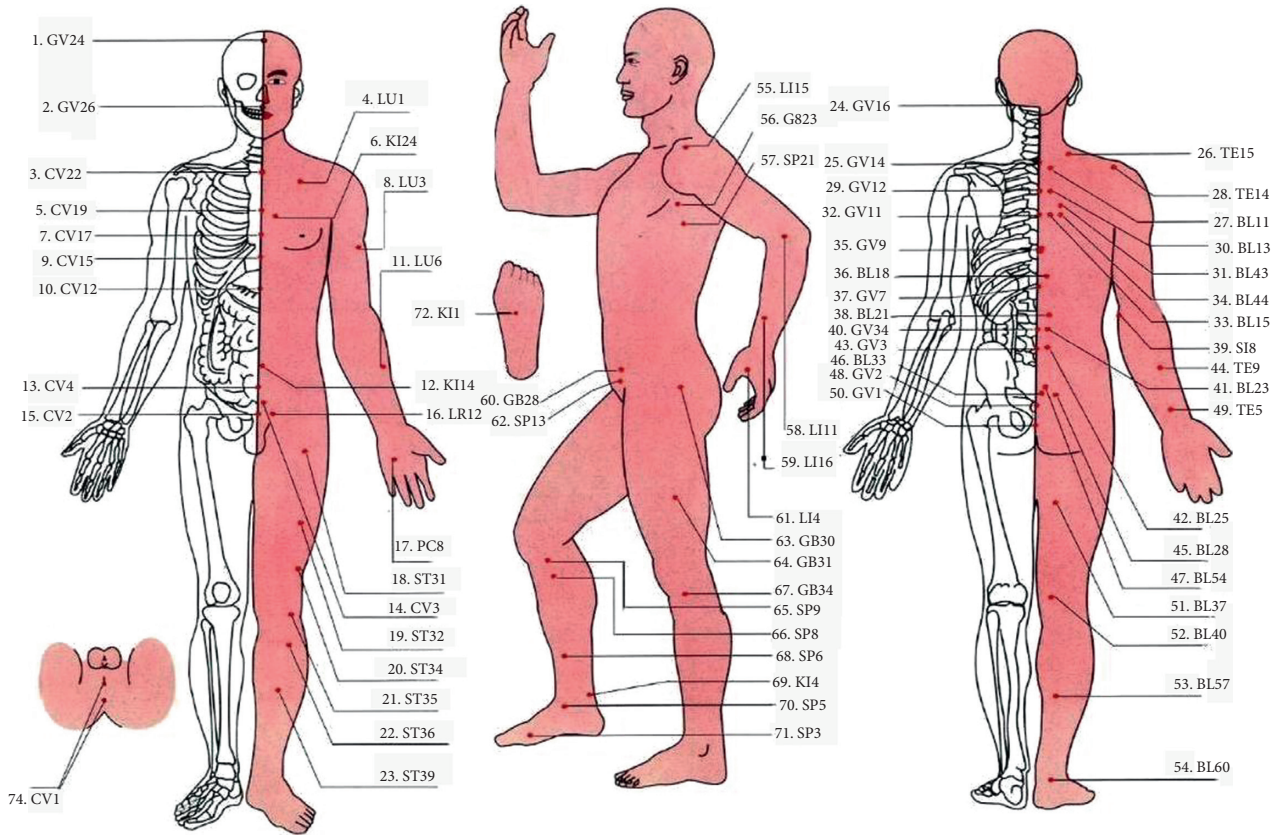

Figure 1: [81]. Human acupoint map GV24 Shengting, GV26 Shuigou, CV22 Tiantu, CV1 Zigong, CV17 Tanzhong, CV15 Jiuwei, CV12 Zhongwan, CV4 Guanyuan, CV2 Qvku, CV1 Huiyin, LU1 Zhongfu, KI24 Lingxu, LU3 Tianfu, LU6 Kongzui, PC8 Laogong, ST31 Biguan, CV3 Dahe, ST32 Futu, ST34 Liangqiu, ST35 Dubi, ST36 Zusanli, ST39 Xiajuxu, GB28 Weidao, SP13 Fushe, KI14 Siman, LR12 Qichong, LI15 Jianyu, GB23 Zhejin, SP21 Dabao, LI11 Quchi, LI16 Pianli, LI4 Hegu, GB30 Huantiao, GB31 Fengshi, GB34 Yanglingquan, SP9 Yinlingquan, SP8 Diji, SP6 Sanyinjiao, KI4 Dazhong, SP5 Shangqiu, SP3 Taibai, GV16 Fengfu, GV14 Dazhui, GV12 Shengzhu, GV11 Shendao, GV9 Zhiyang, BL18 Ganshu, GV7 Zhongshu, BL21 Weishu, GV4 Mingmen, GV3Yangguan, BL33Zhongliao, GV2 Yaoshu, GV1Changqiang, TE15 Tianliao, TE14 Jianliao, BL11 Dashu, BL13 Feishu, BL43Gaohuang, BL44 Shengtang, BL15 Xinshu, SI8 Xiaohai, TE9 Sidu, BL23 Shenshu, TE5 Waiguan, BL25 Dachangshu, BL28 Pangguangshu, BL54 Zhibian, BL37 Yinmen, BL40 Weizhong, BL57 Chengshan, and BL60 Kunlun.

PCOS have not yet made a comprehensive meta-analysis on the changes of menstrual cycle so far. Therefore, more prospective, large-scale, and highly rigorous randomized controlled trials are needed to demonstrate this point in the future basic research and clinical trials.

3.1.2. Mechanism of Acupuncture in the Treatment of PCOS Menstrual Disorders. Acupuncture is divided into two parts, including hand-acupuncture and electro-acupuncture. Hand-acupuncture refers to the method of using the filiform needle to penetrate the skin and stimulate a specific anatomical position of the body, and rapid rotation of the thumb and index finger to stimulate manually to achieve the effect of obtaining qi [88]. Electro-acupuncture refers to a treatment in which the needle is connected to a trace of low-frequency pulsed current by an electroacupuncture machine and the needle obtains qi when it penetrates into the acupoint of the human body [89]. Studies have shown that acupuncture can regulate endogenous regulatory systems, including endocrine, sympathetic nervous system, and neuroendocrine system [62,63], thereby improving women's endocrine disorders, menstrual frequency, and reducing the level of sex hormones [90–92]. The mechanism of acupuncture treatment for menstrual disorders caused by PCOS is considered from the following aspects.

The first point is that acupuncture can improve insulin sensitivity and relieve IR [93–98]. Hyperinsulinemia and IR are the most important endocrine characteristics of PCOS, which can lead to the increasing of androgen in the body and affect the ovulation resulting in menstrual disorders. A study showed [96] that acupuncture can effectively improve the insulin sensitivity of PCOS rats induced by dihydrotestosterone. In this study, ST27, ST28, ST29, SP6, SP9 were selected to acupuncture rats. Compared with the control group without acupuncture, the former was significantly higher than the latter. Johansson et al. [93] carried out low frequency electroacupuncture treatment on PCOS rats model induced by dihydrotestosterone for 5–6 weeks; the acupuncture sites were abdominal and hind limb muscles. Then, the body weight, groin fat, insulin sensitivity, and other related indicators of the rats were measured. It was concluded that repeated low-frequency electroacupuncture can improve the insulin sensitivity in skeletal muscle and

adipose tissue of rats. Increased insulin sensitivity can alleviate IR in PCOS rats, thereby indirectly improving menstrual disorders caused by PCOS.

The second point is that acupuncture can affect the hypothalamus-pituitary-gonadal-axis (HPG), thus affecting the release of hypothalamic gonadotropin-releasing hormone (GnRH) and the secretion of pituitary gonadotropin [86,99].. Patients with PCOS may have abnormal HPG making the pituitary gland more sensitive to GnRH, which results in excessive LH secreted by the ovary, stimulating ovarian stroma and theca cells, thus producing excessive androgen, finally resulting in impaired follicular development and menstrual disorders. Maliqueoet et al. [89] performed acupuncture on DHT-induced PCOS rats at the body nodes corresponding to ovarian nerve insufflations in rectus abdominis and triceps surae. After 5–6 weeks of acupuncture, the LH, T, progesterone, and estrus cycle were evaluated, and it was concluded that acupuncture and moxibustion can improve the level of gonadotropin and regulate the estrus cycle. In one study [100], female rats that were exposed to DHT increased the number of GnRH-expressing cells and the expression of androgen receptor (AR) protein in the hypothalamus. In DHT-induced rats, high-intensity but low-frequency electrical stimulation for 5 days a week, lasting for 4–5 weeks, restored the normal levels of GnRH and AR expression. The regulation of HPG by acupuncture can improve the hormone level of PCOS patients and restore their normal menstrual cycle.

The third point is that low frequency electroacupuncture can also improve ovarian morphology, estrus cycle, and AR protein expression in PCOS rats modeled by regulating sympathetic nervous system activity [101, 102]. Furthermore, acupuncture has a significant effect on reducing body mass index of PCOS women [90], and it can also activate the physiological process similar to physical exercise to reduce obesity, so as to improve the physical quality and endocrine environment of PCOS patients and regulate the menstrual cycle [96].

3.2. The Application of Moxibustion in the Treatment of Menstrual Disorders in PCOS Patients. Moxibustion, as one of the traditional medical therapies in China, prevailed in the spring and autumn periods and the Warring States period, "Yang moves and disperses, so it changes Qi, Yin is quiet and tranquil, so it takes shape." Moxibustion can nourish Qi, warm yang, remove blood stasis, and dredge collaterals. Modern research shows that warm needling is better than single acupuncture [103], and thunder fire Moxibustion plus acupuncture is better than single acupuncture [104]. Clinical studies have shown that moxibustion has a certain role in promoting ovulation [105,106], thus regulating the menstrual cycle. Moxibustion can increase the thickness of endometrium, improve the level of serum sex hormone, and regulate the ovulation cycle in the treatment of PCOS with menstrual disorders. At the same time, it may be related with adjusting the levels of serum tumor necrosis TNF- α and nuclear transcription NF KB in patients with PCOS. A recent randomized controlled trial has shown that heat-sensitive moxibustion can significantly improve ovulation and correct menstrual disorders in women who have ovulation disorders. In this study, 70 patients were randomly divided into the control group and the observation group, with 35 patients in each group. On the basis of the control group, the observation group was added with heat-sensitive moxibustion, and the heat-sensitive moxibustion was carried out at Guanyuan, uterus, Xuehai, and Shenshu, every other day for 10 minutes. After 6 menstrual cycles, the levels of serum T and LH in the observation group were lower than those in the control group, while the E2 level was higher in the observation group than in the control group ($P < 0.05$). However,owing to the lack of the mechanism of moxibustion treating with menstrual disorders, we need more scientific basis researches to guide the clinical application.

3.3. Application of Acupoint Catgut Embedding in PCOS Patients with Menstrual Disorders. Acupoint catgut embedding (ACE) is based on the theory of acupuncture and moxibustion. It can achieve the purpose of stimulating the meridians and balancing Yin and Yang by placing absorbable sheep gut suture at acupoints with needle and medicinal thread. In the view of Western medicine, ACE therapy is considered as an exogenous invasive therapy [13,107], leading to the mechanism of its effectiveness and safety not being clarified; so, the clinical trials related to ACE have totally been completed in China. According to a recent randomized controlled trial, 84 PCOS obese patients with delayed menstruation and IR were selected by Gui-Zhi et al. [108] and divided into the observation group and control group, with 42 patients in each group. The observation group was additionally given acupoints such as BL20, BL23, ST25, GB34, ST40, ST36, SP6, etc., on the basis of the control group. After three months, the BMI of the observation group was lower than that of the control group, and the IR was significantly improved. Related clinical studies [82] reported that the corresponding body segments of acupuncture for ovarian domination were TH12-L2 and S2-S4 [109]. Therefore, ACE was mainly implanted in the muscles of abdomen and lower limbs [13], which inhibited the activity of sympathetic nervous system [110], thus promoting ovulation in PCOS patients, and improving hyperandrogenemia and menstrual disorder. Current animal experiments have shown that ACE may regulate menstruation in PCOS patients by reducing serum T level. The team of Tian et al. and Yi et al. studied the PCOS rats induced by TP and HCG under ACE treatment, and serum LH and T levels were significantly decreased after intervention [13, 111, 112]. Both IR and serum androgen level was decreased [112–114] by regulating the level of reproductive endocrine hormone. At the same time, some studies suggested that ACE may achieve therapeutic effect through the dual stimulation of suture in the process of decomposition and absorption [115]. As for the effective acupoints for catgut embedding, relevant studies [116–118] believed that the main acupoints (ST25, ST40, ST36, SP6, SP9, BL20, BL23, CV4, CV6, CV12) belong to the stomach meridian of Foot Yangming, spleen meridian of foot Taiyin, bladder meridian

of foot Taiyang, and Ren Mai, which provide modern medical basis for the accuracy of acupoint selection. Although ACE therapy has the advantages of simple operation, it still lacks the theoretical basis of evidence-based medicine; in addition, the safety of its operation is still questionable. Consequently, higher quality and more rigorous experimental studies are needed to explore the potential mechanism.

For summarizing the randomized clinical trials, please refer to Table 2 and Figure 1.

4. Other Treatments for PCOS Menstrual Disorders

4.1. Auricular Points. Auricular points, also known as reaction points and stimulation points [81], are the acupoints distributed on the auricle. Local reactions often occur in certain parts of the auricle generally when the human viscera or body is in a state of illness, such as tenderness, nodules, discoloration, electrical conductivity, etc. This phenomenon can be used as a reference for the diagnosis of diseases, or to stimulate these reaction points (ear points) to prevent and treat diseases. The ear is closely related to viscera and meridians; there are corresponding reaction areas (auricular points) in the auricle of viscera. Thus, stimulating auricular points has an effect of partly regulating the corresponding viscera [119]. The main methods of stimulating auricular points are acupuncture, embedding needle, bloodletting, auricular point sticking, magnetic therapy, massage, etc.

The auricular points for treating PCOS with menstrual disorders are: the uterus, the ovary, the kidney, the liver, the spleen, and the pelvic cavity. Auricular acupoints are commonly used for auricular point sticking [120]. The main acupoints include liver, kidney, uterus, ovary, and endocrine, and the matching points are spleen, waist, and pelvic cavity. Appropriate acupoints are selected according to the TCM syndrome differentiation. After the acupoints are disinfected, the auricular plaster is applied to the sensitive parts of auricular points, pressing 3–4 times a day for 30 s each time. Use your fingers to knead, press, pinch, and press the sensitive parts of auricular points to the degree of strong acid, distension, heat, and pain. Change the auricular acupoint plasters every 3 days, 30 days as a course of treatment. Studies have shown that [121] 18 cases of 26 patients with irregular menstruation were cured after undergoing the treatment of accounting the main points of uterus, endocrine, internal genitalia, kidney, and liver, combined with the waist, pelvic cavity, and spleen, accounting for 69.2%. Based on auricular acupuncture and the principle of vagus nerve stimulation (VNS), a noninvasive transcutaneous vagus nerve stimulation (ta-VNS) was established. The results of comparative study on four VNS indications showed that ta-VNS has almost the same effect as VNS. In addition, ta-VNS has the advantages of security, economy, and portability. In the aspect of clinical prevention and treatment of PCOS, polycystic ovary (PCO) is the target organ, and the nerve and vagus nerve are related to it. In general, regulating the reproductive and endocrine disorders of PCOS from the perspective of vagus nerve, introducing at-VNS into the treatment of PCOS, and improving acupuncture therapy such as ear acupuncture may become the new research directions [122].

4.2. Massage. Massag is a method which is based on "the theory of viscera and meridians of TCM" and combined with "the anatomy and pathological diagnosis of Western medicine." The operation method of massage is to select appropriate manipulation and then act on specific parts of the body surface to regulate the physiological and pathological conditions of the body achieving the purpose of physical therapy [123]. Massage has various functions of relaxing muscles and bones, reducing pressure, promoting the blood circulation, detoxification, etc. [124]. The scope of gynecological massage treatment includes menstrual diseases (irregular menstruation, dysmenorrhea), leucorrhea, postpartum diseases (breast carbuncle, etc.), gynecological miscellaneous diseases (hyperplasia of mammary glands, menopausal syndrome), etc. [125].

Modern women's life, work, family, and other aspects of pressure will make some of them suffer from PCOS, resulting in menstrual disorders which are physiological, not necessarily with drug treatment. Thus, PCOS patients with menstrual disorders using massage therapy is an excellent method. The massage methods for treating PCOS with menstrual disorders include: hand acupoints massage for menstrual disorders, foot acupoints massage for menstrual disorders, head acupoints massage for menstrual disorders, ear acupoints massage for menstrual disorders, and whole body acupoints massage for menstrual disorders. In general, whole body acupoint massage is often used to treat menstrual disorders. TCM pays attention to holistic concept and syndrome differentiation and treatment. For acupoint massage, the patients with liver qi stagnation are treated with tonifying the kidney (Guanyuan, Shenshu, Taixi), soothing the liver (Taichong, Guanyuan, Sanyinjiao), warming Yang (Guanyuan, Qihai, moxibustion Yinbai), and activating blood (Diji, Xuehai, Zhongji); those with deficiency of kidney qi are treated with tonifying the kidney (Guanyuan, Shenshu, Taixi), promoting qi (Guanyuan, Qihai, Zusanli, Sanyinjiao), and promoting blood circulation (Diji, Xuehai, Zhongji); Patients with phlegm obstruction are treated with tonifying the kidney (Guanyuan, Shenshu, Taixi), resolving phlegm (Fenglong, Guanyuan, Uterus, Sanyinjiao), and activating blood (Diji, Xuehai, Zhongji). Three consecutive treatments were administered, namely, 3rd day after menstruation, 12th to 15th day of menstrual cycle, 18th to 21st day, and 2nd to 5th day after menstruation. After 3–6 months of continuous treatment, menstrual disorders were significantly improved [126]. The study showed that [127] raising your legs and massaging Sanyinjiao point which is located three inches above from the tip of the medial malleolus of the foot (about four transverse fingers) for 49 times with your thumb meanwhile against the posterior edge of the tibia can help smoothen the flow of Qi and blood and strengthen the spleen at the same time benefiting Qi, which are contributed to improve the symptoms of menstrual disorders.

4.3. Qigong. Qigong is a traditional Chinese method of healthcare, health preservation, and disease elimination. In ancient times, the content of Qigong was very extensive.

Table 2: The summary of randomized studies of acupuncture treatment on PCOS menstrual disorders.

Study ID	Design	Sample size (human)	Interventions	Treatment duration	Outcomes	Limitation
72	RCT	86	Treatment arm: Abdominal acupuncture Control arm: metformin	6 months	1. HOMA-IR were reduced significantly in the two groups ($P < 0.05$).2. Menstrual frequency, HDL-C, follicle-stimulating hormone increased in both groups ($P < 0.05$).	Lack of sham methods
74	RCT	342	Treatment arm: true acupuncture + metformin placebo Control arm: sham acupuncture + metformin, sham acupuncture + metformin placebo	4 months	Treatment arm: HOMA-IR decrease 25% Control arm: HOMA-IR decrease 5%	The limitation of being a single center study without comparison groups
76	RCT	181	Treatment arm: acupuncture group Control arm: same physical therapists	10–13 weeks	Treatment arm: effective rate, 62.5% (10 of 16) Control arm: effective rate, 41.6% (5 of 12)	
77	RCT	60	Treatment arm: Tung's acupuncture group Control arm: CPA/EE group	12 weeks	Treatment arm: LH/FSH ratio −0.66, $P < 0.001$ Control arm: not mentioned	Clinical improvement of symptoms was not examined in this study
78	Nonrandomized, longitudinal, prospective study	60	Treatment arm: electro-acupuncture group Control arm: No intervention	3 months	Treatment arm: effective rate, 37.5 % (9 of 24) Control arm: not mentioned	The sample size is small
80	RCT	84	Treatment arm: low-frequency electro-acupuncture Control arm: no intervention	16 months	Treatment arm: effective rate, 72.9% (43 of 59) Control arm: effective rate, 33.3% (5 of 15)	Low success rate to confirm ovulation (data not shown)
81	RCT	84	Treatment arm: electro-acupuncture group; Physical exercise Control arm: no intervention	32 weeks	Treatment arm: effective rate, 74.4% (32 of 43) Control arm: effective rate, 48.7% (20 of 41)	The sample size is small
82	RCT	32	Treatment arm: acupuncture group Control arm: attention control	4 months	Treatment arm: effective rate, 68.75 % (11 of 16) Control arm: effective rate, 37.5 % (6 of 16)	
88	RCT	143	Treatment arm: true acupuncture Control arm: sham acupuncture	5 months	Treatment arm: effective rate, 57.5% (23 of 40) Control arm: effective rate, 47.7 % (21 of 44)	
109	RCT	70	Treatment arm: heat-sensitive moxibustion plus clomifene citrate capsules Control arm: oral clomifene citrate capsules	6 months	Treatment arm: effective rate, 51.5% (18 of 35) Control arm: effective rate, 20 % (7 of 35)	The sample size is small
111	RCT	84	Treatment arm: metformin and acupoint thread-embedding Control arm: oral metformin	3 months	Treatment arm: effective rate, 87.5 % (35 of 40) Control arm: effective rate, 65.9 % (27 of 41)	The sample size is small

Qigong is characterized by the combination of mind, Qi, and body through the subjective efforts of practitioners. It mainly includes the adjustment of breathing, physical activities, and consciousness, including the means of regulating breath, body, and mind, with the purpose of strengthening the body, preventing and treating diseases, keeping fit and prolonging life, and developing the potential of physical and mental exercise. Heart regulation is to regulate psychological activities, breath regulation is to regulate respiratory movement, and body regulation is to regulate body posture and movement. These three regulations are the basic methods of Qigong exercise and are called the three elements or basic norms of Qigong discipline [128]. Qigong focuses on calmness, relaxation of body and mind, and the peace of mind, which are of great benefit to the harmony of Qi and blood in women, and the smooth flow of Qi also improves the self-regulation of blood. Due to the modern women's fast-paced life, the urgent need is to regulate the pace of life, and to calm down your body and mind. This has a considerable effect on improving PCOS menstrual disorder [129]. The aim of this study [130] is to observe the effect of "fitness Qigong six character formula" on menstrual disorders of female college students [130]. Eighty-six female college students with PCOS menstrual disorders were randomly selected. 46 cases were classified as Qigong group. After 12 weeks of Qigong exercise, the symptoms of menstrual disorders of 41 cases got different degrees of improvement. The total effective rate was 89.1%. Among the 18 students with symptoms of abnormal menstrual cycle, 16 cases were improved, and the effective rate was 89%; in 15 cases of abnormal menstrual volume, 14 cases were improved, and the effective rate was 93%; in 13 cases of abnormal menstrual period, 11 cases were improved, the effective rate was 84%. In the control group, 3 cases among 40 students in the control group improved their symptoms, and the effective rate was 7.5%. The other 37 cases had no obvious improvement. It can be concluded that: Fitness Qigong six character formula can effectively improve the symptoms of irregular menstruation of PCOS female college students.

4.4. Tai Chi. The core of Taiji theory is yin-yang theory. The yin-yang fish in Taiji diagram symbolizes the state of things moving in cycles. The basal body temperature curve of female normal menstrual cycle showed periodic biphasic changes. Similarly, Taiji diagram is often used to show the physiological and pathological changes of menstruation [131]. According to the description of menstrual mechanism in Neijing, the maintenance of menstruation is affected by age, viscera, Tiangui, and other parameters. Besides, the whole set of Taijiquan moves continuously in a gentle and soothing way, like silkworms spinning in spring. And the continuous movement makes the whole body's meridians unblocked, resulting in the smooth circulation of Qi and blood thereby improving the microcirculation of the human body and physique [132]. The characteristics of Taiji are as follows: (1) The combination of will and form, focusing on will. (2) The combination of mobile and interval focuses more on interval. (3) Moving along meridians and exaggerate arm rotation. (4) Selecting the acupoints along the meridians, with fingers instead of needles. (5) In the whole process, removing the disturbance of all the evil thoughts so as to achieve the state of meditation and stabilize your mind, meanwhile, keeping your body highly relaxed [133]. Consequently, Tai Chi is outstanding in regulating Yin and Yang and regulating qi for women. Women are innate with blood. Only when the Yin and Yang are balanced, Qi and blood are smooth, can menstruation naturally occur on schedule; thereby, Tai Chi plays a great role in regulating the menstruation of women, and has a significant effect on PCOS menstrual disorders. Li. et al.'s research showed [133] that Taijiquan involved training for 60 minutes each time, three times a week for 12 weeks, based on the women's initial physical activity level. Each session includes 10 minutes of warm-up and cooling. It adopts 24 kinds of Simplified Taijiquan recommended by the General Administration of sport of China. Finally, the weight, waist/hip circumference, and blood pressure of PCOS patients with menstrual disorders before and after Taijiquan treatment were significantly improved, and the insulin level was significantly improved, which will eventually promote the improvement of menstruation.

4.5. Cognitive Behavioral Therapy. Changes in hormone levels in PCOS and Negative Affectivity (NA) are intricately linked. Relevant experiments showed [134] that LH is significantly negatively correlated with Hospital Anxiety and Depression Scale (HADS), and FSH is significantly negatively correlated with Hospital Anxiety and Depression Scale-Anxiety (HADS-A); a slight increase in free T can reflects a higher degree of depression. Therefore, due to the influence of hormone level, the economic burden of diseases treatment and the potential social doubt, PCOS patients will bear more self-doubt and external disagreement, which may lead to more tension, anxiety, and even depression.

In view of the origin of anxiety, depression, and bad emotions of PCOS patients, Leah Brennan's research team [135] proposed the following psychotherapy strategies: clinicians consciously assist patients to learn and establish the following modes: goal setting, self-monitoring, cognitive reconstruction, problem-solving, and recurrence prevention. In the process of adjuvant treatment, through motivational interview, self-monitoring, and time management strategies, we should teach patients behavior changing skills and coping skills of life and work, so as to improve their ability to deal with bad emotions and restore their mental health; at the same time, we should seek help from psychologists and other professionals as appropriate.

Mindfulness-based therapy, rooted in Buddhist meditation [136], is one of the ways of psychological intervention. By strengthening the relaxation response of the human body, it may effectively prevent common mental diseases (including depression and anxiety), relieve the stress and anxiety in life and work, and assist people to maintain a healthy mental state [137]. In Norio Watanabe's research team [138], experimental groups were given mindfulness

pressure management plan for 8 weeks. The final experimental results were satisfactory; the scores of HADS, perceived stress scale, and quality of daily life questionnaire (Questionnaire on the degree of perplexity of patients with PCOS clinical characteristics, such as hirsutism, obesity, menstrual disorders, etc.) in the experimental group were significantly decreased; and the scores of HADS (Dass 21) were significantly decreased. Dass 21 assesses the state of the sympathetic nervous system rather than the subjective pressure of the patient, which further illustrates the influence of mindfulness therapy on PCOS patients.

Behavioral cognitive therapy is safe and has no side effects, which can effectively relieve the negative emotions of depression and anxiety in patients with PCOS, assist patients to adjust to the overall state of the body from the psychological level, and help shorten the treatment cycle of the disease.

4.6. Establishment of Healthy Life Mode. According to International evidence-based guideline for the assessment and management of PCOS, weight control is one of the main treatment strategies for PCOS [139]. A healthy lifestyle for women with PCOS includes taking a low carbohydrate and ketogenic diet; taking ancient diet or less processed food, cereal, or dairy products; hypoglycemia diet; regular physical exercise; adequate sleep; practicing decompression technology; and various forms of online and written learning courses [140]. However, it should be noted that weight loss through short-term diet and exercise is only the beginning of cultivating a healthy lifestyle. It is the management of long-term lifestyle and the maintenance of healthy eating habits that cannot be ignored. The Mediterranean Diet (MD) is an anti-inflammatory diet, including regular consumption of unsaturated fat, low glycemic index carbohydrates, fiber, vitamins and antioxidants, and an appropriate diet [141]. Luigi Barrea's team [142] found that MD had positive effects on T level and Ferriman-Gallwey score of PCOS patients to varying degrees; and the intake of complex carbohydrates, fiber, monounsaturated fatty acids, and n-3 polyunsaturated fatty acids was positively correlated with C-reactive protein level; compared with the control group, patients with PCOS on a MD had a lower intake of these substances. MD can reduce the inflammatory state, help patients with PCOS-IR and hyperandrogenemia to reduce the inflammatory state of the body. Therefore, it is recommended that the MD be used for PCOS patients.

Apart from reducing exogenous intake, an increase in the amount of exercise is required for weight loss. Relevant experiments have proved that exercise has a certain effect on the recovery of favorable metabolic and ovarian functions [9,143]. The study [144] found that after 12 weeks of exercise, the BMI and Cardiovascular Health Index of the exercise group were improved, and the levels of the Anti-Müllerian Hormone of Ovary (AMH) and Malondialdehyde (MDA) were decreased, while the levels of Superoxide Dismutase (SOD) and Total Antioxidant Capacity (TAC) were increased. Some researchers [145] found that it is the intensity of exercise rather than the amount of the exercise is the key to the patients who want to improve their state by exercising. In addition, the researchers found that patients with PCOS should have at least 120 minutes of vigorous exercise every week for at least 10–12 weeks to better improve the status of PCOS.

4.7. Complementary Alternative Medicine. In recent years, the research on complementary and alternative drugs has made some progress in the treatment of PCOS, such as probiotics, melatonin, fish oil, fatty acid, vitamin D, vitamin K, carnitine, chromium, selenium. They are found to have certain intervention effects on PCOS [146].

The microbial regulation of Lactobacillus may be an effective way to alleviate PCOS. The results showed that Lactobacillus could alleviate the symptoms of PCOS in letrozole model rats [147]. Some *Lactobacillus* may play an important role in improving PCOS by regulating and controlling the sex hormone-related microorganisms. Melatonin [148] has certain effects on reducing oxidative stress, and acts as an antioxidant, anti-inflammatory, and antidepressant agent; fish oil [149] can significantly improve the expression of PPAR - γ, IL-1, and IL-8 genes related to PCOS; omega-3 fatty acids [149] can reduce C-reactive protein and increase adiponectin levels to reduce the inflammatory state of women with PCOS; after providing PCOS patients with vitamin D deficiency supplements including calcium, vitamin D, and K for 8 weeks, insulin metabolism, serum triglyceride, and very low-density lipoprotein cholesterol levels were improved [150]; combining the use of carnitine with chromium [151] can improve the mental health indicators, serum total T, high-sensitivity C-reactive protein, Total Antioxidant Capacity (TAC), and Malondialdehyde (MDA) levels of women with PCOS, as well as the gene expression of Interleukin-6 (IL-6) and Tumor Necrosis Factor alpha (TNF - α); selenium (SE) [152] is a kind of antioxidant and has an insulin-like effect. All of the drugs listed above may have a therapeutic effect on PCOS and provide new direction for the treatment of PCOS.

5. Summary

Owing to the wide range of applications, CAM receives more and more attention and application at home and abroad, and PCOS menstrual disorders are also paid more and more attention by CAM. CAM is a kind of therapy which has less erosion and fewer side effects on patients, providing more beneficial choices for patients and opening up a broad path to health [153]. For PCOS menstrual disorders, TCM and acupuncture are the most commonly used CAM methods, but other CAM medicine therapies also help improve PCOS menstrual disorders [154]. However, at present, CAM is still in the early stage of the trial, which means that there are still some limitations. We should also collect a large number of samples for large-scale clinical research, especially for cognitive behavioral therapy [137], healthy life model [139], replacement drugs [146], etc., and we should further explore its therapeutic potential in the future research [155].

Disclosure

Yuehui Zhang, Xiaozhu Guo, and Shuting Ma are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

The Effects of *Salvia miltiorrhiza* on Reproduction and Metabolism in Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis

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Objective. Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. As a traditional medicine, *Salvia miltiorrhiza* (*S. miltiorrhiza)* has been widely used in the treatment of many gynecological diseases, but the efficacy of *S. miltiorrhiza* in women with PCOS has not been assessed. The purpose of this systematic review and meta-analysis was to evaluate the effectiveness and safety of *S. miltiorrhiza* in women with PCOS. *Methods*. We conducted searches in PubMed, Embase, the Cochrane Library, the China National Knowledge Infrastructure, the Wanfang Database, the Chinese Scientific Journal Database, and the Chinese BioMedical database from inception to December 23, 2020, to identify studies that met the inclusion criteria. The quality of the evidence was estimated using the Cochrane Reviewer Handbook 5.0.0, and the meta-analysis was performed using RevMan 5.3.5 software. *Results*. Six randomized controlled trials (RCTs) involving 390 patients with PCOS were included. The studies suggested that *S. miltiorrhiza* extract combined with letrozole (LET) was more effective in improving pregnancy rate (RR: 2.60, 95% CI: 1.06 to 6.39, $P = 0.04$) compared to LET alone. *S. miltiorrhiza* extract was associated with decreased fasting blood glucose (MD: −0.25, 95% CI: −0.37 to −0.13, $P < 0.0001$), fasting insulin (MD: −1.16, 95% CI: −1.74 to −0.58, $P < 0.0001$), total cholesterol (TC) (MD: −0.58, 95% CI: −0.72 to −0.43, $P < 0.00001$), and triglycerides (TG) (MD: −0.31, 95% CI: −0.35 to −0.26, $P < 0.00001$) compared with placebo, but not with improvements in body mass index or waist-to-hip ratio (MD: −1.41, 95% CI: −4.81 to 2.00, $P = 0.42$; MD: −0.02, 95% CI: −0.05 to 0.01, $P = 0.16$, respectively). There was a significant difference between *S. miltiorrhiza* extract combined with cyproterone acetate (CPA) and CPA alone in terms of decreasing TC (MD: −0.77, 95% CI: −0.89 to −0.65, $P < 0.00001$), TG (MD: −0.43, 95% CI: −0.65 to −0.20, $P < 0.0001$), and low-density lipoprotein cholesterol (MD: −0.49, 95% CI: −0.66 to −0.33, $P < 0.00001$) and increasing high-density lipoprotein cholesterol (MD: 0.30, 95% CI: 0.20, 0.40, $P < 0.00001$). In addition, *S. miltiorrhiza* extract also decreased testosterone, follicle-stimulating hormone, and luteinizing hormone. The studies did not mention any adverse events with *S. miltiorrhiza* extract. *Conclusion*. The current studies indicate that *S. miltiorrhiza* has beneficial effects on reproduction and glucose and lipid metabolism in patients with PCOS, and it is generally safe for clinical application. However, more prospective RCTs with large samples, multiple centers, and longer intervention duration are needed in the future to obtain more reliable conclusions.

1. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder with a prevalence ranging from 6% to 21% in adolescent and reproductive age women depending on the diagnostic criteria that are used [1, 2]. Hyperandrogenemia and metabolic abnormalities are closely related to the occurrence and development of PCOS [3]. Excessive androgens can cause premature follicular atresia, and this results in ovulatory dysfunction and stimulates vigorous sebaceous gland metabolism, subsequently leading to infertility, menstrual disturbances, acne, hirsutism, and other clinical symptoms. More than 60% of PCOS patients will be accompanied by obesity, which can lead to dyslipidemia, abnormal secretion of adipokines, and abnormal steroid metabolism, and obesity further aggravates the occurrence and development of PCOS and increases the risk for type 2 diabetes, hyperinsulinemia, hyperlipidemia, and cardiovascular diseases [4, 5]. Studies have shown that PCOS patients are mainly concerned with reproductive disorders in the early stages of the disease but then become more concerned with the metabolic abnormalities in the later stages [6]. PCOS seriously affects the quality of life of patients and imposes a heavy burden on their families and society as a whole. A Bayesian modelling study in the UK in 2018 showed that women with PCOS require at least £237 million in treatment costs every year [7]. Thus, it is important to develop methods to improve the fertility of PCOS patients, reduce androgen levels, and correct metabolic disorders, and find ways to delay the development of the disease.

At present, western medicine (WM) treatments for PCOS mainly include ovulation-inducing drugs, especially combination oral contraceptives and insulin-sensitization agents, which act on different mechanisms to improve the pathological manifestations of PCOS, but these drugs cause abnormal uterine bleeding, weight gain, gastrointestinal discomfort, liver damage, and other side effects [8]. Thus, many PCOS patients seek complementary and alternative medicine treatments to enhance the efficacy of their treatments and to improve their health. Recent studies have shown that nutritional supplements and herbal medicines as complementary and alternative medicines may help improve the health status of women with PCOS [9], and there is a growing body of evidence that puerarin, semen cuscutae flavonoids, berberine, glycyrrhetinic acid, tanshinone, and other Chinese herbal extracts have advantages in the treatment of this disease [10]. *Salvia miltiorrhiza (S. miltiorrhiza)*, also known as danshen in traditional Chinese medicine, is used for the treatment of cardiovascular and cerebrovascular diseases [11, 12]. The active components of *S. miltiorrhiza* are divided into two groups: water-soluble phenolics [13] and lipophilic tanshinones [14]. Tanshinones (including tanshinone I, tanshinone IIA, tanshinone IIB, cryptotanshinone, and dihydrotanshinone I) exhibit diverse biological activities such as androgen reduction [15] and improvement in glucose and lipid metabolism [16, 17], and they have antitumor [18], cardioprotective [19], and neuroprotective [20] effects. Especially in the last decade, several clinical trials have evaluated the efficacy of *S. miltiorrhiza* for treating PCOS. Cryptotanshinone has been shown to reverse androgen excess and ovarian insulin resistance (IR) in mice by activating the insulin signaling pathway via PI3K and regulating glucose transporter and hormone synthase activity [21]. In addition, Yang et al. [22] demonstrated that cryptotanshinone can effectively reduce serum luteinizing hormone (LH) levels, the LH/follicle-stimulating hormone (FSH) ratio, and testosterone (T) levels, thereby reducing the expression of HMGB1, TLR4, and NFκB/p65 in ovarian tissue and granulosa cells and improving the symptoms of PCOS.

Several randomized clinical trials (RCTs) have investigated the efficacy of *S. miltiorrhiza* in improving the pregnancy rate, fasting blood glucose, fasting insulin, body mass index (BMI), and T levels in patients with PCOS, but the results have been contradictory. Some reports indicated that the clinical pregnancy rate of PCOS patients is significantly increased after tanshinone capsule treatment [23]. However, Wang et al. [24] reported that the tanshinone capsule had no obvious effect on improving the pregnancy rate. In addition, other studies have shown that oral administration of tanshinone capsules in PCOS patients can reduce the fasting blood glucose level [13]. In contrast, Su et al. [25] did not show any effect of tanshinone capsule on fasting blood glucose levels in PCOS patients.

Due to the enthusiasm of PCOS patients for herbal therapy but the lack of strong evidence, evaluating herbal therapy is considered an appropriate research strategy because it may provide relevant answers to the questions of PCOS patients and clinicians. However, RCTs have shown contradictory reproductive and metabolic effects of *S. miltiorrhiza* in patients with PCOS. Moreover, the results of these RCTs may not be sufficient to draw firm conclusions in this regard. Therefore, in order to evaluate the effects of *S. miltiorrhiza* on the reproductive and metabolic outcomes of patients with PCOS more comprehensively and scientifically, we conducted a systematic review of the available RCTs in order to provide evidence-based medical guidelines for clinical practice.

2. Materials and Methods

This review was carried out according to the Cochrane recommendations and was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [26].

2.1. Search Methods. Published articles were searched for in PubMed, Embase, the Cochrane Library, the China National Knowledge Infrastructure (CNKI), the Wanfang Database, the Chinese Scientific Journal Database (VIP), and the Chinese BioMedical database (CBM). The retrieval time was from the establishment of each database to December 23, 2020, and there were no restrictions on the languages nor publication status. Keywords in the literature retrieval included "*Salvia miltiorrhiza*," "tanshinone," "cryptotanshinone," "tanshinone capsule," "danshen," "polycystic

ovary syndrome," "polycystic ovarian syndrome," "PCOS," "polycystic ovary changes," and related synonyms.

2.2. Study Selection. All trials included in our study met the following criteria. (1) Participants were women diagnosed with polycystic ovary syndrome according to the Rotterdam criteria [27] or the recommendation of the American Androgen Excess Society [28]. There was no limitation on nationality, race, physical characteristics, or course of the disease. (2) We accepted RCTs regardless of blinding procedures but only included parallel design studies. (3) The interventions included *S. miltiorrhiza* extract or *S. miltiorrhiza* extract combined with WM (unlimited dosage form, dose, or duration). (4) The control group should be placebo, WM, or placebo combined with WM. (5) The study included the following outcome indicators: reproductive indicators (pregnancy rate); glucose and lipid metabolism indicators (fasting blood glucose, fasting insulin, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C)); clinical symptoms (BMI, waist-to-hip ratio (WHR)); reproductive hormones (T, LH, and FSH); safety and adverse events data.

The exclusion criteria were as follows: (1) repeated publications or articles with unavailable data were excluded; (2) the article was an animal experiment, review, or case report; (3) other traditional Chinese medicine treatments such as acupuncture and massage were used; (4) trials were not RCTs or there were no criteria for how the trial was conducted.;(5) the full text could not be found.

2.3. Literature Screening and Data Extraction. The preliminary articles were imported into NoteExpressV3.3.0 for management. According to the inclusion and exclusion criteria, the titles and abstracts of the articles were read for preliminary screening after eliminating duplicated articles. The full text was then read during rescreening to identify the included articles. Data of each study were collected, including the following: study characteristics (primary author, publication year, study location, study design, and sample size), participant characteristics (mean age), intervention and comparison data (dose and treatment duration), and outcome measures. The results were extracted independently by two authors and then checked for consensus.

2.4. Quality Assessment. The methodological quality of the included RCTs was assessed for risk of bias in accordance with the RCT quality assessment criteria reported in the Cochrane Reviewer Handbook 5.0.0 [29] that focuses on the following six aspects: random sequence generation, assignment concealment implementation, blinding, data integrity, selective reporting with or without results, and other sources of bias, including whether there are clear inclusion/exclusion criteria, whether baseline data are comparable, and whether there is a conflict of interest. For each item, if satisfied, it means there is a "low risk of bias," while contradicted items mean "high risk of bias." When there is not enough information reported in the literature to allow one to make a clear judgment about an item, the item is classified as unclear, implying a moderate risk. The risk of bias for each qualifying study was independently assessed by two reviewers. If there was a disagreement, it was resolved through discussion or with the assistance of a third experienced researcher.

2.5. Data Analysis. RevMan 5.3.5 by the Cochrane Collaboration Network was used for meta-analysis. Binary variables are presented as the risk ratio (RR) with 95% confidence interval (CI), and continuous variables are expressed as the mean difference (MD) or standardized mean difference (SMD) with 95% CI. Heterogeneity was tested by the Q-test, in which I^2 was used to quantitatively estimate the magnitude of heterogeneity. When $I^2 \leq 50\%$ and $P \geq 0.10$, the fixed-effect model was used. If not, a random-effect model was used. If the heterogeneity was too large, the source of heterogeneity was identified and subgroup analysis or sensitivity analysis was conducted to determine the stability of the results of the meta-analysis.

3. Results

3.1. Literature Search Result. The initial search yielded 299 articles that met the search criteria, of which 115, 83, 17, 6, 49, 24, and 5 articles were from the CNKI, Wanfang, VIP, PubMed, CBM, Embase, and Cochrane Library, respectively. After removing duplicates, 199 articles remained. After scanning the titles and abstracts, we discarded 169 animal studies, evaluations, reviews, and clearly unqualified studies, leaving a total of 30 references for full-text review. After assessment according to the inclusion and exclusion criteria, 24 articles were further excluded, and 6 RCTs [23, 30–34] were included for analysis. The literature selection process is shown in Figure 1.

3.2. Features of the Included Literature. The six RCTs were published between 2015 and 2020 and had sample sizes ranging from 48 to 86, and the total number of involved patients was 390, including 195 in the intervention groups and 195 in the control groups. Two studies [32, 33] compared the efficacy of *S. miltiorrhiza* extract with placebo, and four studies [23, 30, 31, 34] compared the efficacy of *S. miltiorrhiza* extract in combination with WM versus WM alone. The characteristics of all the studies included in the meta-analysis are shown in Table 1.

3.3. Study Quality Assessment. Figure 2 summarizes the risk of bias of the six included RCTs. Four of the trials reported the random sequence generation methods, while the remaining two studies only mentioned "randomization" without describing specific randomization methods. Apart from Leila et al. 2020, the allocation concealment of the studies was not clearly defined. One study reported sample size estimates, and one study reported subjects falling off or being lost to follow-up. All studies were assessed as "low risk" for "selective reporting" because they reported the

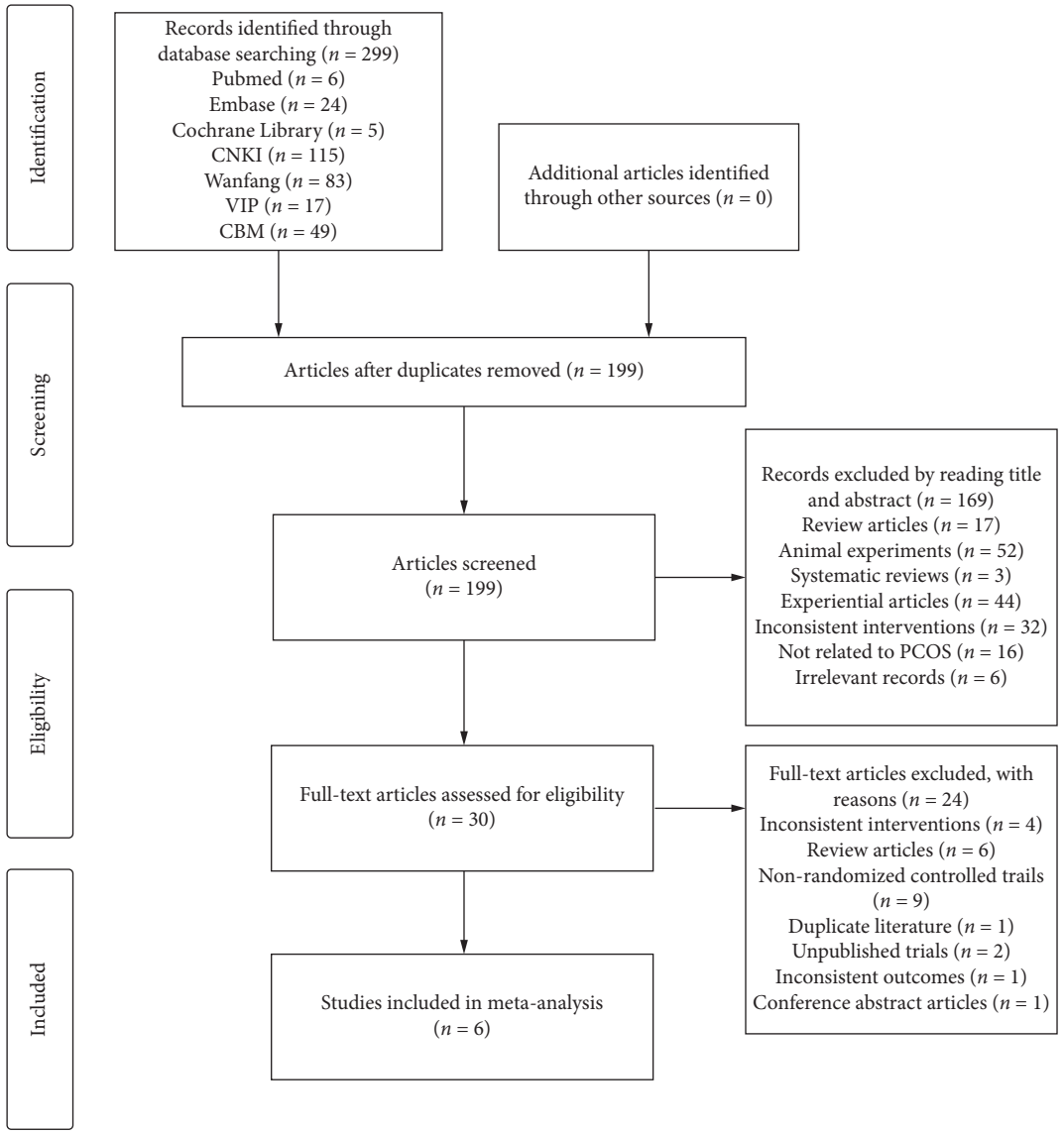


Figure 1: Flow diagram of the study selection process.

Table 1: The details of the included studies.

Authors/ published year	Study location	Study design	Sample size (T/C)	Mean age (T/C)	Intervention	Comparison	Dosage	Duration of treatment	Outcomes
Zhang et al. 2015 [33]	Jiangsu Province, China	RCT	24/24	21–34/20–31	*S. miltiorrhiza* extract	Placebo	100 mg, tid	3 months	BMI, WHR, fasting blood glucose, fasting insulin, TC, TG, HDL-C, LDL-C, T
Amini et al. 2020 [32]	Tehran, Iran	RCT	30/30	28.07 ± 4.18/ 29.23 ± 5.44	*S. miltiorrhiza* extract	Placebo	330 mg, qd	8 weeks	BMI, WHR, fasting blood glucose, fasting insulin

Table 1: Continued.

Authors/ published year	Study location	Study design	Sample size (T/C)	Mean age (T/C)	Intervention	Comparison	Dosage	Duration of treatment	Outcomes
Wu et al. 2016 [30]	Xinjiang Uygur Autonomous Region, China	RCT	36/36	28.2 ± 4.5	*S. miltiorrhiza* extract + CPA	CPA	*S. miltiorrhiza* extract 100 mg, tid CPA 1 pill, qd	3 months	TC, TG, HDL-C, LDL-C, LH
Li 2019 [31]	Henan Province, China	RCT	43/43	29.87 ± 4.23/ 30.02 ± 4.51	*S. miltiorrhiza* extract + CPA	CPA	*S. miltiorrhiza* extract 100 mg, tid CPA 1 pill, qd	3 months	TC, TG, HDL-C, LDL-C, T, LH, FSH
Shi et al. 2016 [34]	Sichuan Province, China	RCT	32/32	25.1 ± 3.8/ 25.0 ± 4.1	*S. miltiorrhiza* extract + CPA	CPA	*S. miltiorrhiza* extract 100 mg, tid CPA 1 pill, qd	2 months	Pregnancy rate, TC, TG, HDL-C, LDL-C
Wang et al. 2016 [23]	Jiangsu Province, China	RCT	30/30	18–37/ 20–38	*S. miltiorrhiza* extract + LET	Placebo + LET	*S. miltiorrhiza* extract 100 mg, tid LET NR	3 months	Pregnancy rate, BMI, fasting blood glucose, TC, TG, T, LH, FSH

CPA: cyproterone acetate; LET: letrozole; BMI: body mass index; WHR: waist-to-hip ratio; TC: total cholesterol; T: testosterone; TG: triglycerides; FSH: follicle-stimulating hormone; LH: luteotropic hormone; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; NR: not reported.

prespecified outcomes in the methods. Overall, the quality of six studies is low or remains unclear due to the high proportion of the unclear risk of biases in most studies.

3.4. Effects of S. miltiorrhiza on Pregnancy Rate. Two studies [23, 34] compared pregnancy rates and found no significant difference between the combination of *S. miltiorrhiza* extract + CPA versus CPA alone in improving the pregnancy rate [RR = 1.36, 95% CI (0.83, 2.21), $P = 0.22$]. However, the combination of *S. miltiorrhiza* extract + letrozole (LET) was superior to LET alone in improving the pregnancy rate [RR = 2.60, 95% CI (1.06, 6.39), $P = 0.04$].

3.5. Effects of S. miltiorrhiza on Reproductive Hormones. *S. miltiorrhiza* extract showed a significant reduction of T [SMD = –3.31, 95% CI (–3.90, –2.72), $P < 0.00001$], LH [MD = –6.40, 95% CI (–8.32, –4.48), $P < 0.00001$], and FSH [MD = –5.70, 95% CI (–6.18, –5.22), $P < 0.00001$] versus the placebo group. Moreover, the combination of *S. miltiorrhiza* extract + CPA significantly decreased T [SMD = –4.13, 95% CI (–4.89, –3.37), $P < 0.00001$] and FSH [MD = –0.97, 95% CI (–1.59, –0.35, $P = 0.002$], while LH was slightly decreased, but not significantly [MD = –2.84, 95% CI (–5.77, 0.08), $P = 0.06$], in comparison to the CPA group (Figure 3).

3.6. Effects of S. miltiorrhiza on Glucose Metabolism. Compared with the placebo group, PCOS patients treated with *S. miltiorrhiza* extract had significantly lower fasting blood glucose [MD = –0.25, 95% CI (–0.37, –0.13), $P < 0.0001$] and fasting insulin [MD = –1.16, 95% CI (–1.74, –0.58), $P < 0.0001$] (Figure 4).

3.7. Effects of S. miltiorrhiza on Lipid Metabolism. Two RCTs [23, 33] in the analysis compared the effect of *S. miltiorrhiza* extract and placebo on lipid metabolism in PCOS patients. As shown in Figure 5, the *S. miltiorrhiza* extract group was more effective in reducing TC [MD = –0.58, 95% CI (–0.72, –0.43), $P < 0.00001$], TG [MD = –0.31, 95% CI (–0.35, –0.26), $P < 0.00001$], and LDL-C [MD = –0.80, 95% CI (–0.97, –0.63), $P < 0.00001$] and increasing HDL-C [MD = 0.23, 95% CI (0.18, 0.28), $P < 0.00001$]. Our meta-analysis showed that the combination of *S. miltiorrhiza* extract + CPA significantly decreased TC [MD = –0.77, 95% CI (–0.89, –0.65), $P < 0.00001$], TG [MD = –0.43, 95% CI (–0.65, –0.20), $P < 0.0001$], and LDL-C [MD = –0.49, 95% CI (–0.66, –0.33), $P < 0.00001$] and increased HDL-C [MD = 0.30, 95% CI (0.20, 0.40), $P < 0.00001$] compared with CPA alone (Figure 5).

3.8. Effects of S. miltiorrhiza on Clinical Symptoms. As shown in Figure 6(a), the three RCTs combined did not show any significant change in BMI in PCOS patients after treatment with *S. miltiorrhiza* extract versus placebo [MD = –1.41, 95% CI (–4.81, 2.00), $P = 0.42$]. In addition, there was no evidence that *S. miltiorrhiza* extract was associated with improved WHR compared to placebo [MD = –0.02, 95% CI (–0.05, 0.01), $P = 0.16$] (Figure 6(b)).

3.9. Safety Outcomes. Only the study by Zhang et al. reported no adverse events, and the other studies did not report any information about adverse events.

3.10. Sensitivity Analysis. Sensitivity analysis revealed that excluding individual studies did not remarkably influence the overall effect size of TC, TG, LDL-C, and HDL-C. In

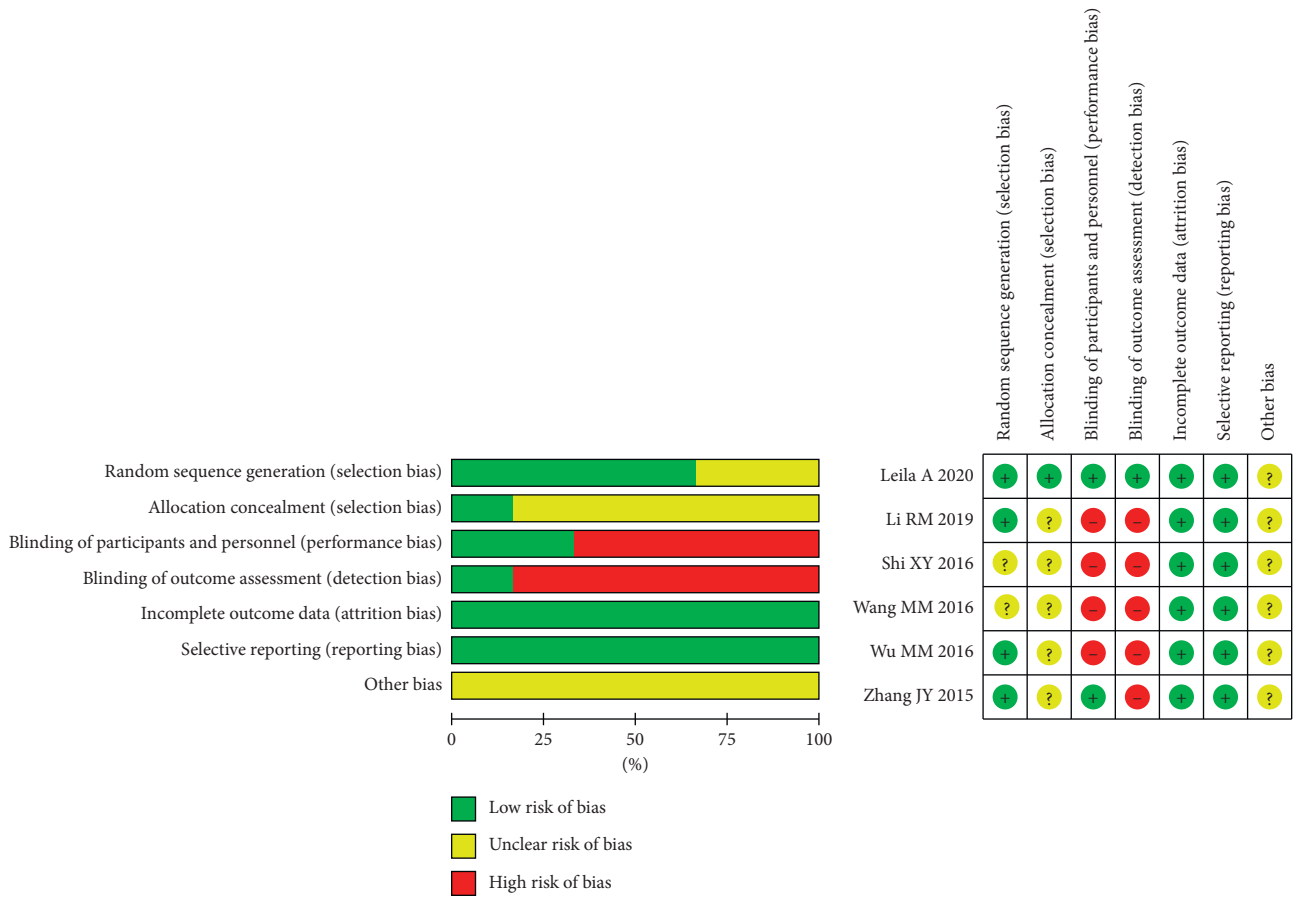


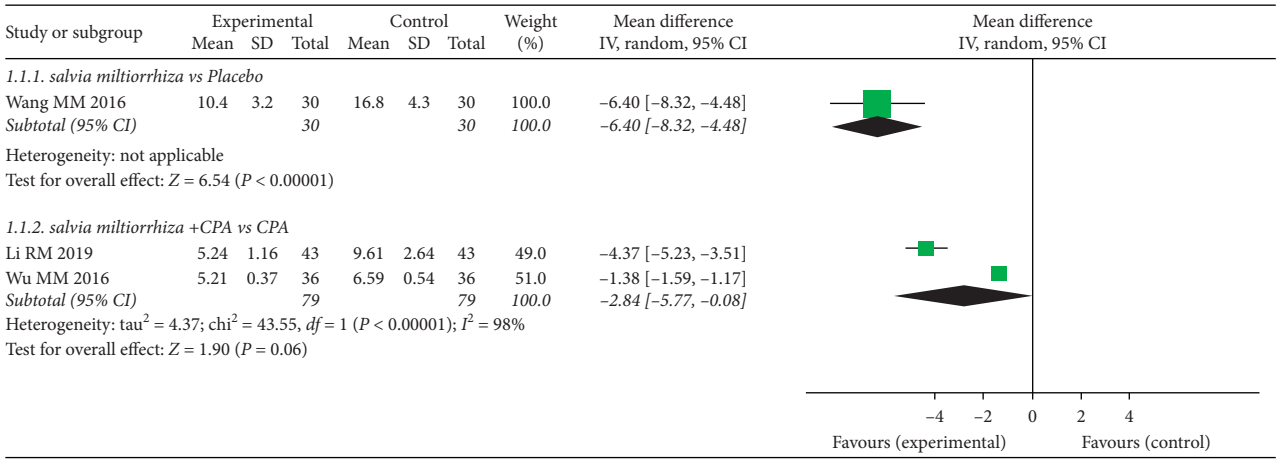
Figure 2: The risk of bias for the included studies.

addition, removing Wu et al. [30], which reported on the effect of *Salvia miltiorrhiza* extract on lipid metabolism, resulted in a decrease in heterogeneity, whereas the result remained significant, TC (95% CI [−0.81, −0.60], $I^2 = 0\%$), TG (95% CI [−0.66, −0.38], $I^2 = 72\%$), LDL-C (95% CI [−0.77, −0.32], $I^2 = 77\%$), and HDL-C (95% CI [0.28, 0.39], $I^2 = 0\%$).

4. Discussion

To the best of our knowledge, this systematic review of six RCTs is the latest and most comprehensive data analysis of *S. miltiorrhiza* in the treatment of PCOS so far, and it was designed to evaluate the reproductive and metabolic effects of *S. miltiorrhiza* in women with PCOS. Compared with the control group, *S. miltiorrhiza* extract may improve pregnancy rate; decrease T, FSH, fasting blood glucose, fasting insulin, TC, TG, and LDL-C; increase HDL-C. However, there is no strong evidence that *S. miltiorrhiza* extract has an effect on BMI and WHR. For patients with PCOS, excessive androgen and insulin resistance can reduce endometrial function leading to decreased fertility [35–37]. Based on current evidence, *S. miltiorrhiza* may be recommended for the treatment of PCOS patients with a desire for fertility and/or those with hyperandrogenism and metabolic disorders.

Based on our results, oral *S. miltiorrhiza* extract appears to have an effect on the pregnancy rate in PCOS patients. *S. miltiorrhiza* may improve fertility through various possible mechanisms. For example, when the endometrial microcirculation is disturbed, the implantation rate is low, and *S. miltiorrhiza* can modify endometrial microcirculation-related indicators, such as the endometrial pulsatility index and resistance index, and thus improve endometrial receptivity to provide a good environment for embryo implantation [38, 39]. It also improves the efficacy of PMSG for increasing the pregnancy rate [40]. In addition, traditional Chinese medicine decoctions containing *S. miltiorrhiza* have been shown to improve reproductive outcomes by regulating the expression of proteins such as integrin, vascular endothelial growth factor, and uncoupling protein 2, which are closely related to endometrial receptivity [41]. Hyperandrogenemia is the main endocrine characteristic of PCOS. Hyperinsulinemia and IR are considered to be the main causes of hyperandrogenemia [35, 36], while excessive androgen leads to increased levels of LH and FSH [37]. In PCOS, theca cells and granulosa cells overexpress mRNA encoding enzymes involved in steroidogenesis, including androgen receptor, CYP11, CYP17, and CYP19, and this can lead to disturbances in ovarian hormone synthesis. The recent studies have shown that *S. miltiorrhiza* extract can reverse reproductive disorders by regulating the expression of androgen receptor, CYP11, CYP17, and CYP19, thus improving reproductive hormone production such as T, LH, and FSH [15, 21].



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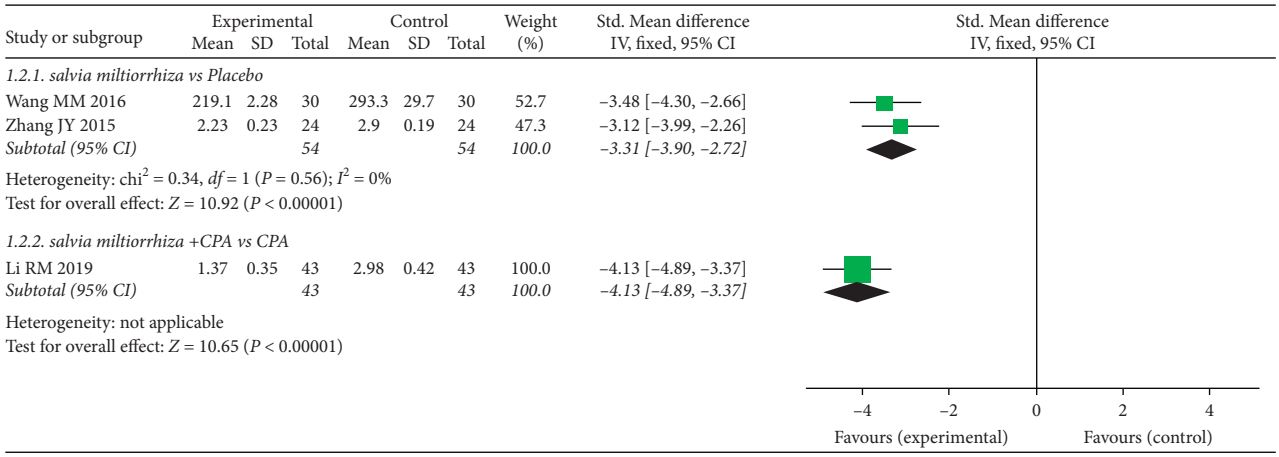
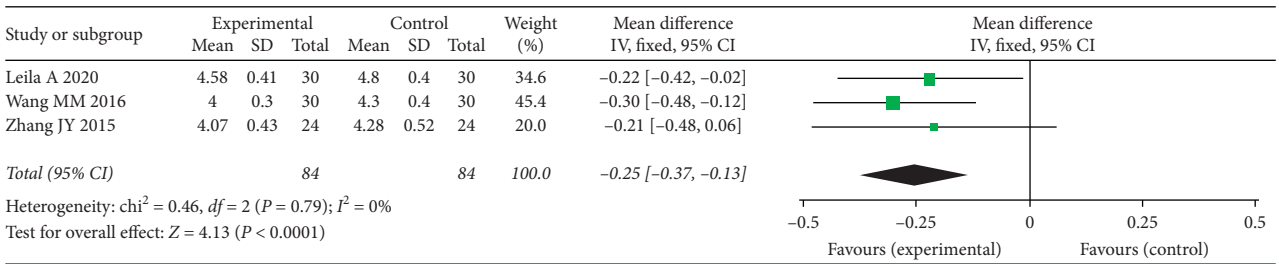


(b)

Figure 3: Meta-analyses of the effect of *S. miltiorrhiza* on reproductive hormones: (a) LH; (b) T.



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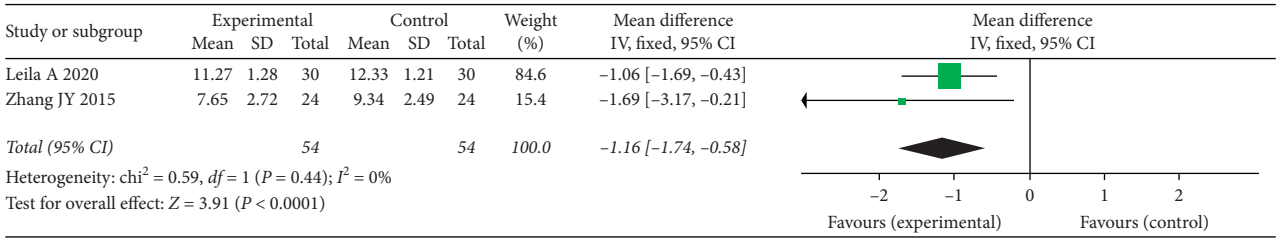

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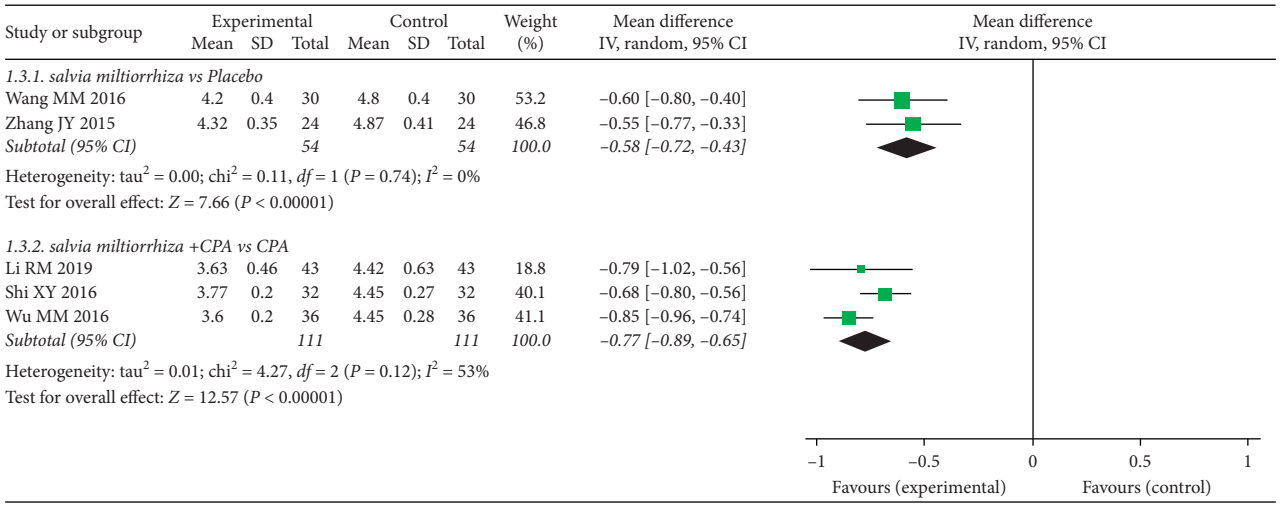
Figure 4: Meta-analyses of the effects of *S. miltiorrhiza* on glucose metabolism indexes: (a) fasting blood glucose; (b) fasting insulin.



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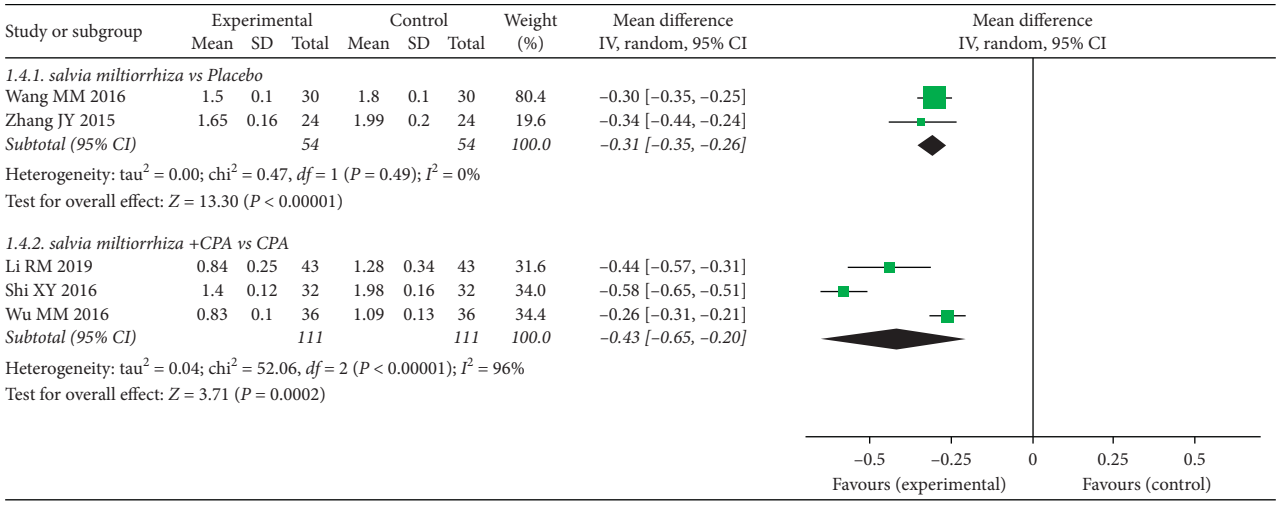

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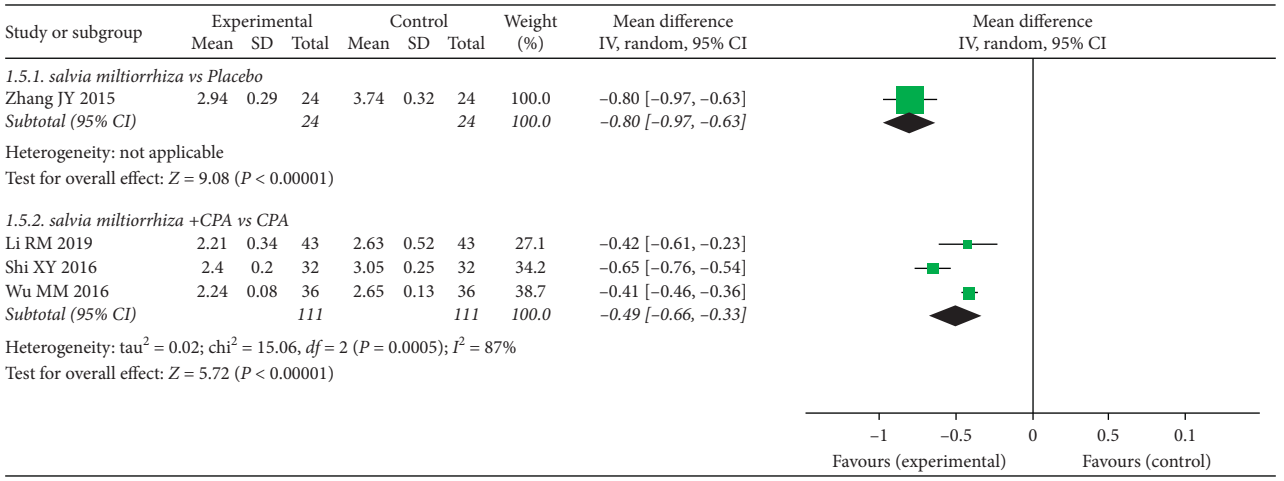
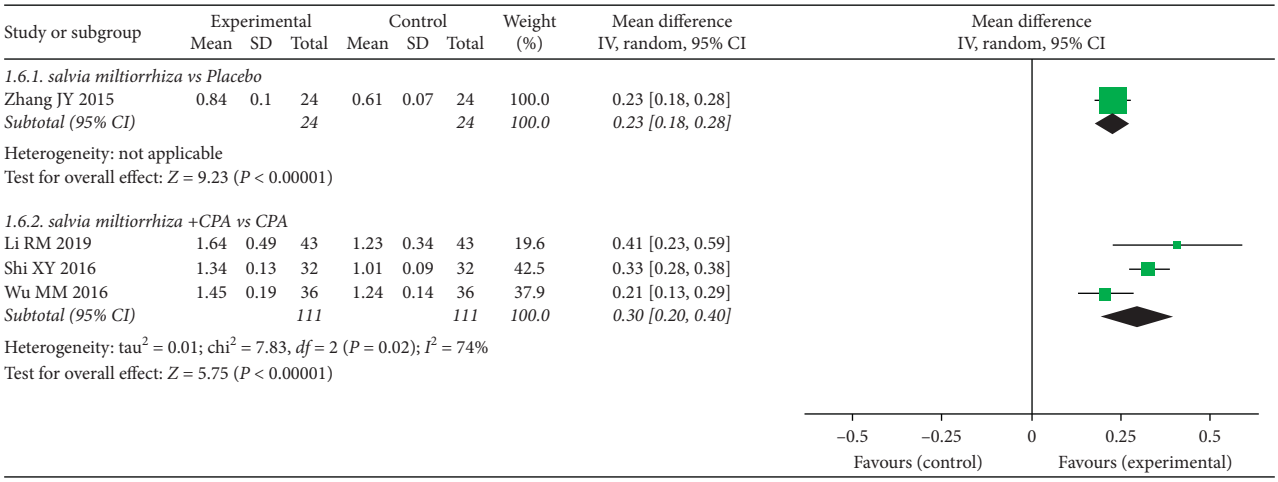


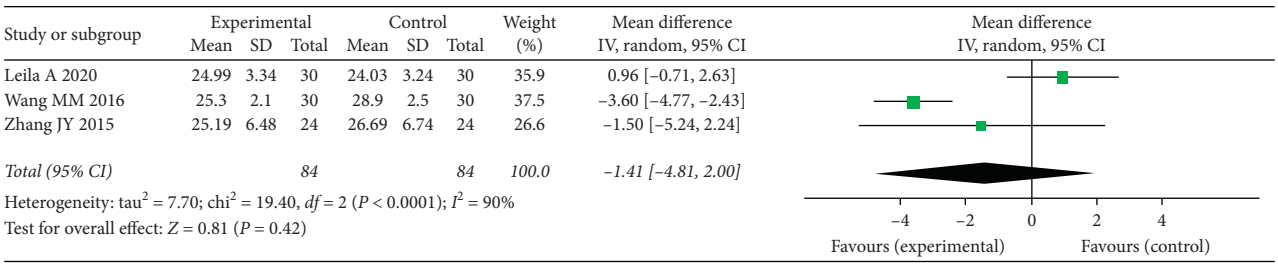
(c)

Figure 5: Continued.

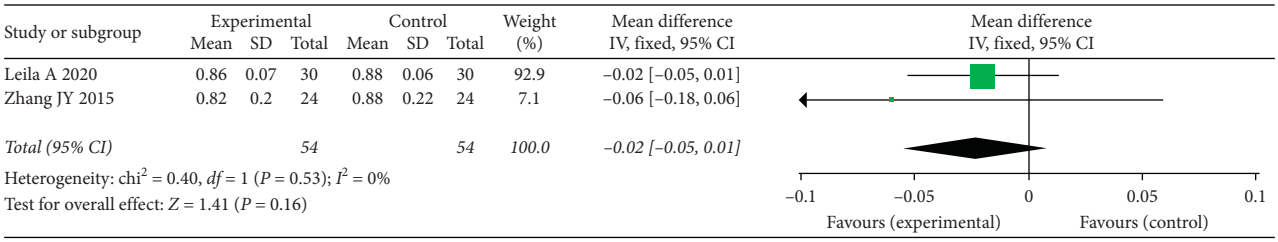


(d)

Figure 5: Meta-analyses of the effects of *S. miltiorrhiza* on lipid metabolism indexes: (a) TC; (b) TG; (c) LDL-C; and (d) HDL-C.



(a)



(b)

Figure 6: Meta-analyses of the effect of *S. miltiorrhiza* on (a) BMI and (b) WHR.

Another important result to take into consideration is the positive effect of *S. miltiorrhiza* extract on lipid profiles in PCOS patients. Animal and experimental studies have shown that *S. miltiorrhiza* has antiobesity effects, and in vitro studies have shown that *S. miltiorrhiza* inhibits adipogenesis in 3T3-L1 preadipocytes, with this inhibition mainly occurring at an early phase of adipogenesis through the attenuation of mitotic clonal expansion via cell cycle arrest at the G1/S phase transition [42]. It also can suppress adipogenesis and reduce obesity-related metabolic disorders by acting on PPARγ, C/EBPα, GATA-2, and GATA-3 [43]. Tanshinone IIA also suppresses fatty acid-induced lipogenesis and TG accumulation in HepG2 cells [44], and a recent study has shown that *S. miltiorrhiza* significantly decreases TC, TG, and LDL-C levels in mice by inhibiting the expression of FAS mRNA and HMGR mRNA [45]. Furthermore, *S. miltiorrhiza* extract reduces the upregulation of SREBP1 and TG induced by high glucose in LO2 cells, which improves lipid metabolism, and the underlying mechanism is probably through the regulation of STAT-3 signaling [46]. Lastly, *S. miltiorrhiza* can activate the estrogen receptor through the ERK signaling pathway, reducing lipid deposition in the aorta [16].

Beyond its effects on lipid profiles, *S. miltiorrhiza* has positive effects on other aspects of PCOS patients' health. PCOS is closely related to metabolic syndrome [47, 48], and several studies have demonstrated the different effects of *S. miltiorrhiza* on hypoglycemia and hypoinsulinemia. Adiponectin (APN) and leptin are closely related to obesity [49]. The imbalance of adipose cytokines such as leptin and adiponectin secreted by adipose tissue in obese patients also aggravates the IR associated with PCOS [50]. *S. miltiorrhiza*

extract increases the sensitivity to insulin by inducing the production and secretion of anti-inflammatory adipokines (such as APN), and it reduces inflammation and the production of proinflammatory cytokines [51, 52]. Tanshinone I may also decrease fasting blood glucose concentrations by decreasing levels of interleukin-6 and tumor necrosis factor-alpha and by reducing the nuclear translocation of NF-κB and the phosphorylation of Ser307 on insulin receptor substrate 1 (IRS-1) [42]. It is also suggested that the hypoglycemic effect of *S. miltiorrhiza* extract is likely to be secondary to an action on carbohydrate metabolism [53]. The observed decrease in fasting blood glucose and fasting insulin concentrations may be a result of cryptotanshinone rescuing the altered protein expressions of IRS-1 and IRS-2, phosphatidylinositol 3-kinase p85α, glucose transporter-4, ERK-1, and 17α-hydroxylase [54]. Lastly, cryptotanshinone stimulates insulin signaling and the regulation of glucose transporters and hormone-synthesizing enzymes, which reverses ovarian IR in mice [21].

There is no strong evidence in our analysis that *S. miltiorrhiza* extract has effects on BMI and WHR. The only exception was the study by Wang et al. [23], who found that the *S. miltiorrhiza* extract group significantly reduced BMI compared with placebo.

Among the included studies, Zhang et al. [33] reported that no adverse reactions occurred during the treatment, such as gastrointestinal discomfort, skin rashes, dizziness and headaches, blood system changes, and liver and kidney damage, while the other studies did not mention anything about adverse events at all. In addition, a more recent study [55] has shown that *S. miltiorrhiza* has the advantages of strong activity, low toxicity, low side effects, and extensive pharmacological effects. Therefore, we believe that *S. miltiorrhiza* is a relatively safe treatment, although more clinical studies are needed to confirm the safety of *S. miltiorrhiza* in long-term treatment.

This review included six recent RCTs (2015–2020), and limitations include the small number of studies, the small sample sizes, the lack of blinding in most of the studies, missing descriptions of the randomization method in some studies, and results with high heterogeneity (e.g., TC, TG, LDL-C, and HDL-C). Bias risk sensitivity analysis was used to investigate the high heterogeneity. After the study by Wu et al. was excluded, TC and HDL-C showed homogeneity. The heterogeneity might therefore be related to the place where the experiment took place, the subjects' dietary habits, and their own constitutions. The interventions included in this study included *S. miltiorrhiza* extract combined with CPA or LET. CPA was used as a combination oral contraceptive and LET was used as an ovulation-induction drug. The mechanism of action and the goal in clinical application of the two are different, so the data from these studies were not combined. The study time was generally short to medium term (mostly 3 months), and there was a lack of follow-up observation of the long-term efficacy of *S. miltiorrhiza*. In addition, the adverse effects of *S. miltiorrhiza* on PCOS are unknown because most trials did not report adverse events, and caution should be used in interpreting the safety of *S. miltiorrhiza*. Finally, we still need a large sample, multiple center, and scientifically validated RCTs to further verify the efficacy of *S. miltiorrhiza* in the treatment of PCOS and to provide solid and reliable evidence for clinical practice.

5. Conclusion

In summary, the results of the present systematic review and meta-analysis indicate that *S. miltiorrhiza* has beneficial effects on reproduction and glucose and lipid metabolism in patients with PCOS. Moreover, our results demonstrate that the clinical application of *S. miltiorrhiza* is generally safe. However, due to the relatively low quality of the included studies, we urge caution in promoting these results. More prospective RCTs with large samples, multiple centers, and longer intervention durations are warranted in the future to obtain more scientific, objective, and reliable conclusions.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Wenjuan Shen, Bao Jin, and Yaguang Han are co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Complementary and Alternative Medicine for the Treatment of Abnormal Endometrial Conditions in Women with PCOS: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Background. Endometrial lesions in patients with polycystic ovary syndrome (PCOS) exhibit complex pathological features, and these patients are at risk of both short-term and long-term complications. Complementary and alternative medicine (CAM), which is gradually becoming more accepted and is believed to be clinically effective, claims to be promising for treating PCOS, and thus its effect on the abnormal endometrium of PCOS patients should be assessed. The present meta-analysis sought to evaluate the efficacy and safety of CAM in treating endometrial lesions in patients with PCOS. *Methods*. Randomized trials on CAM were identified in four Chinese and seven English-language databases from their establishment to January 2020. The present study included patients diagnosed with PCOS and abnormal endometrial conditions who underwent CAM therapy independently or in combination with traditional western medicine. Data were extracted, and the Cochrane "risk of bias" tool was used to assess methodological quality. Effects were expressed as the relative risk (RR) or mean difference (MD/SMD) with 95% confidence interval (CI) as calculated with Rev Man 5.3. *Results*. A total of 13 randomized controlled trials were included, involving 1,297 PCOS patients treated for endometrial abnormalities. Methodological quality was generally unclear or had a low risk of bias. The trials tested four different types of CAM therapies (i.e., traditional Chinese medicine treatment, acupuncture treatment, traditional Chinese medicine in combination with western medicine treatment, and acupuncture in combination with western medicine treatment). CAM treatment could significantly reduce the endometrial thickness in PCOS patients compared to western medicine alone (SMD −0.88, 95% CI [−0.12, −0.57]; $I^2 = 64\%$). Compared with clomiphene treatment for the induction of ovulation, CAM treatment showed a clear improvement in endometrial thickness during ovulation (SMD 2.03, 95% CI [1.64, 2.02]; $I^2 = 48\%$). Moreover, CAM was more effective than western medicine alone in reducing the endometrial spiral artery pulsatility index. No significant difference was seen between CAM and traditional treatment when these were used to improve traditional Chinese medicine syndrome scores. Acupuncture alone or traditional Chinese medicines (taken orally) in combination with western medicine significantly increased the pregnancy rate of PCOS patients (RR 1.59, 95% CI [1.30, 1.93]; $I^2 = 51\%$, $P < 0.00001$), and CAM was more effective than western medicine alone for improving hormone levels. No serious adverse events were reported in 11 of the 13 trials. *Conclusions*. CAM may effectively ameliorate the endometrial condition of PCOS patients, and it can regulate the level of hormone secretion to increase the ovulation rate and the pregnancy rate.

1. Introduction

Polycystic ovary syndrome (PCOS) is a gynecological endocrine disease that is characterized by oligo-ovulation, hyperandrogenemia, and hyperinsulinemia. The prevalence of PCOS has been estimated to be 6–12% in women of childbearing age worldwide [1–3], and the rate in China is about 5.6% [4]. PCOS patients are at increased risk for various complications (e.g., insulin resistance and endometrial abnormalities) along with the typical clinical characteristics of polycystic ovaries, sparse ovulation, and abnormal hormone levels. Changes in the endometrium are among the most common clinical manifestations and complications in PCOS patients. The endometrium of PCOS patients tends to exhibit pathological hyperplasia (e.g., simple hyperplasia, complex hyperplasia, or atypical hyperplasia) [5] due to the long-term exposure to estrogen and the lack of regular progesterone antagonism. As indicated from a previous meta-analysis, PCOS patients are at a higher risk of endometrial cancer, suggesting that long-term pathological endometrial hyperplasia contributes strongly to the development of endometrial cancer [6]. Likewise, PCOS patients suffering from insulin resistance are likely to experience accelerated proliferation of endometrial cells as well as an increased likelihood of long-term complications. In contrast, PCOS patients with infertility suffer from relatively poor endometrial conditions, thereby significantly reducing their pregnancy rate and live birth rate and adversely affecting their health status and their personal family life. When providing treatments for ovulation induction, variations in uterine receptivity have a significant effect on pregnancy outcome [7]. However, there are some indications that modern medical treatments might adversely affect endometrial receptivity in infertile PCOS patients [8]. CAM, which is commonly used to treat PCOS, has been shown to have a positive effect on controlling patients' weight, body mass index, sleep quality, ovulation rate, quality of life, etc. [9–12]. However, no systematic review or research has been conducted on the effects of CAM on the endometrium in PCOS patients, and the endometrium has rarely been discussed as the main outcome index. Therefore, this study undertook a comprehensive literature search on CAM for endometrial intervention in PCOS patients and carried out a systematic review and meta-analysis to supplement the existing evidence in order to determine the contribution of CAM for endometrium abnormalities in PCOS patients and to underpin the clinical treatment of long-term endometrial complications and infertility. Furthermore, only randomized controlled trials (RCTs) were included in the systematic review.

Because PCOS exhibits obvious heterogeneity and because diagnostic standards vary in different regions, this study only included cases that were diagnosed according to the joint criteria of the European Society of Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM) established in Rotterdam in 2003 [13] or according to the Chinese Health Industry Standard WS330-2011: Diagnosis of Polycystic Ovary Syndrome issued by the Chinese Ministry of Health in 2011 [14]. The types of intervention consisted of CAM methods used alone or in combination with traditional western medicine therapy for PCOS.

The concept of CAM has numerous meanings. This study attempted to include as many types of CAM therapies as possible in the literature retrieval in order to avoid any bias in the results due to the omission of therapies. According to the existing research, this study included the following treatment methods within the scope of CAM to treat PCOS: traditional Chinese medicine (TCM), acupuncture, moxibustion, diet suggestions/restrictions, psychological counseling, exercise therapy, and other known CAM methods for treating PCOS [15–19].

2. Method

2.1. Search Strategy. A systematic literature search was conducted in four Chinese databases (CNKI, WANFANG, VIP, and SINOMED) and seven English databases (PubMed, EMBASE, Web of Science, ProQuest Research Library, Medline, Elsevier/ScienceDirect, and The Cochrane Library) from their time of establishment to January 2020. In addition, gray literature was searched (e.g., meeting minutes). We searched using different combinations of key words, including "polycystic ovary syndrome", "endometrium", "complementary and alternative medicine", "traditional Chinese medicine", "acupuncture", "moxibustion", "exercise therapy", and "diet intervention".

2.1.1. Literature Selection and Data Extraction. Two authors (J. Y. Xu and W. J. Fu) independently checked the full text to identify qualified RCTs, and four authors (J. Y. Hu, J. Y. Xu, S. X. Liu, and S. Y. Hu) collaborated with each other to extract data from the included articles according to the predesigned data table. Any conflicts were resolved through discussions with the third author (F. J. Han). The following items were extracted: year of publication, type of study, funding, inclusion/exclusion criteria, diagnostic criteria, research methods, demographic characteristics of the participants, details of the intervention and control, methods of outcome measurement, and adverse events and outcomes.

2.1.2. Quality Assessment. Two authors (J. Y. Hu and J. Y. Xu) used the "risk of bias tool" [20] to assess the methodological quality of the included RCTs. The RCTs were judged as "low risk", "high risk", or "uncertain risk" based on risks involving random sequence generation, assignment concealment, blindness of participants and personnel, blindness of the outcome assessment, incomplete data, selective reporting, and other biases (e.g., drug company funding). Any conflicts were resolved through discussions with the third author (F. J. Han).

2.2. Data Analysis. This study used the Rev Man 5.3 software for all data analysis. For continuous data, the mean difference (MD/SMD) and 95% confidence interval (CI) were calculated, while for binary data the relative risk (RR) and

95% CI were calculated. If similar study designs, participants, interventions, controls, and outcome indicators were found, then those trials were included in a meta-analysis. Mega data were generated by descriptive counting. Other data not suitable for combination analysis were qualitatively synthesized. In compliance with the recommendations of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011), we used the I^2 test for statistical heterogeneity. If I^2 is greater than 50%, this indicates that there may be substantial heterogeneity [20], so we used the random effects model for data pooling with significant heterogeneity ($I^2 \geq 50\%$); otherwise, we used the fixed effect model. If data were available, a subgroup analysis was conducted on the subcategories of CAM and a sensitivity analysis was conducted to explore the impact of the type of RCT (parallel or cross randomized) and the quality of the trial (high or low). If more than ten trials were included in the meta-analysis, a funnel chart was generated to explore possible publication bias.

2.3. Outcomes. The main analysis included the treatment outcome indicators as measured by one or more of the following items: endometrial thickness (ovulation or luteal metaphase); endometrial type and ovulation rate detected by ultrasound; the levels of sex hormones (mainly follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2)) as measured by chemiluminescence immunoassay; the number of pregnancies (or pregnancy rate) as measured by the level of human chorionic gonadotropin (HCG) and by ultrasound; and the type, number, and probability of adverse reactions. The secondary results included the endometrial spiral artery pulsatility index (PI) and endometrial spiral artery resistance index (RI) of the spiral uterine artery as detected by ultrasound, the cervical mucus score as measured by the Insler cervical scoring method, the early spontaneous abortion rate, the proportion of participants with ≥50% improvement in symptoms and signs according to the assessment of clinicians, and the number or probability of patients with a TCM syndrome differentiation type showing improvement in the TCM syndrome score.

3. Results

A total of 1,633 articles were retrieved, including 1,001 in Chinese and 632 in English. After the titles and abstracts were browsed, 1,184 cited trials were excluded due to involving in vitro research, being a dissertation or being non-RCT research, and 352 were duplicates. Among the 97 eligible studies, 34 were excluded for having unreasonable random distribution methods, 12 for having incomplete data or missing outcome indicators, 28 for not mentioning the diagnostic criteria or the inclusion criteria, and 10 for lacking clear methods or criteria for outcome indicators. Finally, 13 trials [21–33] including 1,297 PCOS patients were included in the present review (Figure 1). Twelve of the included trials were in Chinese and one was in English, and all of the studies included patients from mainland China and were carried out by researchers and scholars in mainland China.

The characteristics of the 13 RCTs are shown in Table 1. The sample sizes of the included studies ranged from 56 to 198 participants who ranged in age from 17 to 38 years. In all 13 trials, clinical western medicine treatment for PCOS (e.g., clomiphene and metformin) was used as the western medicine control group, and these included both single drug treatments and multiple drug combinations. In three trials [24, 26, 33], the patients were treated by using CAM alone, with one study using acupuncture alone [33] and two using TCM alone [24, 26]. The remaining 10 trials [21–23, 25, 27–33] used CAM in combination with western medicine. The western medicine control group consisted of clomiphene, metformin, Diane-35, or letrozole alone as well as their combinations with human menopausal gonadotropin (HMG) and HCG.

3.1. Bias Risk in the Trials. Three trials [26, 28, 31] were considered to have "unclear" selection bias risk because they only mentioned "random" without describing any specific method of randomization, while the remaining ten trials [21–25, 27, 29, 30, 32, 33] were considered to have a "low" selection bias risk because the methods for generating the random sequence were mentioned (random number table). In one trial [25] considering a "high" reporting bias risk, a case was withdrawn, and this might have led to incomplete follow-up data. The other 12 trials that did not have any case withdrawals included follow-up information [21–24, 26–33] and were considered to have "low" reporting bias risk. Four trials [25, 26, 29, 32] did not report specific details of sample size calculation and were defined as "unclear" risk of other bias (Figures 2 and 3).

3.1.1. Endometrial Thickness. Four studies comparing CAM with western medicine treatment and involving 511 patients [21, 26, 27, 32] showed that CAM treatment can significantly reduce the endometrial thickness resulting from abnormal hyperplasia in PCOS patients (SMD −0.88, 95% CI [−0.12, −0.57]; $I^2 = 64\%$) (Figure 4). A comprehensive analysis was performed on the nine articles studying endometrial thickness after CAM alone or in combination with western medicine treatment in PCOS patients with infertility [22–25, 28–31, 33], and the results were found to be highly heterogeneous (SMD 1.23, 95% CI [0.50, 1.96]; $I^2 = 95\%$) (Figure 5(a)). A subgroup analysis was conducted, suggesting that CAM treatment compared with clomiphene therapy for ovulation stimulation significantly increased the endometrial thickness during ovulation (SMD 2.03, 95% CI [1.64, 2.02]; $I^2 = 48\%$) (Figure 5(b)) [24, 33].

3.1.2. Type of Endometrium. In three articles involving 358 patients [23, 25, 33], CAM alone (acupuncture) and CAM (oral TCM) in combination with western medicine effectively increased the number of PCOS patients with type A endometrium compared with clomiphene (RR 1.44, 95% CI [1.22, 1.69]; $I^2 = 0\%$, $P < 0.0001$) (Figure 6). In two articles

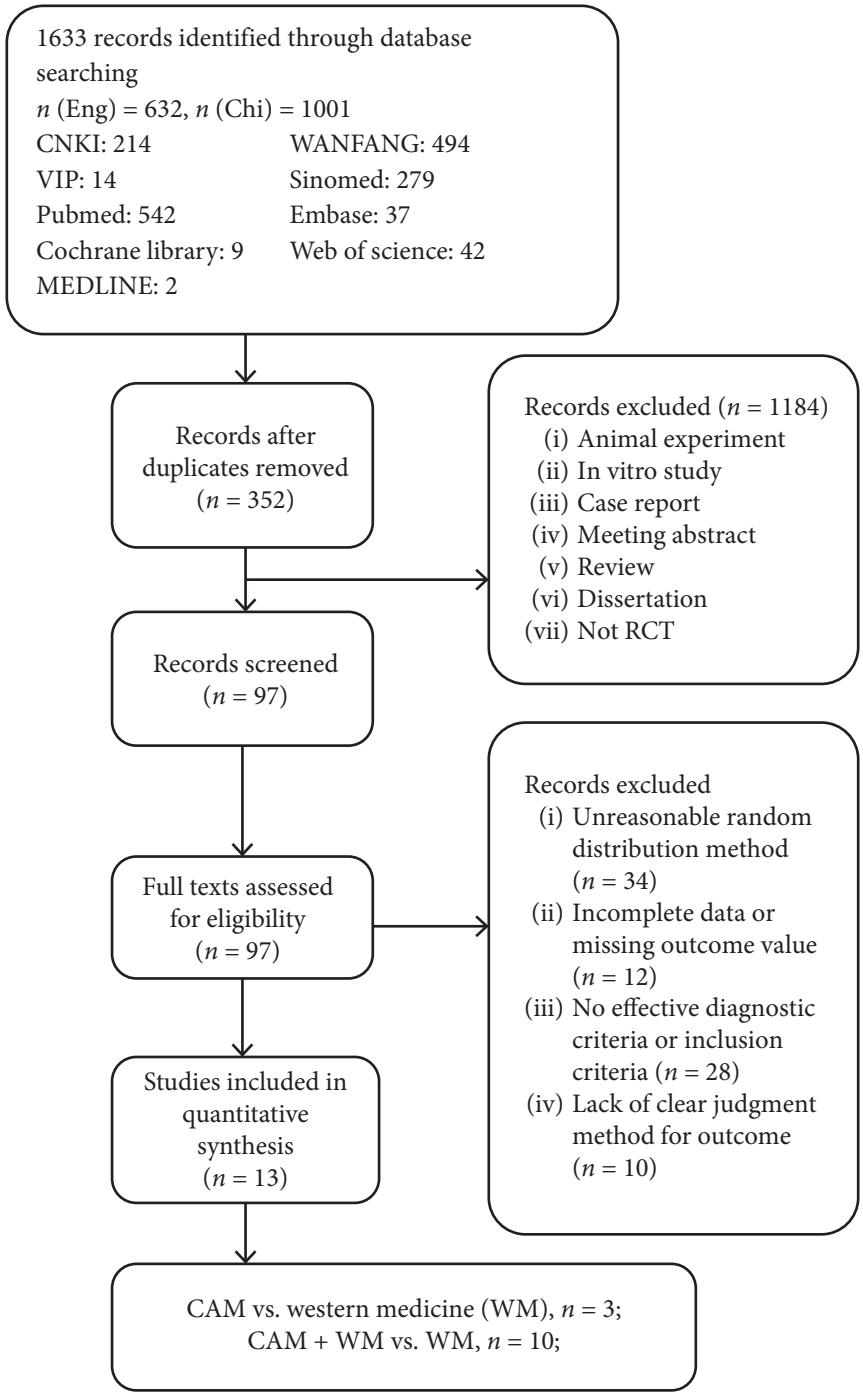


Figure 1: Flow diagram of study selection and different subgroup interventions included in this review.

[23, 33], both acupuncture alone and oral TCM combined with clomiphene significantly downregulated the number of cases of type B and type C endometrium in PCOS patients (RR 0.27, 95% CI [0.10, 0.73]; $I^2 = 60\%$, $P = 0.01$) (Figure 7).

3.1.3. PI and RI. In two trials involving ovulation stimulation in 158 PCOS patients [29, 31], oral TCM in combination with western medicine could effectively reduce the PI compared with western medicine alone (MD −0.27, 95% CI [−0.38, −0.16]; $I^2 = 10\%$, $P < 0.00001$) (Figure 8) but had no significant effect on RI (MD −0.11, 95% CI [−0.22, 0.00]; $I^2 = 0\%$, $P = 0.05$) (Figure 9).

3.1.4. Hormone Levels. Seven trials involving 693 patients [21, 23, 24, 27, 30, 32, 33] showed that CAM alone or CAM in combination with western medicine clearly reduced FSH levels compared with clomiphene and Diane-35 (SMD −0.18, 95% CI [−0.13, −0.33]; $I^2 = 46\%$) (Figure 10). The LH levels in patients treated with TCM or acupuncture in combination with western medicine were not statistically different

Table 1: Characteristics of included randomized clinical trials on CAM therapies for PCOS abnormal endometrial conditions.

Study ID	Sample size	Age	Comparisons	Outcome	Follow-up
CAM vs. WM, 3 studies					
Fang et al. [24]	T: 28°C: 28	T: 27.54 ± 3.65 years C: 28.24 ± 4.36 years	Chinese medicine prescription vs. clomiphene (3 m)	①②③⑧	NR
Li [26]	T: 74°C: 74	T: 27.8 ± 3.50 years C: 28.0 ± 3.50 years	Chinese medicine prescription + progesterone capsule (under certain conditions) vs. HMG + HCG + progesterone capsule (under certain conditions) (3–6 m)	①③⑥⑨	NR
Zhuo [33]	T: 50°C: 50	T: 29 ± 5 years C: 28 ± 5 years	Acupuncture vs. clomiphene (3 m)	①②④⑤⑪⑫	NR
CAM + WM vs. WM, 10 studies					
Chen et al. [22]	T: 30°C: 30	T: 28.63 ± 0.73 years C: 30.13 ± 0.75 years	Ding Kundan + clomiphene + HMG + dydrogesterone (under certain conditions) vs. clomiphene + HMG + dydrogesterone (under certain conditions) (1 m)	①③④⑤⑩	1 m
Chen [21]	T: 32°C: 31	T: 28.63 ± 0.73 years C: 30.13 ± 0.75 years	Yougui Pill (adjusted according to conditions) + Diane-35 vs. Diane-35 (3 m)	①②⑤⑦	6 m
Du [23]	T: 57°C: 57	T: 29.4 ± 5.3 years C: 28.6 ± 5.7 years	Chinese medicine prescription + ethinyl estradiol + clomiphene citrate tablets + HMG vs. ethinyl estradiol + clomiphene citrate tablets + HMG (3 m)	①②④⑤ ⑪ ⑫ ⑬⑯	NR
Hongling and Limian [25]	T: 29°C: 29	T: 25.8 ± 1.8 years C: 26.2 ± 2.4 years	Traditional Chinese medicine + clomiphene citrate tablets + estradiol valerate + HCG vs. clomiphene citrate tablets + estradiol valerate + HCG (3 m)	①④⑤⑩⑪	3 m
Ma et al. [27]	T: 99°C: 99	T: 28.7 ± 5.1 years C: 27.4 ± 14.8 years	Chinese medicine prescription + metformin vs. metformin (3 m)	①②③⑥⑦	NR
Ru et al. [28]	T: 90°C: 90	T: 26.8 ± 4.4 years C: 26.5 ± 5.0 years	Chinese medicine prescription + clomiphene vs. clomiphene (3 m)	①②③④⑤	NR
Tong et al. [29]	T: 40°C: 40	T: 30.58 ± 3.82 years C: 30.23 ± 3.53 years	Chinese medicine prescription + clomiphene + HCG (under certain conditions) + dydrogesterone (under certain conditions) vs. Clomiphene + HCG (under certain conditions) + dydrogesterone (under certain conditions) (3 m)	①④⑤⑭⑮	NR
Xu and Zho [30]	T: 30°C: 30	T: 25.7 ± 4.0 years C: 25.8 ± 4.2 years	Acupuncture + Diane-35 + HMG + HCG vs. Diane-35 + HMG + HCG (2m)	①②④⑤⑨⑩ ⑭	NR
Zhao et al. [31]	T: 38°C: 40	T: 26.21 ± 3.37 years C: 26.30 ± 3.38 years	Letrozole tablets + Tiao Jing Cu Yun pills + aspirin vs. letrozole tablets (only 1 m concluded)	①④⑮	NR
Wenqin and Dianzhou [32]	T: 51°C: 51	T: 28.6 ± 5.14 years C: 29.4 ± 6.14 years	Tiao jing cu yun pill + clomiphene citrate capsules + HCG vs. clomiphene + HCG (3 m)	①②④⑤⑩⑭	NR

① Endometrial thickness, ② hormone levels, ③ clinical efficacy, ④ pregnancy rate (number of cases), ⑤ ovulation rate (number of cases), ⑥ TCM Syndrome Score, ⑦ ovarian volume, ⑧ dominant follicle size, ⑨ dominant follicle count, ⑩ adverse reactions, ⑪ endometrial type, ⑫ cervical mucus score, ⑬ number of menstrual recovery cases, ⑭ abortion rate, ⑮ endometrial blood flow coefficient (PI, RI, etc.), and ⑯ LP leptin. ∗ HMG: human menopausal gonadotropin, HCG: human chorionic gonadotropin, m: month, and NR: no report.

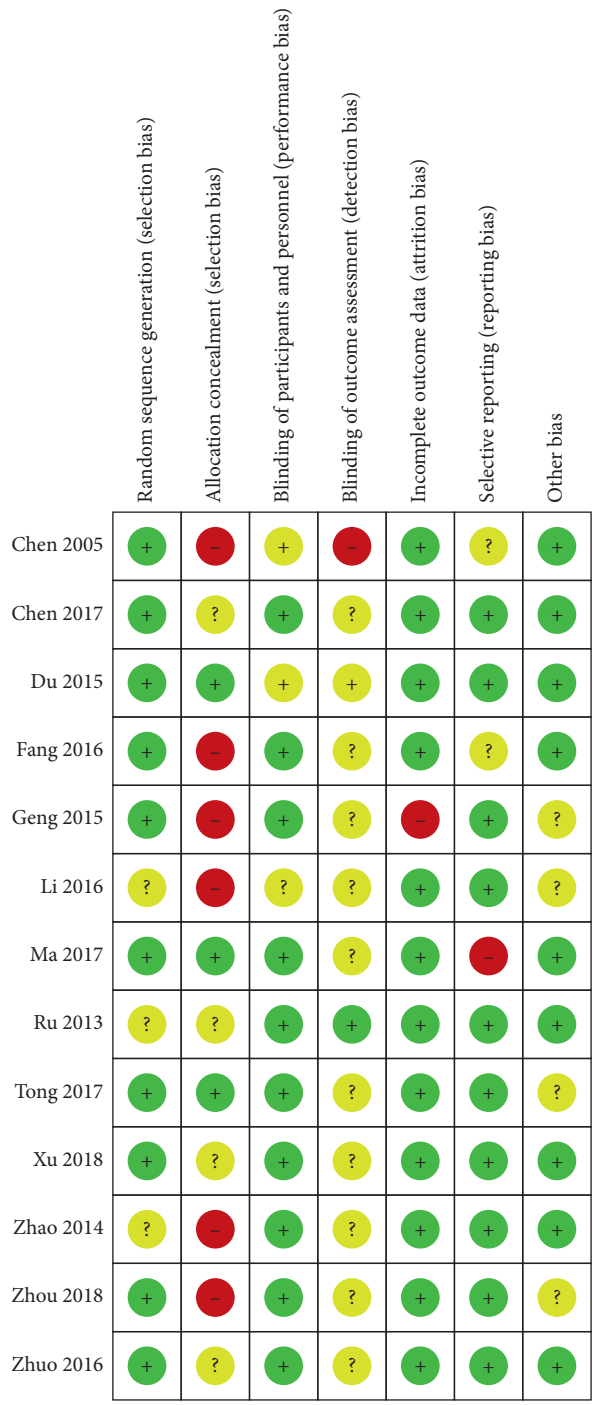


Figure 2: Risk of bias summary.

compared with control patients [24, 33] (Figure 11), while the LH levels in patients treated with acupuncture or TCM in combination with western medicine were significantly improved (SMD −0.33, 95% CI [−0.54, −0.12]; $I^2 = 0\%$, $P = 0.002$)(Figure 12) [21, 28, 33]. Both CAM alone (acupuncture or oral TCM) and CAM (acupuncture or oral TCM) in combination with western medicine performed better in decreasing testosterone levels compared with clomiphene and Diane-35 (SMD −0.68, 95% CI [−1.00, −0.36]; $I^2 = 70\%$, $P < 0.001$) (Figure 13) [21, 23, 24, 28, 30, 33]. However, western medicine alone and CAM alone or in combination with western medicine did not lead to significant changes in E_2 levels (SMD 0.31, 95% CI [−0.04, −0.65]; $I^2 = 81\%$, $P = 0.08$) [21, 23, 24, 27, 28, 30, 33] (Figure 14).

3.1.5. Number of Dominant Follicles. In two trials involving 208 patients, only oral TCM and acupuncture in combination with western medicine effectively increased the

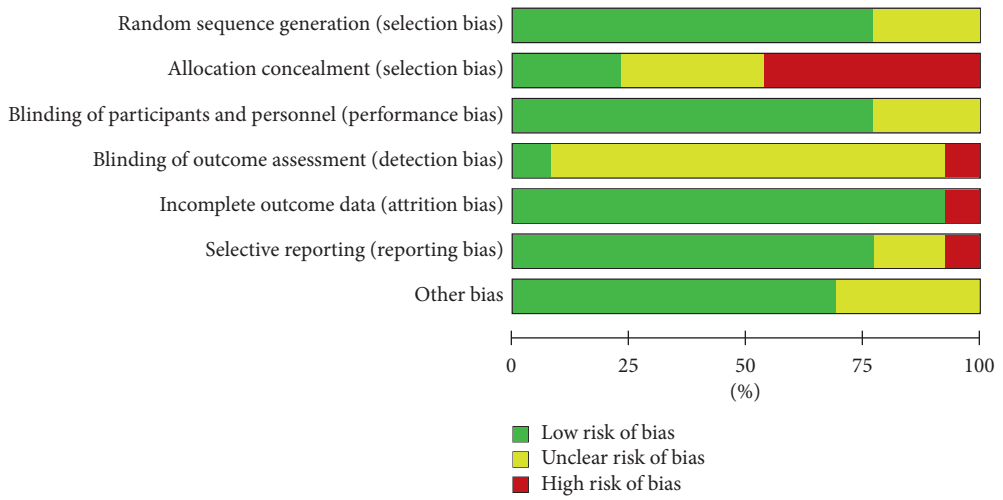


Figure 3: Risk of bias graph.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, random, 95% CI
Chen 2005	5.6	0.18	32	5.8	0.27	31	19.2	−0.86 [−1.38, −0.35]
Chen 2017	10.13	0.23	30	6.98	0.15	30	0.0	16.01 [13.01, 19.02]
Du 2015	9.57	1.03	57	8.61	0.95	57	0.0	0.96 [0.60, 1.32]
Fang 2016	12.03	2.35	28	7.79	1.26	28	0.0	4.24 [3..25, 5.23]
Geng 2015	8.67	1.11	29	8.29	1.06	29	0.0	0.38 [−0.18, 0.94]
Li 2016	1.12	0.15	74	1.2	0.17	74	28.0	−0.50 [−0.82, −0.17]
Ma 2017	6.74	1.35	99	8.32	1.75	99	29.6	−1.01 [−1.30, −0.71]
Ru 2013	8.8	1.4	90	7.8	1	90	0.0	1.00 [0.64, 1.36]
Tong 2017	7.33	0.21	40	7.44	0.2	40	0.0	−0.11 [−0.20, −0.02]
Xu 2018	9.28	3.47	30	9.11	3.24	30	0.0	0.17 [−1.53, 1.87]
Zhao 2014	9.376	1.187	38	8.995	1.371	40	0.0	0.38 [−0.19, 0.95]
Zhou 2018	6.15	1.57	51	8.61	2.39	51	23.2	−1.21 [−1.63, −0.78]
Zhuo 2016	10.23	1.36	50	8.93	1.69	50	0.0	1.30 [0.70, 1.90]
Total (95% CI)			*256*			*255*	*100.0*	*−0.88 [−1.20, −0.57]*

Heterogeneity: tau^2 = 0.06; chi^2 = 8.27, df = 3 (P = 0.04); I^2 = 64%

Test for overall effect: Z = 5.50 (P < 0.00001)

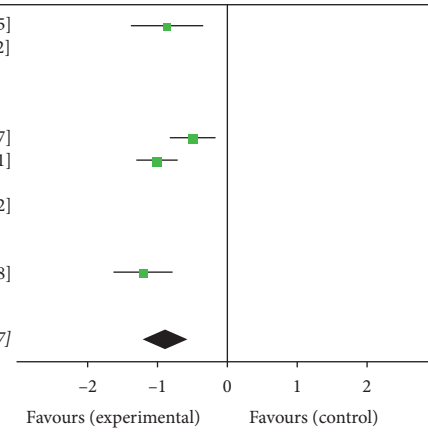
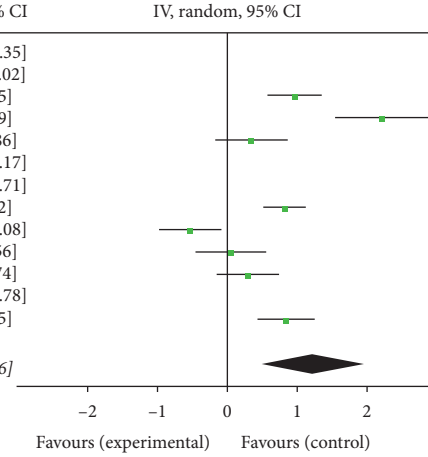


Figure 4: Forest plot of reducing endometrial thickness.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, random, 95% CI
Chen 2005	5.6	0.18	32	5.8	0.27	31	0.0	−0.86 [−1.38, −0.35]
Chen 2017	10.13	0.23	30	6.98	0.15	30	4.0	16.01 [13.01, 19.02]
Du 2015	9.57	1.03	57	8.61	0.95	57	12.2	0.96 [0.57, 1.35]
Fang 2016	12.03	2.35	28	7.79	1.26	28	11.4	2.22 [1.54, 2.89]
Geng 2015	8.67	1.11	29	8.29	1.06	29	11.9	0.38 [−0.17, 0.86]
Li 2016	1.12	0.15	74	1.2	0.17	74	0.0	−0.50 [−0.82, −0.17]
Ma 2017	6.74	1.35	99	8.32	1.75	99	0.0	−1.01 [−1.30, −0.71]
Ru 2013	8.8	1.4	90	7.8	1	90	12.4	0.82 [0.51, 1.12]
Tong 2017	7.33	0.21	40	7.44	0.2	40	12.1	−0.53 [−0.98, −0.08]
Xu 2018	9.28	3.47	30	9.11	3.24	30	11.9	0.05 [−0.46, 0.56]
Zhao 2014	9.376	1.187	38	8.995	1.371	40	12.1	0.29 [−0.15, 0.74]
Zhou 2018	6.15	1.57	51	8.61	2.39	51	0.0	−1.21 [−1.63, −0.78]
Zhuo 2016	10.23	1.36	50	8.93	1.69	50	12.2	0.84 [0.43, 1.25]
Total (95% CI)			*392*			*394*	*100.0*	*1.23 [0.50, 1.96]*

Heterogeneity: tau^2 = 1.08; chi^2 = 161.46, df = 8 (P < 0.00001); I^2 = 95%

Test for overall effect: Z = 3.32 (P = 0.0009)



(a)

Figure 5: Continued.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. mean difference IV, fixed, 95% CI
Du 2005	3.6	0.11	57	2.57	0.07	57		Not estimable
Fang 2016	3.25	1.12	28	1.26	0.19	28	31.0	2.44 [1.74, 3.15]
Ma 2017	−.3.38	0.78	99	−1.86	0.51	99		Not estimable
Ru 2013	1.6	0.2	90	0.8	0.1	90		Not estimable
Zhou 2018	−9.32	0.59	51	−6.5	0.05	51		Not estimable
Zhuo 2016	4.24	0.44	50	2.91	0.91	50	69.0	1.85 [1.38, 2.32]
Total (95% CI)			*78*			*78*	*100.0*	*2.03 [1.64, 2.42]*

Heterogeneity: chi^2 = 1.91, df = 1 (P = 0.17); I^2 = 48%

Test for overall effect: Z = 10.17 (P < 0.00001)

Std. mean difference IV, fixed, 95% CI

−2 −1 0 1 2

Favours (experimental) Favours (control)

(b)

Figure 5: (a) Forest plot of increasing endometrial thickness. (b) Forest plot of increasing endometrial thickness: CAM vs. clomiphene therapy.

Study or subgroup	Experimental Events	Total	Control Events	Total	Weight (%)	Risk ratio M-H, fixed, 95% CI
Du 2015	50	57	30	57	32.1	1.67 [1.28, 2.17]
Geng 2015	46	71	35	73	36.9	1.35 [1.01, 1.81]
Zhuo 2016	38	50	29	50	31.0	1.31 [0.99, 1.74]
Total (95% CI)		*178*		*180*	*100.0*	*1.44 [1.22, 1.69]*
Total events	134		94			

Heterogeneity: chi^2 = 1.78, df = 2 (P = 0.41); I^2 = 0%

Test for overall effect: Z = 4.40 (P < 0.0001)

Risk ratio M-H, fixed, 95% CI

0.2 0.5 1 2 5

Favours (experimental) Favours (control)

Figure 6: Forest plot of endometrial type A.

Study or subgroup	Experimental Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, random, 95% CI
Du 2015	7	57	27	57	48.1	0.16 [0.06, 0.40]
Zhuo 2016	12	50	21	50	51.9	0.44 [0.18, 1.03]
Total (95% CI)		*107*		*107*	*100.0*	*0.27 [0.10, 0.73]*
Total events	19		48			

Heterogeneity: tau^2 = 0.32; chi^2 = 2.50, df = 1 (P = 0.11); I^2 = 60%

Test for overall effect: Z = 2.57 (P = 0.01)

Odds ratio M-H, random, 95% CI

0.005 0.1 1 10 200

Favours (experimental) Favours (control)

Figure 7: Forest plot of endometrial type $B + C$.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Mean difference IV, fixed, 95% CI
Tong 2017	2.07	0.25	40	2.36	0.29	40	86.0	−0.29 [−0.41, −0.17]
Zhao 2014	0.907	0.081	38	1.027	0.944	40	14.0	−0.12 [−0.41, 0.17]
Total (95% CI)			*78*			*80*	*100.0*	*−0.27 [−0.38, −0.16]*

Heterogeneity: chi^2 = 1.1, df = 1 (P = 0.29); I^2 = 10%

Test for overall effect: Z = 4.74 (P < 0.00001)

Mean difference IV, fixed, 95% CI

−1 −0.5 1 0.5 1

Favours (experimental) Favours (control)

Figure 8: Forest plot of PI.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Mean difference IV, fixed, 95% CI
Tong 2017	0.64	0.09	40	0.79	0.46	40	59.0	−0.15 [−0.30, −0.00]
Zhao 2014	0.605	0.043	38	0.659	0.56	40	41.0	−0.05 [−0.23, 0.12]
Total (95% CI)			*78*			*80*	*100.0*	*−0.11 [−0.22, 0.00]*

Heterogeneity: chi^2 = 0.69, df = 1 (P = 0.41); I^2 = 0%
Test for overall effect: Z = 1.94 (P = 0.05)

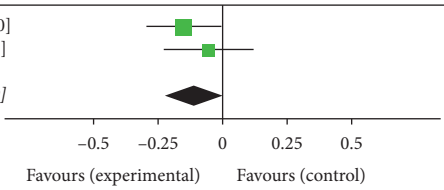


Figure 9: Forest plot of RI.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. Mean difference IV, fixed, 95% CI
Chen 2005	4.83	0.94	32	4.56	0.84	31	9.1	0.30 [−0.20, 0.80]
Du 2015	4.67	1.19	57	4.53	1.08	57	16.6	0.12 [−0.25, 0.49]
Fang 2016	5.41	0.58	28	5.52	0.61	28	8.1	−0.18 [−0.71, 0.34]
Ma 2017	5.39	0.87	99	5.87	1.1	99	28.1	−0.48 [−0.76, −0.20]
Ru 2013	6.01	1.58	90	8.11	3.01	90		Not estimable
Xu 2018	6.86	2.93	30	6.99	1.98	30	8.8	−0.05 [−0.56, 0.45]
Zhou 2018	4.87	1.66	51	5.14	1.47	51	14.8	−0.17 [−0.56, 0.22]
Zhuo 2016	5	1.21	50	5.33	0.99	50	14.4	−0.30 [−0.69, 0.10]
Total (95% CI)			*347*			*346*	*100.0*	*−0.18 [−0.33, −0.03]*

Heterogeneity: chi^2 = 11.14, df = 6 (P = 0.08); I^2 = 46%
Test for overall effect: Z = 2.29 (P = 0.02)

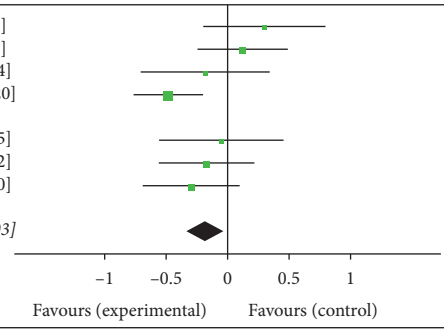


Figure 10: Forest plot of FSH levels.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Std. Mean difference IV, random, 95% CI
Chen 2005	6.02	0.76	32	6.22	0.63	31	0.0	−0.28 [−0.78, 0.21]
Du 2015	6.49	1.16	57	8.03	1.01	57		Not estimable
Fang 2016	7.23	1.47	28	9.78	1.81	28	47.6	−1.53 [−2.13, −0.92]
Ma 2017	4.17	0.75	99	5.56	0.96	99		Not estimable
Ru 2013	6.76	2.43	90	7.66	4.24	90	0.0	−0.26 [−0.55, 0.03]
Xu 2018	8.37	3.16	30	6.35	2.39	30		Not estimable
Zhou 2018	8.6	1.93	51	12.47	1.57	51	0.0	−2.75 [−3.29, −2.20]
Zhuo 2016	9.39	4.62	50	11.61	4.4	50	52.4	−0.49 [−0.89, −0.09]
Total (95% CI)			*78*			*78*	*100.0*	*−0.98 [−2.00, 0.03]*

Heterogeneity: tau^2 = 0.47; chi^2 = 7.96, df = 1 (P = 0.005); I^2 = 87%
Test for overall effect: Z = 1.90 (P = 0.06)

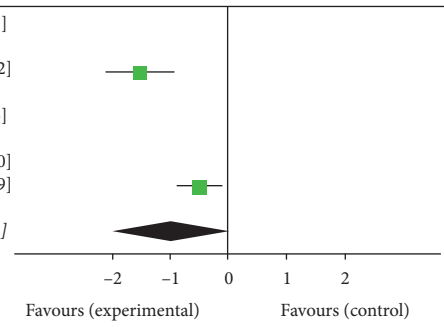


Figure 11: Forest plot of LH levels: TCM or acupuncture + WM vs. WM.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight [%]	Std. Mean difference IV, fixed, 95% CI
Chen 2005	6.02	0.76	32	6.22	0.63	31	18.5	−0.28 [−0.78, 0.21]
Du 2015	6.49	1.16	57	8.03	1.01	57	0.0	−1.41 [−1.82, −0.99]
Fang 2016	7.23	1.47	28	9.78	1.81	28	0.0	−1.53 [−2.13, −0.92]
Ma 2017	4.17	0.75	99	5.56	0.96	99	0.0	−1.61 [−1.93, −1.29]
Ru 2013	6.76	2.43	90	7.66	4.24	90	52.8	−0.26 [−0.55, 0.03]
Xu 2018	8.37	3.16	30	6.35	2.39	30	0.0	0.71 [0.19, 1.23]
Zhou 2018	8.6	1.93	51	12.47	1.57	51	0.0	−2.75 [−3.29, −2.20]
Zhuo 2016	9.39	4.62	50	11.61	4.4	50	28.7	−0.49 [−0.89, −0.09]
Total [95% CI]			*172*			*171*	*100.0*	*−0.33 [−0.54, −0.12]*

Heterogeneity: chi^2 = 0.87, df = 2 [P = 0.65]; I^2 = 0%
Test for overall effect: Z = 3.03 [P = 0.002]

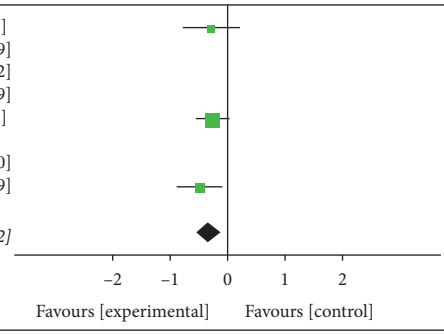


Figure 12: Forest plot of LH levels: acupuncture or TCM + WM vs. WM.

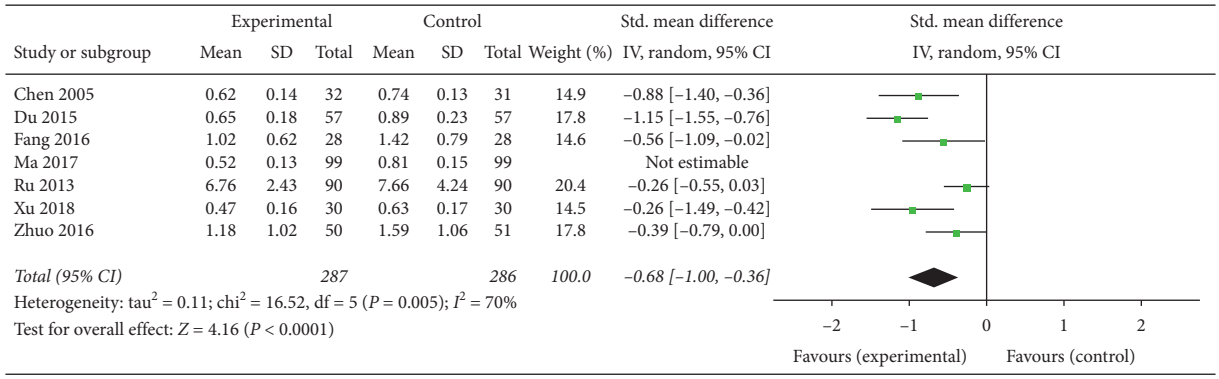


Figure 13: Forest plot of testosterone levels.

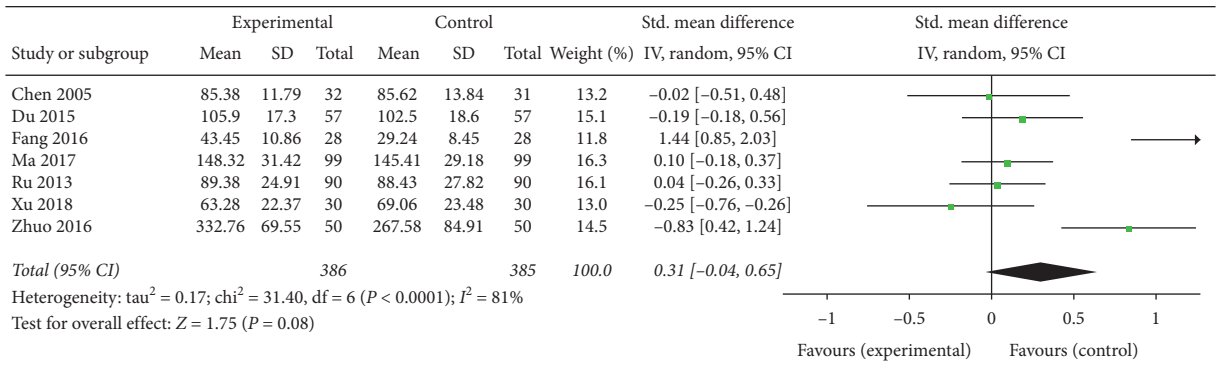


Figure 14: Forest plot of E_2 levels.

number of dominant follicles in PCOS patients after ovulation stimulation treatment (MD −0.12, 95% CI [−0.22, −0.03]; $I^2 = 0\%$, $P = 0.008$) [26, 30] (Figure 15).

3.1.6. Number of Ovulation Cases (Ovulation Rate). Nine trials involving 847 patients [21–25, 28, 30, 32, 33] showed that the number of ovulation cases (as indicated by signs of ovulation monitored by transvaginal ultrasound over a period of at least 2 months) was significantly increased in those treated with CAM (acupuncture or oral TCM) alone or in combination with western medicine compared to controls (clomiphene or Diane-35) (RR 1.34, 95% CI [1.23, 1.46]; $I^2 = 31\%$, $P < 0.00001$) (Figure 16).

3.1.7. Pregnancy Rate. Nine articles involving 923 patients [21, 23, 25, 28–33] showed that acupuncture treatment alone or in combination with western medicine effectively improved the pregnancy rate of PCOS patients (as determined by urine HCG or blood β-HCG positivity along with simultaneous ultrasound showing the gestational sac and fetal heart beat) and that oral TCM in combination with western medicine also showed significant improvements in the pregnancy rate of PCOS patients (RR 1.59, 95% CI [1.30, 1.93]; $I^2 = 51\%$, $P < 0.00001$) (Figure 17).

3.1.8. Abortion Rate. Three articles involving 130 patients [29, 30, 32] found that CAM (oral TCM or acupuncture) in combination with western medicine for ovulation simulation was more effective in inhibiting the occurrence of abortion compared with western medicine treatment alone (RR 0.30, 95% CI [0.09, 0.93]; $I^2 = 0\%$, $P = 0.04$) (Figure 18).

3.1.9. Ovarian Volume. As indicated by two articles involving 261 patients [21, 27], the PCOS patients administered oral TCM in combination with western medicine had greater reductions in ovarian volume compared to metformin or Diane-35 (in MD −2.08, 95% CI [−2.44, −1.71]; $I^2 = 13\%$, $P < 0.00001$) (Figure 19), which was considered a significant improvement in PCOS patients' condition.

3.1.10. Clinical Efficacy. In terms of clinical efficacy, the included trials fell into two groups, namely those that sought to improve the symptoms of PCOS and those that sought to improve the effective pregnancy rate of PCOS patients with infertility. Accordingly, the trials referring to clinical efficacy were integrated, and the clinical efficacy was set as the appearance of effective ovulation in the patients, i.e., the disappearance of mature follicles ≥15 mm or the collapse of the follicle wall as detected through vaginal ultrasound monitoring. Five trials involving 642 patients [22, 24, 26–28]

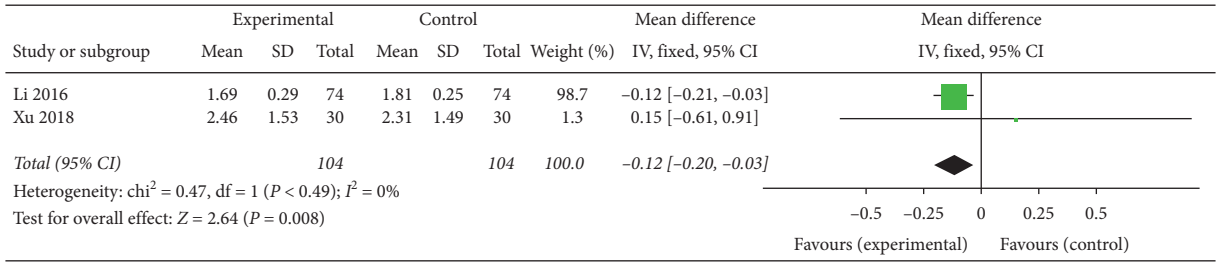


Figure 15: Forest plot of dominant follicle count.

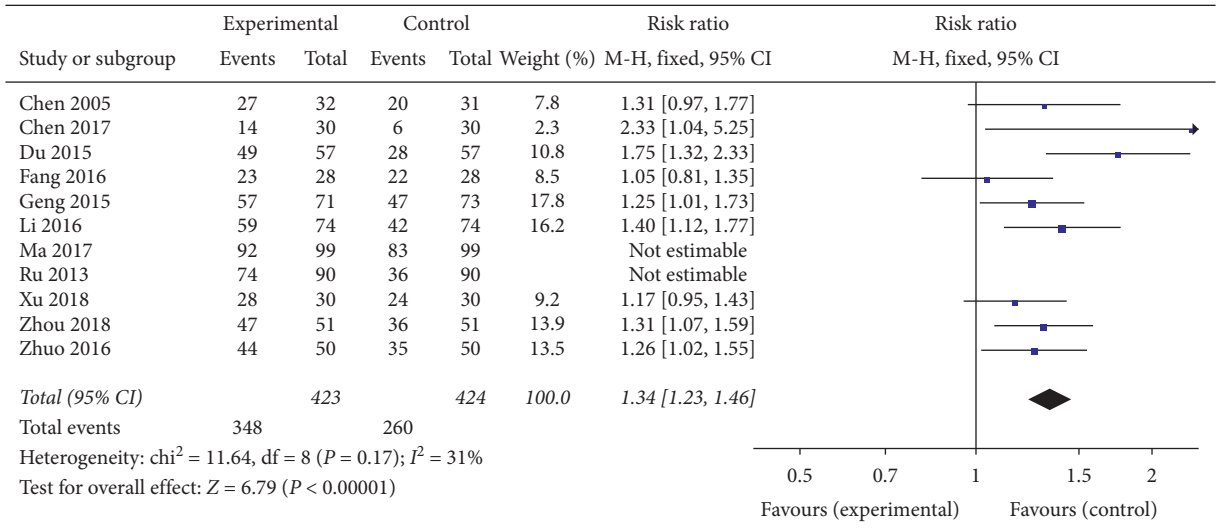


Figure 16: Forest plot of the ovulation rate.

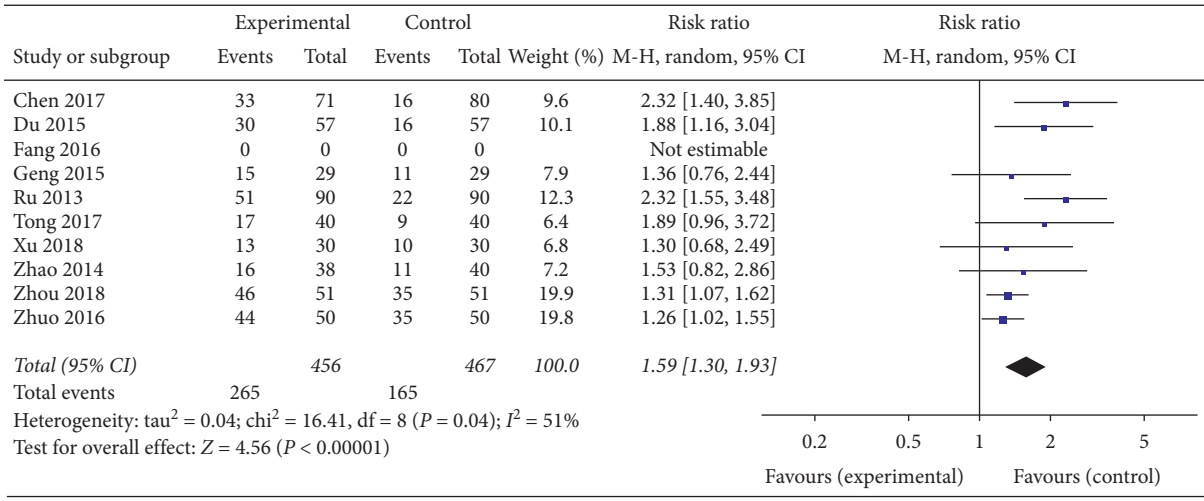


Figure 17: Forest plot of level of the pregnancy rate.

showed that oral TCM alone or in combination with western medicine had an obvious clinical effect in PCOS patients (RR 1.18, 95% CI [1.10, 1.27]; $I^2 = 14\%$, $P < 0.00001$) (Figure 20). Specifically, oral TCM alone [24, 26] was more effective for ovulation simulation compared with western medicine alone (HMG + HCG).

3.1.11. TCM Syndrome Score. Two articles involving 346 patients mentioned the effects of treatments on TCM syndrome differentiation [26, 27], and the TCM syndrome score was determined by a score table as a final indicator of the treatment's effectiveness. However, the results of the two articles for the TCM syndrome score after CAM treatment

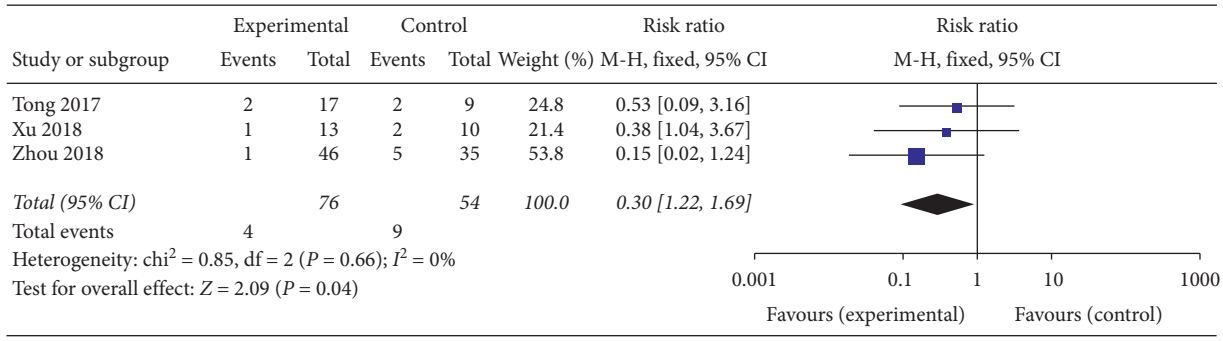


Figure 18: Forest plot of the abortion rate.

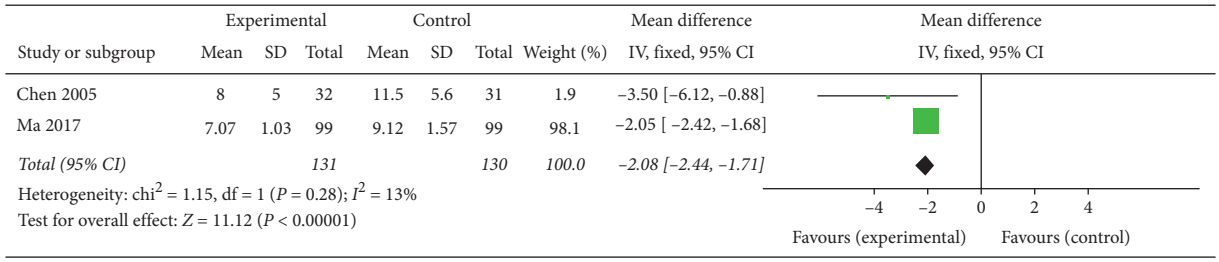


Figure 19: Forest plot of ovarian volume.

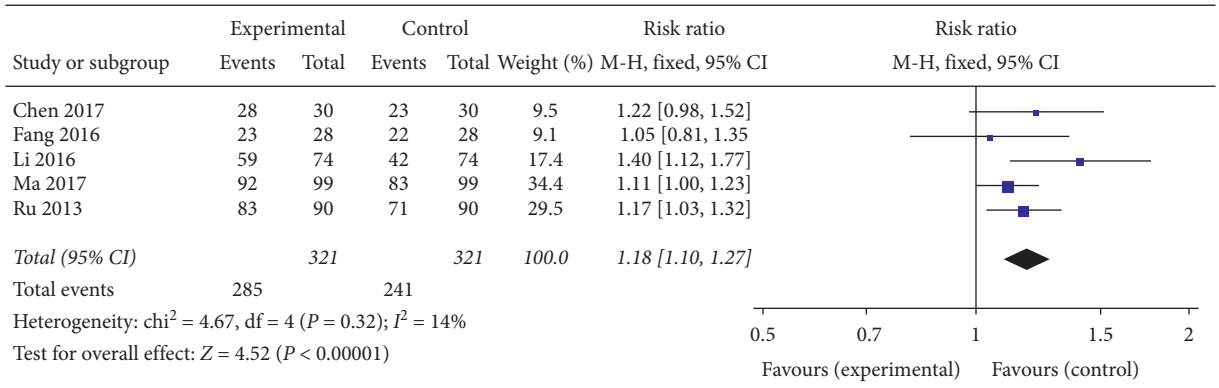


Figure 20: Forest plot of clinical efficacy.

were not significantly different (SMD −4.33, 95% CI [−7.51, −1.16]; $I^2 = 98\%$, $P = 0.007$) (Figure 21).

3.1.12. Cervical Mucus Score. As suggested by two articles involving 214 patients, [23, 33] CAM (acupuncture) or CAM (oral TCM) combined with clomiphene treatment significantly elevated cervical mucus score compared with clomiphene alone (MD 1.73, 95% CI [1.37, 2.09]; $I^2 = 0\%$, $P < 0.00001$) (Figure 22).

3.1.13. Adverse Reactions. Three articles [25, 30, 32] mentioned adverse reactions (e.g., luteinized unruptured follicle syndrome and ovarian hyperstimulation syndrome) during ovulation simulation treatment. Compared with the patients treated with Diane-35 or clomiphene alone, the proportion of adverse reactions in patients administrated with CAM (oral TCM or acupuncture) in combination with western medicine was significantly reduced (RR 0.48, 95% CI [0.31, 0.74]; $I^2 = 50\%$, $P = 0.001$) (Figure 23).

One article [21] reported that TCM in combination with Diane-35 and Diane-35 alone effectively reduced the endometrial thickness and ovarian volume and significantly improved the number of dominant follicles. However, after six cycles after discontinuation of treatment, the endometrial thickness and ovarian volume of the western medicine control group were close to the pretreatment status, while the CAM intervention group effectively maintained the normal levels.

In one article, due to the additional intervention of aspirin in both groups, we could not absolutely attribute the effectiveness to CAM in determining the outcome indicators

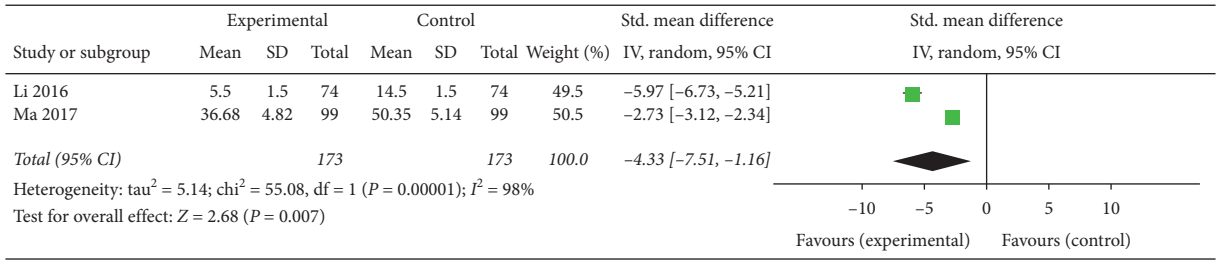


Figure 21: Forest plot of the TCM syndrome score.

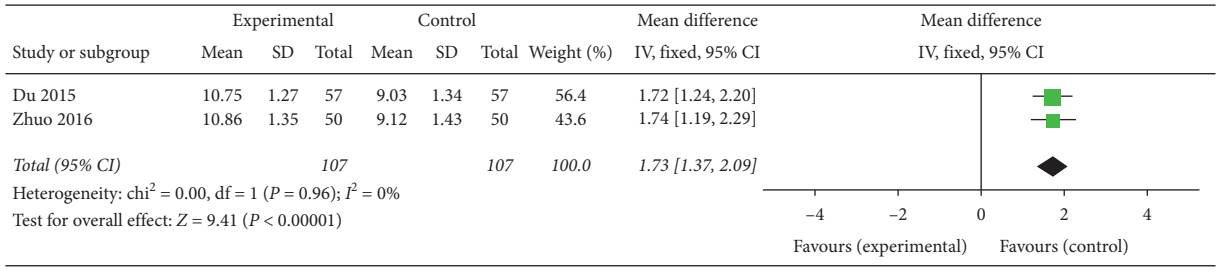


Figure 22: Forest plot of the cervical mucus score.

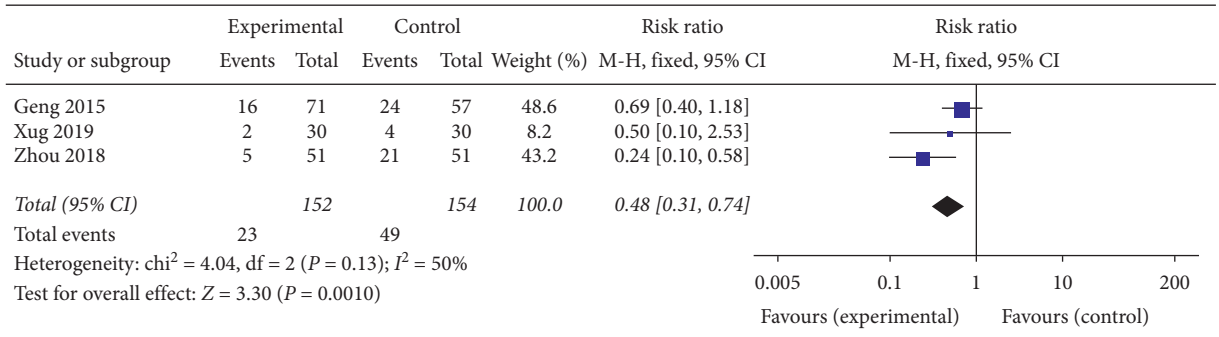


Figure 23: Forest plot of the number of adverse reactions.

when compared with the western medicine control group [31].

3.1.14. Additional Analysis. Because fewer than 10 trials were contained in each comparison, this study could not conduct meaningful funnel chart analysis to determine publication bias. In addition, the heterogeneity of the endometrial thickness and the LH level reached over 70% ($I^2 \geq 70\%$), so a subgroup meta-analysis was conducted. Different types of CAM were used in the included RCTs, and this resulted in a certain level of clinical heterogeneity in the results of this study.

4. Discussion

This review identified 13 RCTs involving 1,297 PCOS patients with abnormal endometrial status. The normal endometrial stages include proliferation, secretion, and menstrual periods, and thus the thickness of the endometrium changes over the course of the menstrual cycle. Most researchers consider that if the endometrium is extremely thin (<8 mm), this might cause unfavorable condition for embryo implantation and thus result in a low clinical pregnancy rate, and extreme thickness (>16 mm) might also reduce the clinical pregnancy rate [34]. Accordingly, as indicated from the results presented here, CAM therapy (including CAM therapies used alone as well as CAM therapies used in combination with traditional western medicine) is capable of effectively reducing the thickness of the endometrium in pathological hyperplasia, while in PCOS patients with infertility it can increase the thickness of the endometrium during ovulation thereby improving the status of the endometrium, increasing the pregnancy rate, and decreasing the abortion rate. Thus, depending on the patient's condition, CAM can reduce or increase the thickness of the endometrium as needed to promote pregnancy more effectively than traditional western medicine and can help to

avoid long-term complications such as endometrial cancer. Compared with western medicine (e.g., clomiphene, Diane-35, and other ovulation simulation treatments), CAM is capable of significantly reducing the adverse reactions associated with ovulation simulation, improving cervical mucus score, increasing ovulation rate, increasing the number of dominant follicles, and increasing the pregnancy rate, thus showing an overall clinical effect of CAM treatments.

Furthermore, this review also focused on the impact of CAM treatment on the hormone levels in PCOS patients. Although E_2 is directly involved in hyperplasia of the endometrium, CAM does not noticeably affect the E_2 level of PCOS patients. This may be related to the small number of articles included. FSH and LH, as gonadotropins, are not directly involved in the cyclic changes of endometrial hyperplasia and secretion, but regulation of their receptors can affect the intracellular function of the glandular epithelium of the endometrium where the receptors are expressed [35]. Gonadotropin receptor levels are positively correlated with the development of endometrial cancer [36], and it is known that exposure to high-level FSH conditions can increase the proliferation, invasion, and metastasis of endometrial cancer cells [37]. The results of the present analysis strongly suggest that CAM is capable of effectively reducing the serum FSH level in PCOS patients and thus reducing the risk of endometrial cancer. Moreover, this study found that CAM can reduce the serum testosterone level in PCOS patients with endometrial abnormalities. The elevation of testosterone is considered one of the common symptoms in PCOS patients, and testosterone, as a type of androgen, suppresses the autoimmune system [38] and can lead to cancer. Patients with endometrial cancer and endometrial adenoma often show elevated levels of testosterone [39, 40], and thus by reducing the level of testosterone CAM might prevent long-term complications such as endometrial cancer in PCOS patients.

The normalization of the endometrium, which is a necessary condition for successful pregnancy, is a critical outcome indicator for PCOS patients. Type A endometrium is more conducive to pregnancy due to its richer blood supply and higher receptivity than types B and C [41], and this review found that CAM significantly increases the occurrence of type A endometrium thus suggesting that CAM can improve the pregnancy rate in PCOS patients. By ameliorating abnormalities of the endometrium, CAM positively affected the pregnancy rate and live birth rate in PCOS patients, thus further proving that PCOS patients with complications (e.g., infertility) can be effectively treated with CAM.

According to the existing literature, the abnormal proliferation of the endometrium in PCOS patients shows a close positive correlation with the expression of prolactin and its receptors [42]. One of the included RCTs involved 80 PCOS patients and 80 matching controls and showed that the visfatin protein in the endometrial tissue of PCOS patients was highly upregulated and that the phosphorylation of AKT and ERK1/2 was also significantly increased, thus indicating that the malignant transformation of the endometrium in PCOS patients might be associated with the visfatin protein and the activation of the AKT and ERK1/2 signaling pathways [43]. Moreover, the abnormal state of the endometrium in PCOS patients can be manifested as insulin resistance contributing to abnormal glucose metabolism [44]. It has been reported that the endometrium of PCOS patients might suffer from abnormal amino acid metabolism in the tryptophan, tyrosine, and phenylalanine pathways, thereby causing abnormal cell proliferation and decreasing endometrial receptivity [45]. In the trials included here, however, none studied the effects of CAM on the factors that have been reported to cause endometrial abnormalities, and thus it remains unclear if CAM can effectively ameliorate the abnormalities of the endometrium after the related pathogenic factors mentioned above have been appropriately regulated.

While the endometrial problems in PCOS patients have aroused huge attention [46–49], CAM has not been valued as a primary method to improve the endometrial status in these patients. However, CAM has been found to be increasingly employed in controlling body weight and improving hormone levels and ovulation rates in PCOS patients [10, 50, 51]. Most of the studies included here have bias risk in numerous areas (e.g., distribution concealment, blindness, data loss, and sample size calculation), and thus the effectiveness of CAM remains unclear.

4.1. Strengths and Limitations. The present meta-analysis systematically evaluated the efficacy and safety of CAM in treating endometrial lesions in patients with PCOS. Although this study searched as many trials as possible, we still cannot be sure that we have covered all the evidence, and there might still be unanalyzed or unpublished data that might influence our conclusions. Additionally, in the retrieval process factors such as exercise, diet intervention, and psychological influences were not considered, so omissions and deficiencies might have occurred in the retrieval of RCTs studying CAM therapy. The final 13 RCTs included here focused on TCM treatment, and the research subjects and researchers almost exclusively originated from mainland China, and thus there was a lack of research information about other regions, which may have caused other bias. Moreover, most of the included RCTs lacked clear double-blind design methods, resulting in the low quality of the included RCTs. Furthermore, due to the statistical heterogeneity and variability of the CAM methods, subgroup meta-analysis, meaningful sensitivity analysis, and funnel chart analysis could not be conducted. Thus the present systematic review is limited in terms of the validity and universality of its conclusions, and this suggests that future RCTs should be designed as multicenter, double-blind placebo-controlled trials with more indicators of effectiveness, and they should be reported in accordance with the CONSORT (Consolidated Standards for Reporting Trials) criteria [52].

5. Conclusion

This systematic review suggests that CAM has potential for improving endometrial thickness, endometrial type, serum hormone level, and pregnancy rate in PCOS patients. However, due to the limited quantity and the general low quality of the methodology of the included trials, more in-depth research is required before CAM can be applied more widely in clinical practice. Thus more rigorous double-blind, placebo-controlled trials should be conducted to confirm the efficacy of CAM in improving endometrial condition in PCOS patients.

Data Availability

The data for this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no relevant conflicts of interest.

Authors' Contributions

Jiayu Hu designed the study, analyzed the data, and drafted the manuscript. Wenhua Shi assisted in writing the manuscript and corrected the grammar and writing. Jiayue Xu and Shaoxuan Liu collected the data. Siya Hu and Wenjing Fu collected the data and assessed the methodological quality of the included trials. Jing Wang and Fengjuan Han conceptualized the study and reviewed the protocol for important intellectual content. Jiayu Hu and Wenhua Shi contributed equally to this work.

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Review Article

The Efficacy of Complementary and Alternative Medicine in the Treatment of Female Infertility

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Female infertility is a state of fertility disorder caused by multiple reasons. The incidence of infertility for females has significantly increased due to various factors such as social pressure, late marriage, and late childbirth, and its harm includes heavy economic burden, psychological shadow, and even marriage failure. Conventional solutions, such as hormone therapy, in vitro fertilization (IVF), and embryo transfer, have the limitations of unsatisfied obstetric outcomes and serious adverse events. Currently, complementary and alternative medicine (CAM), as a new treatment for infertility, is gradually challenging the dominant position of traditional therapies in the treatment of infertility. CAM claims that it can adjust and harmonize the state of the female body from a holistic approach to achieve a better therapeutic effect and has been increasingly used by infertile women. Meanwhile, some controversial issues also appeared; that is, some randomized controlled trials (RCTs) confirmed that CAM had no obvious effect on infertility, and the mechanism of its effect could not reach a consensus. To clarify CAM effectiveness, safety, and mechanism, this paper systematically reviewed the literature about its treatment of female infertility collected from PubMed and CNKI databases and mainly introduced acupuncture, moxibustion, and oral Chinese herbal medicine. In addition, we also briefly summarized psychological intervention, biosimilar electrical stimulation, homeopathy, hyperbaric oxygen therapy, etc.

1. Introduction

The World Health Organization defines infertility as a failure of a couple to conceive after one year of regular unprotected intercourse. Nearly 15% of couples of childbearing ages worldwide suffer from infertility, most of whom are residents of developing countries [1, 2]. Nearly 30% of infertility factors are associated with males, about 40% with both males and females, and approximately 20–70% with females [3, 4]. Women, therefore, play more important roles in infertility. The primary causes of female infertility usually include ovulation disorders, fallopian tube problems, uterine lesions, and endometriosis [5]. The conventional treatments of infertility include sex hormone therapy (follicle-stimulating hormone, human chorionic gonadotropin, etc.), tubal plastic surgery, and assisted reproductive technology. These therapies, however, have unavoidable side or/and adverse effects. Hormone therapy, for example, can give rise to ovarian hyperstimulation syndrome (OHSS) or mental illness [6, 7]. IVF was initially utilized as an assisted reproductive technology to solve tubal obstruction and now is used for treating infertility. It has been 42 years since the first IVF baby was born in 1978 [8]. Although the live rate of embryo transfer has increased during the past years, the result is still

lower than the expectation of patients. Meanwhile, its extremely high cost also makes most infertile couples in the world unable to afford it [9].

CAM called "Unconventional Medicine" or "Unorthodox Medicine" covers various treatments, including not only traditional medicine and folk therapies but also many new therapies that cannot be covered by medical insurance. CAM treatments consist of Chinese medicine (Chinese herbal medicine, acupuncture, and moxibustion, Qigong), Indian medicine, medicinal foods, health foods, aromatherapy, vitamin therapy, diet therapy, psychotherapy, spa, oxygen therapy, etc. CAM is a commonly used adjuvant therapy widely accepted by infertility patients. Some RCTs have found that these interventions are helpful for the conception of infertility patients [10, 11]. Although there are many overviews on CAM treatment for infertility, its therapeutic effect and mechanism are still controversial. This review summarizes CAM treatment of infertility and briefly generalizes its mechanism.

2. Overview of Acupuncture and Moxibustion Treatment of Infertility

Acupuncture and moxibustion, an essential part of Traditional Chinese Medicine (TCM), have been protecting the health of the Chinese people for five thousand years. As a symbol of TCM, it is being accepted by most countries in the world. Guided by meridian and acupoint theory, acupuncturists take the human body as a whole and employ acupuncture and moxibustion, a unique clinical technique, to treat and prevent diseases. This technique uses needles and Artemisia as tools and raw materials, through inserting the needles and burning the leaves to stimulate the specific parts of the body, to adjust the balance of the body for disease treatment and prevention [12–14]. This therapy can be traced back at least 3000 years ago. With the modernization of TCM, acupressure, electroacupuncture (EA), moxibustion, and laser acupuncture have branched out from the original model. At present, about 180 countries around the world apply acupuncture and moxibustion to the treatment and prevention of diseases, out of which more than 50 countries consider acupuncture and moxibustion as CAM [15].

In recent years, acupuncture and moxibustion have become ideal treatments for infertility due to many of their superiorities. Because of the complex etiology, the treatment of infertility commonly takes a long time and the success rate is relatively low. The effect of acupuncture and moxibustion, however, is rapid and significant, because they can reinforce body function and improve the disease resistance of the body. Meanwhile, they are easy to operate and economical. Besides, there are fewer side effects, which can be avoided by careful operation. Furthermore, they can also be used as adjuvant therapy in combination with conventional therapies. The efficacy has aroused the interest of many clinicians and medical scientists. Clinical or animal studies have been conducted to evaluate the effect of acupuncture and moxibustion on infertility and papers have been published to elaborate the mechanism. However, the results across studies varied widely, some RCTs show that the treatment for infertility is beneficial, while others indicate otherwise. And the mechanism of acupuncture and moxibustion for infertility is still controversial.

2.1. The Application of Acupuncture in Infertility. Acupuncture is one of the most studied CAM interventions that were related to the improvement of reproductive outcomes [16]. Acupuncture has a long history of treating gynecological diseases. An increasing number of researches have indicated that acupuncture can regulate menstruation and assist female pregnancy without the risk of multiple pregnancies [17, 18]. According to The Yellow Emperor's Inner Classic: The Spiritual Pivot Nine Needles and Twelve Source Points: "The most crucial thing in acupuncture is to get the needling sensation. When it presents, the curative effect will be better" [19]. Needling sensation, also known as obtaining qi, refers to the feeling that needles are punctured into acupoints and apply with manipulation. It usually manifests as soreness, numbness, pain, and other reactions. Furthermore, better efficacy can be achieved by giving stimulation after obtaining qi [20]. Clinical practice showed that different interventions can produce different amounts of stimulus, which directly affected clinical efficacy [21]. Manual acupuncture and EA are currently the most popular acupuncture protocols. Manual acupuncture refers to rotating the needles with fingers, while electroacupuncture is the combination of acupuncture and electrical stimulation, both of them aiming to increase the therapeutic effect. There is no research to show which stimulus is more effective for reproductive function [22].

2.2. Clinical Effect of Acupuncture on Infertility. Most western countries' cognition of acupuncture came after President Nixon visited China in the 1970s. Since then, a completely new understanding of TCM was gained in western countries, and the remarkable effect of acupuncture in treating diseases fascinated western practitioners [23]. Although acupuncture has been accepted for treating ache, it has no substitute for anesthesia [24]. In recent years, acupuncture has been increasingly utilized as an auxiliary method for infertility and has been widely used in various circumstances during pregnancy. However, from the perspective of physiology, it is difficult to reach a consensus on the mechanism of acupuncture in the treatment of diseases [25]. Before the 21st century, there were few reports on the research of acupuncture in reproductive medicine, especially large-sample RCT. In 2002, Paulus et al. carried out an RCT, which reported the effect of acupuncture on the pregnancy rate of IVF for the first time. The 160 recruited patients randomized to the control group and the acupuncture group received acupuncture treatment 25 minutes before and after embryo transfer. Compared with the control group, the pregnancy rate in the acupuncture group significantly increased (42.5% versus 26.3%; $P < 0.03$) [26]. Subsequently, in 2006, Stefan Dieterle et al. conducted an RCT to investigate the effect of acupuncture during the luteal phase on IVF/ICSI outcomes. 225 infertile patients were randomized to the

treatment group and the control group. In the treatment group, acupuncture was performed in line with the principles of TCM, while the control group chose the placebo acupoints for comparison. The results showed that the pregnancy rate and implantation rate in the treatment group were 29.4% and 12.6%, respectively, while those in the control group were 8.2% and 3.2% ($P < 0.01$), which concluded that acupuncture in the luteal phase has a positive effect on IVF/ICSI [27]. Recently, LY et al. conducted a clinical trial to observe the effect of acupuncture combined with Chinese medicine on infertility patients with thin endometrium. 60 patients were randomized to the treatment group receiving acupuncture combined with Chinese medicine and the control group received estradiol valerate tablets. The results showed that endometrium-thickness and pregnancy rate in the treatment group were significantly higher than those of the control group, and the difference was statistically significant ($P < 0.05$). Therefore, it can be concluded that acupuncture combined with Chinese medicine can promote the growth of endometrium and improve the clinical pregnancy rate [28]. With more positive reports, acupuncture for infertility is gradually accepted by countries all over the world. In the past few years, several meta-analyses on acupuncture for infertility have been published. Although these studies have different degrees of bias risk, the conclusions still add weight to acupuncture as a substitution for western medical therapy. Among them, a meta-analysis was conducted to evaluate the efficacy of acupuncture or clomiphene (CC) or acupuncture combined with CC in treating anovulatory infertility for the first time. Compared with CC, acupuncture had better treatment results in pregnancy rate and maximum follicle diameter. At the same time, this meta-analysis also pointed out that CV3, CV4, CV6, ST36, SP6, and EX-CA1 are the most commonly selected for treating anovulatory infertility women, and it suggests that the above acupoints should give priority in future treatment [29].

While numerous articles have reported the positive effect of treating infertility with acupuncture, some scholars still questioned and denied the efficacy. In the acupuncture RCT, sham acupuncture or placebo acupuncture is usually the control group, which is needling on nonacupoint. Some scholars believe that even stimulating nonacupoints can produce therapeutic effects [21, 23]. In 2017, the team of Professor Wu published an article on JAMA entitled "Effect of Acupuncture and Clomiphene in Chinese Women with Polycystic Ovary Syndrome: A Randomized Clinical Trial." This is a multicenter RCT to explore whether acupuncture or acupuncture combined with CC can increase the fertility rate of women with PCOS. The results showed that there was no significant difference in the live birth rate between active acupuncture and control acupuncture (29.4% versus 28.0%, 13.9% versus 16.8%). Finally, the conclusion is as follows: "compared with acupuncture plus placebo, acupuncture with or without CC could not improve the live birth rate of Chinese women with PCOS." Therefore, using acupuncture for infertility in such patients was not supported [30, 31]. The research results of Madaschi et al. showed that giving acupuncture immediately before and after embryo transfer did not affect the outcome in general [32]. The results of Rashidi et al. showed that although acupuncture did not affect the IVF/ICSI results of women with PCOS, it has a beneficial efficacy on embryo quality at the early stage of oocyte recruitment. Further research is needed to prove how to transform the improvement of embryo quality into a high pregnancy rate. Multiple systematic reviews and meta-analyses have shown that there is insufficient evidence to support acupuncture in promoting live birth, pregnancy, and ovulation [33]. However, it found that acupuncture can promote the recovery of the menstrual cycle and reduce the levels of luteinizing hormone and testosterone in patients with PCOS [34].

The reasons for these different results may be related to the heterogeneity of clinical trials such as the experience of acupuncturists, the selection and positioning of acupoints, whether to use electrical stimulation or other manipulations, and the course of treatment. Other possible reasons, such as fewer subjects and non-RCT, may also lead to different results. Therefore, shortly, some larger sample, prospective, double-blind, placebo-controlled RCTs are urgently needed to clarify it. We have listed some RCTs in Table 1.

2.3. Mechanism of Acupuncture in Treating Infertility. Studies have shown that acupuncture can induce reactions that activate nerve, endocrine, and immune signaling pathways by inserting the skin [35]. The possible mechanisms are listed as follows. First of all, acupuncture makes the gonadotropin (GN) and steroid hormone cycles work together via the hypothalamic-pituitary-ovarian axis (HPOA) to promote the selection of dominant follicles and prepare for embryo implantation. Secondly, acupuncture can improve abnormal ovarian perfusion and the state of diminished ovarian reserve and enhance the quality of oocytes. Finally, acupuncture provides suitable conditions for embryo implantation by improving endometrial morphology, promoting endometrial microcirculation, and regulating estrogen and progesterone receptors in both directions [36–38]. Now, we will discuss these aspects.

2.3.1. Acupuncture Regulates Hypothalamic Function. The hypothalamus regulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by secreting gonadotropin-releasing hormone (GnRH), thus controlling the secretion of estrogen and progesterone [36]. Under the action of GN, the ovaries ovulate periodically, accompanied by cyclical secretion of E and P. By regulating the release of hypothalamic neurotransmitters and the secretion of GnRH and GN, acupuncture can improve the abnormal function of HPOA in infertile patients and restore the menstrual cycle, ovulation, and fertility [39]. Studies have shown that acupuncture can regulate the production and secretion of inhibitory neurotransmitters, including dopamine, gamma-aminobutyric acid, and β-endorphin (β-EP). They have an inhibitory effect on hypothalamic activity, among which β-EP is one of the main inhibitors. β-EP directly inhibits GnRH neuron activity by binding to its receptor, thereby inhibiting GnRH secretion. Stener-

Victorin et al.'s research on animals and patients with PCOS pointed out that acupuncture could regulate the production and secretion of central and peripheral β-EP, thereby affecting the release of GnRH and GN [40]. Acupuncture has a bidirectional regulation effect. It can also regulate the release of excitatory neurotransmitters, including leptin and glutamate, to stimulate the function of the hypothalamus. Kamyabi et al. proposed that high leptin might harm the internal environment required for ovarian function and embryonic development, which could be one cause of infertility [41, 42]. Meanwhile, a study has found that obese mice are infertile due to low leptin, and their reproductive function has been improved after leptin injection [43]. After six weeks of auricular point intervention by Hsu CH, they found that the level of leptin was significantly reduced [44]. Therefore, acupuncture can effectively adjust the HPOA function.

2.3.2. Acupuncture Regulates Ovarian Function. Apart from regulating the HPOA, acupuncture can also directly affect the ovary and other peripheral tissues [45–47]. Research conducted by Julia Johansson et al. showed that repeated acupuncture results in a higher ovulation frequency in lean/overweight women with PCOS [48]. The main function of the ovarian artery is to provide nutrients and transmit related hormones to the ovary. Ovarian artery blood mainly supplies nutrients needed for the growth and development of follicles, so it will directly affect the growth of follicles. The research conducted by Manni et al. suggested that the effectiveness of EA in the regulation of ovarian responsiveness indicates that EA could be an alternative approach to preventing and/or overcoming sympathetic-related anovulation in women with PCOS [49]. The study done by Dolz et al. pointed out that the main cause of anovulation in PCOS patients was the decreased vascular resistance index of the ovarian interstitium and increased interstitial blood vessels [50]. Low-frequency EA at proximal and distal acupoints has proven to be effective in promoting ovulation and increasing the possibility of pregnancy [51]. It can improve the angiogenesis of ovarian vessels and antral follicles. An experiment proved that EA promoted the angiogenesis of antral follicles in PCOS-like rats induced by DHT, thus promoting follicular maturation, ovulation, and luteal formation [52]. Stener-Victorin et al. designed a rat experiment to determine whether EA can increase ovarian blood flow. They found that the change of ovarian blood flow depended on the acupoint and frequency [51, 53]. Besides, acupuncture can affect the secretion of AMH. AMH is secreted by granulosa cells and participates in the development of follicles. Sverre C Christiansen et al. confirmed that anti-Müller's hormone was positively correlated with follicle count and seemed to be a reliable indicator for predicting follicle count [54]. The study of Shi et al. has demonstrated that EA improved follicular arrest by decreasing the excessive expression of AMH to regulate FSH and AMH imbalance in granulosa cells in PCOS [55].

2.3.3. Acupuncture Regulates Uterine Function. Finally, acupuncture can improve the endometrial morphology, promote the microcirculation of the endometrium, and bidirectionally regulate the estrogen and its receptor, which provide good conditions for embryo implantation and improve the pregnancy rate. Endometrial receptivity (ER) refers to the ability of the endometrium to accept the embryo implantation changing with menstruation. ER correlates with infertility, and a good ER is a prerequisite for blastocyst implantation [56]. Embryo implantation is closely related to endometrial thickness, morphology, and blood supply [57]. The thin endometrium is one of the most critical factors for low ER and low pregnancy rates [58, 59]. The endometrium usually divides into three types: A, B, and C. The thinner endometrium of type B and C is not conducive to embryo implantation and development, while type A with a thickness greater than 8 mm is more suitable for embryo implantation and development [60, 61]. Studies have shown that acupuncture can change the type of endometrium, and after treatment, the percentages of type A and B are higher than before. Li selected LI4, LR3, KI3, SP6, and other acupoints to treat IVF-ET patients. The results showed that compared with the control group, acupuncture could increase the endometrial thickness and the pregnancy rate [62]. It can be seen that acupuncture can improve the morphology of the endometrium and can also increase the thickness of the endometrium and the clinical pregnancy rate. The blood supply of the endometrium includes the uterine artery, endometrial, and endometrial blood flow [63]. Studies have shown that reducing the blood flow impedance of bilateral uterine arteries and endometrium can significantly improve the blood flow parameters of the uterine artery [64, 65] and increase uterine blood flow and endometrial thickness, thereby improving ER. It has a positive impact on embryo implantation rate and clinical pregnancy rate [66, 67]. Steer et al. found that when the uterine artery pulsatility index >3.0, the pregnancy rate would be decreased [68]. Meanwhile, Stener-Victorin et al. confirmed that the uterine artery pulsatility index decreased after a series of acupuncture treatments [69]. The results of Ho et al. also confirmed that the pulsatility index of the uterine artery in the acupuncture group was significantly reduced [70]. Besides, acupuncture can also regulate estrogen and progesterone and their receptors. The endometrium is the main target organ of estrogen and progesterone. An appropriate amount of them is conducive to pregnancy. However, the imbalanced ratio of estrogen and progesterone can decrease ER and cause blastocyst-implantation failure. The result of Mu et al. predicted that acupuncture could increase ER and proposed that the potential molecules promoting ER were HSA-Mir-449a, HSA-Mir-3135b, and HSA-Mir-345-3p [38].

2.4. Safety of Acupuncture in the Treatment of Infertility. It is well known that acupuncture is relatively safe with fewer adverse events compared with western medicine. Most of

them are transient, such as skin erythema, bruising, bleeding, and pain, which can be avoided by careful manipulation; in addition, the reports of serious complications are rarely [71, 72]. In an RCT of IVF, 152 women had adverse events; all of them were mild discomfort or bruises [73]. In another clinical trial involving more than 200,000 patients receiving acupuncture for ache, the incidence of adverse events was only 8.6%. In short, adverse events do occur in acupuncture, but to a large extent, they are mostly minor compared with nonacupuncture-related interventions [74]. In a word, although the existing studies show that acupuncture has a positive effect on infertility and its mechanism is relatively explicit; there are still some limitations, such as insufficient sample size and lack of high-quality evidence in the existing studies. Therefore, more large-scale RCTs are needed to clarify the efficacy and mechanism of acupuncture in infertility.

3. Clinical Efficacy and Mechanism of Moxibustion on Infertile Women

Moxibustion refers to burning or fumigating acupoints or lesions by moxibustion to prevent diseases. From the perspective of TCM, moxibustion has the functions of warming meridians and dispersing cold, strengthening the body, and eliminating diseases. It is commonly used in treating infertility, dysmenorrhea, premature ovarian failure, and other gynecological diseases [75–78]. Heat-sensitive moxibustion and drug-separated moxibustion on the umbilicus are commonly used in treating infertility. Heat-sensitive moxibustion is to use the ignited moxa stick to produce a heat-sensitive effect to the heat-sensitive acupoints, which can go directly to the disease site, promote local pelvic blood circulation, accelerate local drug absorption, improve hydrosalpinx to restore its function, improve endometrial thickness, and regulate endometrial receptivity [79, 80]. Several researchers used heat-sensitive moxibustion combined with TCM decoction or western medicine to treat PCOS infertility patients. The results showed that the intervention group was significantly better than the control group in reducing ovarian volume, improving endometrial thickness, reducing LH and T, and increasing E2 and pregnancy rate, which may be related to the decrease of NF-κB and TNF-a [81, 82]. Heat-sensitive moxibustion also has a significant effect on infertility resulting from ovulation disorder and hydrosalpinx [83, 84]. Some researchers use drug-separated moxibustion on the umbilicus combined with CC in treating ovulation disorder infertility; the result showed that the maximum follicle diameter and the endometrial thickness could be significantly increased. The total effective rate, clinical cure rate, estradiol and progesterone levels, and TCM syndrome scores have been greatly improved in luteal insufficiency infertility treated with drug-separated moxibustion on umbilicus combined with oral Chinese medicine [85, 86]. In the theory of TCM, the umbilicus is RN8, which is exterior-interior related to the Du Channel. Moxibustion can invigorate the deficiency and warm the yang and dispel cold while applying moxibustion across the medicine powder for warming the kidney and promoting yang can increase the power of promoting yang. It can be seen that the medicine-separated moxibustion on the umbilicus is an organic combination of moxibustion, acupoints, and drugs. Besides, some studies have shown that moxibustion can inhibit ovarian cell apoptosis and enhance antioxidant defense capacity to improve ovarian function [87]. Modern pharmacological studies have shown that the effective components in Artemisia can activate blood vessels, accelerate blood circulation, improve ovarian artery blood supply, and increase ovarian blood flow perfusion, which can significantly improve ovulation rate and pregnancy rate [88–90].

4. Overview of Oral Chinese Herbal Medicine (CHM) in the Treatment of Infertility

TCM is by far the most complete, widely used, and influential medical system in the world [91]. The legend of "Shennong tasting hundreds of herbs" dates back to the commune days. Taking CHM orally is also one of the important CAMs for treating infertility. It is guided by the theory of Yin Yang, Five-Phase, Viscera and Bowels, Qi-Blood, Fluid-Humor, etc., and based on the principle of syndrome differentiation and treatment, which provides individualized treatment for infertility. In recent years, with the improvement of the clinical efficacy evaluation system and the development and implementation of scientific research, more studies have proved that CHM has the advantages, such as significant efficacy and high security in treating infertility. The number of infertile couples seeking TCM for infertility (including oral CHM and Chinese herbal diet) is also increasing.

4.1. The Application of Oral CHM Administration for Infertility. TCM, a kind of CAM, seems to be a more popular protocol for treating infertile women. A study conducted by Hung YC showed that among 8766 infertile women, 96.17% of them used TCM for the treatment of infertility in addition to conventional therapies. They also noted the female infertile patients who suffered from the diseases such as endometriosis, uterine fibroids, or irregular menstrual cycles were more willing to seek TCM treatment [92]. CHM has a significant effect in treating infertility, which is mainly achieved by improving follicular development, reducing the inflammatory environment of the uterine cavity, and improving hormone levels, etc.

4.2. Clinical Effect of Oral CHM Administration for Infertility. Many RCTs showed that administrating conventional therapies combined with oral CHM could greatly improve the ovulation rate, clinical pregnancy rate, etc. of infertile patients. Wan YT et al. randomly assigned 150 infertile patients caused by ovulation disorder into three groups. All three groups were given CC. On this basis, one group was given CHM and the other group was given aspirin. After three menstrual cycles of treatment, the results showed that the ovulation rate and pregnancy rate in the CHM combined with the CC group were significantly higher than the other

two groups ($P < 0.05$) [93]. Tian et al. found that compared with the hormone alone, hormone combined with CHM can significantly improve the pregnancy rate ($P < 0.05$) [94]. Modern pharmacological studies have shown that Dodder seed, a kind of CHM, can regulate the function of HPOA, promote the secretion of estrogen and progesterone in rats with embryo implantation dysfunction, and thus improve ER [95]. CHM also has advantages in improving follicular development. Cai et al. found that compared with the group without CHM treatment, CHM treatment can significantly promote follicular development and ovulation ($P < 0.05$) without increasing the incidence of adverse reactions [96]. The blood-activating and stasis-resolving medicine, Sichuan lovage root, in this prescription has the effect of improving the hemorheology and microcirculation of ovary and uterus, facilitating follicular development and ovulation, and improving ER [97]. Liu's experiment also reached the same conclusion by using another prescription [98]. CHM can also treat salpingitis, thereby greatly increasing the fertility rate of patients with infertility caused by fallopian tube factors [99]. Gao administrated CHM to infertile patients caused by chronic pelvic inflammation. The results showed that the combination of western medicine and CHM was better than western medicine alone in improving tubal adhesion, hydrops, pregnancy rate, etc. ($P < 0.05$) [100]. Feng randomized 80 patients with tubal obstructive infertility into the observation group (receiving hydrotubation combined with CHM) and control group (receiving hydrotubation). The results showed that the effective rates of the two groups were 92.50% versus 75.00% respectively, and the symptoms in the observation group were significantly alleviated compared with the control group [101]. Zhai and Lang also confirmed that CHM treatment can improve the inflammatory state, thereby improving the pregnancy rate [102, 103]. Premature ovarian failure is also a major cause of infertility. Premature ovarian failure is also a major cause of infertility. Wang KL studied 56 cases of infertility caused by premature ovarian insufficiency (POI) and found that Bushen Culuan Decoction could effectively improve ovarian reserve in patients with POI [104]. Oral CHM treats infertility by improving hormone levels. Gong et al. divided 80 infertile patients with PCOS into the control group ($n = 40$), and the observation group ($n = 40$) added CHM based on the control group. After treatment, the levels of T and LH in the observation group decreased more significantly than those in the control group ($P < 0.05$), and the pregnancy rate in the treatment group was much higher than that in the control group (85% versus 65%) ($P < 0.05$) [105]. Men found that the efficacy of bromocriptine combined with CHM was significantly better than bromocriptine alone for infertile patients with hyperprolactinemia. After treatment, all indicators in the two groups were improved; however, the efficacy of the combined group was better than the bromocriptine alone (95.0% versus 77.5%) ($P < 0.05$) [106]. Related research showed that the raw germinated barley in this prescription had the effect of reducing prolactin [107]. TCM takes pattern differentiation and treatment as the principle and gives corresponding prescriptions based on patients' pathological states. Zhao et al. conducted a multicenter RCT, which confirmed that taking CHM could significantly improve the pregnancy rate and live birth rate of infertile patients with endometriosis after laparoscopic surgery ($P < 0.05$) [108]. Liu's trial reached the same conclusion and found that the abortion rate could be reduced as well ($P < 0.01$) [109]. Table 2 lists the prescription composition of the above protocols and some protocols not mentioned [110–113].

Most RCTs have shown that CHM is beneficial in treating infertility, but there are also a small number of trials showing that it is ineffective. Lan et al. conducted an RCT on 80 infertile patients with follicular dysplasia. The control group of 40 patients was given CC combined with estradiol valerate, and the treatment group of 40 patients was given CC combined with estradiol valerate additionally CHM orally. There was no significant difference in pregnancy rate between the two groups after treatment [114]. Moreover, Zhou et al. made a systematic review on the treatment of PCOS with oral CHM. But they failed to collect enough high-quality literature to indicate that CHM had a positive effect on the live birth rate of infertile women with PCOS. Although some literature suggested that the addition of CHM to CC might improve the pregnancy rate, due to the small sample size, wide confidence interval, and other reasons, the quality of the literature was low and there was insufficient evidence to demonstrate the absolute safety of CHM [115]. Due to the heterogeneity of the patient's age, etiology of infertility, previous treatments, and different interventions, the results across studies varied widely. Therefore, larger-sample, multicenter, double-blind, placebo-controlled trials are needed to verify the efficacy and safety of oral CHM in infertility treatment in the future.

4.3. Mechanisms of CHM in Treating Infertility

4.3.1. CHM Regulates Uterine Function. Improving ER is one of the mechanisms of oral CHM in treating infertility. Taking CHM can increase endometrial thickness and ER through molecular pathways and gene expression changes, thus improving the pregnancy rate. Liu et al. conducted a study on 120 patients. They found the expression of Hox10 mRNA and ER in the CHM group was increased, which suggested that the treatment of CHM improved ER by increasing the expression of Hox10 mRNA in the endometrium [116]. Yang et al. also reached a similar conclusion in the study of SD rats with implantation disorders [117]. Xin et al. studied the effects of CHM on ER and endometrial angiogenesis in rats and concluded that CHM can promote ER recovery and endometrial angiogenesis by regulating the expression of PI3K, HIF-1A signaling pathway, and VEGF [118].

4.3.2. CHM Regulates Ovarian Function. CHM can improve autophagy or apoptosis of ovarian granulocyte and protect ovarian function by regulating the molecular signaling pathway and molecular expression. Gao studied the effect of CHM on follicular development in rats with follicular dysplasia. He thought that it may be through activating

Table 1: Summary of randomized studies of the effect of acupuncture on infertility outcomes.

Study ID	Design	Sample size	Interventions	Outcomes	Limitation
26	RCT	160	Treatment arm: acupuncture intervention control arm: no intervention	Treatment arm: PR, 42.5% [34 of 80]* control arm: PR, 26.3% [21 of 80]	Not mentioned blindness Small sample size
27	Double-blind, RCT	225	Treatment arm: acupuncture intervention control arm: placebo acupuncture intervention	Treatment arm: PR, 33.6%; OPR, 15.6%* control arm: PR, 28.4%; OPR, 13.8%	Small sample size
28	RCT	60	Treatment arm: acupuncture combined with TCM intervention control arm: estradiol valerate tablets intervention	Treatment arm: PR, 26.7% [8 of 30]* control arm: PR, 6.7% [2 of 30]	Not mentioned blindness Small sample size
31	Double-blind, RCT	1000	Treatment arms: active acupuncture plus clomiphene group; active acupuncture plus placebo group control arms: control acupuncture plus clomiphene group; control acupuncture plus placebo group	There were no significant differences in outcomes of LBR between treatment arms and control arms	
34	RCT	62	Treatment arm: acupuncture intervention control arm: no intervention	There were no significant differences in outcomes of OPR between the two groups	Not mentioned blindness Small sample size
47	Single-blind, RCT	60	Treatment arm: auricular acupuncture intervention control arm: sham auricular acupuncture intervention	Auricular acupuncture revealed a significant increase in ghrelin level and decrease in leptin level than sham auricular acupuncture	Single-blind trial Small sample size

Note: RCT: randomized clinical trial; PR: pregnancy rate; OPR: ongoing pregnancy rate; LBR: live birth rate. *$P< 0.05$ versus treatment arm.

PI3K/Akt/mTOR signaling pathway, reducing the apoptosis-related molecule cleaved Caspase-3 and the apoptosis rate of rat ovarian granulosa cells (GCS), which provides a new idea for the treatment of follicular development disorders [119]. Li used a similar rat model to study and found that CHM may increase the expression of IGF-1R and HIF-1A in the rats' ovarian and downregulate the expression of proapoptotic factor FOX03a, to inhibit excessive follicular atresia and to promote the growth and development of follicles [120]. Chen used circrNAS chip technology to screen the differentially expressed circrNAS in plasma of POI patients and normal people. 35 differentially expressed circrNAS were screened and partially differentially expressed circrNAS were verified by quantitative RNA (QRT-PCR). The results showed that HSACirC0000367 was downregulated in POI patients, suggesting that HSA-CirC0000367 may play an important role in the POI process [121]. Shi and Sun et al. also reached the same conclusion [122, 123].

4.3.3. CHM Decreases Tubal Inflammation. Hydrotubation is often used in tubal obstructive infertility, but the efficacy cannot meet expectations. TCM takes the damp heat and obstructs the uterus as the etiology and pathogenesis of this disease, which leads to the failure of fertilized ovum formation. The treatment is usually based on invigorating blood and dissolving stasis. The treatment of CHM can significantly reduce the inflammatory factors, thus improving the pregnancy rate. Qiu's research found that the mechanism of CHM in treating tubal obstructive infertility may be through regulating the gene of TLR2, MyD88, and NF-κB and inhibiting the NF-κB Effects of serum-containing Tongguan Pill on TLR2, MyD88, and NF-κB gene expression in macrophage inflammatory models [124]. A study has shown that high concentrations of TNF-α were detected in the tubal fluid of patients with tubal inflammation infertility, which was believed to be a certain role in the occurrence and development of tubal inflammatory infertility [125]. Ma et al. conducted an RCT on 82 patients with tubal obstructive infertility and found that hysteroscopic tubal fluid drainage combined with CHM could effectively improve the efficacy and significantly reduce the TNF-α [126].

4.3.4. CHM Improves Hormone Levels. The endocrine disorder is the main factor leading to infertility. Oral CHM has an obvious effect on improving hormone disorder in infertile patients. Numerous studies have found that oral CHM could improve pregnancy rates by regulating HPOA, improving insulin resistance, etc. Cao DD treated POI rats with CHM and found that it could improve impaired ovarian function and regulate sex hormones mainly through the MAPK pathway [127]. Jiang gave Bushen Cuyun Recipe (BCR) for the DOR rats. After treatment, the ovarian morphology, follicle, corpus luteum, and serum AMH of the DOR rats were significantly improved. Through network pharmacologic analysis, they found that the possible

Table 2: Summary of randomized studies of the effect of CHM on infertility outcomes.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitation
97	RCT	150	Treatment arm: group B : CC + Zhushi Tiaojing Cuyun formula control arm: group A : CC; group C : CC + aspirin	Treatment arm: PR, group B: 52% [26 of 50]* control arm: PR, group A: 18.0% [9 of 50] PR, group C: 32.0% [16 of 50]*	Zhushi Tiaojing Cuyun formula: radix codonopsis (Dang Shen), astragalus root (huang Qi), *Angelica sinensis* (Dang Gui), prepared rehmannia root (Shu Di Huang), Morinda officinalis (Ba Ji Tian), Epimedium (Yin Yang Huo), Dodder (Tu Si Zi), Raspberry (fu Pen Zi), Photinia leaf (Shi Nan Ye), acorus tatarinowii (Shi Chang Pu), Salvia (Dan Shen), Safflower (hong Hua), Human placenta powder (Zi He Che Fen), Citrus (Chen Pi)	Not mentioned blindness and drop-out rate
98	RCT	80	Treatment arm: Bushen Peiyuan Yanggong decoction + estrogen + progesterone control arm: estrogen + progesterone	Treatment arm: PR, 57.50% [23 of 40]* control arm: PR, 37.50% [15 of 40]	Bushen Peiyuan Yanggong decoction: astragalus (Huang Qi), prepared rehmannia root (Shu Di Huang), Dodder (Tu Si Zi), Cyathula root (Chuan Niu Xi), Fructus Lycii (Gou Qi Zi), *Angelica sinensis* (Dang Gui), Danshen root (Dan Shen), Epimedium (Yin Yang Huo), Ligusticum wallichii (Chuan Xiong), roasted liquorice (Zhi Gan Cao)	Not mentioned blindness and drop-out rate Small sample size
100	RCT	120	Treatment arm: Yuyin Ling + clomiphene control arm: clomiphene	Treatment arm: PR, 46.7% [28 of 60]* control arm: PR, 20.0% [12 of 60]	Yuyin Ling : Yam (Shan Yao), prepared rehmannia root (Shu Di Huang), Chinese herbaceous peony (Shao Yao), Dodder (Tu Si Zi), *Angelica sinensis* (Dang Gui), Eucommia (Du zhong), Placenta (Zi he che), Cyperus (Xiang fu), Danshen root (Dan shen), achyranthes bidentata (huai niu xi), Tortoise shell (Gui jia), Bupleurum (Chai hu) Modifications: Severe phlegm dampness: Add Citrus (Chen pi), acorus tatarinowii (Shi chang pu) Severe blood stasis: Add Ligusticum wallichii (Chuan xiong), Trogopterus dung (Wu ling zhi), *Angelica sinensis* (Dang gui)	Not mentioned blindness and drop-out rate

Table 2: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitation
102	RCT	76	Treatment arms: Huoxue Quyu formula + ciprofloxacin control arms: ciprofloxacin	Treatment arm: PR, 39.5% [15 of 38]* control arm: PR, 18.4% [7 of 38]	Huoxue Quyu formula: Red peony (Chi Shao), Dried ginger rhizome (Gan Jiang), Peach kernel (Tao Ren), Safflower (hong Hua), Ligusticum wallichii (Chuan Xiong), Tree peony bark (Dan Pi), Fennel (Xiao Hui Xiang), Radix aucklandiae (Mu Xiang), Herba Patriniae (Bai Jiang Cao)	Not mentioned blindness and drop-out rate Small sample size
103	RCT	80	Treatment arm: Wenjing Tongluo decoction + Tubal hydrotubation control arm: Tubal hydrotubation	Treatment arm: PR, 67.5% [27 of 40]* control arm: PR, 40% [16 of 40]	Wenjing Tongluo Decoction: Evodia rutaecarpa (Wu Zhu Yu), White peony root (Bai Shao), Dwarf lilyturf (Mai Dong), Ligusticum wallichii (Chuan Xiong), cassia twig (Gui Zhi), Moutan (Mu Dan Pi), hide gelatin (E Jiao), Ginger (Sheng Jiang), *Angelica sinensis* (Dang Gui), Pinellia ternate (Ban Xia), Licorice (Gan Cao)	Not mentioned blindness and drop-out rate Small sample size
106	RCT	56	Treatment arm: Bushen Culuan Decoction control arm: estradiol valerate tablets/ estradiol cyproterone tablets (clement) +clomiphene	There was no significant difference in outcomes of PR between two groups	Bushen Culuan Decoction: Dodder (Tu Si Zi), Ligustrum (Nv Zhen Zi), Medlar (Gou Qi Zi), Mistletoe (Sang Ji Sheng), Radix dipsaci (Xu Duan), cyathula root (Chuan Niu Xi), Red peony (Chi Shao), *Angelica sinensis* (Dang Gui), Lycopus lucidus (Ze Lan), Danshen root (Dan Shen), Rhizoma cyperi (Xiang Fu), Cattail pollen (Pu Huang)	Not mentioned blindness Small sample size

Table 2: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitation
107	RCT	80	Treatment arm: self-designed Bushen huoxue decoction + ethinylestradiol cyproterone tablets + clomiphene citrate capsules control arm: ethinylestradiol cyproterone tablets + clomiphene citrate capsules	Treatment arm: PR, 85% [34 of 40]* control arm: PR, 65% [26 of 40]	Self-designed Bushen huoxue Decoction: *Angelica sinensis* (Dang Gui), Ligusticum wallichii (Chuan Xiong), Epimedium (Yin Yang Huo), Danshen root (Dan shen), Dodder (Tu Si Zi), prepared rehmannia root (Shu Di Huang), Dried radix rehmanniae (Sheng Di Huang), Red peony (Chi shao), Ligustrum (Nv Zhen Zi), Eclipta (Mo Han Lian), Cyathula root (Chuan Niu Xi), Morinda officinalis (Ba Ji Tian), Herba leonuri (Yi Mu Cao), Safflower (hong Hua), Bupleurum (Chai Hu), Licorice (Gan Cao)	Not mentioned blindness and drop-out rate Small sample size
108	RCT	80	Treatment arm: Shugan Jianpi formula + bromocriptine control arm: bromocriptine	Treatment arm: PR, 42.5% [17 of 40]* control arm: PR, 17.5% [7 of 40]	Shugan Jianpi formula: bupleurum (Chai Hu), raw malt (Sheng Mai Ya), *Angelica sinensis* (Dang Gui), White peony root (Bai Shao), Indian bread (Fu Ling), atractylodes (Bai Zhu), achyranthes bidentata (Niu Xi), Licorice (Gan Cao)	Not mentioned blindness and drop-out rate Small sample size
109	Multicenter double-blind placebo parallel controlled RCT	202	Treatment arm: ①Before ovulation: Huoxue Xiaoyi granule; ②After ovulation: Bushen Zhuyun granule control arm: Placebo treatment	Treatment arm: PR, 44.6% [45 of 101]* LBR, 34.7% [35 of 101]* control arm: PR, 29.7% [30 of 101] LBR,20.8% [21 of 101]*	Huoxue Xiaoyi granule: radix bupleuri (Chai Hu), Cyperus (Xiang Fu), Salvia Miltiorrhizae (Dan Shen), Rhizoma Curcuma (Jiang Huang), Radix Paeoniaerubra (Shaoyao) Bushen Zhuyun Granule: Radix Bupleuri (Chai Hu), Indian bread (Fu Ling), Ligustrum lucidum (Nv Zhen Zi), Eclipta (Mo Han Lian), Rhizoma atractylodes (Bai Zhu) Radix dipsaci (Xu Duan)	

Table 2: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitation
110	RCT	62	Treatment arm: Bushen Yangjing granule + letrozole control arm: compound packaging of estradiol tablets/estradiol and progesterone tablets + letrozole	Treatment arm: PR, 67.7% [21 of 31]* control arm: PR, 35.5% [11 of 31]	Bushen Yangjing granule: prepared rehmannia root (Shu Di Huang), *Angelica sinensis* (Dang Gui), White peony root (Bai Shao), Ligusticum wallichii (Chuan Xiong), Dodder (Tu Si Zi), Fructus Lycii (Gou Qi Zi), Semen plantaginis (Che Qian Zi), the fruit of Chinese magnoliavine (Wu Wei Zi), Fructus rubi (Fu Pen Zi), Cyathula root (Chuan Niu Xi), Cyperus (Xiang Fu), Fried Fructus aurantia (Chao Zhi Qiao), Radix codonopsis (Dang Shen), Epimedium (Yin Yang Huo), Salty anemarrhena asphodeloides (Yan Zhi Mu), Herba leonuri (Yi Mu Cao) Modifications: Postmenopausal: Add the amount of Dodder (Tu si zi), and add fallopia multiflora (He Shou Wu), remove Herba leonuri (Yi Mu Cao) Intermenstrual period: Add Morinda officinalis (Ba Ji Tian), The seed of cowherb (Wang Bu Liu Xing), Liquidambar formosana hance (Lu Lu Tong); Premenopausal: Add amethyst (Zi Shi Ying), Radix dipsaci (Xu Duan); Menstrual period: Add Semen persicae (Tao Ren), Safflower (Hong Hua)	Not mentioned blindness and drop-out rate Small sample size

Table 2: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitation
112	RCT	60	Treatment arm: Bushen huoxue formula control arm: oral estradiol valerate	Treatment arm: PR, 47.7% [14 of 30]* control arm: PR, 20.0% [6 of 30]	Bushen huoxue formula: Bupleurum (Chai Hu), Dodder (Tu Si Zi), Raspberry (fu Pen Zi), Curculigo orchioides (Xian Mao), Psoralea (Bu Gu Zhi), prepared rehmannia root (Shu Di Huang), Epimedium (Yin Yang Huo), *Angelica sinensis* (Dang Gui), Rhizoma Dioscoreae (Shan Yao), Indian bread (Fu Ling), Ligusticum wallichii (Chuan Xiong), Cyperus (Xiang Fu), Dwarf lilyturf (Mai Dong), Roasted liquorice (Zhi Gan Cao), Parched hawthorn fruit (Jiao Shan Zha)	Not mentioned blindness and drop-out rate Small sample size
113	RCT	120	Treatment arm: Bushen Quyu decoction + laparoscopic surgery, and then gestrinone treatment control arm: laparoscopic surgery, and then gestrinone treatment	Treatment arm: PR, 68.3% [41 of 60]* control arm: PR, 43.3% [26 of 60]	Bushen Quyu decoction: polygonatum (Huang Jing), fallopia multiflora (He Shou Wu), Yam (Shan Yao), Epimedium (Yin Yang Huo), prepared rehmannia root (Shu Di Huang), Ligusticum wallichii (Chuan Xiong), Dodder (Tu Si Zi), Citrus (Chen Pi), Moutan (Dan Pi), Placenta (Zi He Che), Sliced deerhorn (Lu Jiao Pian)	Not mentioned blindness and drop-out rate
114	RCT	70	Treatment arm: Jinlinzi powder + sini powder + conventional western medicine control arm: conventional western medicine	Treatment arm: PR, 82.86% [29 of 35]* control arm: PR, 60% [21 of 35]	Jinlinzi powder + Sini powder: Jinlingzi, Bupleurum (Chai Hu), Radix aucklandiae (Mu Xiang), White peony root (Bai Shao), fruit of citron or trifoliate orange (Zhi Shi), Corydalis tuber (Yan Hu Suo), Inner layer of cinnamon (Gui Xin), Roasted liquorice (Zhi Gan Cao)	Not mentioned blindness and drop-out rate small sample size

Table 2: Continued.

Study ID	Design	Sample size	Interventions	Outcomes	Composition	Limitation
115	RCT	60	Treatment arm: Jianpi Bushen Zhuluan formula + letrozole control arm: letrozole	Treatment arm: PR, 56.7% [17 of 30]* control arm: PR, 30% [9 of 30]	Jianpi Bushen Zhuluan formula: Dodder (Tu Si Zi), Radix codonopsis (Dang Shen), Dried radix rehmanniae (Sheng Di Huang), Yam (Shan Yao), prepared rehmannia root (Shu Di Huang), Lotus fruit (Lian Zi Rou), Radix scutellariae (Huang Qin), Radix glehniae (Bei Sha Shen), Dendrobe (Shi Hu), polygonatum (huang jing), rose (Mei Gui Hua), Sargentodoxa cuneata (Hong Teng), Citrus (Chen Pi), Tangerine leaf (Ju Ye)	Not mentioned blindness and drop-out rate Small sample size

Note: RCT: randomized clinical trial; PR: pregnancy rate; LBR: live birth rate. *$P< 0.05$ versus treatment arm.

mechanism of BCR for infertility was the regulation of HPOA and prevention of ovarian granulosa cell apoptosis [128]. Zhang administrated CHM to PCOS-like rats and found that this medicine could effectively reduce the weight of rats and improve endocrine disorders [129]. After treatment with Liuwei Dihuang Pills, Qiu ZX found that the polycystic morphology of the ovaries of the PCOS-like rats was significantly restored. The possible mechanism could be the upregulation of CYP19A1 to restore follicular development and PI3K/Akt signaling pathway to reduce insulin resistance [130]. Yao treated the rats with hyperprolactinemia with CHM and found that the symptoms improved significantly. The mechanism may be through increasing the expression of IP3, PKC, and CaMK in the hypothalamus of rats to open the Ca2+ channel, thus further strengthening the signal transduction of dopamine D2 receptor [131].

All in all, the mechanism of oral CHM in treating infertility is pretty complex. To clarify the more accurate mechanism of its treatment of infertility, more research studies are needed in the future to provide uniform and accurate guidance for clinical treatment.

4.4. Clinical Efficacy and Mechanism of Chinese Herbal Diet Therapy in Treating Infertility. Food is the foundation of human existence. Since ancient times, there has been a saying in TCM that medicine and food are homologous. In addition, The Yellow Emperor's Inner Classic has clear requirements on the quality, quantity, time, cold or hot food, and compliance with the four seasons [132]. Chinese herbal diet therapy has also played an indispensable part in disease prevention and treatment. In the process of treating disease, corresponding herbal diet therapy is given based on the physical condition and disease pathology of patients [133]. Herbal diet therapy can also treat infertility. For patients who need to take medicine for a long time, adjuvant dietary therapy can reduce the burden of the digestive system [134]. When treating infertility, professor Ban XW usually adds mutton, soybean, duck, sea cucumber, etc. to CHM to increase the efficiency of medicine [135]. When treating infertility, professor Ban XW usually adds mutton, soybean, duck, sea cucumber, etc. to CHM to increase the efficiency of medicine. The theory of "taking the viscera to nourish the viscera" is also a treatment proved by thousands of years' practice. For example, pig liver, chicken liver, and other animal livers cooperated with Chinese herbs to treat infertility caused by liver depression; common yam rhizome, fleeceflower root, etc. to cure infertility of yin deficiency [134, 135].

Kang J treated DOR infertility with CHM and supplemented it with medicinal food, which effectively improved the clinical efficacy [136]. Wang et al. conducted an RCT to investigate the efficacy of the medicinal diet recipe "Warm Uterus Bao" combined with letrozole in the treatment of PCOS ovulatory infertility. After three menstrual cycles, the results showed that the effective rate of the treatment group was 90.00%, much higher than the control group (76.67%), with a statistically significant difference ($P < 0.05$). The ovulation rate and the pregnancy rate of the treatment group and control group were 81.18% versus 47.73% ($P < 0.01$) and 33.33% versus 10.0%, respectively ($P < 0.05$). This indicated that the combination of the medicated diet "Warm Uterus Bao" with letrozole had a better effect in treating PCOS dysfunction infertility, which can effectively improve the symptoms of patients and increase the ovulation rate and pregnancy rate [137]. Huang ZT studied 75 patients with anovulatory infertility and found that sea cucumber could promote endometrial growth, thereby increasing the pregnancy rate [138]. There are few studies on the mechanism of dietary therapy in infertility. Relevant articles discussed that fish, carrots, sesame, walnut, and other foods described in

Essentials from the Golden Cabinet are antiaging and longevity [139]. It is hoped that there will be more research studies on the effects of diet therapy on infertility to better guide patients with a healthy diet and play an auxiliary role in treating infertility.

5. Overview of Other CAM in the Treatment of Infertility

In addition to acupuncture and moxibustion and oral CHM, there are still other CAMs in treating infertility, such as Chinese medicine enema therapy, psychological intervention, and bionic electrical stimulation.

5.1. The Application of TCM Retention Enema in the Treatment of Infertility. The efficacy of TCM retention enema on tubal obstructive infertility is particularly significant. A warm enema containing Chinese medicine is administered before going to bed to treat fallopian tube adhesion. The drug can be absorbed directly by rectal mucosa, which is beneficial to improve the congestion, edema, adhesion, and hyperplasia of local tissues, and thus restoring the function of the fallopian tube [140, 141]. Xu conducted a clinical trial by giving TCM decoction retention enema to patients with tubal infertility, which had obvious efficacy. He believed that the structure of the rectum was close to the uterus, with a large number of venous plexuses and thin walls. Chinese medicine could penetrate the pelvic cavity through venous plexuses, improve the local microenvironment and blood circulation, and reduce inflammatory exuding [142]. Some researchers used TCM retention enema after a hysteroscopy to reduce the levels of TNF-α, IL-6, and IL-8, effectively slow down the chronic inflammatory response, improve the patency of fallopian tubes and the abnormal leucorrhea, and lower abdominal pain, thereby promoting the recovery of fertility [143–146]. Some researchers also used the external application of TCM and TCM retention enema after tubal interventional recanalization, which had a significant influence on hemorheology and also promoted the fertility of patients [147]. TCM retention enema combined with acupuncture can also improve the patency of fallopian tubes and pregnancy rates [148, 149]. Some researchers also treated the patients for thin-endometrial infertility with TCM retention enema combined with acupuncture, which improved the blood supply and morphology of the endometrium, enhanced ER, and further increased the pregnancy rate [150]. In addition, for infertility caused by endometriosis, western medicine is prone to relapsing with laparoscopic surgery alone, so it is necessary to use follow-up drugs. Some researchers used TCM retention enema after laparoscopic surgery to improve symptoms such as dysmenorrhea, menorrhagia, and dyspareunia, improve pregnancy rate, and regulate the balance of MMP-9 and TIMP-1 [151]. All studies mentioned above have been listed in Table 3.

5.2. The Application of Psychological Interventions on Patients of Infertility. Infertile patients, under pressure from society and family, have an urgent expectation of pregnancy, so they are prone to produce various negative emotions. Patients who particularly failed in IVF-ET have a higher degree of anxiety, which will further lead to reproductive endocrine dysfunction, thus affecting the success rate of treatment [152, 153]. Psychological interventions for infertility include health education, psychological counseling, relaxation training, and mindfulness-based stress reduction. First, it is beneficial to relieve patients' pressure, improve patients' mental health and somatization symptoms, restore fertility function, and increase the success rate of conception [154, 155]. Secondly, psychological intervention can also regulate the mood of infertile patients and improve sleep and life quality, thereby improving the pregnancy outcome of assisted reproductive technology [156, 157]. However, some studies have failed to confirm the effectiveness of the psychological intervention on the pregnancy outcome of assisted reproductive technology, so further studies are needed [158]. Thirdly, studies have confirmed that yoga, a form of relaxation training, can not only regulate physical and mental state and improve ART outcome but also reduce vaginal assisted delivery, relieve pain, and improve fetal outcome [159].

5.3. The Application of Biosimilar Electrical Stimulation on Patients of Infertility. Biosimilar electric stimulation can regulate nerve reflex and muscle tension, improve local blood perfusion, and promote tissue regeneration by stimulating pelvic nerve and muscle with various frequency currents. It is usually used to treat postpartum repair, uterine prolapse, urinary incontinence, and infertility. Otherwise, studies have shown that biosimilar electrical stimulation can be used to treat thin-endometrial infertility which can promote endometrial growth, increase blood perfusion, and improve EA and pregnancy rate [160, 161]. Biosimilar electrical stimulation can also accelerate the recovery of ovarian reserve function [162, 163].

5.4. The Application of Homeopathy on Patients of Infertility. Homeopathy is to activate the body's ability to heal itself and the immune system to facilitate the recovery process by using a low dose of homeopathic medicine. It is characterized by treating the body as a whole and addressing the causes of the disease rather than focusing on individual symptoms. Homeopathy is effective for infertility caused by psychological problems, ovulation disorders, sperm abnormalities, and unknown causes [164, 165]. Meanwhile, homeopathy is effective in improving patients' health, sperm quality, and hormone levels [166, 167]. Parveen used individualized homeopathy to successfully deliver a healthy newborn in an infertile patient with endometriosis complicated with fallopian tube abnormality and insufficient ovarian reserve, which suggested that homeopathy has a positive effect on infertility [168].

5.5. The Application of Hyperbaric Oxygen Therapy on Patients of Infertility. Hyperbaric oxygen therapy can increase blood oxygen content and oxygen partial pressure and improve the

Table 3: Clinical studies on retention enema in infertility treatment.

Study ID	Design	Sample size	Interventions	Outcomes	Limitation
146	RCT	92	Treatment arm: penqiangyan prescription and TCM retention enema control arm: routine treatment	Treatment arm: TE 80.43%* control arm: TE95.65%	Not mentioned drop-out rate
147	RCT	53	Treatment arm: hysteroscopy and TCM retention enema control arm: hysteroscopy	Treatment arm: PR 55.56%* control arm: PR23.08%	Not mentioned drop-out rate Small sample size
148	RCT	50	Treatment arm: laparoscopic surgery and TCM retention enema control arm: laparoscopic surgery	Treatment arm: PR72%* control arm: PR44%	Not mentioned drop-out rate Small sample size
149	RCT	86	Treatment arm: hysteroscopy and TCM retention enema control arm: hysteroscopy	Treatment arm: PR 62.7%* control arm: PR41.8%	Not mentioned drop-out rate
150	RCT	98	Treatment arm: tubal interventional recanalization and TCM retention enema control arm: tubal interventional recanalization	Treatment arm: PR53.1%* control arm: PR20.4%	Not mentioned drop-out rate
153	RCT	60	Treatment arm: TCM retention enema, acupuncture and estradiol tablets control arm: estradiol tablets	Treatment arm: TE 83.3%* control arm: TE50.0%	Not mentioned drop-out rate Small sample size

Note: RCT: randomized clinical trial; PR: pregnancy rate; TE : total effective rate; $^*P < 0.05$ versus treatment arm.

state of the whole-body organs. It can be used to treat infertility, acute kidney injury, and wound nonunion of malignant tumor [169]. Studies have shown that hyperbaric oxygen therapy can improve uterine hemodynamics and ER, improve ovum quality, and improve the reproductive capacity of infertile patients [170, 171]. However, studies have shown that hyperbaric oxygen therapy did not promote endometrial thickening but increased serum AMH level, which still needs further research [172].

6. Summary

In recent years, infertility has become the third disease after cardio-cerebrovascular disease and tumors. Women with infertility are also at increased risk of developing mental illness. Many patients and doctors are not satisfied with the efficacy of the conventional treatment. CAM, widely accepted as adjuvant therapy for infertility in many Western countries, has met a medical need in the infertile population. However, its effectiveness and safety are still controversial. Acupuncture and moxibustion and CHM are the most commonly used CAM for infertility. Besides, enema therapy and psychological intervention, etc. are also mentioned in this review. At present, there are some limitations with CAM treatment of infertility, such as small sample size, low quality, and lack of uniform standards. Therefore, the validation of CAM's effectiveness has been hindered. Therefore, we look forward to more high-quality studies on CAM in the treatment of infertility.

Conflicts of Interest

The authors declare that there are no conflicts of interest or financial interest regarding the publication of this paper.

Authors' Contributions

Jiaxing Feng, Jing Wang, and Yuehui Zhang contributed equally to this work.

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Review Article

The Treatment of Complementary and Alternative Medicine on Premature Ovarian Failure

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It has been confirmed by growing evidence that common hormone replacement therapy is associated with an increasing risk of causing cardiovascular disease and cancer, while complementary and alternative medicine (CAM) is gaining popularity and application in more and more patients with premature ovarian failure (POF). Although there is little data concerning the clinical safety and efficacy of CAM, the literature includes application studies on the phytoestrogen-rich herbal, acupuncture treatment and intervention therapy. This article reviews recent literature on CAM therapy for POF, aiming to provide theoretical support for clinical application.

1. Instruction

Premature ovarian failure (POF) refers to a condition in women who are in the normal range of menarche age and the normal development of secondary sexual signs before the age of 40 with ovarian dysfunction or even failure. The hormone is characterized by high gonadotropin and low estrogen, especially FSH, FSH > 40 u/L [1]. Moreover, the main clinical manifestations are menstrual cycle disorders, amenorrhea, fertility decline, and even infertility. At the same time, it is also paralleled with hot flashes, night sweats, insomnia, psychological distress, and sexual dysfunction that are similar to the symptoms of menopausal transition. The reduction of estrogen levels can also increase the incidence of osteoporosis, ischemic heart disease, autoimmune diseases, Alzheimer's disease, etc., as well as the risk of death, bringing many serious consequences [2–4]. The incidence of POF in patients over 40 years old is about 1% and over 30 years old is about 0.1% [1]. With the enlargement of social pressure, the incidence of POF is on the rise, and the age of onset is also younger than before. The exact etiologies of POF are still unclear, and most of them are believed to be related to iatrogenic factors, chromosomal/genetic defects, autoimmune diseases, infections, environmental factors, congenital enzyme deficiency, idiopathic factors, etc. [2, 3, 5–7].

Most of the symptoms of POF are associated with estrogen deficiency; therefore, the general treatment in western medicine of POF is hormone replacement therapy (HRT) simulating physiological hormone release. HRT can relieve perimenopausal symptoms and reduce the incidence of ischemic cardiovascular disease, osteoporosis, urinary symptoms, and Alzheimer's disease, improving the quality of life and prolonging life [8, 9]. POF patients cannot normally ovulate mature follicles in order to naturally conceive because of the ovarian follicle depletion; however, egg donation is a common method for treating POF infertility and the in vitro activation (IVA) and bone marrow-derived stem cells also have potential in treating POF infertility [10–12]. Additionally, melatonin supplement therapy is also playing an active role in preventing and treating ovarian dysfunction caused by chemotherapy [13]. Although HRT can relieve some clinical symptoms, it is not effective in

restoring ovarian function and fertility and may increase the risk of ovarian cancer, breast cancer, endometrial cancer, thrombotic disease, meningioma, and other diseases [14–18]. Therefore, it is necessary to find an alternative therapy to supplement or replace the conventional western medicine treatment in order to reduce the adverse reactions brought by the conventional western medicine treatment.

CAM is defined as "a group of diverse medical and healthcare systems, practices, and products that are not generally considered part of conventional medicine" [19]. The National Center for Complementary and Alternative Medicine (NCCAM) classified CAM treatments into five major categories: traditional medical practices, such as whole medical systems; mind-body interventions; biological substance–based practices; manipulative and body-based practices; and energy medicine. The use of CAM is related to many factors such as patients' ethnic education, perceived behavior, positive attitude, credibility, and cultural embedment [20–22]. The global usage rate of CAM reaches 9.8%–76.0%, varying in different countries. In the United States, the adult usage rate is 38%, while in Trinidad and Tobago the usage by nurses is as high as 92.4% [20].

Complementary medicine refers to methods used with conventional medicine; on the contrary, alternative medicine refers to methods that could replace conventional medicine. CAM may be beneficial to women who have failed or have contraindications to conventional treatment [23]. It has played a great role in the treatment of gynecological diseases, and some CAM therapies have great value in the treatment of breast cancer [24]. The use of CAM in the treatment of endometriosis can effectively shrink the lesion, inhibit the symptoms, and reduce the recurrence rate [25]. CAM can improve menstruation and ovulation in patients with POF and at the same time improve perimenopausal symptoms such as hot flashes, sweating, insomnia, anxiety, and osteoporosis. Consequently, the treatment of POF with CAM is a problem worthy of discussion. This paper reviews the efficacy of CAM in the treatment of POF by means of retrieval of literature on herbal therapy, acupuncture, massage, psychotherapy, dietary supplementation, vitamin, calcium, exercise therapy, bioelectric therapy, and other treatments.

2. Herbal Remedies

The term "herbal" is derived from the Latin "herba," translated as "grass." It is one of the oldest healthcare methods and made a great difference in ancient times. Plants have long been recognized for their therapeutic properties. Indigenous cultures around the world have used traditional herbal medicine to treat a myriad of maladies [26]. In the western world and many Asian countries, including China and India, patients often use complementary medicines in the form of herbal remedies. Herbs are widely used in the treatment of POF in East Asian countries, and herbal medicine is also widely practiced in clinics in China [27]. Herbal medicine is one of the most common forms of traditional Chinese medicine (TCM) and has always been considered the best among the treatment methods in Chinese medicine. Studies have shown that CHM is effective in treating POF [28]. There are numerous reports in the Chinese literature about the treatment of POF with herbal remedies, but only abstracts are available in English. TCM's holistic theory, "pattern identification," diagnoses symptoms based on diseases and development and treats them based on individual needs. The concept of "kidney" takes a basic role in the physiology of female menstruation and pregnancy in TCM's theories; most clinical physicians also believe that kidney deficiency is the main etiology and pathogenesis of the disease. The basic method of treating POF is to invigorate the kidney; commonly used methods include invigorating the kidney, promoting blood circulation, and invigorating the kidney and spleen, which are confirmed effective through modern research on the treatment of POF [29–31].

2.1. Compound Chinese Medicine. Through experiments and clinical experiences, Chinese clinical workers have found that TCM prescription of the POF treatment has many advantages, with effect being significant [32–34]. Furthermore, a section of researchers found that Bushen Huoxue decoction can effectively improve the clinical symptoms of patients with POF, by adjusting FSH levels and FSH/LH ratios, increasing AMH levels, and adjusting ANA-ACA-AOA, ACT-INH-FS, and other pathways to alleviate symptoms through clinical randomized controlled trials (RCT). Zuogui pill (ZGP) is a classic prescription of TCM, which has been widely used in the treatment of POF. Although there is no prominent difference in pregnancy rate after receiving ZGP treatment, the oestrus cycle, ovarian ultrastructure, follicular number, and corpus luteum are significantly improved. ZGP resulted in a significant decrease in serum follicle-stimulating hormone (FSH) concentration and an increase in estradiol (E2). In addition, after administration of ZGP, a significant downregulation of Bax and cytochrome c (Cyt-c) and an upregulation of B-cell lymphoma/leukemia 2 (Bcl-2) were observed at both the gene and protein levels; thus, it is assumed that the effect may be achieved by inhibiting mitochondrial-dependent apoptosis in follicles [35]. Moreover, Bushen Shugan decoction, Yulinzhu, Guilu Erxian decoction, Bushen Tiaojing decoction, and Bazhen decoction have been also proven to have therapeutic effects on POF (Tables 1–3) [36–44].

2.2. The Monomer of Herbal Medicine

2.2.1. Cistanche. In addition to the prescriptions, single herbal medicine is also effective in the treatment of POF. *Cistanche salsa* is the dry fleshy stem with scaly leaves of *Cistanche* of Orobanchaceae family. It is an extremely valuable TCM which is known as "desert ginseng." The use of *Cistanche salsa* in medicine was first seen in Classic of *Shennong Materia Medica*. During the Northern and Southern dynasties, *Cistanche salsa* was used in dietotherapy to tonify deficiency with the functions of tonifying kidney yang, tonifying essence and blood, moistening intestines, and purging bowels; thus, it was often used in the treatment of impotence and infertility [45]. The main chemical

Table 1: The summary of compound Chinese medicine on POF.

Classical prescription of TCM	Components	References
Bushen Huoxue decoction	*Codonopsis* 20 g, danshen root 20 g, *Angelica* 20 g, *Astragalus* 20 g, *Epimedium* 15 g, *Morinda* 12 g, *Rehmannia* 12 g, *Cuscuta*, *Rubus idaeus* 12 g	[32]
Zuogui pill (ZGP)	*Rehmannia* 20 g, yam 15 g, wolfberry 15 g, *Cornus alba* 10 g, *Cuscuta* 10 g, Lujiao 10 g, testudo gum 10 g, *Codonopsis* 20 g, *Ligustrum vulgare* 20 g, *Eclipta prostrata* 20 g, Chuan *Achyranthes* 10 g, *Angelica* 10 g, Xiangfu 10 g, *Caulis spatholobi* 20 g, turmeric 10 g	[36]
Guilu Erxian decoction	Testudo gum 6 g, Gum cornibus extant 6 g, wolfberry 10 g, *Angelica* 10 g, yam 15 g, Taizishen 15 g, *Cuscuta* 15 g, arbor aglaophotis 10 g, album aglaophotis 10 g, *Cornus* 10 g, *Rubus idaeus* 15 g, teasel 10 g, *Rehmannia* 10 g, *Ligustrum vulgare* 10 g, semina pirorum 10 g, Flumen purpura Car 6 g	[37]
Bushen Shugan decoction	*Bupleurum falcatum* 12 g, *Angelica* 15 g, Frixum album aglaophotis 12 g, *Cuscuta* 15 g, *Atractylodes fricta* 12 g, *Rehmannia* 15 g, motherwort 15 g, danshen root 12 g, licorice 6 g, gingiberi 6 g	[38]
Yulin decoction	Taizishen 15 g, *Cuscuta* 15 g, *Rehmannia* 10 g, *Atractylodes fricta* 10 g, *Poria* 10 g, *Cnidium* 3 g, *Angelica* 10 g, *Ligustrum vulgare* 15 g, *Eclipta prostrata* 15 g, *Eucommia* 10 g, *Polygonatum* 10 g, Chuanxiong 5 g, Cnecos 5 g,	[39]
Bushen Tiaojing recipe	*Rehmannia* 15 g, *Cornus* 10 g, yam 15 g, wolfberry 12 g, *Cuscuta* 12 g, *Ligustrum vulgare* 9 g, *Poria* 10 g, fleeceflower root 12 g, moro 10 g, Chuan *Achyranthes* 10 g, *Angelica* 9 g, teasel 10 g, *Eclipta prostrata* 9 g, danshen root 9 g, motherwort 10 g, arbor aglaophotis 10 g, *Cistanche* 12 g	[40]
Bazhen recipe	*Bupleurum falcatum* 6 g, *Angelica* 10 g, Chuanxiong 6 g, album aglaophotis 10 g, *Rehmannia* 10 g, *Codonopsis* 10 g, *Atractylodes* 6 g, *Poria* 10 g, licorice 3 g, Cremor cornibus extant 10 g, Xiangfu 10 g, Chuan *Achyranthes* 10 g,	[41]

Table 2: The summary of randomized clinical studies of compound Chinese medicine on POF.

Prescription	Design	Sample size	Interventions	Outcomes	References
Bushen Shugan recipe	RCT	$n = 117$	Control group A : Bushen Shugan recipe, control group B : artificial cycle therapy: estradiol valerate tablet + progesterone capsule, treatment group: Bushen Shugan recipe combined with artificial cycle therapy	Bushen Shugan recipe combined with artificial cycle therapy can significantly improve serum hormone levels, clinical syndrome scores, and clinical symptoms in patients with POF with kidney deficiency and liver depression syndrome.	[38]
Bushen Tiaojing decoction	Single blind, RCT	$n = 110$	Control group: hormone replacement therapy, treatment group: kidney-tonifying prescription	Bushen Tiaojing recipe can effectively reduce the symptoms and signs related to POF, improve reproductive axis status, upregulate GDF-9 and BMP-15 levels, and help to reduce the risk of long-term recurrence.	[40]
Yulin decoction	RCT	$n = 60$	Control group: sequential estrogen therapy (oral estradiol valerate tablets + progesterone capsules), treatment group: Yulinzhu	In the treatment of POI (kidney qi deficiency type), Yulinzhu could significantly improve the clinical symptoms, decrease the levels of FSH and LH, increase the level of E2, and increase the number of follicles in the basal antrum of ovary and the thickness of endometrium.	[43]

components of *Cistanche salsa* are phenylethanoid glycosides, iridoid, lignan, polysaccharide, etc. and are also rich in phytoestrogens. Modern pharmacological studies have shown that it has many biological activities, for instance, antiaging, protecting liver, relieving physical fatigue, antiosteoporosis, and moistening bowels [46]. Pan et al. [47] discovered through animal experiment that the decoction of *Cistanche* could improve the POF caused by cisplatin by interfering with the apoptosis of ovarian granulosa cells induced by cisplatin, which is of therapeutic significance to POF after chemotherapy. *Cistanche* can reduce follicular atresia and apoptosis, by regulating the level of sex hormones and inhibiting the expression of TNF-α and IFN-γ, and then slow down the rate of ovarian failure. *Cistanche* deserticola can also upregulate Bcl-2/Bax. It is suggested that *Cistanche* deserticola can inhibit POF, and its mechanism may be related to the level of sex hormone and the expression of TNF- α, IFN- γ, and apoptosis-related protein Bcl-2/Bax in ovary [48].

2.2.2. Cuscuta. *Cuscuta australis* R.Br or *Cuscuta chinensis* Lam. is the dry mature seed of the southern *Cuscuta australis*. This product mainly contains triterpenoid acids, sugars, saponins, starch, etc. [49]. The functions of *Cuscuta* basically include tonifying liver and kidney, consolidating concentration and shrinking urine, strengthening muscles and bones, promoting saliva and thirst quenching, and tranquilizing spirit [50]. The total flavonoids from dodder

Table 3: The summary of basic studies of compound Chinese medicine on POF.

Prescription	Experimental type	Sample size	Interventions	Outcomes	References
Zuogui pill (ZGP)	SD rats	$n = 54$	Control group, model group, three ZGP groups (3.2, 1.6, and 0.8 g/kg), and triptorelin group	After treating with ZGP, though the rate of pregnancy showed no significant difference, the estrous cycle, ovarian ultrastructures, and numbers of follicles and corpora lutea were improved significantly.	[35]
Guilu Erxian decoction	SD rats	$n = 40$	Control group, model group, wild yam compound nutrition soft capsule group, TCM group	Compared with the control group, modified Guilu Erxian decoction can effectively increase the number of primordial follicles and primary follicles in the ovarian tissue of POI rats, and its mechanism may be related to the inhibition of ovarian miR-190 expression.	[37]
Bushen Huoxue recipe (BHR)	SD rats	$n = 45$	Normal group, POF model group, and BHR group, with 15 mice in each group	The results demonstrated treatment efficacy of BHR on POF mice and revealed that BHR might repair the dysfunction of germline stem cells in the bone marrow and thus help to improve the ovarian reserve and enhance the ovarian function of POF mice through neogenesis.	[42]
Modified Bazhen decoction, (MBD)	SD rats	$n = 24$	Control group, POF group, MBD treatment group, and Fufang Ejiao Syrup (FES) treatment group	Compared with the control group, XIAP expression was significantly lower, and miR-23a and miR-27a expression were significantly higher in the POF group. XIAP expression was significantly higher, and miR-23a and miR-27a expression were significantly lower in the MBD group. MBD may be a useful TCM for the treatment of POF.	[44]

(TFSC) can significantly restore the ovarian function of rats with POF, increase the ovarian weight and the number of follicles, raise estrogen levels, and have an obvious effect on POF [51]. The experiment of Li et al. [52] proved that a variety of active ingredients extracted from dodder-*Lycium barbarum* drugs may have an effect on treating POF through the PI3K/AKT signaling pathway, MAPK, and other pathways, and dug out the potential active ingredients like sterol, sesamin, and potential target IL-6 and TNF simultaneously.

2.2.3. Epimedium. *Epimedium*, as one of the medicinal plants, comes from Berberidaceae, perennial herbs, also known as Xianling spleen. Compendium of Materia Medica believed that it has the effect of invigorating qi, strengthening muscles and bones, tonifying waist and knee, strengthening heart, etc. *Epimedium* is often used to treat female infertility, irregular menstruation, POF, and other diseases. Herba Epimedii mainly contains flavonoids, polysaccharides, lignin, phenolic glycosides, alkaloids, and other kidney-enhancing and aphrodisiac biological active components [53, 54]. The studies of Zhang et al. [55] found that *Epimedium* is closely related to the vitamin D axis, assuming that *Epimedium* may be used to treat female reproductive system diseases by regulating the expression level of vitamin D axis. Vitamin D axis may be a potential target for Chinese Herba *Epimedium* to interfere with female reproductive system diseases.

2.2.4. Maca. Maca is the rhizome of the cruciferous plant Maca solo, also known as beet root or Peruvian ginseng. Native to the Andean plateau of South America, its fleshy root is short conical and the outer skin is purple, cream, or yellow [56]. Maca mainly contains macaramide, macaenes, alkaloids, glucosinolates, thiohydantoin, sterols, and other chemical components [57], which play a role in sexual function, spermatogenesis, female reproductive function, memory, depression, anxiety, energy, benign prostatic hyperplasia, osteoporosis, and metabolic syndrome [58]. The experiment conducted by Wang et al. [59] used POF model of kidney yang deficiency syndrome in SD rats with domestic Maca, imported Maca from Peru, and evaluated the effect and mechanism of Maca in warming kidney yang and maintaining ovary. It was concluded that both kinds of Maca could improve kidney yang deficiency syndrome and maintain ovary in SD female rats, being effective after two weeks of Maca and more obvious after four weeks. The mechanism by which Maca can improve the maintenance of ovary of kidney yang deficiency syndrome is related to the effect of warming and tonifying kidney yang, invigorating spleen, and tonifying qi and has biaxial regulation on kidney-Tiangui-Chongren-cell axis and hypothalamus-pituitary-ovary-uterus reproductive axis.

2.2.5. Muniziqi. Muniziqi, a Chinese medicine, has the effect of preventing and treating POF and effectively changing the tissue morphology and hormone level of gonadal axis. Some

studies believed that [60] chronic stress can lead to POF. Histopathological changes of the ovary and hormonal disorders (E2, FSH, LH) may be among the important mechanisms. Muniz can improve the early lesions of POF, and its pharmacological mechanism may be related to the regulation of the disordered hormone levels (E2, FSH, LH) and the downregulation of the expression of related proteins PFN1 and CFL1 [61].

3. Acupuncture and Moxibustion

In recent years, acupuncture and moxibustion therapy has been widely used in the clinical treatment of various gynecological diseases due to its good clinical efficacy and few side effects [62, 63]. Since the pathogenesis of POF is mostly related to liver, spleen, kidney, and blood essences, acupuncture and moxibustion therapy can dredge meridians, regulate qi and blood, balance yin and yang, and harmonize the functions of viscera. Research has shown that acupuncture can regulate the hypothalamic-pituitary-ovarian axis (HPOA) function by activating the dopamine system in the brain, adjusting the function of HPOA so as to restore the dynamic balance of reproductive endocrine system back to normal physiology. Moreover, it is also the most promising treatment method to improve menopausal symptoms and reduce serum follicle-stimulating hormone and luteinizing hormone levels [63, 64]. Acupuncture and moxibustion therapy have been fully recognized in POF treatment and have proved to be superior to western medicine [64, 65]. According to the clinical study of acupuncture and moxibustion for primary ovarian insufficiency (POI), the intervention methods of acupuncture and moxibustion include filiform needle, electroacupuncture, acupoint catgut-embedding therapy, padding moxibustion, moxibustion, and auricular point sticking therapy [66].

3.1. Acupuncture

3.1.1. Acupoints. According to the statistics, the proportion of each type of POF from high to low in proper order is as follows: disharmony of lesser yin, disharmony of lesser yin and reverting yin, disharmony of lesser yin and greater yin, disharmony of greater yin, and disharmony of reverting yin [67]. This indicates that deficiency of kidney essence is the key to POF; qi dynamic stagnation and dual deficiency of qi and blood are also the main factors. Therefore, these commonly used acupoints should have the functions of tonifying the kidney, nourishing the liver and invigorating the spleen, and nourishing the three yin meridians and blood. Acupuncture and moxibustion acupoints of POF are mostly distributed in the back, lower limbs and abdomen [68]. Shenshu (BL23), Pishu (BL20), and Ganshu (BL18) of Foot Taiyang Channel of Bladder are commonly used in clinic on the acupoints of nape. Acupoints of the lower limbs such as Sanyinjiao (SP 6), Xuehai (SP 10), and Yinlingquan (SP 9) in Spleen meridian; Taixi (KI3), Yongquan (KI1), and Fuliu (KI7) in kidney meridian; and Zusanli (ST36), Fenglong (ST40), and Shangjuxu (ST37) in stomach meridian are mainly used [69]. Guanyuan (RN4), Zhongji (RN3), Qihai (RN6), etc. in Conception Vessel are the main used points in clinic [67, 70, 71]. Among them, acupoints of the lower limbs were used most frequently, accounting for 33.92%, and lower back was used most frequently, accounting for 32.76%. Among all the acupoints, Sanyinjiao (SP6), Guanyuan (RN4), and Shenshu (BL23) ranked the top 3, with the support degrees of 62.65%, 57.83%, and 49.40% [72].

3.1.2. Acupuncture Method. Acupuncture has its unique advantages in treating POF because of its various treatment methods. Zhang et al. [73] showed that acupuncture has certain advantages in the treatment of POF and can achieve a similar effect to estrogen; its mechanism may be related to the upregulation of gene and protein expression in the PI3K/Akt/mTOR signaling pathway. Yao et al. [74] randomly divided 104 patients with POF into acupuncture group and western medicine group. The results showed that the total effective rate of the acupuncture group was 90.4%, which was higher than the western medicine group's 67.3%; the acupuncture group's FN-γ and expression of TNF-α were significantly lower than those of the western drug group ($P < 0.05$). It effectively adjusted the levels of serum LH, FSH, and E2 and improved the pituitary and ovarian endocrine in patients with POF. Wu et al. [75] reported that the menstrual recovery rate of POI patients treated with Fang's regulating menstruation and pregnancy promoting acupuncture method is 86.7%, which can effectively adjust the menstrual cycle of patients with early-onset ovarian insufficiency, increase endometrial thickness, effectively reduce serum FSH levels, and improve symptoms of ovarian function and low estrogen. Zhang et al. [76] and Guo et al. [77] found that the acupuncture is superior to hormone therapy in improving clinical symptoms and menopausal recurrence and reducing serum FSH and LH levels in patients with POF. In addition, the latter can significantly increase the rate of menstruation, improve the clinical effective rate, and create better conditions for pregnancy, and the curative effect is stable. Wang et al. [78] adopted a prospective case sequence study design and used preacupuncture intervention to interfere with POF [79]. The results showed that the levels of FSH, LH, and E2 in patients were adjusted and improved in varying degrees, and the total effective rate after treatment was 86.7%. The clinical pregnancy rate of the latter reached 31.8%. In the study of Shang et al. [80], it was concluded that acupuncture can improve the serum hormone level of patients with early-onset ovarian insufficiency. There is a correlation between menstruation and pregnancy outcome in patients with POI. The possibility of pregnancy in patients with oligomenorrhea is higher than that in patients with amenorrhea, and acupuncture can improve the level of estrogen in obese patients more obviously.

3.1.3. Acupuncture Point Embedding Therapy. As the development and extension of acupuncture therapy, acupoint catgut-embedding therapy is an improved needling method relying on the needle retaining theory [81]. On the basis of

inheriting the short-term acupuncture effect, while strengthening the sense of acupuncture and prolonging the action time, it has been paid more and more attention in the treatment of gynecological diseases. Li et al. [82] through clinical randomized controlled study found that the acupoint catgut implantation combined with artificial periodic therapy achieved remarkable improvements in the clinical symptoms of POF in patients. Acupoint embedding could effectively improve estradiol level [83]. In the experiment conducted by Chen et al. [84], 18 patients with POF were given acupoint catgut-embedding therapy, with an average treatment period of 6 months and total effective rate of 72.2%. Studies have shown that acupoint catgut-embedding therapy combined with Kuntai capsule [85] and that combined with artificial menstrual cycle therapy [86] can be filled with kidney essence, promote the transformation of Tiangui, promote the prosperity of Chong and Conception Channels, and further coordinate the "kidney-Tiangui-Chongren-Baogong" axis. It can regulate the hormone level in ovary and promote the recovery of ovarian function. In Chen et al.'s clinical study [87] on the treatment of POF by combining TCM for tonifying the kidney and soothing the liver with acupoint catgut embedding, it was suggested that this combination had accurate efficacy, low recurrence rate, and few adverse reactions. In addition, in the experimental study conducted by Xia et al. [88] on the decline of ovarian reserve function and POF in female rats treated by acupoint cat embedding and TCM, the results suggested that cat embedding and TCM have the effect of improving the ovarian reserve function and have a positive effect on the prevention of the decline of ovarian reserve function and the treatment of POF.

3.1.4. Ventro-Acupuncture. Ventro-acupuncture is a microneedle therapy to treat systemic diseases by acupuncture at abdominal acupoints. Regarding the regulation of the Shenque system as the core, it treats diseases by regulating the body's meridians and nervous system and regulating the balance of visceral functions. Li et al. [89] used "Zhang's abdomen three needles" to treat POF. The total effective rate of the treatment group was 93.33%, which achieved definite curative effect. Cao et al. [90] observed the clinical efficacy of Guishen pills combined with ventro-acupuncture in the treatment of POF. Compared with the control group, the treatment group had better efficacy. At the same time, the treatment group was excellent in the improvement of E2, FSH, and LH.

3.1.5. Auricular Acupuncture. Auricular acupuncture is a traditional Chinese meridian therapy, including ear embedding therapy, ear sticking therapy, auricular bleeding, and automatic ear acupuncture therapy equipment. It achieves the purpose of preventing or curing diseases by stimulating auricular acupoints. Luo et al. [91] conducted a systematic review and meta-analysis of the efficacy and safety of ear acupuncture in the treatment of POI by searching multiple databases before August 2020. The results showed that stimulation of specific ear points can enhance the physiological functions of specific parts of the human body, promote qi and blood circulation, and regulate the reproductive function of the hypothalamus-pituitary-ovarian axis. Yang et al. [92] explored the clinical effect of body acupuncture combined with ear acupuncture in the treatment of kidney deficiency and liver depression POF; the total effective rate in the treatment group was 80%, which was superior to 66.67% in the control group. Meanwhile, the ear bean combined TCM therapy is widely applied in clinical practice. For example, Yang et al. [93] selected 90 patients with POF and divided them into 3 groups: 30 cases in Chinese medicine group, 30 cases in auricular group, and 30 cases in Chinese medicine combined with auricular group, with three menstrual cycles as a course of treatment and three courses of consecutive treatment. The results showed that the total effective rate for the auricular point combined with CHM decoction was 90.00%, for the Chinese medicine treatment group was 83.33%, and for the auricular acupoint treatment group was 76.66%. In the experiment conducted by Shen et al. [94], selected 60 patients were divided into two groups including Guishen pill with auricular point treatment group (combined treatment group) and artificial cycle group, with 30 patients in each group. Compared with the artificial cycle group (80.0%), the total effective rate (93.3%) of the treatment group was significantly higher ($P < 0.05$). Jin et al. [95] selected 70 patients with POF of kidney deficiency and liver depression type and then randomly divided them evenly into treatment group and control group, with 35 cases each; the control group was administered Marvelon orally, while the treatment group was treated with Bushen Tiaojing decoction combined with auricular acupoint pressing. The result showed that the total effective rate of the treatment group was 84.85% after 3 months of treatment, while that of the control group was 52.94% with significant differences.

3.1.6. Electroacupuncture. Electroacupuncture (EA) therapy uses density wave, and its low-frequency pulse current stimulates acupoints through filiform needle. It can adjust human body function, promote blood circulation, and stimulate muscles and nerves. Zhang et al. [96] found that the effect of EA on PI3K/Akt/mTOR signaling pathway may be one of the mechanisms involved in the attenuation of POF in mice. Tan et al. [97] first estimated that transcutaneous electrical acupoint stimulation (TEAS) can be used as a potential therapy to reduce radiation-induced ovarian failure by inhibiting the loss of primordial follicles, increasing the secretion of serum AMH, and inducing antioxidant and antiapoptotic systems. In clinic, electroacupuncture and heat sensitive moxibustion are also used to treat premature ovarian failure. Heat sensitive moxibustion is a kind of therapy guided by meridian theory. It uses burning moxa stick to moxibustion heat sensitive acupoints to stimulate acupoints to produce heat sensitive moxibustion feeling. Moxibustion pays attention to qi sensation, dredges meridians, regulates viscera, and improves curative effect. Tian et al. [98] treated 60 patients with POF of kidney deficiency and liver depression type by

electroacupuncture combined with heat sensitive moxibustion, with a total effective rate of 93.3%.

3.2. Moxibustion. Moxibustion refers to a kind of external treatment method that uses moxa leaf and other inflammable materials or drugs to burn or fumigate and iron acupoints or affected area after being ignited, so as to achieve the purpose of disease prevention and treatment through its warm stimulation and drug effect. Studies have shown that there is no significant difference in the clinical efficacy of different moxibustion methods in the treatment of POF, but all of them can be used for treating POF because of their safety and efficacy [99].

3.2.1. Warm Acupuncture. Warm acupuncture is a method of combining acupuncture and moxibustion. It can simultaneously adjust viscera and bowels, warm qi and blood, and balance yin and yang with the help of filiform needle, drug, and heat through the conduction of meridians and collateral. Wu et al. [69] study found that warm acupuncture at Zusanli (ST36) and Guanyuan (CV4) combined with ginger moxibustion at Baliao point had significant effect on POF patients and on the improvement of FSH/LH, PSV, and AFC. Clinically, warming acupuncture combined with traditional Chinese medicine decoction has achieved good curative effect on POF. Wei et al. [100] studied the effect of Yikun Tiaojing decoction combined with warm acupuncture on POF and its influence on ovarian blood flow state. Patients in each group were randomly divided into observation group and control group. All patients were given hormone therapy. The control group was given warm needling on this basis, and the observation group was given Yikun Tiaojing decoction on the basis of the control group. With continuous medication for 28 days as a course of treatment, two groups of patients were treated for 3 courses. The treatment effect, ovarian artery blood flow status, sex hormone levels, and ultrasonic examination indexes were compared between the two groups. The results showed that the total effective rate of the control group was 73.53% and that of the observation group was 94.12%. It was concluded that the observation group was significantly better than the control group.

3.2.2. Umbilicus Moxibustion. Umbilicus moxibustion combines the advantages of moxibustion, drugs, and acupoints, which can stimulate, mobilize, and strengthen the body's own regulatory system; promote the blood circulation of uterus and ovary; and promote the development and maturation of follicles. Yu et al. [101] discussed the clinical efficacy of umbilical acupuncture combined with Hunyuan moxibustion in the treatment of POF. 90 patients with POF were randomly divided into research group and control group; the research group was treated with acupuncture at navel, and Hunyuan moxibustion was performed at Shenque point after the acupuncture. The control group was given hormone cycle replacement therapy. The total effective rate of the research group was 93.33% compared with 71.11% of the control group. It has achieved good results in rat experiments and has considerable development prospects. In the study conducted by Jia et al. [102], they observed the effect of drug-separated moxibustion on the expression of Bcl-2 and Bax in rats with POF and the effect on the expression of VEGF and AMH in rats with POF [103], and they found that drug-separated umbilical therapy had significant promoting effects on the number of follicles and ovarian reserve function in the rats with POF and could also improve the ovarian function.

4. Other Treatments for POF

4.1. Massage. In clinical practice, traditional Chinese medicine combined with massage has quite good curative effect. For example, the experiment conducted by Liu et al. [104] discussed the clinical efficacy of Bushen Tiaojing decoction combined with acupoint massage therapy, the 60 patients were randomly divided into A and B groups, and patients in both groups were treated with artificial cycle hormone replacement therapy. On that basis, group B was treated with Bushen Tiaojing decoction and acupoint massage therapy, and the therapeutic effect and serum sex hormone levels of the two groups were compared. The results showed that the total effective rate of B group was higher than that of A group, the levels of FSH (follicle-stimulating hormone) and LH (luteinizing hormone) in B group were lower than those in A group, and the E2 (estrogen) levels in B group were higher those that in A group. Wu et al. [105] observed the clinical efficacy of Yishen Huoxue decoction combined with massage in the treatment of 33 patients with POF in the treatment group taking self-made Yishen Huoxue decoction orally, conducting acupoints (Dazhui (DU14), Tao Dao (DU13), Shenzhu (DU12), Lingtai (DU10), Yongquan (KI1), Zigong (EX-CA1), Sanyinjiao (SP6), and Zusanli (ST36)) massage therapy. In the control group, 26 patients were given Tibolone tablets once a day, and 8 mg of progesterone was added after 21 days for five days; both groups were treated for 6 months. Changes of E2, FSH, and LH level and improvement of clinical symptoms were observed before and after treatment. The results suggested that there was significant difference between the two groups before and after treatment, and the curative effect of the treatment group was more obvious than that of the control group.

4.2. Psychotherapy. POF has a negative effect on the quality of life and psychological well-being of patients. The modern integrative approach to the treatment of disease includes biological, modern ways of treating diseases with the integration of physical, mental, biological, psychological, and social factors, which may make people more inclined to promote or continue the medical efficacy. It is not assumed that there is a direct causal relationship between mental and physical phenomena, but that biological, psychological, and social factors influence each other at the same time [106, 107]. Recently, through clinical trials [108], some scholars have observed that the cure rate of patients with POF is significantly increased due to the combination of

conventional medical treatment and psychological treatment, which can not only improve the clinical symptoms, but also reduce the subjective burden and mental pressure and improve the quality of life. A large number of clinical experimental studies have shown that [109, 110] psychological nursing of patients with POF can significantly improve the adverse mood of the patients, play a good role in promoting the treatment effect, and have clinical promotion and application value. More and more doctors realize the benefits of adapting the combination of biopsychosocial therapy to treat diseases, and combine psychosomatic therapy with modern medical treatment based on their clinical expertise, which may greatly contribute to the treatment and prognosis of POF.

4.3. Dietary Supplements. TCM has always believed that medicine and food are homologous, having the same use and effect. A considerable part of common clinical medicine is not only medicine but also food. In the long development of TCM, food therapy has always attached great importance to nutrition. Zhang Xichun, a famous modern doctor, once said, "Diet therapy can not only cure diseases but also satisfy one's hunger. What's more, the diet therapy is more palatable than the traditional drugs, and it is believed that diet therapy is of no harm for treating disease, which means that if the chosen diet therapy is matched with the disease, the time of recovering from disease will be shorten, even if it is not, the disease will not get worse." Chinese medicine scholar Doctor Wei [111] followed the ancient admonition, repeatedly practiced, carefully selected the medicine bait, and made a dietetic prescription by himself. The prescription is based on four kinds of medicine, namely, Nansha ginseng, yam, lotus seeds, and *Dendrobium candidum*. The blood deficiency person removes *Dendrobium* and *Angelica sinensis*; qi deficiency person adds *Astragalus membranaceus*. All the carefully selected medicines are food and medicine. Stewing the medicine together with the tube bone, the soybean, the radish, and the mushroom has the guiding significance and the practical value in the clinical treatment of POF. In a model study conducted by BaezaI et al. [112], a polyphenol-supplemented diet (from flavonoids such as soybeans and green tea) was used to treat sham surgery and ovariectomized mature mice. It was found that dietary polyphenols can be used to restore immune function in elderly mice [113]. Some nutrients are protective factors of ovarian function like legume food, which can reduce the level of blood follicle-stimulating hormone in women and thus protect ovarian function. So far, there is little research on the relationship between dietary nutrition and POF which means that further research is needed to determine the risk factors that may affect the incidence of POF in dietary nutrition, thus guiding the rational diet structure, so as to prevent the occurrence of POF to a certain extent. Although there are limited studies on the efficacy of dietary supplements in POF, the results of these studies show promising evidence.

4.4. Vitamin Supplement Therapy. Vitamin D is a steroid hormone which is mainly produced by the skin after being exposed to sunlight while slightly taken in food, combining with calcium to relieve osteoporosis caused by POF. Vitamin *D* is found naturally in some food, such as certain fish (salmon, herring, and sardines), egg yolks, and mushrooms [114]. Many scholars have studied the relationship between serum vitamin *D* level and the influence of ovarian-related diseases [115–118], showing that vitamin *D* concentration in POF patients is lower than that in healthy people [119]. The experiment of Chang et al. [120] showed that vitamin *D* may enhance ovarian function by regulating androgen activity to treat POF. Most experts agree that vitamin *D* supplementation is necessary when the serum concentration of vitamin *D* is below 50 nmol/L (the normal range is 125 nmol/L), especially in women with diminished ovarian reserve undergoing [121]. Cheng et al. [122] studied the combination of Zishen Buyi Shujing decoction with vitamin E in the treatment of POF, which can effectively relieve clinical symptoms, promote menstrual cycle recovery, and help to improve the level of serology.

4.5. Calcium. POF leads to a decrease in estradiol concentration and an accelerated loss of bone density [123]. In the case of bone density loss, nondrug therapy and lifestyle changes are first-line interventions. The National Osteoporosis Foundation strongly recommends several ways to keep bones healthy. Quitting smoking, weight-bearing, strengthening muscle exercise, avoiding excessive alcohol consumption, and properly supplementing calcium and vitamin *D* [124] are the main living principles. WHO guidelines indicate that 1,000 mg of calcium per day is sufficient to maintain the appropriate bone density, while consumption of more than 1,200 to 1,500 mg per day may increase the risk of renal calculus or cardiovascular diseases, with no positive effect on bone mass. With regard to vitamin *D*, an intake of 800 IU per day is sufficient and vitamin *D* supplementation should be controlled under 25(OH)D (the recommended serum concentration is above 30 ng/ml) [124, 125].

4.6. Exercise Therapy. Exercise combined with progressive relaxation training can effectively improve the mental health status of patients with POF as it is simple and easy to implement without increasing the economic burden of patients, with high patient acceptance and strong operability [126]. The application value of exercise therapy and psychological counseling in patients with POF is extremely high, with obvious improvement of symptoms, normal sex hormone level, improvement of psychological state, and high satisfaction, which is worthy of promotion and extension [127]. Ana et al. [128] studied the effects of angiotensin-converting enzyme (ACE) inhibition and aerobic exercise on the heart of elderly female rats with POF (10 weeks) and found that physical exercise and electromagnetic therapy had similar beneficial effects on the morphology and heart function of elderly female rats with POF. Although the reason still remains a question, both treatments promote a reduction in myocardial contractile

power, and the reduced hydroxymethyl-epinephrine sensitivity suggests that both treatments may reduce the sympathetic action of the heart. In conclusion, the clinical effect will be more significant when the exercise therapy is combined with other therapies, especially the psychological intervention therapy.

4.7. Biomimetic Electrotherapy. With the continuous improvement of medical level, pelvic floor rehabilitation technology has been introduced in China and gradually used in clinical practice, becoming an important treatment in gynecology at the present stage step by step. The application of pelvic floor EMG imitating bioelectric stimulation in the decline of ovarian reserve function is gradually known. The principle [129] is to strengthen the whole pelvic floor muscle group by stimulating the electrode placed in the perineum at different frequency, stimulate the innervation of the pelvic floor muscle, and restore the nerve function. Li et al. [130] explored the clinical effect of TCM combined with biomimetic electrical stimulation on patients with decreased ovarian reserve function. The results showed that it could effectively regulate the endocrine and ovarian related indexes of the patients, improve the decline and even deficiency of estrogen in patients, at the same time significantly improve the symptoms of bone metabolism and osteoporosis, and reduce the physical and mental pain, anxiety, and depression of patients in order to achieve the purpose of enhancing the quality of life. Guo et al. [131] retrospectively analyzed the clinical data of 80 patients with POF in order to explore the effect of biomimetic electrical stimulation combined with sequential therapy of estrogen and progesterone in the treatment of POF. Studies have shown that biomimetic electrical stimulation is effective in patients with POF on the basis of sequential therapy of estrogen and progesterone, which can further promote the improvement of sex hormone level and bilateral ovarian hemodynamics, and does not increase the risk of adverse reactions with good safety.

Bionic electrical stimulation therapy is a kind of physical therapy, which is safe to use and easy to operate and has high compliance. It can be used as an adjuvant treatment for patients with premature ovarian failure, and its exact mechanism needs to be further explored.

Apart from that, many other treatments such as hypnosis, meditation, and practice have also proved to have good curative effects in gynecological diseases including dysmenorrhea [132, 133], ovarian cancer [134], endometriosis [25], and pelvic inflammation [135], but the curative effect in POF remains to be further studied and observed.

5. Discussion

In conclusion, although the effectiveness of herbal therapy and acupuncture therapy has been well documented, it is difficult to make definitive recommendations for clinical guidance. Most of these studies are hindered by a small sample size or limited to one ethnic group, and there are few studies on adverse reactions, so it is not clear whether the effects of these treatments span all ethnic groups and nationalities. Therefore, before making any clinical recommendations, it is necessary to conduct a large number of clinical randomized controlled trials, entirely composed of POF participants with different ethnic backgrounds. Existing studies have shown that interventions such as psychotherapy, exercise therapy, and dietary supplement therapy can reduce the related symptoms caused by POF, and there are almost no adverse reactions, which seems to provide safe treatment options worth considering; however, due to the limited number of studies, the specific treatment mechanism is not clear and there is lack of empirical support.

CAM is an increasingly mature treatment system. In future studies, more high-quality randomized controlled trials are needed for each CAM intervention to make the results more reliable and professional. There have been a large number of studies on herbal medicine and acupuncture therapy, and the treatment is relatively mature. Randomized controlled trials based on standardized preparations should be used in a consistent way to conduct a more systematic and professional review of these interventions and meta-analysis of the results, so as to show that they have clinical efficacy value. POF can be accompanied by mild or severe sexual dysfunction, eventually leading to depression or infertility, so it is more important for patients with POF to correctly dredge their psychology on the basis of respecting their privacy. Therefore, we suggest that, in today's fast-paced life, we should fully respect women, especially the lover, whether in life or work to give his wife tolerance and love of encouragement.

In the treatment of POF, in addition to mainstream medicine, CAM will continue to be widely used to better control the physical and mental diseases caused by POF, including preventing deterioration, reducing symptoms, and providing a healthy lifestyle. However, further clinical studies are needed to evaluate the effectiveness, safety, and cost-effectiveness of CAM in POF. A large number of complex genetic mutations and the interaction of today's fast-paced living environment play an important role in POF. CAM therapy combined with conventional therapy is expected to find a new treatment strategy for POF.

Disclosure

Jing Lin, Denghui Wu, and Liyan Jia are co-first authors

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

The Underlying Molecular Mechanisms Involved in Traditional Chinese Medicine *Smilax china* L. for the Treatment of Pelvic Inflammatory Disease

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Smilax china L. (SCL) is extensively used in the treatment of pelvic inflammatory disease (PID). This study aimed to clarify the potential active ingredients of SCL and mechanisms on PID. SCL was widely distributed in Japan, South Korea, and China, which was traditionally considered heat-clearing, detoxicating, and dampness-eliminating medicine. Systems pharmacology revealed that 32 compounds in SCL may interact with 19 targets for immunoenhancement, antiapoptosis, anti-inflammation, and antioxidant activity of the PID model. Molecular docking revealed that isorhamnetin, moracin M, rutin, and oxyresveratrol may have higher binding potential with prostaglandin-endoperoxide synthase 2 (PTGS2), mitogen-activated protein kinase 1 (MAPK1), siderocalin (LCN2), tumor necrosis factor (TNF), and matrix metalloprotein-9 (MMP9), respectively. Molecular dynamics simulation showed that the binding modes of moracin M-MAPK1, rutin-TNF, and oxyresveratrol-MMP9 complexes were more stable, evidenced by relatively smaller fluctuations in root mean square deviation values. Conclusively, SCL may treat PID by inhibiting inflammatory factors, antitissue fibrosis, and microbial growth.

1. Introduction

Pelvic inflammatory disease (PID), the infection and inflammation of the female upper genital tract, is a common cause of infertility, chronic pain, and ectopic pregnancy [1]. Diagnosis and management are challenging, largely due to a polymicrobial etiology that is not fully delineated [2]. Reportedly, it is estimated that 2.5 million American women aged between 18 and 44 have received a PID diagnosis in their lifetime [3], and one in eight women with a history of PID encountered difficulties in getting pregnant [4]. PID treatment is mainly based on broad-spectrum antibiotic regimens and surgical treatment [2,5]. Antibiotics are effective in lessening short-term morbidity but have no effects on long-term complications, due to the disease's complex mechanism and long-term process [6]. Although the incidence of PID has decreased because of screening for gonorrhea and chlamydia and the early intervention of broad-spectrum antibiotics, damage to the reproductive system caused by infection has not been ameliorated [7]. Therefore, the therapeutic goal for the treatment of PID ought to include both short-term microbiological effects

and long-term prevention of sequelae [6]. Also, the use of antibiotics is limited by the emergence of antibiotic resistance and PID without an identified pathogen. To inhibit progress, alleviate the long-term sequelae of PID, and avoid antibiotic resistance, it is often used in conjunction with traditional Chinese medicine (TCM) [8, 9]. Recent studies showed that the anti-inflammatory and immune mechanisms of PID were related to T cells, B cells, IG, cytokines (e.g., IL-6, TNF-α, and IL-1β), prostaglandin-endoperoxide synthase 2 (PTGS2), matrix metalloprotein-9 (MMP9), and TLRs signaling pathways [8, 10].

From the theoretical perspective of TCM, the internal pathogenesis of PID is the disharmony of yin-yang (a general term for all kinds of pathological changes due to imbalance and incoordination of yin and yang) and insufficiency of healthy qi, and the external is that the dampness-heat and heat toxin invaded the thoroughfare and conception vessels, uterus, and uterine vessels. It caused blood blockage and abdominal pain (Figure 1). Hence, the core excitation of PID onset is the blood stasis induced by dampness-heat (dampness-heat syndrome) [11, 12]. *Smilax china* L. (SCL), a Liliaceae plant, commonly known as "Baqia" (or "Jin Gang Teng"), is widely distributed in Asia (Figure 2). From 1887 to 2020, it was found 7636 times (Figure 2(a)) according to GBIF online database (https://www.gbif.org/species/5295472/metrics) and mainly distributed in Japan (3630 times) (Figure 2(b)), South Korea (2458 times) (Figure 2(c)), and China (1487 times) (Figure 2(d)). Its original plant is shown in Figure 3, including the form of lamina and stem (Figure 3(a)), Rhizoma pieces (Figure 3(b)), and fruits (Figure 3(c)). Its diversified styles of freehand sketching were recorded in *Chongxiu Zhenghe Jingshi Zhenglei Beiyong Bencao* in 1249 AD (Figure 3(d)), *Compendium of Materia Medica* in 1552 AD (Figure 3(e)), and *Flora of China* in 2004 AD (Figure 3(f)). Up to now, SCL has been included in Chinese pharmacopeia [13] with the effects of heat-clearing, detoxicating, and dampness-eliminating. It has still been widely used in TCM for the treatment of PID and formulated into granules, syrup, pills, and capsules, demonstrating a good curative effect [9, 14]. Based on the previous studies, steroid saponin, flavonoids, glycosides, and stilbenes are the principal chemical compounds in SCL and demonstrated anti-inflammatory effect via TLR-4-mediated signaling pathway [14–16]. The flavonoid derivatives such as engeletin, isorhamnetin, and quercetin are the main constituents for the treatment of PID by inhibiting extracellular regulatory protein kinase and SMAD2/3 protein phosphorylation, thereby relieving the degree of fibrosis in the uterus via ERK1/2 and TGFβ-SMAD2/3 signaling pathways [17]. However, most of these studies showed the rough mechanisms of extract in SCL against PID, and the main active compounds and how these compounds interact with PID-related targets to interfere with relevant signaling pathways are still unclear.

TCM is designed to maintain the balance of the body's functions utilizing a lot of intricate compounds in herbs. Because multiple constituents may produce synergistic regulation on different targets, elucidating the mechanisms of TCM always takes lots of time and resources. There is no doubt that systems pharmacology has recently emerged as a new field including physiology, genetics, biochemistry, and molecular simulation via integrating various research methods to investigate the complicated mechanisms of multiple compounds [18, 19]. Nowadays, systems pharmacology has been applied for revealing the pharmacological mechanism of TCM from the perspective of entirety. For instance, most of the ingredients from well-researched herbs are carried out by molecular simulation, such as pharmacophore matching and inverse-docking to clarify the candidate targets which is available for researchers to further illustrate the integral mechanism of TCM [20–22]. Otherwise, the particle interaction, a role of the fundament of integral regulation, is the same concerned. Thus, static molecular docking and calculation of molecular mechanics-generalized Born surface area (MM-GBSA) free binding energy will provide a view of good binding pose and binding free energy to ensure that the complexes of compounds and targets possess enough energy to engender reaction of biochemistry [23, 24]. Finally, molecular dynamics (MD) simulation is utilized to evaluate the stability of protein-ligand complexes obtained from molecular docking using root mean square deviation (RMSD) and explore the noncovalent interaction between the active ingredients of herbs and the predicted targets, such as hydrogen bond (H-bond) and decomposition of molecular mechanics-Poisson Boltzmann surface area (MM-PBSA) energy of amino acid residues [25]. Overall, a schematic representation of the workflow in this study is shown in Figure 4, which makes it possible to systematically decode the active compounds and mechanism of TCM in the network. Hence, we evaluated the whole candidate targets of active compounds and provided a perspective of the integral mechanism via enriching the functions of targets and dissected the molecular mechanism of SCL in the treatment of PID using computational systems pharmacology.

2. Methods

2.1. Screening of Potentially Active Compounds in SCL. SCL compounds were systematically listed as ligands from published paper mining [26, 27], TCMID (http://www.megabionet.org/tcmid/) [28], SymMap (https://www.symmap.org/detail/SMHB00008) [29], and TCMSP databases (http://tcmspw.com/tcmsp.php) [30]. All compound structures from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [31] were filtered by utilizing the "Lipinski rules" of the Molinspiration database (https://www.molinspiration.com/cgi-bin/properties) [32]. In the field of drug discovery, the Lipinski rules were used to screen the compound database to eliminate molecules that were unsuitable for drug use, including n·OHNH ≤ 5, n·ON ≤ 10, MW ≤ 500, and miLogP ≤ 5. Compounds that met the Lipinski rules and others that did not but possessed good bioactivity were used in systems pharmacology and molecular docking [33, 34]. The 2D structures (.sdf format) of all compounds were generated by ChemBioOffice2014 [35].

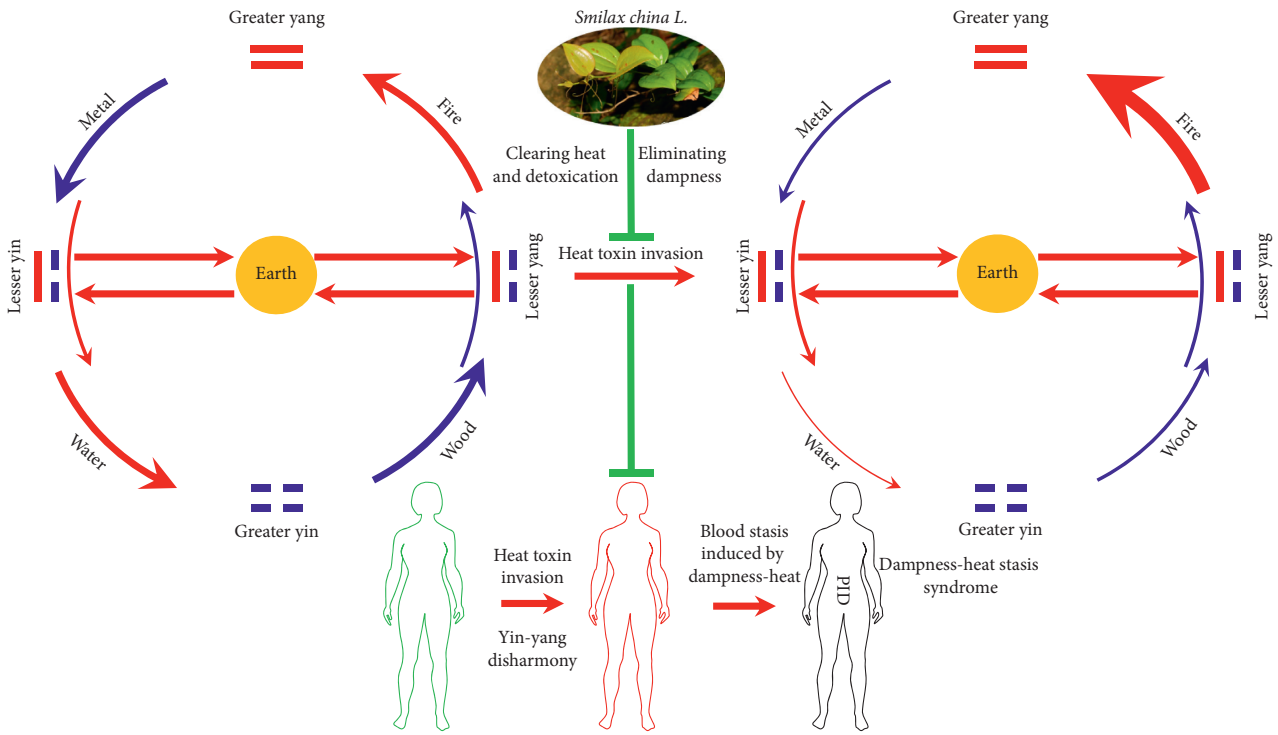


Figure 1: Effects of SCL on PID from view point of traditional Chinese medicine. From the theoretical perspective of TCM, the left and right circles represent the development process from physiological state of human (arrows of five phases are of the same size) to pathological state of PID patients (fire phase increased and water phase decreased). And the main interfering process of SCL against PID is between two circles. Above all, the internal pathogenesis of PID is the disharmony of yin-yang (a general term for all kinds of pathological changes due to imbalance and incoordination of yin and yang) and insufficiency of healthy qi (a collective designation for all normal functions of the human body and the abilities to maintain health, including the abilities of self-regulation and adaptation), and the external is that the dampness-heat and heat toxin invaded the thoroughfare and conception vessels, uterus, and uterine vessels. It caused blood blockage and abdominal pain. Hence, the core excitation of PID onset is the blood stasis induced by dampness-heat.

Figure 2: Distribution map on *Smilax china* L. by GBIF Secretariat online database.

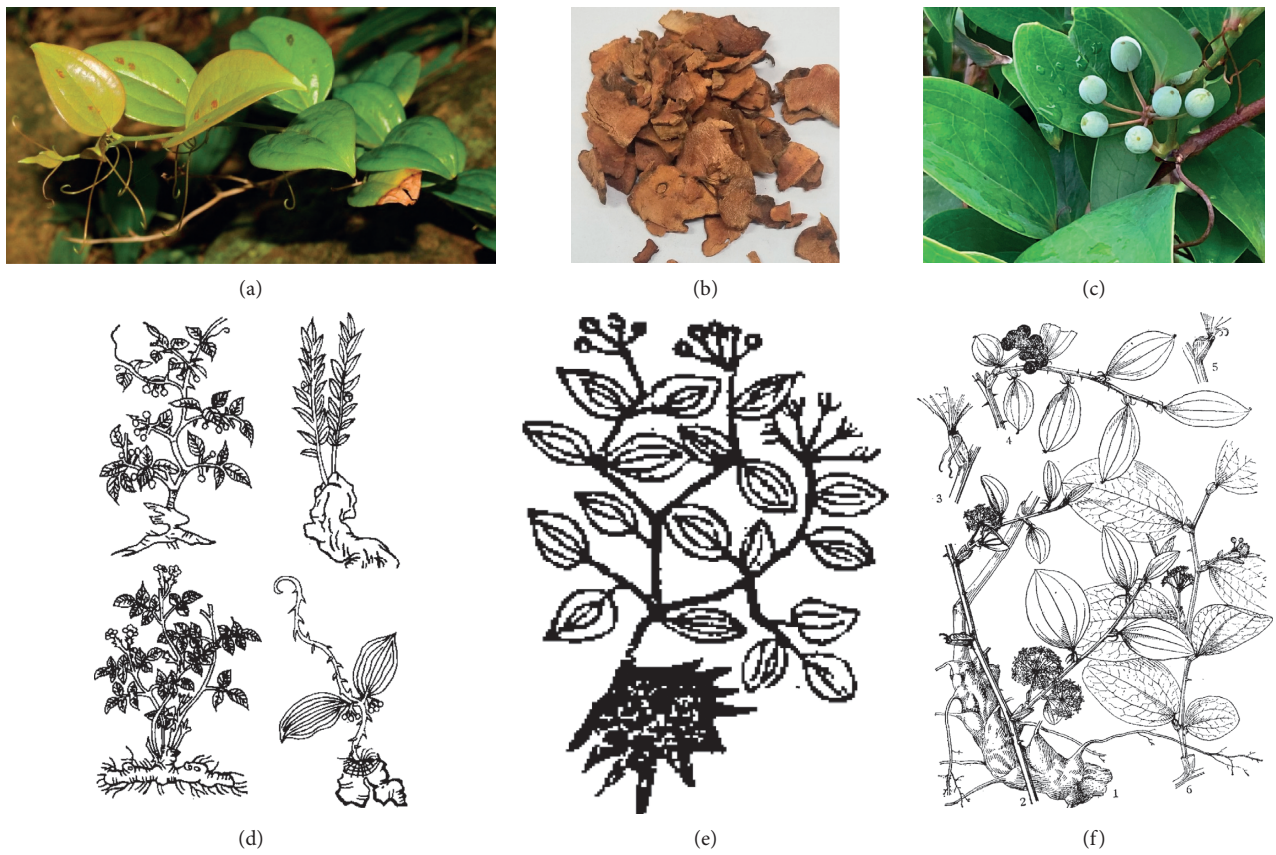
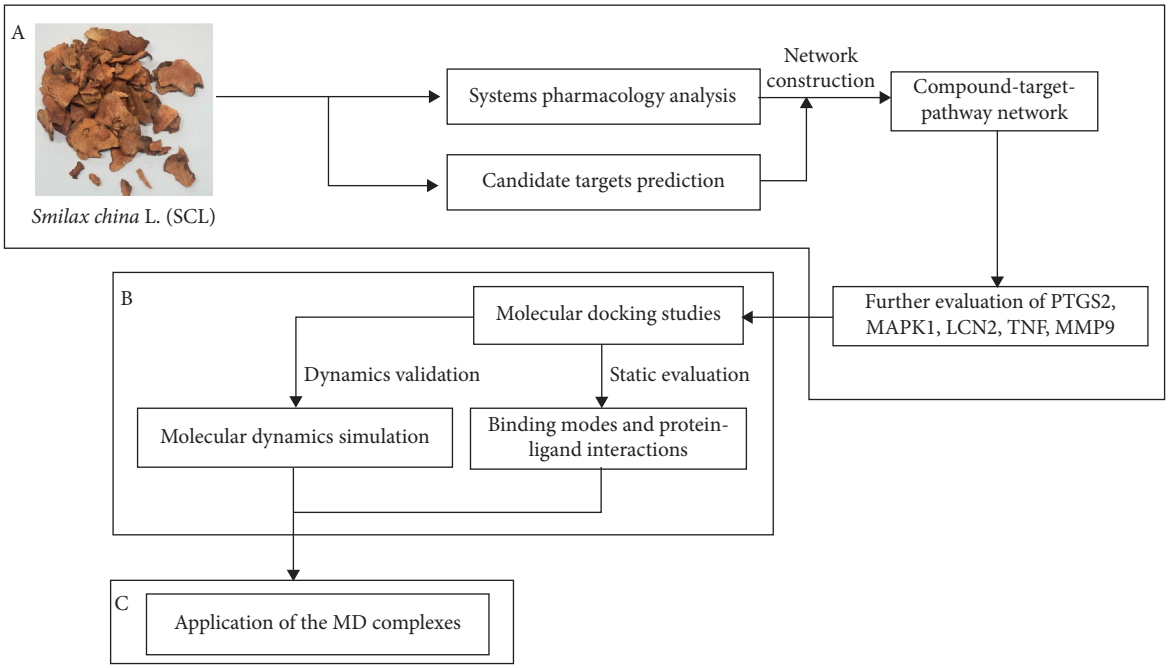


Figure 3: Original botany map and illustration of SCL plants in materia medicas. (a) The form of lamina and stem in SCL; (b) The Rhizoma pieces; (c) fruits. Its diversified styles of freehand sketching were recorded in *Chongxiu Zhenghe Jingshi Zhenglei Beiyong Bencao* in 1249 AD (d), *Compendium of Materia Medica* in 1552 AD (e), and *Flora of China* in 2004 AD (f).

2.2. Candidate Targets of Active Compounds in SCL. Compounds that satisfied Lipinski rules were uploaded to SwissTargetPrediction (http://www.swisstargetprediction.ch/) [36], PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) [37], and SEA (http://sea.bkslab.org/) [38] to obtain preliminarily candidate targets. All the genes should be from "*Homo sapiens*" to clarify the function of critical targets and were proofread by the UniProt database (https://www.uniprot.org/) [39].

2.3. Network Construction of Compound-Target-Pathway in SCL for PID Treatment. Systems pharmacology was applied to analyze the interaction between SCL and PID and the selection of critical targets. To identify the intersection of targets between PID and SCL, the title of "pelvic inflammatory disease" was placed in GeneCards (https://www.genecards.org/) [40], DisGeNET (https://www.disgenet.org/) [41], DrugBank (https://www.drugbank.ca/) [42], and existing research [43,44] to obtain gene names of PID targets, which manually confirmed that each target had a clinical study in PID. Additionally, the intersection genes (compound- and disease-related targets) were used to perform annotation analysis of the obtained crossover genes by using Gene Ontology (GO) and the KEGG pathway analysis functions in the STRING platform (https://string-db.org/) [45], with the intersection genes directly mapped to the pathway. Cytoscape [46] was used to visualize a network of "Compound-target-pathway (C-T-P network)". In this network, each compound, target, and pathway was indicated by nodes, and the interactions between each node were described by edges. The network was established to project an overview of the interactions among compounds, targets, and pathways.

2.4. Protein-Protein Interaction (PPI) Analysis of Crucial Targets in SCL for PID Treatment. To reveal the direct and indirect roles active compounds of SCL played in the pelvic inflammatory targets, the intersection targets were introduced to the STRING platform and a graphical network of PPI was generated [47]. In the network, each node represents all the proteins produced by a single, protein-coding gene locus, and edges represent protein-protein associations which are meant to be specific and meaningful, i.e., proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other. Ultimately, to clarify the interfering mechanism, compounds broke into PPI network targets with a high degree and relating to the critical pathway in the PPI network were selected to operate molecular docking.



A: SCR-target-pathway network

B: analysis of protein-ligand interactions

C: significant amino acid residues during binding process can guide the relevant drug design

Figure 4: A schematic representation of workflow in this study.

2.5. Docking between PID Crucial Targets and Active Compounds in SCL. Due to the algorithm defect of the database on target prediction, a thorough docking was performed to improve the credibility of systems pharmacology. The crystal structures (.PDB) of selected targets were obtained from RCSB (http://www.rcsb.org/) [48], and the crucial targets were docked with active compounds.

Schrödinger Glide was used to pretreat the 3D protein structure for docking, including adjusting the bond orders to ensure the stability of the chemical bonds between the atoms, adding the missing hydrogen atoms and amino acid residues, optimizing the orientation of amino acids and hydrogen atoms, optimizing the distribution of H-bonds, and removing water molecules and heterogeneous molecules. Finally, energy minimization with a force field OPLS-2005 was supplied.

At the end of pretreatment, the Receptor Grid Generation module was employed to select the ligand-binding cavity and generate a grid in protein. Next, the active compounds were introduced to Maestro and optimized by the liquid simulation of OPLS-2005 all-atoms force field in the LigPrep module, as well as combined into a ligand package. The docking accuracy was evaluated by standard precision (SP), as well as flexible docking. At this point, the docking preparation was completed, the scaling factor and partial charge cutoff of van der Waals radius scaling 1.0 and 0.25 were used to generate the grids on active sites, and ligand package was selected to perform molecular docking in the Ligand Docking module. Next, the generated Glide G-score was used to assess the affinity between compounds and proteins. Moreover, crucial targets were docked with their self-ligands to set positive contrast, and their Glide G-scores were used to measure whether compounds possessed a good affinity to the protein and standardize the score of compounds to be visualized as a heat map by MeV [49] (step 1: normalized genes/rows; step 2: hierarchical clustering: average dot product-complete linkage clustering).

2.6. Binding Free Energy Calculation (MM-GBSA) Based on SP Docking. Good poses and good scores were obvious by SP docking, but what was the binding free energy of the docked complex was another problem. Docking results showed the active compounds did bind to the active site of the protein, but could this association last long enough to elicit any potential biological response, as biological response largely depends upon the binding free energy of the association. Therefore, the docked complexes in SP mode were subjected to binding free energy calculation (MM-GBSA) using the Prime module of Maestro [50]. A total of 14 active compounds were selected for this analysis.

2.7. Molecular Dynamics Simulation. MD simulation is a powerful method in the analysis of the target-ligand interactions by considering the flexibility of binding pose. A good pose of complexes, an essential premise of MD, is

obtained by molecular docking. Otherwise, all atoms of the system in complexes are sanctioned to motion and interact for a fixed period (~45 ns), and the trajectories of atoms and molecules are defined through Newton's equations of motion. In this study, the best poses of SP docked ligand/protein with an excellent binding free energy were employed to operate explicit solvent MD simulation. GROMACS [51] as a computational tool of MD simulation was used for this purpose.

Due to five complexes that would perform MD simulation, we had to protocol slightly various systems to reach our expectation as shown in Supplementary Table 1. In general, coordinates and charge of ligands were generated using PRODRG 2.5 [52], and protein and SPC water model were described by the GROMOS96 43A1 force field [53] and defined in a solvent box with 8.5 nm × 8.5 nm × 8.5 nm. Na^+ and Cl^- ions were added to ensure the overall neutrality of the systems. Each MD simulation system was first relaxed to remove possible steric crashes by the steepest descent energy minimization algorithm and stopped minimization when the maximum force is <100.0 KJ/mol. In the second step, a 100 ps simulation was performed utilizing the canonical ensemble (NVT ensemble) using the modified Berendsen thermostat with a slowly ascending temperature from 0 K to 300 K, a fast temperature relaxation constant of 0.1 ps, and a temperature coupling of protein and ligand to prevent system bursting. Next, the periodic boundary condition was employed to produce the constant temperature and pressure (NPT) ensembles. The pressure was set at 1.0 bar and was controlled by the isotropic pressure scaling protocol applied in GROMACS. Moreover, no cutoff limit was used for electrostatic forces by employing the particle mesh Ewald (PME) algorithm. All bonds were constrained using the LINCS algorithm. Then the simulation time for each system was 45 ns, and the trajectories of simulated systems were saved every 10 ps. Finally, disintegrated calculation of binding free energy was hired to qualitatively analyze the principal force of interaction between protein and ligand. All results were visualized by QtGrace (https://sourceforge.net/projects/qtgrace/), VMD (http://www.ks.uiuc.edu/Research/vmd/), and LigPlot$^+$ (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/).

3. Results

3.1. Compound-Target-Pathway Network of SCL in the Treatment of PID. After filtering was operated for Lipinski rules, thirty-two in sixty-eight compounds were screened as active compounds in SCL and are listed in Supplementary Table 2. Chemical formats of smile and SDF were generated to predict the potential targets. Then 718 potential targets (some were duplicates) associated with compounds were screened from the SwissTargetPrediction, PharmMapper, and SEA server, respectively (Supplementary Table 3). Otherwise, eighty-six PID-related targets were retrieved from databases (Supplementary Table 4). Then, to clarify the relationship between herb and disease, a network of compounds, targets, and pathways was visualized by Cytoscape. We found that a total of 32 compounds could act on 19 key targets and associate with 16 relevant GO annotations and 10 effective pathways (Figure 5).

3.2. PPI Network Analysis. 19 intersection targets were analyzed using the PPI network in the STRING platform (Figure 6). Relevant parameters of the network were as follows: (1) number of nodes and edges: 19 and 91; (2) average node degree and expected number of edges: 9.58 and 26; and (3) PPI enrichment p value: $<1.0e-16$. Otherwise, red nodes (MMP9, tumor necrosis factor (TNF), interleukin-6 (IL-6), PTGS2, neutrophil gelatinase-associated lipocalin (LCN2), mitogen-activated protein kinase 1 (MAPK1), interleukin-2 (IL-2), and signal transducer and activator of transcription 3 (STAT3)) were used to highlight the IL-17 signaling pathway and Th17 cell differentiation. All highlighted targets except IL-2, STAT3, and IL-6 were selected as core targets to illuminate the SCL interfering mechanism against PID utilizing molecular docking and MD simulation.

3.3. Molecular Docking with Binding Free Energy. The heat map was employed to stick out the features of 32 active compounds as shown in Figure 7 (original data are presented in Supplementary Table 5). Compounds that had a high activity clustered together excellently, with a high affinity to PTGS2, LCN2, TNF, MAPK1, and MMP9. Rutin (10), isorhamnetin (18), oxyresveratrol (30), and moracin M (44) were found to occupy the top score, which exceeded or neared the original ligands in verified docking. Furthermore, compounds demonstrated a binding affinity to one or several targets. According to the distance metric of average dot product in Mev, 14 capital protein-ligand molecular interactions were analyzed in Table 1.

Further analysis (Figure 8) demonstrated that rutin possessed a strong binding ability to the carboxymycobactin binding cavity [54] of LCN2 and inhibitor binding cavity [55] of TNF (Figure 8(c): rutin and LCN2; Figure 8(d): rutin and TNF). Otherwise, as shown in Figure 8(a), isorhamnetin (–51.06 kcal/mol) formed four H-bonds with GLN 192, PHE 518, TYR 385, and SER 530 indicating that was matched well in the rofecoxib (a COX-2 inhibitor) binding pocket [56] of PTGS2. Figure 8(b) demonstrates that moracin M (–37.01 kcal/mol) separately formed one interaction of pi-cation and three H-bonds with LYS A 45, GLU A 62, GLN A 96, and GLU B 360, affecting the biding cavity of allosteric and ATP-competitive inhibitor [57] of MAPK1. Finally, as for the inhibitor binding activity [58] of MMP9, Figure 8(e) indicated that oxyresveratrol formed two pi-pi stacking with TYR 179 and PHE 192 and three H-bonds with ALA 191, HIS 210, and GLY 233, which illustrated the formation process of a good pose.

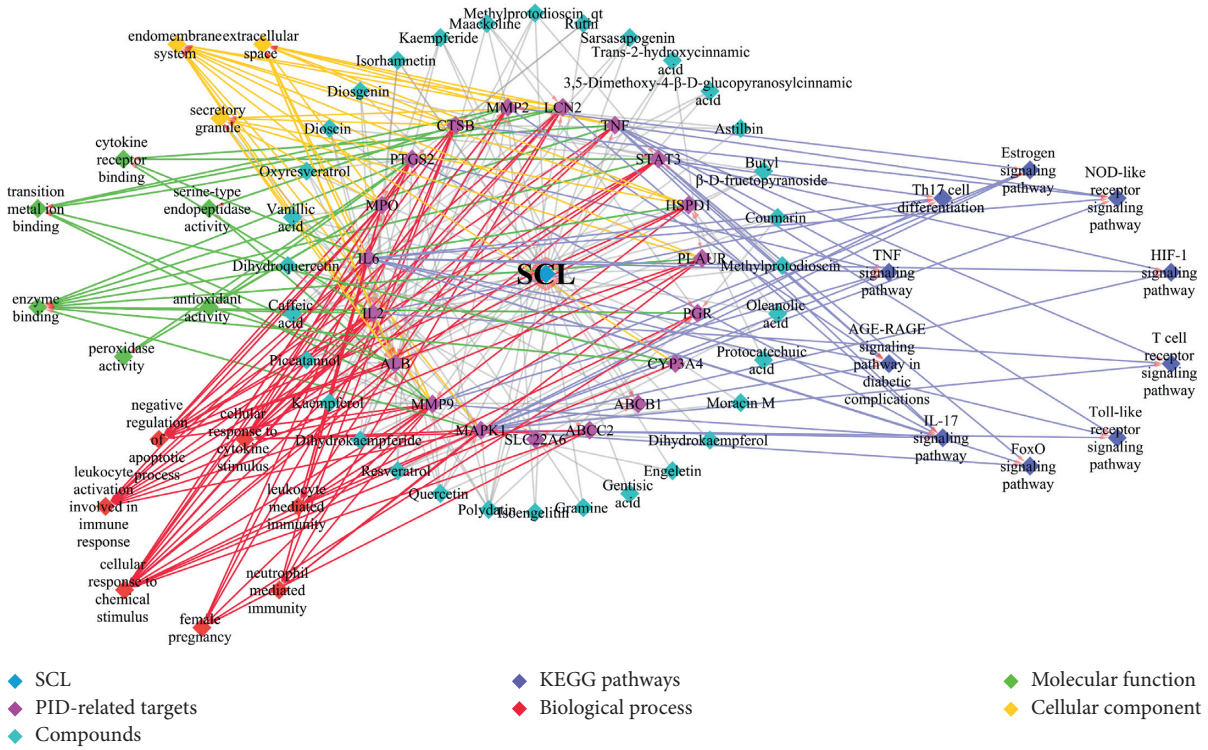


Figure 5: Compound-target-pathway (C-T-P) network. The network consists of compounds, targets, and pathways, including 78 nodes and 285 edges. 32 components interact with 19 target proteins and are associated with PID through 10 pathways and 16 GO annotations.

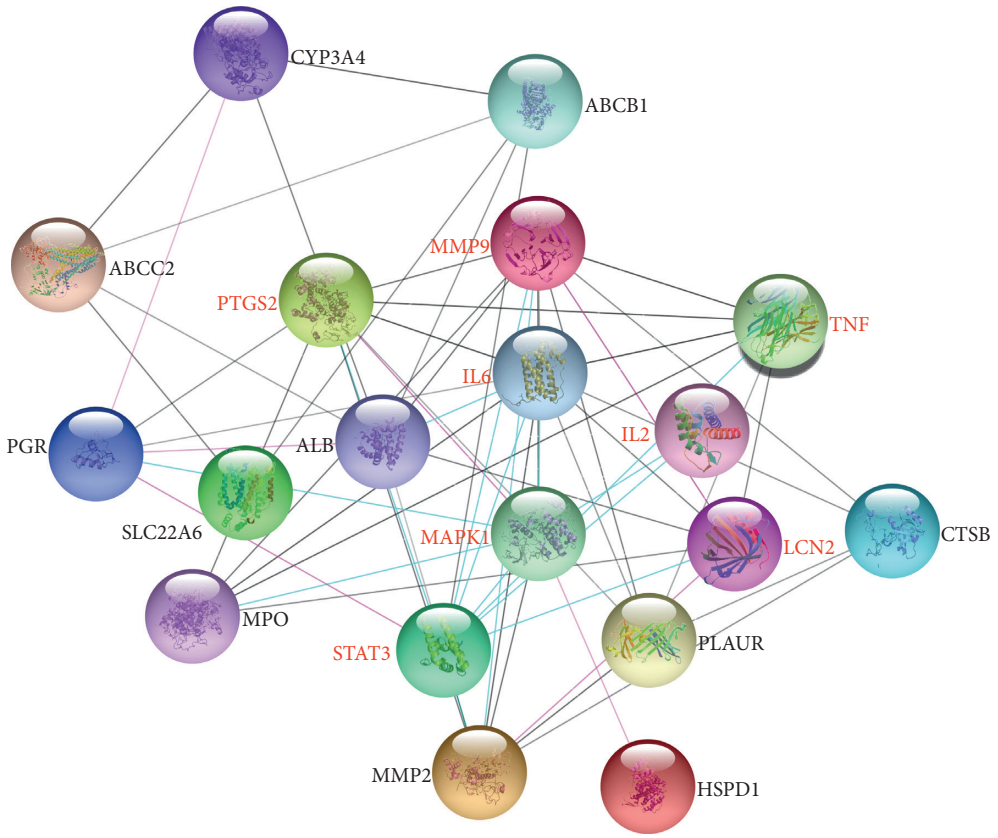


Figure 6: Protein-protein interaction analysis of 19 PID-related targets. Red labels were used to highlight the IL-17 signaling pathway (MMP9, TNF, IL6, PTGS2, LCN2, and MAPK1) and Th17 cell differentiation (IL2, MAPK1, IL6, and STAT3).

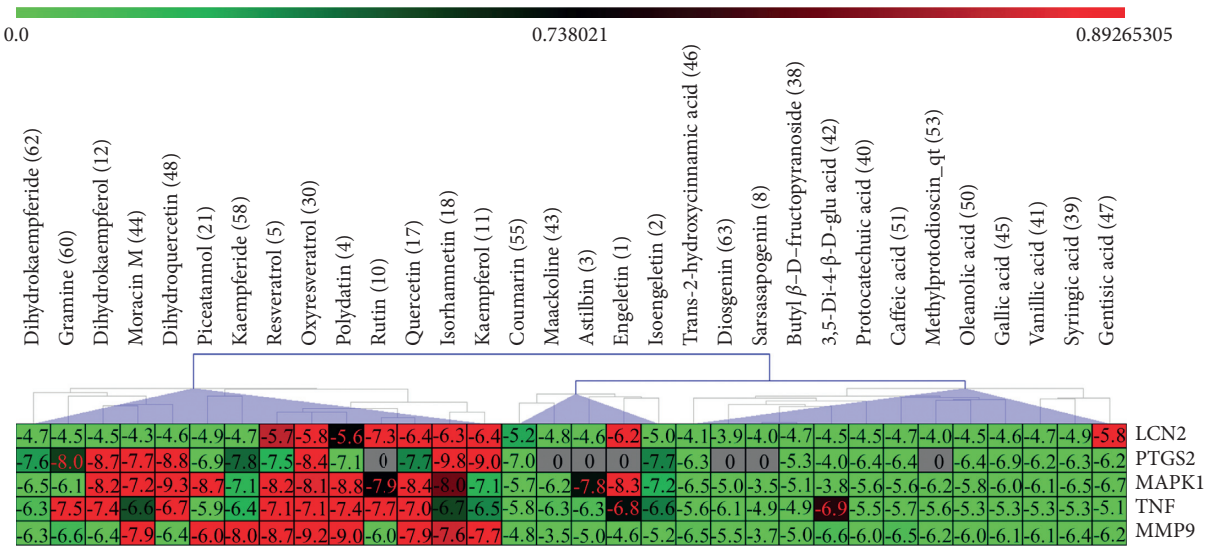


Figure 7: Clustering heat map between compounds and PID-related targets. All compounds with a docking score of proteins were divided into 3 clusters from left to right considering the affinity. 14 active compounds on the right were operated MM-GBSA binding free energy. "Di": dimethoxy; "glu": glucopyranosyl cinnamic.

Table 1: Docking table with bonding characterization and binding energies in kcal/mol with 14 active compounds involved in five therapeutic targets.

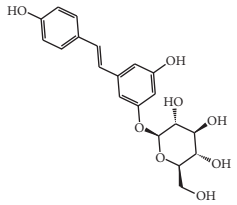
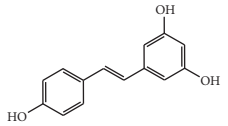
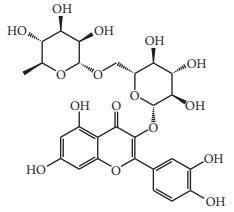
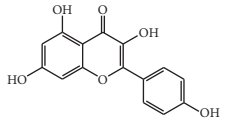
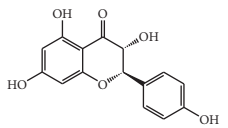
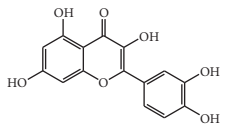
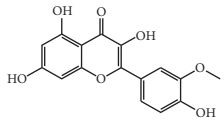
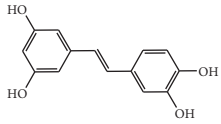
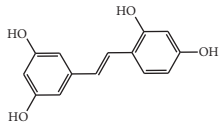
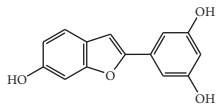
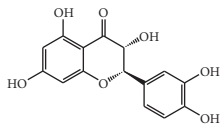
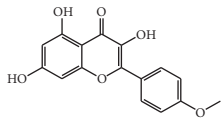
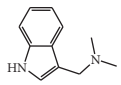
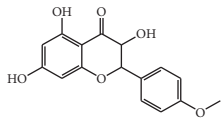
No.	Name	Structure	Glide G-score	MM-GBSA dG bind (kcal/mol)	Bonding interaction	Bond type	Binding protein
4	Polydatin		−8.846	−44.64	ASP A 158, GLU A 62, GLU B 360, ASN A 145, SER A 144 TYR A 27, GLN A 45 LYS A 45	H-acc H-don Pi-cation	MAPK1 (PDB ID: 5ax3 native ligand G-score: −8.713)
5	Resveratrol		−7.539	−37.18	GLN B 192, SER B 530 HIE B 90 TRP B 387	H-acc H-don Pi-pi stacking	PTGS2 (PDB ID: 5kir native ligand G-score: −9.800)
10	Rutin		−7.320	−53.30	SER A 68 ARG A 81 TYR A 106 LYS A 125	H-acc H-don Pi-pi stacking Pi-cation	LCN2 (PDB ID: 1x89 native ligand G-score: −7.190)
11	Kaempferol		−7.734	−47.73	ALA B 191, GLY B 233 HIS B 230 PHE B 110	H-acc H-don Pi-pi stacking	MMP9 (PDB ID: 5ue4 native ligand G-score: −8.661)
12	Dihydrokaempferol		−8.167	−32.82	GLU A 62, ASP A 158, GLU A 24, MET A 99 GLN A 96 LYS A 45	H-acc H-don Pi-cation	MAPK1
17	Quercetin		−8.367	−38.06	ASP A 158, GLU B 360, GLU A 62 GLN A 96 LYS A 45	H-acc H-don Pi-cation	MAPK1

Table 1: Continued.

No.	Name	Structure	Glide G-score	MM-GBSA dG bind (kcal/mol)	Bonding interaction	Bond type	Binding protein
18	Isorhamnetin		−9.757	−51.06	SER B 530, GLN B 192 PHE B 518, TYR B 385	H-acc H-don	PTGS2
21	Piceatannol		−8.673	−40.61	GLU B 360, GLU A 62, ASP A 158, GLN A 96 LYS A 45	H-acc H-don Pi-cation	MAPK1
30	Oxyresveratrol		−9.241	−56.71	ALA B 191, HIS B 230, GLY B 233 PHE B 110, TYR B 179	H-acc Pi-pi stacking	MMP9
44	Moracin M		−9.326	−37.01	GLU A 62, GLU B 360, MET A 99 GLN A 96	H-acc H-don	MAPK1
48	Dihydroquercetin		−7.020	−41.83	TYR D 151 TYR D 59	H-acc Pi-pi stacking	TNF (PDB ID: 2az5 native ligand G-score: −7.879)
58	Kaempferide		−7.962	−47.23	GLY B 233, ALA B 191 HIS B 230 PHE B 110	H-acc H-don Pi-pi stacking	MMP9
60	Gramine		−7.452	−30.51	GLY A 121	H-acc	TNF
62	Dihydrokaempferide		−7.638	−38.65	GLN B 192 TYR B 385	H-acc H-don	PTGS2

MM-GBSA dG bind: the binding energy of the receptor and ligand as calculated by the prime energy, a molecular mechanics + implicit solvent energy. Function (kcal/mol) = prime energy (optimized complex) − prime energy (optimized free ligand) − prime energy (optimized free receptor).

3.4. Molecular Dynamics Simulation of Five Docking Poses

3.4.1. Root Mean Square Deviation and Total H-Bonding Change Analysis. RMSD is of importance to quantify the structural stability of protein-ligand complexes within a fixed time frame [59]. In this study, five complexes were used to calculate the RMSD within 45 ns. Firstly, RMSD analysis and complexes in solvent depicted that PTGS2 (Figures 9(a) and 9(b)) started to stabilize after 10 ns, and it maintained 20 ns stability until 30 ns and slightly increased after 30 ns. Interestingly, a similar fluctuation was captured in the change of total H-bonds at 30 ns (Supplementary Figure 1A), and the average number of H-bonds before 25 ns was 2.7 but increased to 3.5 during the last 15 ns. This fluctuation indicated that the stable growth of the H-bond and the transition of one stabilized configure to another. The ligand (Figure 9(b)) started stabilizing after 10 ns and maintained to 45 ns. The average RMSD of protein and ligand was 0.312 and 0.082 nm, respectively.

Secondly, MAPK1-moracin M complex (Figures 9(c) and 9(d)) gained stability at around 10 ns, and the RMSD pattern of ligand suddenly increased at 27 ns and maintained to 45 ns. At the same time, the corresponding change appeared in the number of total H-bonds (Supplementary Figure 1B). The average number of H-bonds before 27 ns was 3.7 and increased to 4.1 during the last 18 ns. The average RMSD of protein and ligand was 0.286 and 0.116 nm, respectively.

Thirdly, for the LCN2-rutin system (Figures 9(e) and 9(f)), stable protein-ligand interaction was also observed. The protein and rutin were both equilibrated at ~11 ns with a slight fluctuation. The main fluctuation of protein was observed at 27~30 ns and equilibrated during the last 15 ns. In the view of total H-bond number (Supplementary Figure 1C), the H-bonds of complex initially increased before ~22 ns (average number: 2.8), reduced by 0.7 at 22~32 ns, and stabilized at 3.2 during the last 13 ns. The average RMSD of LCN2 and rutin was 0.247 and 0.164 nm, respectively.

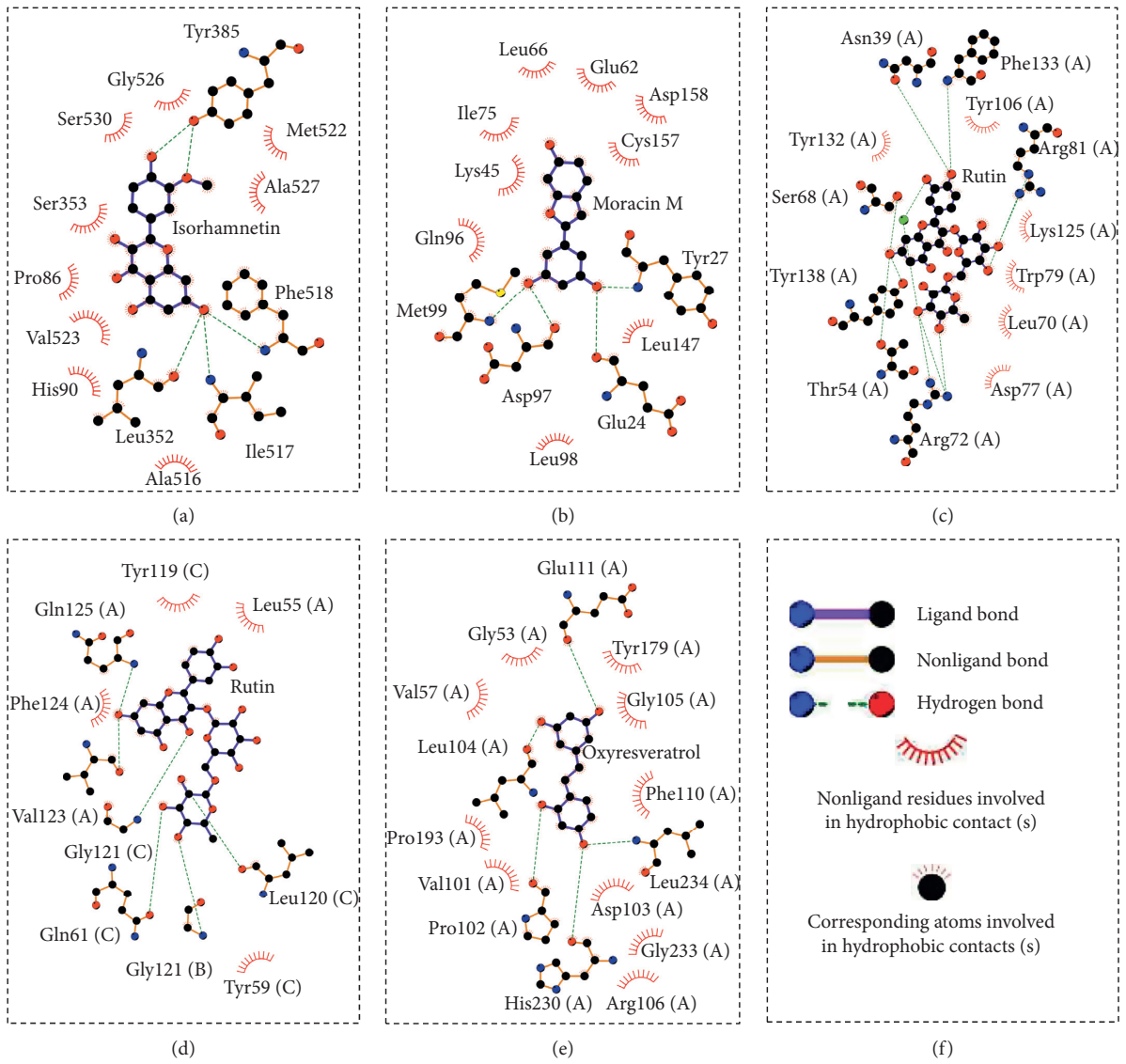


Figure 8: Docking poses between compounds and PID-related targets. (a) Isorhamnetin and PTGS2; (b) Moracin M and MAPK1; (c) Rutin and LCN2; (d) Rutin and TNF; (e) Oxyresveratrol and MMP9. (a–e) 2D binding models within 4 Å residues.

Fourthly, for multiple chain (four chains) system of TNF-rutin (Figures 9(g) and 9(h)), it was time-consuming to equilibrate. Therefore, a stable protein-ligand interaction was observed during the last 15 ns. The RMSD of TNF constantly rose before ~26 ns. The ligand initially stabilized at ~16 ns with a slight fluctuation and ultimately stabilized at 30 ns, and a sudden increase in the RMSD of ligand lasted 3 ns was captured. The total H-bond number was selected for analysis during the last 20 ns. A constant change and a large span of the H-bond number were recorded before 30 ns, which indicated an unstable H-bonding. Then, the span of H-bond variation gradually reduced, and 2~4 stable H-bonds were retained to the end. The average RMSD of TNF and rutin was 0.389 and 0.189 nm, respectively.

Finally, for the MMP9-oxyresveratrol system (Figures 9(i) and 9(j)), the RMSD analysis depicted that the protein achieved stability at around 7 ns and fluctuated slightly at ~16 ns, and the ligand equilibrated at 1 ns and maintained until 40 ns with a slight fluctuation at ~ 17 ns. Meanwhile, the change of the H-bond number gradually stabilized in the last 25 ns. The average RMSD of MMP9 and oxyresveratrol was 0.282 and 0.097 nm, respectively. Overall, the RMSD and total H-bond change analysis of five MD simulation complexes illustrated that the all above systems maintained a period of stability with ligands.

3.4.2. Intermolecular H-Bonding. Hydrogen bonding is among the most essential parameters to understand the binding affinity of small molecules towards a biomacromolecule (e.g., protein). A large number of H-bonds present in between protein and small molecules signify a strong binding affinity. In this regard, H-bonds between the natural molecules and disease-related proteins were monitored over the MD simulation time.

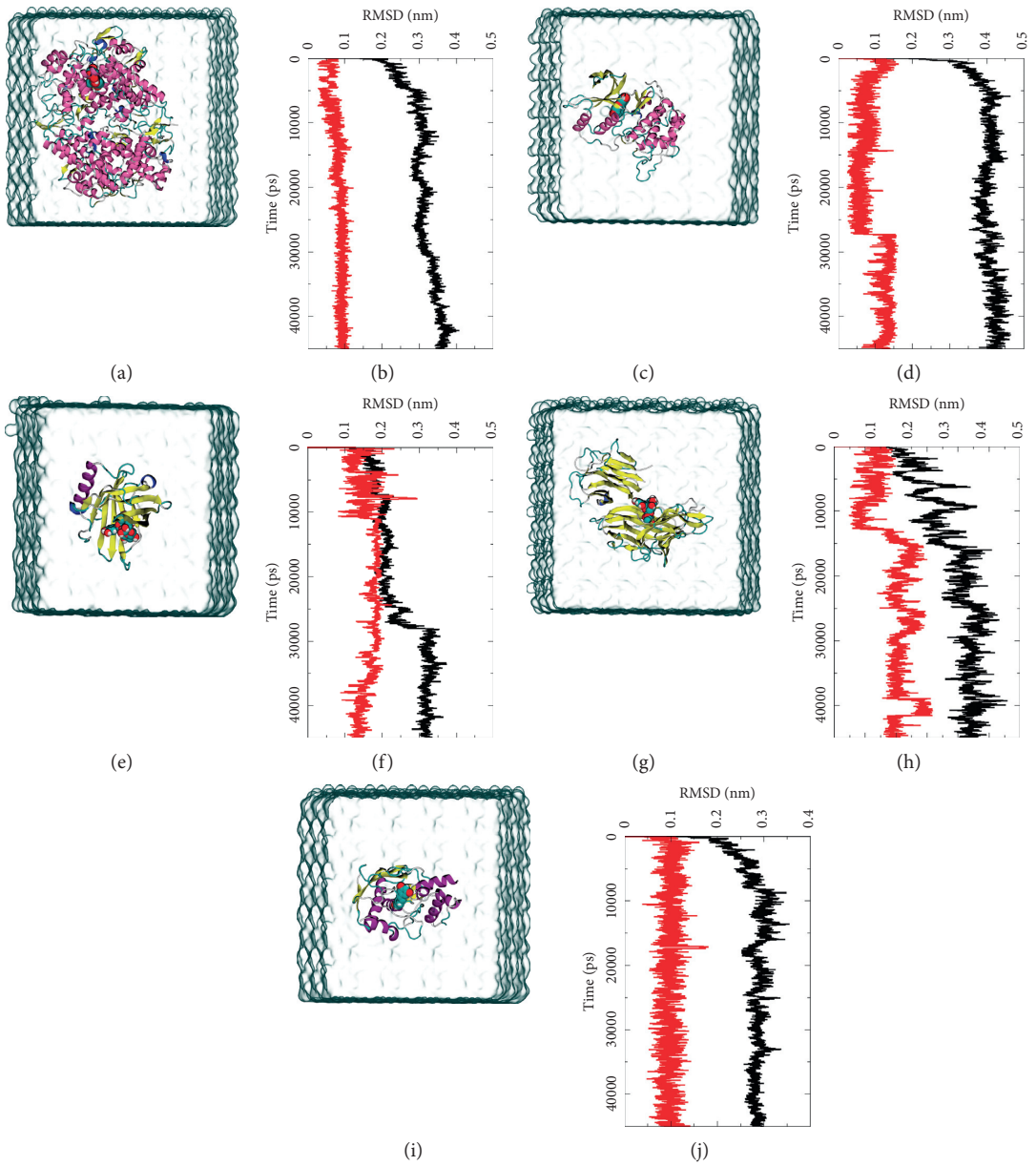


Figure 9: The 45 ns molecular dynamics simulation and calculated root mean square deviations (RMSD) of the backbone atoms and ligands referenced to the topology documents of five complexes. (a, b): PTGS2-isorhamnetin; (c, d): MAPK1-moracin M; (e, f): LCN2-rutin; (g, h): TNF-rutin; (i, j): MMP9-oxyresveratrol. (a, c, e, g, i) Simulation system for the protein-ligand model complex, with water in cyan box, protein in new cartoon of secondary structure, and ligand in VDW representation. (b, d, f, h, j) RMSD fluctuation for proteins and ligands.

The H-bond analysis results are shown in Tables 2 and 3. In the PTGS2-isorhamnetin complex, a total of 18 different H-bonds (H-bonds between ligands and different residues in all frames) were detected. On average, there were 2.5 H-bonds (average number of hydrogen bonds as a function of time), and the distance and angle were 2.94 Å and 14.88°, respectively. The amino acid residues on the side chain including H-bond donor (Phe 518, Ile 517, and Ser 530) possessed high occupancy to isorhamnetin.

In the case of MAPK1, a total of 19 different H-bonds were detected. On average, there were 4.0 H-bonds, and the distance and angle were 2.85 Å and 16.15°, respectively. The H-bond occupancy of important amino acid residues on the main chain and side chain including acceptor (Glu 62 and Asp 97) and donor (Tyr 27 and Met 99) was all greater than 60%, and the occupancy of Glu 62 with moracin M was 124.50% which showed that one more H-bond existed. And this result was in agreement with molecular docking.

In the case of LCN2, a total of 45 different H-bonds were detected. On average, we observed 2.8 H-bonds, and the distance and angle were 2.85 Å and 14.59°, respectively. The amino acid residues on the side chain including H-bond donor (Ser 68 and Arg 81) and acceptor (Tyr 106 and Tyr 138) possessed good occupancy and were also in agreement with molecular docking.

Table 2: Time-averaged H-bond properties obtained from MD simulation of five complexes.

Protein	Ligand	H-bond (number)	H-bond distance (average, Å)	H-bond angle (average, °)	Detected H-bonds
PTGS2	Isorhamnetin	2.5	2.94	14.88	18
MAPK1	Moracin M	4.0	2.85	16.15	19
LCN2	Rutin	2.8	2.85	14.59	45
TNF	Rutin	2.2	2.94	16.93	25
MMP9	Oxyresveratrol	4.3	2.78	14.78	20

Table 3: Properties of H-bond between five complexes, including occupancy, distance, and angle.

Protein	Donor and acceptor	Occupancy (%)	Distance (Å)	Angle (°)
PTGS2	Phe518 main~isorhamnetin	73.23	3.06	12.72
	Ile517 main~isorhamnetin	43.31	2.89	15.01
	Ser530 side~isorhamnetin	32.92	2.80	14.10
MAPK1	Moracin M~Glu62 side	124.50	2.82	14.40
	Tyr27 main~moracin M	67.10	2.90	16.60
	Moracin M~Asp97 main	63.76	2.74	15.95
	Met99 main~moracin M	63.29	2.85	16.23
LCN2	Ser68 side~rutin	63.14	2.77	12.53
	Arg81 side~Rutin	37.55	2.98	18.48
	Rutin~Tyr106 side	36.64	2.72	12.10
	Rutin~Tyr 138 side	35.70	2.69	11.52
TNF	Rutin~Val123 main	30.74	2.84	13.80
	Rutin~Tyr119 side	18.45	2.88	16.50
	Ser60-main~Rutin	17.91	3.03	19.91
MMP9	Oxyresveratrol~Leu104 main	87.28	2.70	11.43
	Oxyresveratrol~His230 main	82.36	2.69	14.86
	Oxyresveratrol~Pro102 main	69.60	2.72	15.57
	Leu234 main~oxyresveratrol	66.74	2.94	15.81

Occupancy = number of snapshots with H-bond between amino acid residues and ligand during the period of equilibrium/(total number of snapshots during the period of equilibrium (PTGS2:20 ns, MAPK1:30 ns, LCN2:35 ns, TNF:20 ns, MMP9: 25 ns)).

In the TNF-rutin complex, 25 different H-bonds were detected. On average, we observed 2.2 H-bonds, and the distance and angle were 2.94 Å and 16.93°, respectively. Due to a constant change and large span of the H-bond number mentioned in "Section 3.4.1", the occupancy of amino acid residues including H-bond receptor (Val 123 main and Tyr 119 side) and donor (Ser 60 main) was generally lower.

As for the MMP9-oxyresveratrol complex, 20 different H-bonds were detected. There were on average 4.3 H-bonds, and the relevant distance and angle were 2.78 Å and 14.78°, respectively. Three H-bond receptors (e.g., Leu 104 main, His 230 main, and Pro 102 main) and one donor (Leu 234 main) were bound to oxyresveratrol stably. These results hinted that the natural molecules interacted effectively towards the active site of PID-related proteins with a significant property of H-bonds.

3.4.3. Energy Decomposition of MM-PBSA. To explore the interaction between proteins and their ligands, energy decomposition of each complex was performed by using g_mmpbsa [60] module of GROMACS software. A total of 100 snapshots were extracted from the stable and continuous trajectories for the free energy calculation. The binding free energy could be divided into van der Waals Interaction (ΔEvdw), electrostatic energy (ΔEele), polar solvation interaction (ΔEpol), and solvent-accessible surface area (ΔEsasa). The results were listed in Table 4 and Figure 10. To understand the energy contribution, ΔBind (−146.535 ± 1.934 KJ/mol) of the PTGS2-isorhamnetin complex was decomposed to each amino acid residue. Figure 10(a) hinted that the important residues including Val 523, Leu 352, Phe 518, and His 90 involved in the binding site of PTGS2 (Figure 8(a)) showed a positive tendency for binding. As Figure 10(b) shows, Lys 145, Glu 62, and Asp 158 in MAPK1 showed a negative tendency to bind, and the latter two residues were within 4 Å of ligand, but Glu 62 was the important residue which formed excellent H-bond interaction with the ligand. Otherwise, residues (e.g., Val 30 and Ile 75) that showed positive binding tendency were within 4 Å to the ligand. The results showed that residues which had a positive binding tendency were closer to ligands (within 4 Å) and even formed H-bonds and pi-pi stacking interaction, and the negative residues were apt to far away from the ligand as shown in Figures 10(c)–10(e). All the above results were consistent with our previous docking results.

4. Discussion

Traditionally, in modern medicine, drugs were designed to target specific proteins relevant to the disease. However,

Table 4: Molecular mechanics-Poisson Boltzmann surface area (MM-PBSA) binding free energy results for five complexes (kJ mol^{-1}).

	PTGS2	MAPK1	LCN2	TNF	MMP9
ΔEvdw	−216.440 ± 3.246	−162.988 ± 1.077	−254.221 ± 3.069	−224.329 ± 1.717	−164.405 ± 5.045
ΔEele	−34.967 ± 1.184	−59.667 ± 0.842	−56.01 ± 3.385	−40.788 ± 1.380	−50.800 ± 1.845
ΔEpol	122.151 ± 3.055	134.190 ± 1.299	178.273 ± 4.009	180.285 ± 2.108	92.767 ± 3.313
ΔEsasa	−17.279 ± 0.243	−14.699 ± 0.075	−23.279 ± 0.144	−22.452 ± 0.171	−13.464 ± 0.391
ΔBind	−146.535 ± 1.934	−103.164 ± 1.533	−155.246 ± 4.579	−107.284 ± 1.485	−135.904 ± 4.201

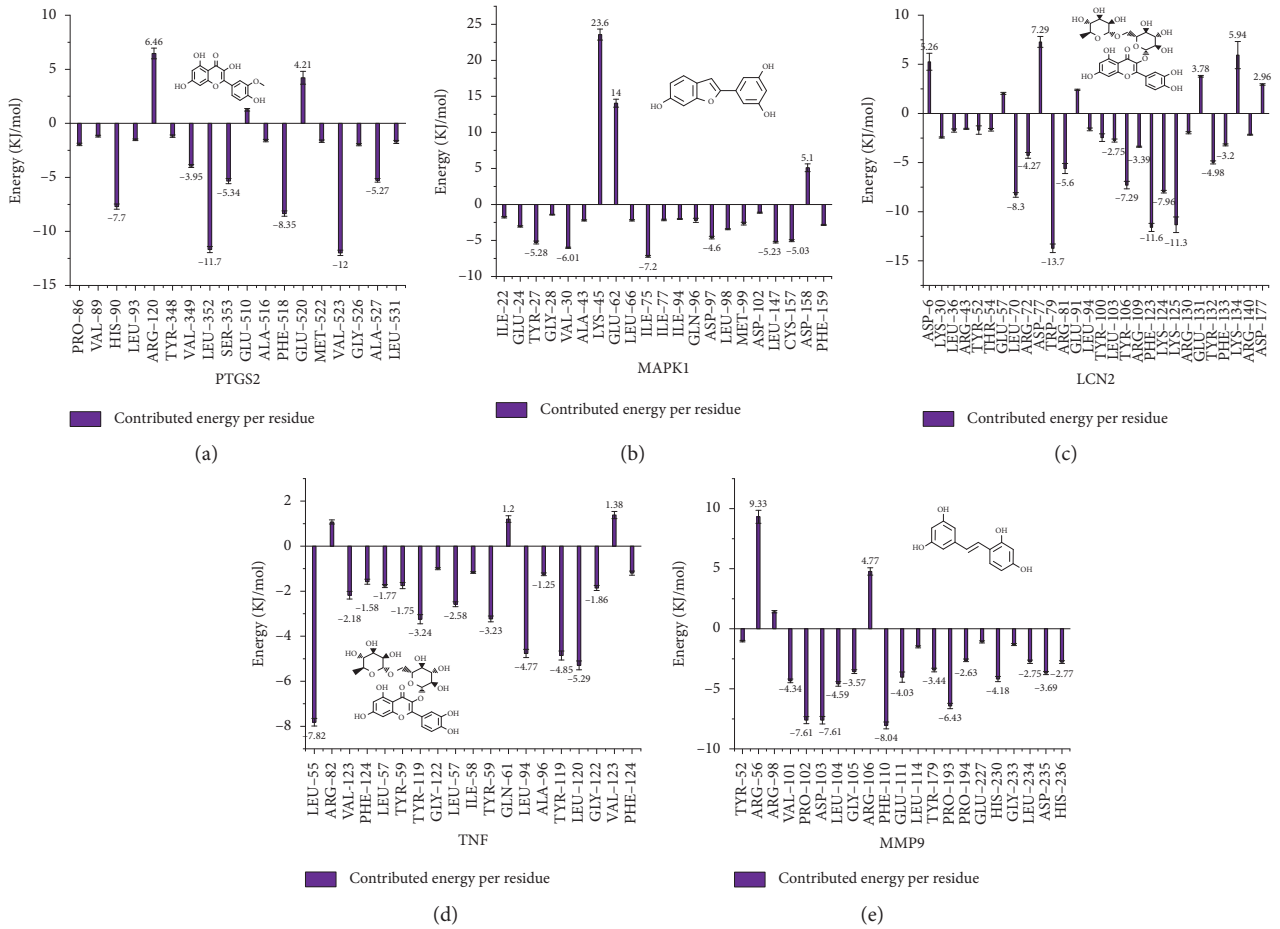


Figure 10: The energy contribution of important amino acid residues of five protein-ligand complexes. The significant residues of energy contribution (e.g., Val 523 and Leu 352 on PTGS2, Ile 75 on MAPK1, Trp 79 on LCN2, Leu 55 on TNF, and Phe 110 on MMP9). (a–e) The positive residues and negative residues (only residues which were out of the range: -1~1 KJ/mol remained). Most of the residues within 4 Å play positive roles for lower energy saving Lys 45 & Glu 62 on MAPK1 and Arg 56 & Arg 106 on MMP9.

herbs could possess even hundreds of compounds with multiple targets in TCM, which presented a significant obstacle to the exploration of drug mechanisms [61]. Fortunately, systems pharmacology, molecular docking, and MD simulation provided a holistic perspective to clarify the potential mechanisms for illustrating the integral regulation-based active compounds of TCM and conducting the consequent experiment [19]. Therefore, the above methods were employed to reveal the intricate mechanisms of SCL in the treatment of PID.

According to the C-T-P network (Figure 5), we proposed a simple inference as shown in Figure 11 (details are presented in Supplementary Figure 2), with the IL-17 signaling pathways (the highest value of –log10 (FDR)) considered potentially efficacious utilizing in-depth excavation of the above network. IL-17 signaling pathway reportedly had dual regulatory roles in proinflammatory and host defense processes [62, 63]. On the one hand, excessive secretion of IL-17A and IL-17F from Th17 cells can induce massive inflammatory factors including IL-6, IL-1β, and TNF-α. IL-17 signaling pathway can also synthesize prostaglandin E2 (PGE2) by inducing PTGS2, and the vasodilator effect of PGE2 also promoted inflammatory cells to enter the site of inflammation, so the activation of IL-17 signaling pathway had a strong proinflammatory effect [64]. In previous studies, SCL downregulated the expression of IL-6,

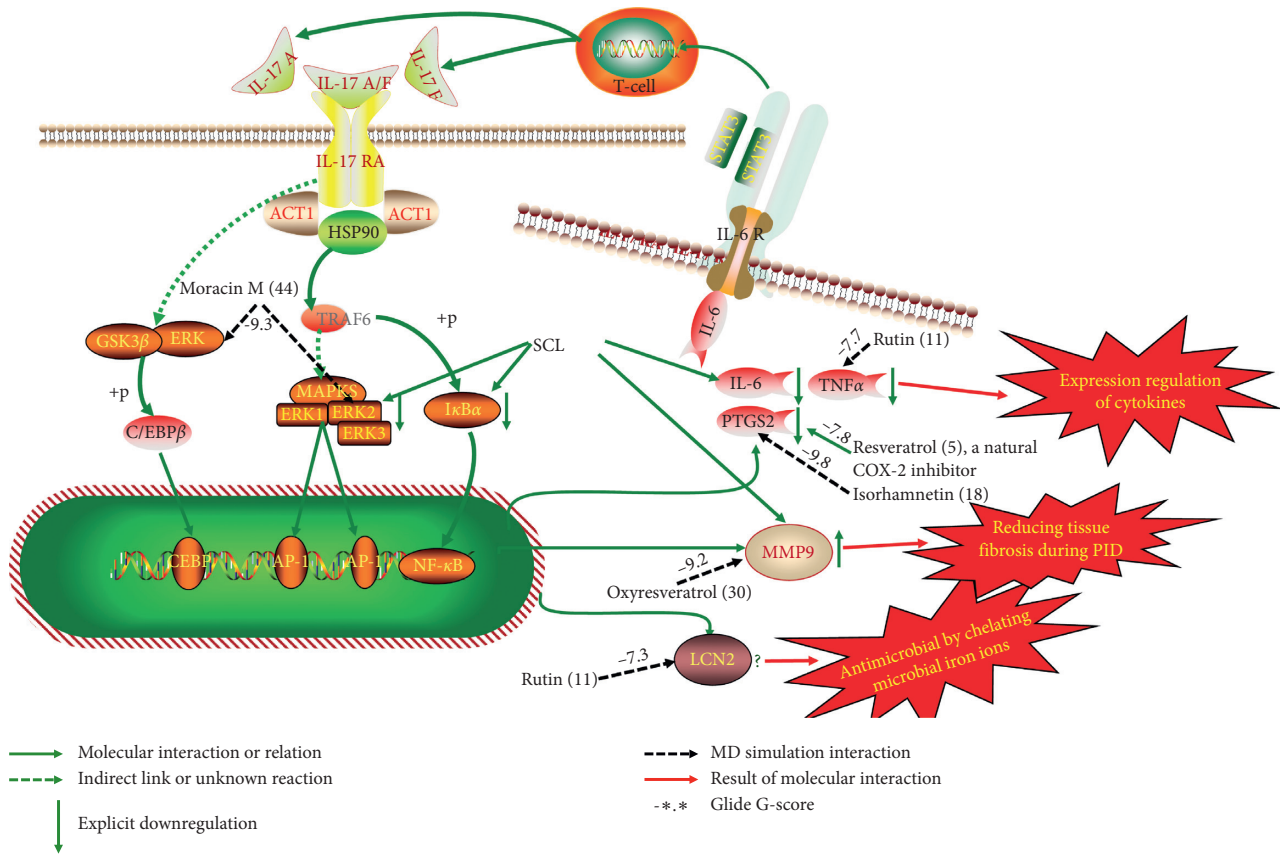


Figure 11: Computational simulation and experimentally validated regulation mechanism of SCL in PID model.

interleukin-1 beta (IL-1β), TNF-α, IL-2, and PTGS2 in rats of the PID model, but its regulatory mechanisms involved remain unclear [17, 26]. However, combined with the results of this study, this may be the SCL inhibition of the IL-17 signaling pathway.

On the other hand, in the process of immunoregulation, the regulation of IL-17 signaling pathway can recruit neutrophil to the inflammatory region, releasing myeloperoxidase (MPO) [65] and inducing gene expression of LCN2 and matrix metalloproteinases (MMPs) [63, 66]. These proteins make important impacts in host defense, which may contribute to alleviating PID symptoms by enhancing the immune function of the body. For instance, MMP9 was activated to promote embryo formation, wound healing, and transfer of inflammatory cells [66–68]. Interestingly, it was reported that SCL upregulated the expression of MMP-2 and MMP-9 in rats of the PID model and downregulated the MMPs inhibitor TIMP-1, thereby restoring the balance between MMPs and TIMP-1 and reducing tissue fibrosis during PID [17]. Collectively, these results demonstrated the multitarget regulation of compounds in SCL.

Although the above network provided a clear view of the integral regulation of SCL, there was still a barrier to validate the facticity of each research data and it was difficult to consider the critical effect of each result. Hence, how to select a crucial result (targets or pathways) to concern and validate was an issue worth pondering carefully for researchers. Therefore, PPI analysis could play an essential role which helps us find the key targets and pathways and explore critical radioactive targets in the network. In this study, IL-17 signaling pathway and Th17 cell differentiation-related targets were considered focuses in the PPI. As previously reported, PTGS2 is inducible and usually produces inflammatory prostaglandins, which mediate responses to physiological stress (infection and inflammation), stimulate chronic inflammation, and is a target for nonsteroidal anti-inflammatory drugs (NSAIDs) [56]. As shown in Table 1 and Figure 8, the binding capacity of isorhamnetin to PTGS2 (Glide G-score: −9.757) was very close to rofecoxib (−9.800), suggesting that isorhamnetin may be a potential novel COX-2 inhibitor somewhat analogous to rofecoxib. Additionally, all the active compounds (Figure 7) mostly belonged to flavonoids and stilbenes. Based on existing pharmacodynamic investigations, these flavonoids and stilbenes have achieved obvious anti-inflammatory effects and ameliorated fibrosis in PID animal models by inhibiting the synthesis or release of histamine, 5-hydroxytryptamine (5-HT), and PGE2, as well as enhancing the production of MMP9 in uteri [17, 69]. Particularly, engeletin (1), polydatin (4), and resveratrol (5) have inhibited the release of IL-6 and TNF-α [70]. Rutin (10) inhibited the release of IL-2 and TNF-α [26]. These results and existing experiments indicated that active ingredients in SCL, including isorhamnetin (18), polydatin (4), oxyresveratrol (30), and piceatannol (21), could inhibit the activity of PTGS2 to decrease the synthesis of PGE2 [71], which contributes to the restoration of PID by inflammatory inhibition. What is more, MD simulation of five complexes

during 45 ns was utilized to understand the dynamics binding process between proteins and ligands, which corresponded to molecular docking. Protein complexes (Supplementary Table 1) whatever single chain (LCN2 and MMP9) and multiple chains (PTGS2, MAPK1, and TNF) could stabilize ultimately (Figure 9). The fluctuation of various systems was significantly relevant to H-bonding change. As shown in Table 2, the properties of H-bond in different systems were decoded. For the MAPK1-moracin M and MMP9-oxyresveratrol systems, the average H-bond numbers were greater than the LCN2-rutin and TNF-rutin systems, but the detected H-bonds were lower than them. The structures of rutinose and flavonoid aglycones on rutin were easy to form H-binding interaction to surrounding amino acid residues in the protein. Otherwise, the H-bonding interaction and energy contribution of crucial residues were analyzed (Table 3 and Figure 10); the significant H-bond-related residues including Phe 518 on PTGS2, Glu 62 on MAPK1, Ser 68 on LCN2, Val 123 on TNF, and Leu 104 on MMP9 were of great importance to maintain the system's stability. The significant residues of energy contribution (e.g., Val 523 and Leu 352 on PTGS2, Ile 75 on MAPK1, Trp 79 on LCN2, Leu 55 on TNF, and Phe 110 on MMP9) indicated the van der Waals interaction and electrostatic energy were the same important. So far, a part of the results in network pharmacology has been validated and discussed via existing experiments, molecular docking, and MD simulation, which can yet be regarded as an effective method to elucidate the active compound of SCL and relevant mechanisms against PID. In our study, the protein LCN2 which can inhibit microorganisms by chelating the iron ions [72] was found to have an important impact and phenolic compounds (e.g., moracin M) were also considered as key compounds in treating PID. Mechanistically, compared to previous research studies on the main constituents for the treatment of PID by relieving the degree of fibrosis in the uterus via ERK1/2 and TGFβ-SMAD2/3 signaling pathways, we reported the critical active compounds and relevant binding modes of IL-17 signaling pathway and Th17 cell differentiation-related targets in the treatment of PID by inhibiting inflammatory factors, antitissue fibrosis, and microbial growth.

However, some tough problems associated with disease targets and traditional herbs still bother investigators. The first thing is that the vague and a small number of valuable targets based on current research studies cannot orient to the whole disease. Therefore, systems pharmacology and molecular simulation are used to reveal the partly network mechanisms and molecular actions rather than the whole disease network mechanisms. And similar to the current drug discovery strategies, compared with the number of disease targets, the quality of the targets (druggability and crystal reliability) is paid more heed by researchers. Secondly, as for molecular docking, due to the limitation of computational power and force field algorithm, a newly and generally applicable force field to improve the accuracy of molecular docking and fast methods of the binding free energy calculation for virtual screening [73] are urgent to develop. Enough computational accuracy provides researchers enough confidence to conduct subsequent experiments. Finally, the specific components in TCM are needed to extract and identify completely to enrich the efficacious material basis. It is comparatively easy to illustrate the molecular mechanism once obtaining reliable targets and adequate ingredients. All above, there is a shortcut to uncover the overall network mechanism of TCM against diseases through cross cooperation among pharmacology, medicinal chemistry, and computational chemistry.

5. Conclusions and Further Prospect

Considering the promising treatment potential of SCL on PID, efforts are in demand to reveal the acting targets for SCL and the unclear mechanisms behind the therapeutic potentials. In this study, the computational systems pharmacology method was applied to explore the active ingredients of SCL and provided an integral view of the mechanism against PID. The principal 32 potent ingredients for the treatment of PID were uncovered to regulate 718 candidate targets. Furthermore, in the PPI and C-T-P network analysis, 8 of 19 PID-related targets were mapped to the IL-17-signaling pathway and Th17 cell differentiation. We focused on five reported PID-related targets PTGS2, MAPK1, LCN2, TNF, and MMP9. The interactions between active compounds and PID-related targets were described with static and dynamic evaluations. A total of 14 active compounds, including rutin (−40.46 kcal/mol), isorhamnetin (−51.06 kcal/mol), oxyresveratrol (−56.71 kcal/mol), and moracin M (−37.01 kcal/mol), showed greater binding force to the therapeutic targets. At the same time, the amino acid residues in the hydrophobic cavity which played an important role in the process of complexes were revealed to guide the design of relevant drugs.

Overall, active ingredients of SCL exhibited a strong affinity to therapeutic targets of PID, thereby contributing to decreasing inflammation, ameliorating fibrosis, and inhibiting or eliminating microorganisms via bidirectional regulation of the IL-17 signaling pathway. However, it was a time-consuming and risky process to draw this kind of conclusion. Analysis of the network had to be up against a problem on how to select the principal results to focus, which is a big challenge but a core in systems pharmacology. Therefore, valid validation of results (e.g., static molecular docking, MD simulation, animals, and biochemistry) is equally important. Through the analysis of this study, IL-17 pathway was found to probably play a critical role in the development and treatment of PID, but relevant research was lacking and incomplete. Hence, serum, integrated pharmacodynamics, and pharmacokinetics will be utilized to clarify the components in serum and relevant therapeutic mechanisms of SCL on PID.

Abbreviations

C-T-P: Compound-target-pathway
ΔEele: Electrostatic energy
ΔEpol: Polar solvation interaction
ΔEsasa: Solvent-accessible surface area
ΔEvdw: van der Waals interaction
GO: Gene Ontology

H-bond: Hydrogen bond
5-HT: 5-Hydroxytryptamine
KEGG: Kyoto Encyclopedia of Genes and Genomes
LCN2: Siderocalin
MAPK1: Mitogen-activated protein kinase 1
MD: Molecular dynamics
MM-GBSA: Molecular mechanics-generalized born surface area
MM-PBSA: Molecular mechanics-Poisson Boltzmann surface area
MMP9: Matrix metalloprotein-9
MMPs: Matrix metalloproteinases
MPO: Myeloperoxidase
NSAIDs: Nonsteroidal anti-inflammatory drugs
NVT: Canonical ensemble
NPT: Constant temperature and pressure
OPLS-2005: Optimum polarized ligand simulation-2005
PME: Particle mesh Ewald
PID: Pelvic inflammatory disease
PPI: Protein-protein interaction
PGE2: Prostaglandin E2
PTGS2: Prostaglandin-endoperoxide synthase 2
RMSD: Root mean square deviation
SCL: *Smilax china* L.
SEA: Similarity ensemble approach
SP: Standard precision
TCM: Traditional Chinese Medicine
TCMID: Traditional Chinese Medicine Integrated Database
TCMSP: Traditional Chinese Medicine System and Pharmacology
TNF: Tumor necrosis factor.

Data Availability

The data used to support the findings of the study are included within Supplementary Materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xiaobo Wang, Yi Zhang, Gang Fan, and Yunsen Zhang designed this study; Yutong Fu, Wenxiang Wang, Qi Li, Xuanhao Li, Zikuang Zhao, and Huimin Chen collected the relevant data; Xiaobo Wang and Yunsen Zhang drawn the figures; Yunsen Zhang drafted the manuscript; all authors proofread the manuscript.

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Supplementary Materials

The detailed documents are available in the Supplementary Materials. (*Supplementary Materials*)

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Review Article

The Effect of Acupuncture on Glucose Metabolism and Lipid Profiles in Patients with PCOS: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Objective. To evaluate the effectiveness of acupuncture on glucose metabolism and lipid profiles in patients with polycystic ovary syndrome (PCOS). *Methods*. Databases, including the China National Knowledge Infrastructure (CNKI), the China Science and Technology Journal Database (VIP), Wanfang, PubMed, and the Cochrane Library were searched for the relevant literature, with the retrieval deadline being February 2020. Two reviewers independently screened, selected, and extracted the data and validated the results. The methodological quality of the included studies was evaluated with the risk of bias tool, and the meta-analysis was performed using the RevMan 5.3.5 software. *Results*. A total of 737 patients with PCOS from 10 randomized controlled trials were included in the meta-analysis. A pooled analysis showed significant decreases in body mass index (mean difference (MD) = −1.47, 95% CI −2.35 to −0.58, $P < 0.001$) and waist-to-hip ratio (MD = −0.04, 95% CI [−0.06, −0.02], $P < 0.001$) in the acupuncture group along with significant improvements in fasting plasma glucose (MD = −0.38, 95% CI [−0.70, −0.07], $P = 0.02$), homeostasis model assessment of insulin resistance (MD = −0.22, 95% CI [−0.41, −0.02], $P = 0.03$), and triglycerides (MD = −0.26, 95% CI [−0.48, −0.04], $P = 0.02$). No significant differences were observed in the Ferriman–Gallwey score, 2 h fasting plasma glucose, fasting insulin, 2 h fasting insulin, serum total cholesterol, low-density lipoprotein cholesterol, or high-density lipoprotein cholesterol. *Conclusion*. Acupuncture is relatively effective and safe in improving glucose metabolism and insulin sensitivity in patients with PCOS. The included studies were generally of not bad methodological quality, but further large-scale, long-term randomized controlled trials with rigorous methodological standards are still warranted.

1. Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder in reproductive-age women with an incidence of 6% to 21% globally and 5.6% to 11.2% in Chinese women [1]. PCOS generally manifests as hyperandrogenism, oligoanovulation, and/or polycystic ovaries [2], and patients with PCOS are at an increased risk for metabolic disturbances such as insulin resistance (IR), impaired glucose tolerance (IGT), dyslipidemia, insulin-induced metabolic syndrome predisposing them to type 2 diabetes mellitus (T2D), and cardiovascular disease [3–5]. Furthermore, hyperinsulinemia is considered to be associated with the mechanism of ovulatory dysfunction in women with PCOS [6].

IGT, obesity, and T2D are more prevalent in women with PCOS women than in the general population [6–8], and it is estimated that among obese women with PCOS about 30%–40% are diagnosed with IGT and 5–10% suffer from T2D [9, 10]. Moreover, metabolic syndrome is three times more frequent in women who present with classic PCOS symptoms [11]. The metabolic disturbances present in women with PCOS not only involve an increased risk for cardiovascular disease but

also might worsen many of the typical PCOS symptoms, and thus they have become an important target for therapy [12].

Lifestyle measures, including diet, exercise, and behavioral modification, are the first-line treatments in obese women with PCOS for improving metabolic parameters and endocrine abnormalities [13]. Nevertheless, lifestyle changes might not be easy to sustain and might not be sufficient for significant weight loss. Thus, pharmaceutical methods targeting both reproductive and metabolic disorders might be needed.

Biguanides (metformin) and thiazolidinediones (rosiglitazone and pioglitazone) are insulin-sensitizing agents that are currently in clinical use [8]. Metformin has a few safety concerns and is the most widely used insulin-sensitizing agent for treating women with PCOS of reproductive age [6, 14]. Metformin is an oral antihyperglycemic biguanide drug that enhances glucose uptake in skeletal muscle and adipocytes by increasing insulin sensitivity, and it is often used as a first-line pharmacological treatment for women with PCOS presenting with IR [15–17]. However, gastrointestinal symptoms like diarrhea and abdominal discomfort are common adverse effects of metformin [18, 19]. In addition, rosiglitazone has been withdrawn from the market in many countries due to concerns of increased risk of congestive heart failure [20].

Pharmacological approaches are often effective, but there are issues with adverse effects and patient compliance due to the prolonged treatment required by PCOS patients [19]. Thus, additional nonpharmacological treatment strategies such as acupuncture should be considered in treating PCOS [19]. Acupuncture, which has been used to treat diseases for more than 2500 years in China [21], has become increasingly popular worldwide in recent years for its convenience and low incidence of adverse effects. Acupuncture is widely applied in clinical practice for treating PCOS [19], and studies have concluded that acupuncture is effective against metabolic disturbances associated with IR such as overweight [22], hyperglycemia [23, 24], and hyperlipidemia [25] by improving insulin sensitivity [26]. It has also been reported that acupuncture may improve glycemic outcomes in women with PCOS [27]. Our prospective pilot study [28] showed that homeostasis model assessment of insulin resistance (HOMA-IR) was decreased after 6 months of treatment with acupuncture, and HOMA-IR remained significantly decreased at 3 months of follow-up. In addition, electroacupuncture has been shown to decrease HOMA-IR and improve IR in a rat model of PCOS [29]. However, the designs of these studies were all considerably different and thus it is difficult to draw strong conclusions, and the effects of acupuncture on glucose and lipid metabolism in PCOS have not been systematically analyzed.

To fill this knowledge gap, this systematic review aimed at comparing the effectiveness of acupuncture to that of standard therapy (lifestyle management or insulin-sensitizing agents) in the treatment of metabolic dysfunction in patients with PCOS.

2. Methods and Materials

2.1. Eligibility Criteria. We only included randomized controlled trials (RCTs) using accepted interventions involving acupuncture (manual acupuncture or electroacupuncture) alone, with unrestricted acupoints or intensity, compared with placebo (placebo or sham acupuncture) or with standard therapy (lifestyle management, including weight reduction by diet and exercise or insulin-sensitizing agents such as thiazolidinediones and metformin). The subjects were adult patients with PCOS. Only studies with the outcomes of HOMA-IR, HbA1c, glucose, and insulin levels or lipid profiles were included.

2.2. Literature Search. We searched for published literature in the China National Knowledge Infrastructure (CNKI), Wanfang, the China Science and Technology Journal Database (VIP), PubMed, and the Cochrane Library databases. We also retrieved the completed but unpublished studies from the clinicaltrials.gov website and tracked the results of these studies. Only Chinese and English articles were retrieved, and the last search was carried out on 29 February 2020. The search words included "polycystic ovary syndrome", "PCOS", "acupuncture", "electroacupuncture", "needle", "needling", "scalp acupuncture", "abdominal acupuncture", "ear acupuncture", "wrist-ankle acupuncture", "warm acupuncture-moxibustion", "homeostatic model assessment", "HOMA", "glucose", "insulin", "insulin sensitivity", "metabolic", "glycemic control", "OGTT", "lipid profile", "HbA1c", "triglycerides (TG)", "total cholesterol (TC)", "high-density lipoprotein cholesterol (HDL-C)", "low-density lipoprotein cholesterol (LDL-C)", "randomized controlled trial", "clinical trial", "RCT", "random", "randomize", and "randomization". Depending on the characteristics of different databases, search strategies including both subject words + free words and keywords + full text were used.

2.3. Literature Screening. The identified articles were initially imported into NoteExpress, and the initial screening was performed based on the inclusion/exclusion criteria after reading the titles and abstracts. In the next step, full-text articles were acquired and checked for eligibility prior to including them in the final analysis. All duplicated articles and any papers that did not meet our inclusion criteria were excluded.

2.4. Data Extraction. A data extraction table was designed to collect the data to be analyzed, including the following aspects: (a) basic characteristics of the included studies; (b) research methods and possible biases; (c) participant characteristics; (d) interventions; (e) outcome measures; (f) research findings; and (g) other required information.

Two investigators (Zheng and Qing) independently extracted the data and assessed the quality of all relevant RCTs. The following data were extracted from the published RCTs: the first author's name, year of publication, the country where the trial was conducted, the type of study, the intervention and placebo groups, the frequency of acupuncture or the dosage of pharmaceutical interventions (mg/day), the duration of intervention, the sample size, the

mean and standard deviation of the glucose metabolism outcomes, and the mean and standard deviation of the lipid profile outcomes. Microsoft Excel with standard spreadsheets was used for data extraction.

2.5. Quality Evaluation. The risk of bias among the included studies was evaluated using the tool developed by the Cochrane Collaboration [30]. The results were cross-referenced, and any disagreements were resolved by discussion or consultation with a third evaluator with rich experience.

2.6. Outcome Measures. The primary outcome of interest was HOMA-IR. The secondary outcomes were fasting plasma glucose (FPG), fasting plasma insulin (FINS), 2 h fasting plasma glucose (2hFPG), 2 h fasting insulin (2hFINS), TC, TG, HDL-C, LDL-C, body mass index (BMI), waist-to-hip ratio (WHR), and the Ferriman–Gallwey score (FGS). The safety indicator was any adverse event.

2.7. Data Analysis. The quantitative analysis was carried out using the Cochrane Collaboration software RevMan 5.3.5. If the included studies used the same measurement scales, the continuous variables were described using the mean difference (MD) and 95% confidence intervals (CIs). For the heterogeneity test, the chi-square test was performed first, and based on those results, the estimates of heterogeneity (I^2) were applied. A fixed-effect model was used when the I^2 was ≤50% and the P value was ≥0.1, and a random-effect model was applied when the I^2 was >50% or the P value was <0.10. If heterogeneity was high, the source of heterogeneity was explored, and subgroup analysis or sensitivity analysis was performed to investigate the stability of the meta-analysis.

3. Results

3.1. Literature Search and Screening Flowchart. A flow chart of the study selection is shown in Figure 1. In all, 1077 articles were retrieved in our initial search. After removing duplications and screening the titles and abstracts, we obtained 39 full-text articles. Finally, 10 studies were included in the systematic review and meta-analysis.

3.2. Characteristics of the Included Literature. A total of 737 patients were included in the 10 RCTs [31–40]. All participants were diagnosed with PCOS according to Rotterdam criteria [41], and they were treated with acupuncture alone or with placebo or metformin. The main acupoints used in these studies included CV3, RN4, RN6, ST25, ST28, ST36, SP9, and SP6. The specific features of these studies are summarized in Table 1.

3.3. Quality Evaluation of the Articles. Among these 10 studies, the patients were randomized by using a random number table in six studies [31, 33–35, 37, 38], while the remaining four studies only mentioned "random" or "randomization" without describing the specific randomization methods. Only three articles described allocation concealment [31, 33, 40]. For participant and personnel blinding, five trials were at low risk of bias [31, 32, 38–40], and the remaining studies [33–37] were given a high risk of bias due to the loss of blinding during implementation. Measurements were generally made by third parties other than the researchers, so the blinding of outcome assessment was defined as low risk. Two articles failed to describe the missing data, while in the remaining eight articles, the number of patients in all randomized groups was consistent with the number of subjects in the statistical analysis. The articles reported both the glucose metabolism and lipid profile indicators. The quality of the literature included in our analysis was average, and the details of the evaluation are shown in Figure 2.

3.4. Results of the Meta-Analysis

3.4.1. General Indicators. All included studies compared BMI and WHR, while only five studies reported FGS. There was evidence for a decrease in BMI in the acupuncture groups versus the control groups (MD = –1.47, 95% CI [–2.35 to –0.58], $P < 0.001$). The overall effect showed a significant improvement in WHR between the groups (MD = –0.04, 95% CI [–0.06, –0.02], $P < 0.001$). No significant difference was observed in FGS. The results of the meta-analysis are shown in Figure 3.

3.4.2. Glucose Metabolism Indicators. Nine articles reported HOMA-IR and eight articles reported FPG and FINS, but only four articles reported 2hFPG and only two reported 2hFINS. The pooled results from nine studies showed a significant difference in HOMA-IR in the acupuncture groups compared with the control groups (MD = –0.22, 95% CI [–0.41, –0.02], $P = 0.03$). The pooled analysis showed a decrease in FPG in the acupuncture group (MD = –0.38; 95% CI [–0.70, –0.07], $P = 0.02$). There was an improvement in FINS between the groups (MD = –0.99, 95% CI [–2.03, 0.04], $P = 0.06$), but this was not statistically significant. No significant differences were observed for the other outcomes. The results of the meta-analysis are shown in Figure 4.

3.4.3. Lipid Profile. Six studies with 370 participants were included to compare TC, TG, LDL-C, and HDL-C between groups. There was no significant difference in TC or HDL-C between the acupuncture and control groups, but a significant decrease was observed in TG in the acupuncture group (MD = –0.26, 95% CI [–0.48, –0.04], $P = 0.02$). The results of the meta-analysis are shown in Figure 5.

3.4.4. Adverse Events. Six of the studies reported the presence of adverse events. Of these, two studies [31, 40] reported that there were no adverse events, while the remaining four studies [33, 36–38] reported gastrointestinal problems in the metformin groups such as nausea, vomiting, mild diarrhea, slight dizziness, or weakness. There were no adverse events found in the acupuncture groups of these

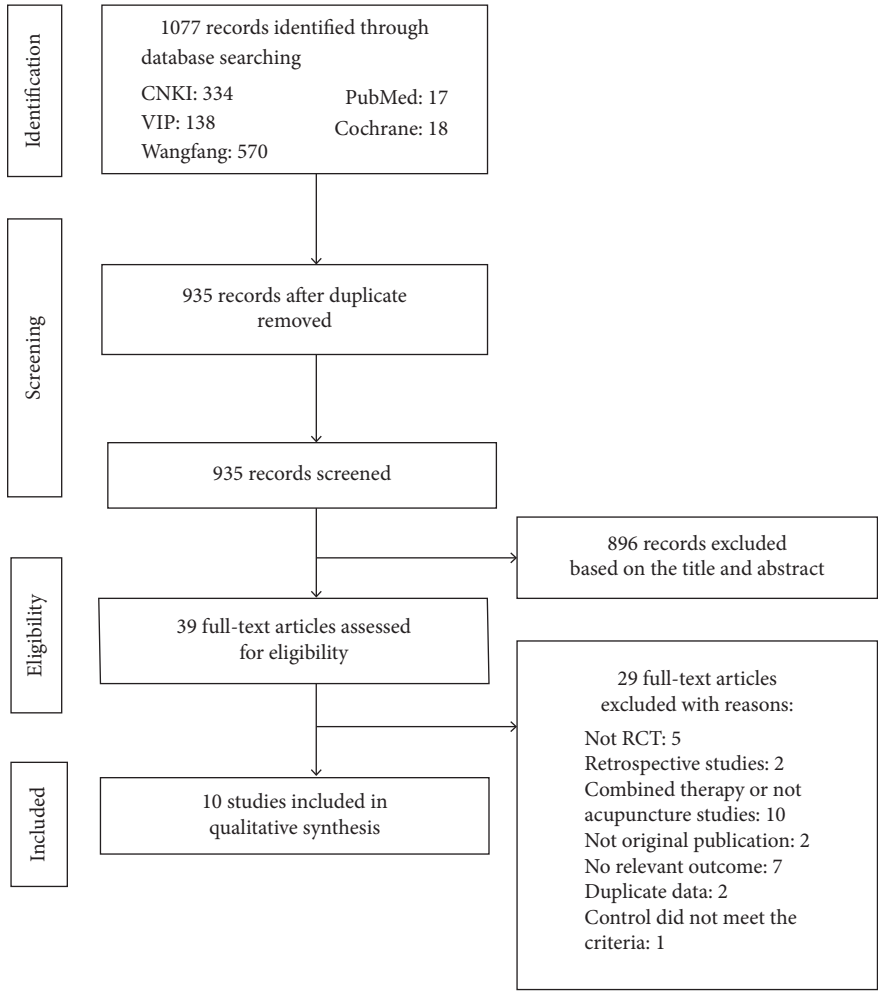

Figure 1: Literature search and screening flowchart.

studies except for one study [37] in which one patient had mild bleeding at the site of needling.

4. Discussion

4.1. Principle Findings. The objective of this review was to summarize and evaluate the use of acupuncture to improve glucose metabolism and lipid profiles in patients with PCOS. Overall, we found that acupuncture was closely associated with decreased BMI, WHR, FPG, and HOMA-IR and that acupuncture could significantly improve HOMA-IR and the level of fasting glucose in patients with PCOS, which confirms previous reports [27, 28]. It is also reported that acupuncture can decrease BMI and WHR [28, 42, 43]. FINS, 2hFPG, and 2hFINS were reduced in the acupuncture groups, but these differences were not significantly different. The results of the lipid profile were reported in a few studies, and we found that acupuncture significantly improved TG levels, while the differences in TC, LDL-C, and HDL-C were not significant. Acupuncture seems to be associated with a few adverse events, and the reported adverse events, such as bleeding, were mild and transient, demonstrating that acupuncture is safe and reliable. Thus, we conclude that acupuncture, compared with standard therapy, is more effective and safer in improving glucose metabolism and insulin sensitivity in patients with PCOS.

4.2. Limitations. First, this meta-analysis only included clinical studies comparing acupuncture alone versus placebo (placebo or sham acupuncture) or standard therapy. Studies on other combined therapies were not included because the combined therapies are more complex than monotherapy. Indeed, if acupuncture alone can achieve a curative effect without significant cost or time, then additional therapies might not be necessary. Second, there are certain heterogeneities among these studies. On the one hand, PCOS itself is heterogeneous by nature in terms of its clinical and biochemical features, and different distributions of ethnicity and age contribute to different manifestations of PCOS [44]. On the other hand, the disease severity and the dosages of metformin differed among the studies. Finally, some of the included studies did not describe the specific randomization method or did not adopt blinding. Therefore, these studies have just an average methodological quality.

Table 1: Features of the included studies.

Study ID	Sample size	Course of PCOS	Participant characteristics	Age	Treatment versus control	Outcome
Stener-Victorin, 2009	15			T: 29.9 ± 4.5 C: 30.4 ± 5.5	Electroacupuncture group: the needles were inserted into acupoints including CV3, RN6, bilateral ST29, SP6, and LI4 (or PC6 alternatively every second time) and retained for 30 min. The procedure was administered two times a week for 2 weeks, once a week for 6 weeks, and then once every second week for 8 weeks, giving a total of 14 treatments over 16 weeks. Control group: this group was given information about the importance of physical activity and a healthy diet.	BMI, WHR, FGS, FPG, FINS, HOMA-IR, TC, TG, LDL-C, HDL-C
Zheng, 2012	86	T: 3.5 ± 2.8 C: 3.6 ± 2.2	Obesity-type PCOS	T: 26.5 ± 3.0 C: 24.9 ± 4.9	Acupuncture group: the needles were inserted into acupoints including RN4, RN6, RN10, CV12, ST21, ST25, and ST28 and retained for 30 min. The procedure was administered twice a week for 6 months. Control group: metformin was taken with food for 6 months. In the first week of the study, patients received 250 mg three times daily; thereafter, the metformin dose was 500 mg three times daily.	BMI, WHR, FGS, FGP, FINS, 2hFPG, 2hFINS, HOMA-IR, TC, TG, LDL-C, HDL-C
Yao, 2018	97	T: 3.1 ± 0.9 C: 3.2 ± 0.8	Obesity-type PCOS	T: 27.8 ± 4.8 C: 28.2 ± 4.5	Acupuncture group: the needles were inserted into acupoints including RN17, CV12, RN4, bilateral BL18, ST25, RN19, ST36, LR14, SP6, and LR3 and retained for 30 min. The procedure was administered three times a week for 6 months. Control group: metformin was taken at 500 mg three times daily with food for 6 months.	BMI, WHR, FGS, 2hFPG, HOMA-IR
Kong, 2015	55		Obesity-type PCOS	T: 28.1 ± 3.8 C: 27.8 ± 3.4	Electroacupuncture group: the needles were inserted into acupoints including DU20, CV3, RN6, bilateral LI4, SP6, ST29, and SP9 and retained for 30 min. The needles were then inserted into acupoints including DU20, CV3, bilateral ST29, PC6, SP6, ST25, and LR3 and retained for 30 min. The procedure was performed two or three times a week for a total of 32 sessions. Control group: Sham acupuncture was used, and virtual electroacupuncture was used at the acupoint.	BMI, WHR, FGP, FINS, HOMA-IR, TC, TG, LDL-C, HDL-C
Peng, 2017	100	T: 2.5 ± 0.8 C: 2.4 ± 0.9		T: 28.6 ± 3.8 C: 28.8 ± 3.3	Acupuncture group: the needles were inserted into acupoints including SP6, ST36, ST40, and ST25 and retained for 30 min. The procedure was administered three times a week for 3 months. Control group: Sham acupuncture was administered three times a week for 3 months.	BMI, WHR
Li, 2014	100				Acupuncture group: the needles were inserted into acupoints including CV3, RN4, bilateral ST36, SP6, KI7, and RN6 and retained for 30 min. The procedure was performed every day except during the menstrual period. Control group: both sham acupuncture and metformin were used. Metformin was taken as a single tablet with food three times daily for 6 months.	BMI, WHR, FGS, FGP, FINS, HOMA-IR, TC, TG, LDL-C, HDL-C

Table 1: Continued.

Study ID	Sample size	Course of PCOS	Participant characteristics	Age	Treatment versus control	Outcome
Cai, 2016	50		Obesity-type PCOS		Acupuncture group: the needles were inserted into acupoints including CV12, ST21, ST25, GB26, RN6, RN4, ST28, SP10, ST34, ST36, ST37, and SP6 and retained for 30 min. The procedure was performed three times a week for 3 months. Control group: metformin was taken at 500 mg three times daily with food for 6 months.	BMI, WHR, FGP, FINS, HOMA-IR
Lai, 2012	120	T: 2.5 ± 0.6 C: 2.5 ± 0.7		T: 26.7 ± 2.7 C: 26.5 ± 2.7	Acupuncture group: the needles were inserted into acupoints including CV12, RN10, RN6, RN4, ST25, and ST28 and retained for 30 min. The procedure was performed once every 3 days for 4 months. Control group: metformin was taken at 500 mg three times daily with food for 4 months.	BMI, WHR, FGP, FINS, 2hFPG, 2hFINS, HOMA-IR
Guo, 2014	38		PCOS-IR	T: 27.8 ± 3.2 C: 29.3 ± 2.9	Electropuncture group: the needles were inserted into acupoints including CV3, RN6, DU20, bilateral ST29, SP6, SP9, and LI4 and retained for 30 min. The needles were then inserted into acupoints including CV3, RN6, DU20, bilateral ST25, ST29, SP6, LR3, and PC6 and retained for 30 min. The procedure was performed once every 2 days for 2 months. Control group: Sham acupuncture was used and retained for 30 min. The procedure was performed once every 2 days for 2 months.	BMI, WHR, FGS, FGP, FINS, HOMA-IR, TC, TG, LDL-C, HDL-C
Gu, 2019	76	T: 4.6 ± 3.6 C: 4.3 ± 3.3	PCOS-IR	T: 27.0 ± 4.5 C: 28.6 ± 4.0	Electroacupuncture group: the needles were inserted into acupoints including CV3, RN6, DU20, bilateral ST29, SP6, SP9, and LI4 and retained for 30 min. The needles were then inserted into acupoints including CV3, RN6, DU20, bilateral ST25, ST29, SP6, LR3, and PC6, and retained for 30 min. The procedure was performed twice a week for a total of 32 sessions. Control group: Sham acupuncture was used and retained for 30 min. The procedure was performed twice a week for a total of 32 sessions.	BMI, WHR, FGP, 2hFPG, FINS, 2hFINS, HOMA-IR, TC, TG, LDL-C, HDL-C

BMI: body mass index; WHR: waist-to-hip ratio; FGS: Ferriman–Gallwey score; FPG: fasting plasma glucose; 2hFPG: 2 h fasting plasma glucose; FINS: fasting insulin; 2hFINS: 2 h fasting insulin; HOMA-IR: homeostasis model assessment of insulin resistance; TC: serum total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

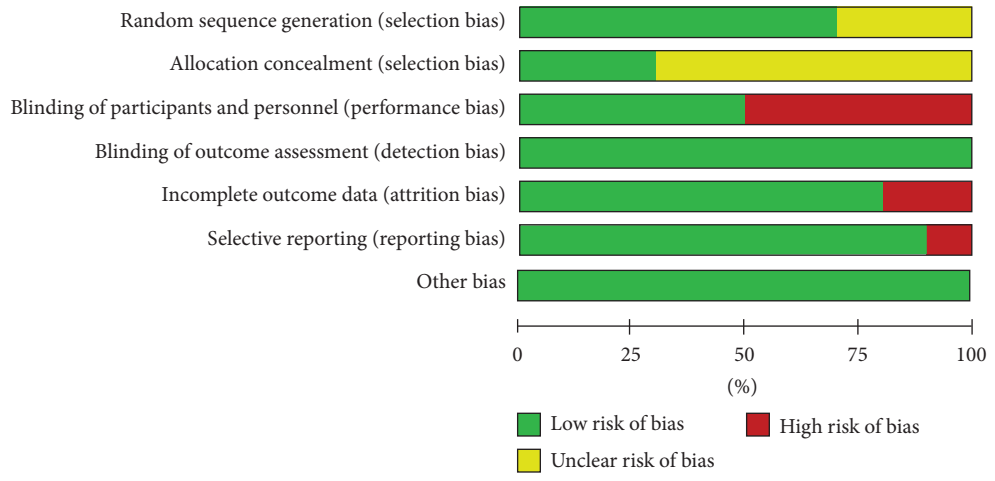

Figure 2: Evaluation of the risk biases of the included studies.

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight (%)	Mean difference IV, random, 95% CI
1.1.1. BMI								
Cai XB 2016 China	24.15	1.84	25	24.49	2.16	25	11.9	−0.34 [−1.45, 0.77]
Elisabet SV 2009 Sweden	27.6	8.8	9	28.5	6.2	6	1.2	−0.90 [−8.49, 6.69]
Gu Y 2019 China	26.03	4.55	38	27.77	2.7	38	9.6	−1.74 [−3.42, −0.06]
Guo NJ 2014 China	24.96	2.46	19	27.28	3.95	19	8.0	−2.32 [−4.41, −0.23]
Kong XJ 2015 China	25.84	3.84	29	26.75	3.09	26	9.0	−0.91 [−2.74, 0.92]
Lai MH 2012 China	20.6	3.06	60	24.63	3.03	60	12.0	−4.03 [−5.12, −2.94]
Li L 2014 China	24.5	1.9	49	24.6	2.2	51	13.1	−0.10 [−0.90, 0.70]
Peng Y 2017 China	22.48	2.64	50	24.25	2.19	50	12.5	−1.77 [−2.72, −0.82]
Yao M 2018 China	26.8	2.1	49	27.4	1.9	48	13.1	−0.60 [−1.40, 0.20]
Zheng YH 2012 China	22.3	3.7	43	24.2	4.2	43	9.6	−1.90 [−3.57, −0.23]
Subtotal (95% CI)			*371*			*366*	*100.0*	*−1.47 [−2.35, −0.58]*
Heterogeneity: $\text{tau}^2 = 1.39$; $\text{chi}^2 = 41.18$, $df = 9$ ($P < 0.00001$); $I^2 = 78\%$								
Test for overall effect: $Z = 3.25$ ($P = 0.001$)								
1.1.2. WHR								
Cai XB 2016 China	0.83	0.03	25	0.9	0.03	25	12.1	−0.07 [−0.09, −0.05]
Elisabet SV 2009 Sweden	0.8	0.09	9	0.8	0.06	6	5.2	0.00 [−0.08, 0.08]
Gu Y 2019 China	0.85	0.05	38	0.88	0.05	38	11.5	−0.03 [−0.05, −0.01]
Guo NJ 2014 China	0.887	0.023	19	0.922	0.034	19	11.9	−0.04 [−0.05, −0.02]
Kong XJ 2015 China	0.88	0.26	29	0.93	0.36	26	1.6	−0.05 [−0.22, 0.12]
Lai MH 2012 China	0.73	0.06	60	0.84	0.07	60	11.4	−0.11 [−0.13, −0.09]
Li L 2014 China	0.73	0.06	49	0.76	0.03	51	11.9	−0.03 [−0.05, −0.01]
Peng Y 2017 China	0.81	0.03	50	0.82	0.04	50	12.3	−0.01 [−0.02, 0.00]
Yao M 2018 China	0.84	0.06	49	0.84	0.04	48	11.7	0.00 [−0.02, 0.02]
Zheng YH 2012 China	0.69	0.08	43	0.73	0.06	43	10.5	−0.04 [−0.07, −0.01]
Subtotal (95% CI)			*371*			*366*	*100.0*	*−0.04 [−0.06, −0.02]*
Heterogeneity: $\text{tau}^2 = 0.00$; $\text{chi}^2 = 82.00$, $df = 9$ ($P < 0.00001$); $I^2 = 89\%$								
Test for overall effect: $Z = 3.37$ ($P = 0.0008$)								
1.1.3. FGS								
Elisabet SV 2009 Sweden	15.1	10.1	9	10.8	5	6	0.4	4.30 [−3.42, 12.02]
Guo NJ 2014 China	4.63	2.22	19	4.63	2.31	19	9.9	0.00 [−1.44, 1.44]
Li L 2014 China	5.6	2.1	49	5.2	1.9	51	23.7	0.40 [−0.39, 1.19]
Yao M 2018 China	3.13	0.94	49	2.98	0.96	48	43.2	0.15 [−0.23, 0.53]
Zheng YH 2012 China	4.8	1.6	43	5.6	2.2	43	22.8	−0.80 [−1.61, 0.01]
Subtotal (95% CI)			*169*			*167*	*100.0*	*−0.00 [−0.50, 0.49]*
Heterogeneity: $\text{tau}^2 = 0.11$; $\text{chi}^2 = 6.40$, $df = 4$ ($P = 0.17$); $I^2 = 37\%$								
Test for overall effect: $Z = 0.02$ ($P = 0.99$)								

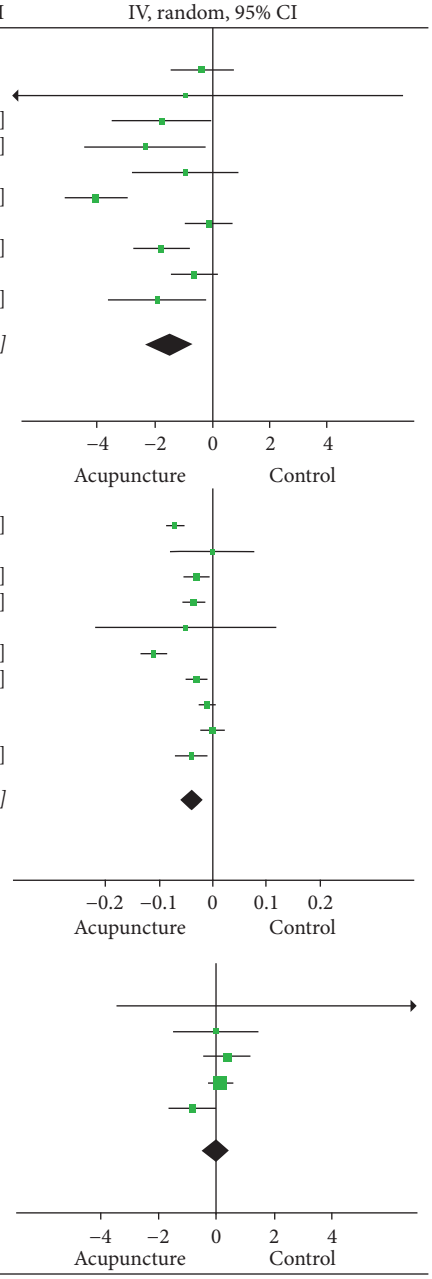

Figure 3: Comparison of the BMI, WHR, and FGS between the acupuncture and control groups in the treatment of PCOS.

4.3. Implications for Clinical Practice and Further Research. The results of this meta-analysis suggest that acupuncture alone has better efficacy than control interventions. Because traditional Chinese medicine departments have been established in most maternity hospitals and general hospitals in China, it is easy and convenient to apply such treatments. As a safe and simple therapy, acupuncture might be an alternative or a good adjunct therapy for PCOS, especially for overweight patients and patients with IR. However, we have no information on how long the effect of acupuncture might last. Thus, more long-term follow-up studies are needed to examine the effectiveness of acupuncture in improving insulin resistance and depressing BMI and WHR and to assess the sustainability of their effects.

Several meta-analyses have been performed regarding the effectiveness of acupuncture for treating PCOS, specifically focusing on rates of ovulation, pregnancy, and live birth [45–47], but articles about how acupuncture affects metabolic-related indexes in patients with PCOS, particularly lipid profiles, are lacking.

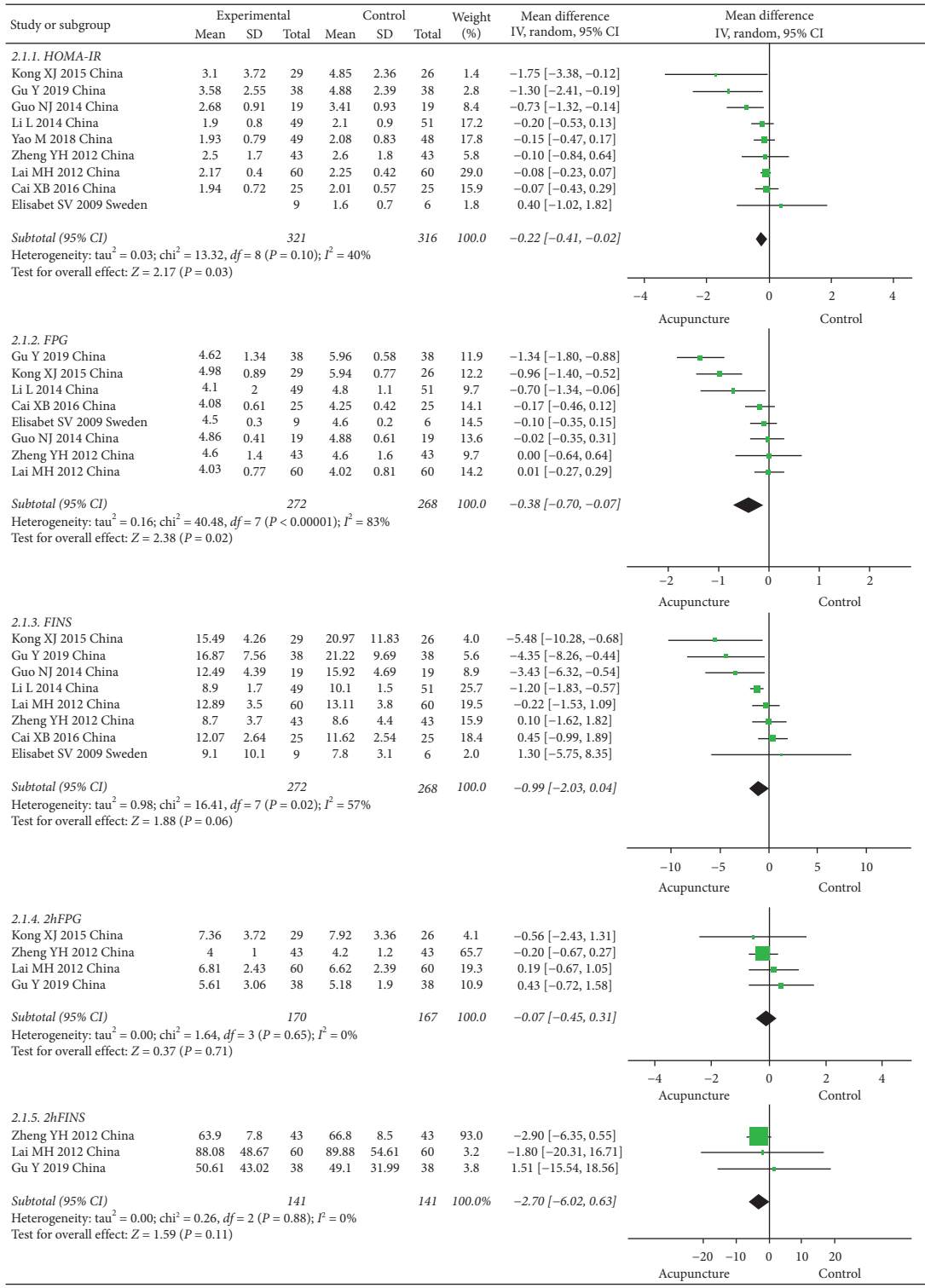

Figure 4: Comparison of glucose metabolism indicators between the acupuncture and control groups in the treatment of PCOS.

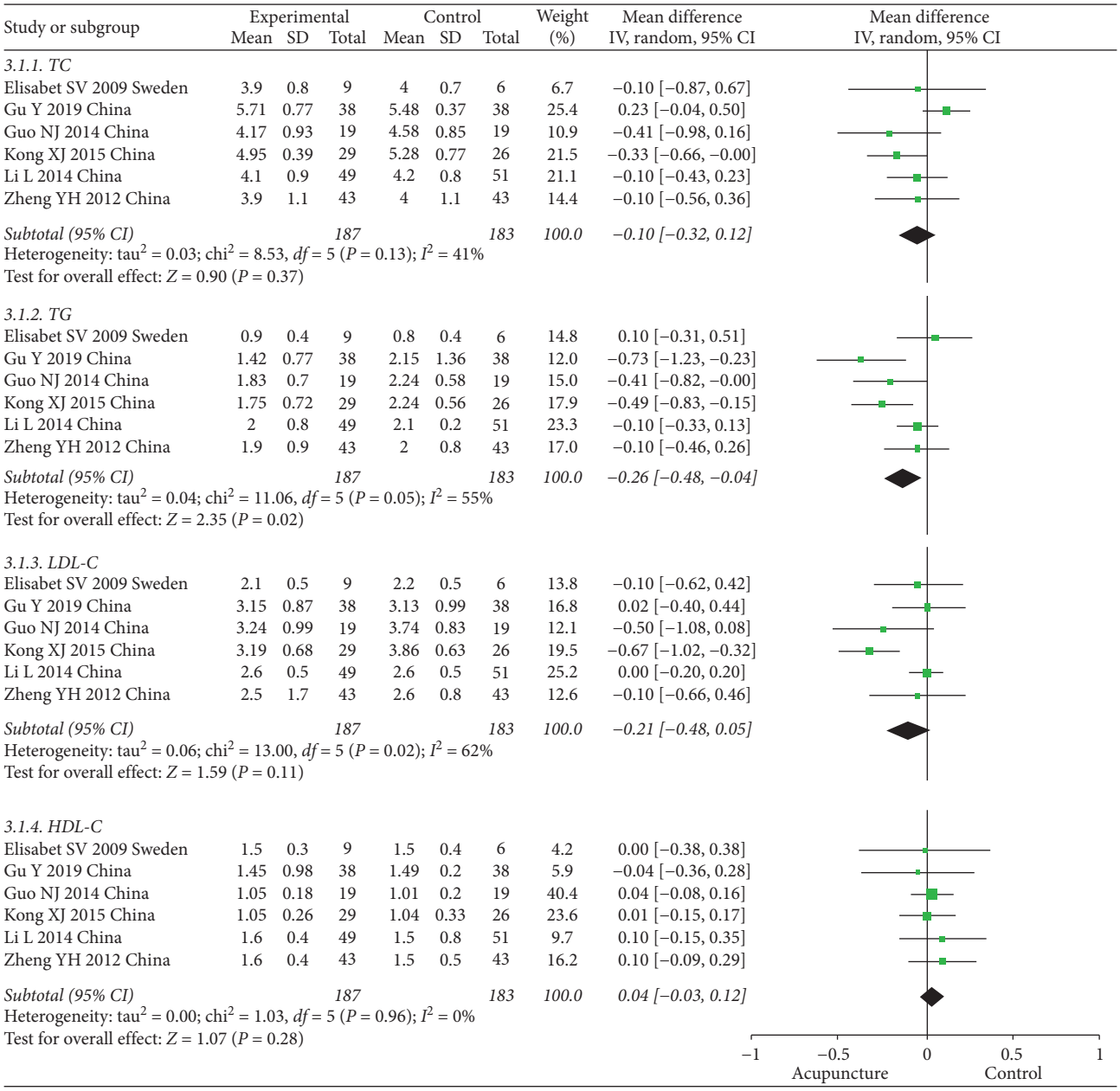

Figure 5: Comparison of lipid profiles between the acupuncture and control groups in the treatment of PCOS.

5. Conclusion

There is insufficient evidence to support that acupuncture can improve lipid profiles except for TG levels. However, this review of 10 RCTs shows that acupuncture could improve BMI and WHR as well as HOMA-IR in patients with PCOS. The included studies are inconclusive because of their moderate level of evidence, and further large-scale, long-term RCTs with rigorous methodological standards are still warranted.

Disclosure

RZ and PQ are the co-first authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

JL and HM contributed equally to this work. JL conceptualized and designed the study. RZ drafted the manuscript. RZ and PQ collected and analyzed the data. MH, JS, MH, and HM reviewed the protocol for important intellectual content and revised the manuscript critically. JL and HM sought funding. All authors contributed to the further editing of the manuscript and approved the final version of the manuscript accepted for publication. Ruqun Zheng and Peng Qing contributed equally.

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Review Article

Complementary and Alternative Medicine for Threatened Miscarriage: Advantages and Risks

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Threatened miscarriage is one of the most common complications causing pregnancy loss, and it affects approximately 20% of confirmed pregnancies. More and more women are seeking treatment with complementary and alternative medicine (CAM) for this common complication, and it has been reported that women have had successful pregnancies after threatened miscarriage when being treated with CAM, which mainly includes Chinese herbal medicines, acupuncture, and nutritional supplements as well as psychological interventions and other approaches. However, many experts are concerned about the safety and adverse events of certain CAM approaches in women with threatened miscarriage. Therefore, this review focuses on the status of CAM for threatened miscarriage and presents the potential therapeutic efficacy and safety of CAM based on some clinical and experimental studies. We thus hope to provide some instructive suggestions for the application of CAM for treating threatened miscarriage in the future.

1. Introduction

Threatened miscarriage is one of the most common pregnancy complications and is indicated by vaginal bleeding before the 20th week of gestation. It affects up to 20% of confirmed pregnancies, and approximately 50% of cases end in pregnancy loss [1–3]. Even if the miscarriage is avoided, women who suffer from threatened miscarriage remain at high risk for adverse pregnancy outcomes such as premature birth, antepartum hemorrhage, low birth weight, and neonatal death, as well as psychological anomalies [4–8], and thus threatened miscarriage is a physically and psychologically traumatic experience for women and their families. Many factors cause threatened miscarriage, including chromosomal defects, immunological dysfunction, maternal thrombophilic disorders, endocrine abnormalities, and uterine structural anomalies. Additionally, maternal age, previous miscarriage, environmental pollutants, infectious agents, and previous clinical interventions also contribute to a high risk of miscarriage [9–12], and the thorough evaluation of the intrauterine condition with sonography and maternal serum markers might help to diagnose threatened miscarriage or predict subsequent pregnancy outcomes [13]. Bed rest does not significantly reduce the risk of miscarriage, and progesterone and human chorionic gonadotropin (hCG) are most commonly prescribed in women with threatened miscarriage even though little evidence supports their effectiveness [11–13]. Rh prophylaxes like heparin plus aspirin and other regimens such as buphenine hydrochloride have been suggested to be beneficial for threatened miscarriage, but they are associated with adverse events and their effects have not been verified (Figure 1).

The use of complementary and alternative medicine (CAM) for promoting health and treating ailments is an increasing trend worldwide. Studies have reported that women use CAM more than men, and over 80% of women in the UK, 50% of women in Australia, 90% of women in Canada, nearly 25% of women in Denmark, and nearly 50%

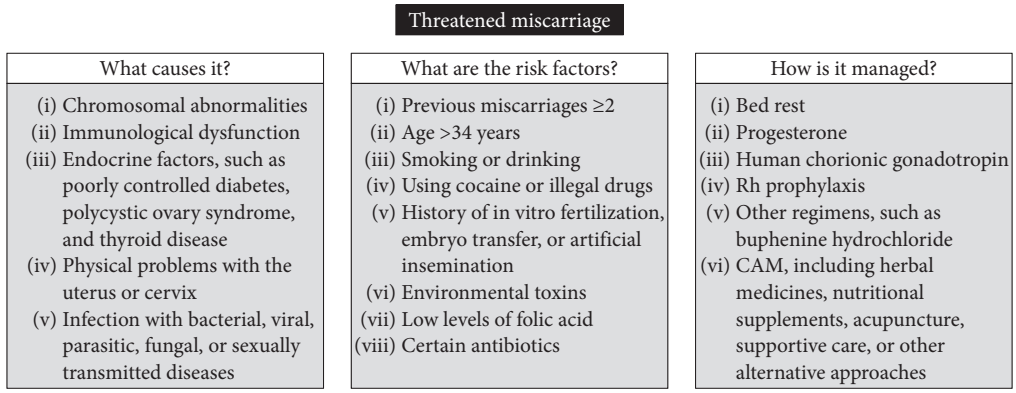

Figure 1: Possible etiology and current management of threatened miscarriage.

of women in the USA are users of CAM [8, 14–17]. Pregnant women use CAM at similar rates to those of nonpregnant women, and health professionals are increasingly recommending that pregnant women use CAM [18–20]. A systematic review found that women commonly use CAM alone or in combination with other approaches during pregnancy, with 5.8–74.2% of pregnant women taking herbal or natural treatments and 12–95% of pregnant women using vitamins, with most of them using CAM during the first trimester [19]. The women in the reviewed studies assumed that CAM can promote maternal and fetal health, enhance the efficacy of western medicines, and relieve specific pregnancy-related conditions, but there is poor evidence to support such effects of CAM. In line with this, a series of studies have been performed regarding the safety of CAM for pregnant women [17, 21–23]. To date, several studies have reported on women who have had successful pregnancies after threatened miscarriage when being treated with CAM, mainly including Chinese herbal medicine (CHM), acupuncture, nutritional supplements, and psychological interventions [8, 10, 11, 24]. This review briefly summarizes the current progress in preventing and treating threatened miscarriage with diverse forms of CAM and discusses the potential risks of such treatments.

2. CHM in Threatened Miscarriage

The prevalence of herbal medicine use by pregnant women ranges between 7% and 79.9% depending on different geographic, social, cultural, and ethnic factors [21, 22, 25, 26]. CHM has a history of about 5000 years, and it has become one of the main therapies in East Asia and has spread to western countries. CHM is widely accepted as an alternative form of medicine, and it is considered beneficial for preventing and treating miscarriage with fewer adverse events compared to current conventional western medicine [10, 27, 28]. There have been a number of cases of treating threatened miscarriage using CHM according to the theory of traditional Chinese medicine (TCM). However, the safety and side effects of using certain herbal medicines during pregnancy is still a matter of debate, and here we review the efficiency and safety of CHM in the treatment of threatened miscarriage.

2.1. Efficiency of CHM for Threatened Miscarriage. Most of the literature regarding CHM use during pregnancy has focused on miscarriage, and the majority of the publications are in Chinese [10, 28, 29]. It has been suggested that the effectiveness of CHM ranges from 79.22% to 100% in the treatment of threatened miscarriage according to randomized and semirandomized trials comparing CHM, conventional western medicine, and combinations of the two (Table 1). However, there is a lack of well-designed placebo-controlled randomized clinical trials. A meta-analysis of 44 randomized clinical trials indicated that CHM alone has a similar efficacy to that of conventional western medicines alone in the treatment of threatened miscarriage, and CHM in combination with other pharmaceuticals was found to be superior to pharmaceuticals alone in maintaining pregnancy after 28 gestational weeks in women with threatened miscarriage [28]. CHM alone or combined with conventional western medicines has also been shown to be more beneficial in improving vaginal bleeding, lower back pain, and abdominal pain [30]. Moreover, a majority of randomized clinical trials and some systematic reviews have demonstrated that a combination of CHM and other pharmaceuticals or psychotherapy might maintain pregnancy and increase the live birth rate for women with recurrent miscarriage [29, 31]. These studies all suggest that CHM has the potential to prevent threatened miscarriage in subsequent pregnancies, but most studies have neglected to investigate the duration, follow-up, adverse events, or toxicity of CHM, and the sample sizes of the interventions have been small.

2.2. Common Formulas and Single CHM for Threatened Miscarriage. A CHM formula contains one or more compositions of herbal raw material and can be available in different preparations such as decoctions, pills, powders, and extracts in order to obtain optimized efficiency. At present, the most commonly used classic prescription for threatened miscarriage is Shoutai Pill [10], which was first described in the book *Integrating Chinese and Western Medicine* from the late Qing dynasty around 200 years ago. The Shoutai Pill is composed of four ingredients, with *Herba Taxilli*, *Semen Cuscutae*, and *Radix Dipsaci* replenishing the liver and kidney and strengthening the bones and muscles to maintain

Table 1: Commonly used CHM formulas for the treatment of threatened miscarriage.

Formula	Main compositions and intervention	Comparison	Effective rate	Adverse outcomes∗	References
Shoutai pill	*Semen Cuscutae* (10–15 g), *Herba Taxilli* (10–20 g), *Radix Dipsaci* (10 g), *Colla Corii Asini* (10 g) PO, QD/BID, 7–28 days, alone or combined with comparators	(A) Progesterone, 20 mg, IM, QD, 7–21 days; (B) progesterone, 40 mg, PO, every other day, to 10 completed weeks of gestation; followed by once every three days to weekly until 12 completed weeks of gestation	90.00–93.34%	Premature rupture of fetal membranes, gestational diabetes mellitus, gestational hypertension, postpartum hemorrhage, low birth weight infant, neonatal malformation	[34–37]
Zishen Yutai pill	Radix Codonopsis, Radix Dipsaci, Rhizoma Atractylodis Macrocephalae, Radix Morindae Officinalis, Fallopia multiflora, *Cortex Eucommiae* make a pill at 5 g PO, TID, 7–21 days, alone or combined with comparators	(A) Progesterone, 20–40 mg, IM, QD, 14–21 days; (B) progesterone, 40 mg, PO, at the first time, followed by 10 mg, Q8H/Q12H, until symptoms disappear; (C) dydrogesterone, 10 mg, PO, BID, 7 days; (D) allylestrenol, 10 mg, PO, QD, 7 days	85.33–100%	Colporrhagia, soreness of waist, abdominal pain, hypogastralgia, nausea, dry mouth, poor appetite, constipation, dizziness, headache, and edema	[38–44]
Gushen Antai pill	*Semen Cuscutae, Herba Taxilli, Radix Dipsaci, Fallopia multiflora,* Rehmannia glutinosa, *Cistanche, Uncaria rhynchophylla, Radix Paeoniae Alba, Rhizoma Atractylodis Macrocephalae, Radix Scutellariae* make a pill at 6 g PO, TID, 14–28 days, combined with comparators	(A) Progesterone, 20–40 mg, IM, QD, or 100 mg, PO, BID, 14 days; (B) progesterone, 100 mg, PO, BID, 14 days; (C) dydrogesterone, 40 mg, PO, at the first time, followed by 10 mg, PO, Q8H, 14 days	87.64–93.90%	Preterm delivery and miscarriage	[45–48]
Andian Ertian decoction	*Radix Dipsaci* (15 g), *Semen Cuscutae* (15 g), *Herba Taxilli* (15 g), *Pseudostellaria heterophylla* (15 g), *Radix Rehmanniae Praeparata* (10–15 g), *Rhizoma Atractylodis Macrocephalae* (15–20 g), *Dioscoreae Rhizoma* (15 g), *Fructus Corni* (10–15 g), *Dolichos Lablab* (15 g), *Cortex Eucommiae* (9-10 g), *Fructus Lycii* (6 g), *Radix et Rhizoma Glycyrrhizae* (3–6 g) PO, QD/QOD, 7–14 days	Progesterone, 20 mg, IM, QD, 7–90 days	93.33–97.14%	Preterm delivery, miscarriage within 28 weeks	[49, 50]
Baoyin decoction	Rehmannia glutinosa (12 g) (6–15 g), *Rehmanniae Radix Praeparata* (6–15 g), *Radix Scutellariae* (4.5–10 g), *Phellodendron chinense Schneid* (4.5–10 g), *Radix Paeoniae Alba* (6–10 g), *Radix Dipsaci* (4.5–10 g), *Dioscorea opposita* (4.5–15 g), *Radix et Rhizoma Glycyrrhizae* (3–5 g) PO, BID/TID, 7–21 days, combined with progesterone 100–150 mg PO, BID, 7 days, or dydrogesterone as comparator	(A) Dydrogesterone, 20 mg, PO, Q12H, 21 days; (B) hCG, 2000 IU, IM, QD, 7 days	90.00–97.40%	Not reported	[51, 52]

Table 1: Continued.

Formula	Main compositions and intervention	Comparison	Effective rate	Adverse outcomes∗	References
Baotai decoction	Rehmannia glutinosa (6–15 g) (12 g), *Semen Cuscutae* (12 g), *Herba Taxilli* (12 g), *Radix Scutellariae* (9 g), *Poria cocos* (9 g), *Radix Dipsaci* (9 g), *Dioscoreae Rhizoma* (10 g), *Rhizoma Atractylodis Macrocephalae* (15 g), *Cinnamomum cassia Presl* (6 g), *Radix Aconiti Lateralis Preparata* (3 g) PO, QD, 14 days	Dydrogesterone, 40 mg, PO, once; followed by 10 mg, PO, Q8H, 14 days	95.00%	Not reported	[53]
Bushen Antai decoction	*Semen Cuscutae* (10–30 g), *Cortex Eucommiae* (10–15 g), *Herba Taxilli* (10–18 g), *Radix Dipsaci* (10–15 g), *Pseudostellaria heterophylla* (10–15 g), *Radix Astragali* (10–15 g), *Rhizoma Atractylodis Macrocephalae* (10–15 g), *Radix Scutellariae* (15 g), *Radix Paeoniae Alba* (10 g), *Rehmanniae Radix Praeparata* (10 g), *Boehmeriae Radix* (10 g), *Colla Corii Asini* (11 g) PO, BID, 10 days, combined with comparator	Progesterone, 20 mg, IM, QD, 10 days	79.22–96.00%	Constipation, mouth sores, and asitia	[54, 55]

CHM: Chinese herbal medicine; TCM: traditional Chinese medicine; PO: per os; IM: intramuscular; QD: quaque die; Q8H: quaque 8 hours; Q12H: quaque 12 hours; BID: bis in die; TID: ter in die; hCG: human chorionic gonadotropin. ∗Adverse events occurred when using CHM alone or combined with conventional medicine.

pregnancy and *Colla Corii Asini* nourishing the yin and blood and further strengthening Qi [32]. The four ingredients are documented in the Chinese Pharmacopeia and have been approved by the World Health Organization (WHO) for use in clinical trials. The prescription used alone or in combination with western medicines has been shown to be superior to western medicine alone for preventing miscarriage in the first trimester of pregnancy, and no adverse events have been reported [33–37] (Table 1).

On the basis of the Shoutai Pill, professional practitioners have further developed the recipe according to individualized clinical presentations for threatened miscarriage, for example, the Bushen Antai decoction. In addition, the generally applied prescriptions for threatened miscarriage include Baotai decoction, Zishen Yutai Pill, and other recipes that have been shown to be effective. With regard to the theory of TCM, these regimes are considered to regulate Qi and the blood and to improve kidney and spleen function in order to maintain pregnancy. We summarize the studies on the application of common CHM formulas for threatened miscarriage in Table 1 [38–55].

According to TCM theory, threatened miscarriage is due to insufficiency of the spleen and kidney, deficiency of Qi and blood, or both stasis and heat based on syndrome differentiation. Thus, herbal medicines that are frequently used to prevent miscarriage include those that tonify the kidney (e.g., *Semen Cuscutae*, *Herba Taxilli*, and *Radix Dipsaci*), those that tonify Qi and the blood (e.g., *Colla Corii Asini* and *Codonopsis pilosula*), those that nourish Qi to invigorate the spleen (e.g., *Radix Astragali* and *Rhizoma Atractylodis Macrocephalae*), and those that clear heat and cool the blood (e.g., *Radix Scutellariae*). Importantly, the proper concerted application of individual CHMs can have synergistic effects. The most commonly used combination is *Semen Cuscutae* and *Herba Taxilli*, which are contained in most of the formulas described above and can strengthen the kidney to prevent miscarriage. The most commonly used single compositions of CHM in formulas used to treat threatened miscarriage and prevent further pregnant complications are listed in Table 2 [10, 17, 56–77].

2.3. Potential Mechanism of CHM for Threatened Miscarriage. To explore the molecular mechanism of CHM in threatened miscarriage, many studies have been performed in humans and in diverse animal models both in vivo and in vitro. First, CHM might improve uterine function to retain pregnancy, and it has been reported that Shoutai Pill may reduce the miscarriage rate, increase serum progesterone and estrogen levels [33, 78], enhance endometrial thickness, and regulate the hemodynamic parameters of the uterine spiral artery in order to improve endometrial receptivity and promote implantation [79]. Second, CHM potentially contributes to the development of trophoblasts and reduces the miscarriage rate. For example, Shoutai Pill can regulate the bioactivity behavior of trophoblasts to prevent spontaneous miscarriage by regulating trophoblast proliferation, invasion, and migration capacity, β-hCG secretion by trophoblasts, and apoptosis of trophoblasts in vitro [31, 80]. Furthermore, CHM has the effect of regulating immunological function and anti-inflammatory and antioxidation activities, which is consistent with TCM theory on tonifying the kidney and preventing miscarriage [79, 81, 82]. Shoutai Pill can improve pregnancy outcomes by reducing placental damage and regulating oxidative stress, and Shoutai Pill increases serum IL-2 and IL-6 levels and placental glutathione and superoxide dismutase (SOD) levels and decreases 3,4-methylenedioxyamphetamine and reactive oxygen species (ROS) levels [82]. Another study found that CHM can treat threatened miscarriage by regulating the expression of the inflammatory factors IFN-γ and IL-10 [83]. Overall, the application of CHM may improve endometrial function, inhibit uterine contraction, regulate immune response, and promote the development of the embryo. However, there are limited numbers of studies into the mechanisms through which individual CHMs or combinations have an effect on threatened miscarriage, and few of the reports are in English.

2.4. Safety and Adverse Events of CHM for Threatened Miscarriage. Almost half of pregnant women in the world use CHMs or other herbal medicines to support their pregnancy, to ameliorate disturbing symptoms and complications, and to reduce the need for western medication, and they widely consider that these treatments are safe and effective. However, all regimes, including CHM, have the potential risk for pregnancy and fetal development, but only a few studies have evaluated the safety of these interventions. CHMs are commonly used in threatened miscarriage in order to prevent pregnancy loss and further complications, and most clinical trials have reported no adverse events or significant risks. Some systematic reviews have concluded, however, that no data are available to demonstrate the safety of CHM for the mother or the infant [29, 31]. In turn, a few studies have found adverse outcomes in relation to CHM, including gastrointestinal reactions, preterm birth, premature rupture of membranes, stillbirth, asphyxia, and infections that cause neonatal death [27]. None of these clinical studies further explained the reasons for these adverse events, and there are limited data available for identifying the risks of CHM. A systematic review and meta-analysis indicated that CHM combined with conventional medicines had lower intervention failures for women with threatened miscarriage, and no obvious differences were found between the combination of CHM and conventional medicine and CHM alone regarding adverse events and toxicity in terms of pregnancy and perinatal outcomes [27]. In the pooled randomized controlled trials, 3.1–22.3% had intervention failure; 2–10% had dry mouth, constipation, and insomnia; 3% had diabetic complications; 5% had preterm delivery; and 1.8% had neurodevelopmental morbidity. Notably, some individual herbal medicines were reported to have potent risks during pregnancy; for example, a Danish prospective cohort study suggested that licorice causes an increase in blood pressure in pregnant women [17] (Table 2).

Moreover, various animal studies have indicated reproductive toxicity with the frequently used CHMs for threatened miscarriage, including fetal resorption, growth

Table 2: Commonly used single CHMs in formulas for threatened miscarriage.

English name	Latin name	Efficacy based on TCM theory	Mechanism of action	Adverse events	References
Baikal skullcap root	*Radix Scutellariae*	Clearing heat and stopping bleeding to prevent miscarriage	It can have an antiabortive effect through inhibition of maternal-fetal interface immunity, and it contains baicalein, which has anti-inflammatory effects and elevates progesterone levels to prevent miscarriage.	Treatment with 32 g/kg/day led to potential maternal toxicity and major limb abnormalities in mice.	[56–59, 75]
Chinese Angelica	*Radix Angelicae Sinensis*	Replenishing and promoting blood circulation, regulating menstruation, relieving pain, and acting as a laxative	The water-based extracts can restrain the mobility of uterine smooth muscle and rectify the excitation of oxytocin.	Inhibited embryonic growth and development in rats and mice.	[60, 75]
Chinese Dodder seed	*Semen Cuscutae*	Tonifying the kidney to prevent miscarriage, benefiting essence, and nourishing yin	It promotes progesterone secretion, improves trophoblast function, regulates decidual proliferation and apoptosis, and acts as an antioxidant.	It increased antepartum and postpartum maternal mortality, decreased embryonic development, and minor limb abnormalities, mainly polydactyly and oligodactyly, in mice.	[61–63, 75]
Chinese Taxillus twig	*Herba Taxilli*	Nourishing the liver and kidney, strengthening the waist and knees to prevent miscarriage, dispelling wind, and removing dampness	It is rich in elements such as zinc and manganese that promote fetal development, inhibits uterine contraction, and reduces platelet aggregation.	It had embryotoxic effects with high dose in rats, reduced maternal weight gain and increased early fetal resorption rate, shortened pregnancy duration, increased congenital malformation and postnatal mortality rate, and reduced postnatal weight gain in mice.	[64, 65, 75]
Donkey-hide glue	*Colla Corii Asini*	Nourishing and regulating the blood to prevent miscarriage, and relieving pain	It contains iron, zinc, and other essential trace elements and thus can also stop vaginal bleeding, increase circulating calcium levels, and contribute to embryonic development.	Not reported.	[66]
Eucommia bark	*Cortex Eucommiae*	Replenishing the liver and kidney and strengthening bones and muscles to prevent miscarriage	It can weaken pituitrin secretion and suppress uterine contraction.	It impacted embryonic growth and development.	[67, 68, 75]
Himalayan Teasel root	*Radix Dipsaci*	Nourishing the liver and kidney, regulating blood vessels, and strengthening bones and muscles	It suppresses uterine contraction during pregnancy, improves ovarian function, and promotes uterine and embryonic development.	Aqueous extracts at the dosage of 8 or 32 g/kg/d might cause adverse impacts on maternal health and embryonic/fetal development, including maternal death before delivery.	[69, 70, 75]
Largehead Atractylodes rhizome	*Rhizoma Atractylodis Macrocephalae*	Tonifying Qi and strengthening the spleen	It contains volatile oils that can promote fetal development and maturity, inhibit uterine contraction, and maintain pregnancy.	Potential reproductive toxicity in pregnant animals within the clinical dose equivalent to that used by humans, such as postpartum maternal mortality, embryonic developmental delay, and major fetal limb abnormalities.	[56, 71, 75, 76]

Table 2: Continued.

English name	Latin name	Efficacy based on TCM theory	Mechanism of action	Adverse events	References
Licorice root	*Radix et Rhizoma Glycyrrhizae*	Tonifying and regulating Qi and the blood and relieving urgency and pain	It is anti-inflammatory and antioxidative and enhances endogenous steroids. It is also used to relieve abdominal pain and muscle spasms.	It can increase blood pressure in pregnant women, and it contains glycyrrhizin (≥500 mg/week) that may result in lower gestational age, increased postpartum maternal mortality, reduced maternal weight gain, and increased early fetal resorption rate in mice.	[10, 17, 75, 77]
Milkvetch root	*Radix Astragali*	Tonifying Qi and yin, promoting body fluid production, and lifting depression	It elevates placental blood supply and restrains the placental immune response in order to prevent pregnancy complications, and it has benefits for embryonic development.	Not reported.	[72]
Szechwon Tangshen root/ Pilose Asiabell root	*Radix Codonopsis*	Enriching blood and promoting fluid production, strengthening the spleen and benefiting the lung	Its polysaccharide component can strengthen immunity and promote compensatory hematopoiesis of the spleen.	Antepartum maternal mortality increased in mice.	[73, 75]
White Peony root	*Radix Paeoniae Alba*	Enriching blood, astringing yin, and relieving pain	It might downregulate fetal Th1/Th2/Th17 cytokines and receptors, which might benefit embryonic survival and development.	Antepartum maternal mortality and perinatal mortality increased in mice.	[74, 75]

CHM: Chinese herbal medicine; TCM: traditional Chinese medicine.

restriction, and congenital malformations [27]. For example, Wang et al. found 17 individual CHM extracts that seemed to be toxic to embryos and fetuses in pregnant mice [75]. Li et al. investigated the reproductive toxicity of *Largehead Atractylodes Rhizoma* in mice, rats, and rabbits [76], and the single composition increased prenatal and postnatal mortality and at high doses increased congenital malformations. Although these studies indicated that CHMs can have reproductive toxicity during pregnancy, the evidence is not sufficient. The experiments were conducted in normal pregnant animals rather than miscarriage models, and thus the data are not reliable sources for deriving pregnancy risks in humans for the tested CHMs, and this might explain why the results are in stark contrast to the outcomes after treatment in humans [84]. In addition, large doses of astragaloside, the main component of *Astragalus membranaceus*, can induce fetal toxicity, and *Eucommia ulmoides Oliv.* and total glucosides from peonies may lead to gene mutations and are suspected to be teratogenic [74, 85, 86]. In practice, TCM practitioners generally utilize CHM formulas in the treatment of threatened miscarriage, not single crude CHM extracts like those used in the animal experiments. For example, Zishen Yutai Pill, which is a common CHM to treat threatened miscarriage, shows no toxicity in the perinatal period in pregnant rats [87]. CHM synergies based on TCM theory might reduce the toxic effects, and there is a close correlation between the safety and efficacy of CHMs and the quality of the source materials used in their production.

So far, there is limited evidence for adverse events when using CHM to treat threatened miscarriage. Most CHMs are in a similar situation to that of the majority of pharmaceuticals available today, and neither their safety nor their risks during pregnancy have been verified. The current evidence from the evaluation of the safety of CHM in clinical use is valuable, but large-scale randomized placebo-controlled trials showing an unremarkable impact of pregnancy are warranted.

3. Acupuncture and Moxibustion in Threatened Miscarriage

3.1. The Role of Acupuncture in the Treatment of Threatened Miscarriage. Acupuncture and moxibustion are recognized nonpharmacological and alternative approaches, and they are increasingly used for reproductive conditions worldwide [88, 89]. Based on the theory of TCM, together these treatments have the function of dredging the meridian and stimulating the energy response of the Qi and blood, thereby strengthening the body's resistance to disease and eliminating pathogenic factors. Traditional acupuncture has specific theories relating to promoting optimal early pregnancy responses, and it is recommended as a treatment modality for threatened miscarriage in acupuncture texts. Although the therapies exist in textbooks, there is yet no quality research to support the use of acupuncture for threatened miscarriage. Within fertility research, acupuncture has been shown to improve hormonal responses with decreased miscarriage rates, thus raising the possibility that acupuncture might promote specific beneficial effects in early pregnancy [90, 91]. Acupuncture might thus be a potential alternative option for threatened miscarriage under proper treatment control [11].

The safety of acupuncture in pregnancy is reasonably well accepted, but there is still a lack of high-quality scientific research to validate the safety and effectiveness of acupuncture for threatened miscarriage [92]. Many experts have raised concerns about the safety of acupuncture for treating women in early pregnancy [93, 94], and there remains a debate regarding the needling points that are historically considered to be forbidden during pregnancy, like SP6, LI4, BL60, BL67, GB21, and LU7. Nevertheless, electroacupuncture at these forbidden points did not aggravate the miscarriage rate or fetal loss over the course of gestation in pregnant rats [95]. Furthermore, there is no reliable evidence to suggest that acupuncture can induce miscarriage or premature delivery, and the adverse events with the use of forbidden points during pregnancy were similar to the control interventions [95]. The incidence of adverse events probably related to acupuncture during pregnancy was 1.3% according to a systematic review, and most of these were mild adverse events such as needling pain. Severe adverse events and fetal complications due to preterm delivery were rare, and the miscarriage rate was 5%, which was lower than that for control interventions [96]. A recent retrospective cohort study in South Korea reported consistent observations as previous studies, and there were no significant differences in delivery outcomes between pregnancies in the acupuncture and control groups [97]. Even though many of these studies have had small sample sizes and the methodologies have had a high risk of bias, they consistently suggest the safety of acupuncture during pregnancy.

Nowadays, about 4%–13% of pregnant women in Europe receive acupuncture for pregnancy and childbirth issues, and most treatments are given in the first trimester [98, 99]. Acupuncture has been shown to reduce the risk of miscarriage by increasing blood flow to the uterine lining and by aiding implantation, and thus it has potential for the treatment of threatened miscarriage. Professional practitioners are more and more using acupuncture during pregnancy, and surveys on acupuncture use conducted in the UK, Australia, and New Zealand showed that over a half of the acupuncturists had treated women for threatened miscarriages [91]. In a randomized feasibility trial with semistructured participant interviews with 40 women with threatened miscarriage, the participants received a pragmatic acupuncture and moxa protocol or touch intervention with medical self-care advice. The women who received acupuncture on the basis of TCM theory reported reduced vaginal bleeding, cramping, and back pain. These findings demonstrated that acupuncture was a feasible and pragmatic intervention that may be effective for women experiencing threatened miscarriage [92] (Table 3). However, although the application of a pragmatic acupuncture treatment protocol allowed flexibility for diagnosis and treatment, reflecting a treatment approach applicable to the real world, the protocol is difficult to use because the protocol designs do not provide information regarding the specific acupuncture points or needling effects. In addition, it was

Table 3: Commonly used acupuncture treatments for threatened miscarriage.

Approaches	Acupoints	Intervention	Comparison	Study design	Efficacy rate	Outcomes	Adverse events	References
Pragmatic acupuncture	LU7, LI11, HT7, LR2, LR3, GB30, GB34, GB41, SP1, SP4, ST36, ST37, KI6, KI9, KI21, KI27, BL20, BL23, BL57, BL62, PC6, TE4, TE5, TE6, CV4, GV4, GV20, EX-HN 3	Needles, moxibustion, and self-care advice. Weekly visits until 12 completed weeks of gestation	Touch intervention and medical self-care advice	A mixed methods study involving a RCT and semistructured interviews	Pregnancy loss: 16.7% vs. 23.8%; pregnancy complication: 11.1% vs. 14.2%	It causes reduced bleeding, cramping, and back pain.	Not reported	[92]
Acupoint sticking	CV 8	Dingtai decoction (*Panax ginseng* 30 g, *Radix Angelicae Sinensis* 10 g, *Radix Paeoniae Alba* 15 g, *Cortex Eucommiae* 15 g, *Rehmannia glutinosa Libosch* 15 g, *Rhizoma Atractylodis Macrocephalae* 10 g, *Pericarpium Citri Reticulatae* 5 g, *Radix Glycyrrhizae Preparata* 20 g, *Radix Scutellariae* 15 g, *Semen Cuscutae* 15 g, *Herba Taxilli* 15 g, *Radix Dipsaci* 15 g, *Artemisia Argyi* 15 g, *Amomum Villosum* 3 g), PO, BID, with the herbal residue placed on CV 8 for 6 h for 5 days until vaginal bleeding stops; combined with comparators	hCG, 3000 IU, IM, QD, 3 days; after vaginal bleeding stops, reduce to 3000 IU, IM, QOD; vitamin E, 50 mg, PO, BID; Folic acid, 0.4 mg, PO, QD, 0.4 mg, PO, QD	RCT	93.3%	The combination is beneficial for improving the symptoms of threatened miscarriage.	Not reported	[103]
Acupoint sticking	CV 8	CHM (*Colla Corii Asini* 1 g, *Folium Artemisiae Argyi* 1 g, *Cortex Eucommiae* 1 g, *Fructus Psoraleae* 1 g) placed at CV 8 for 4–6 h per day	Progesterone, 20 mg, IM, QD; hCG, 2000 IU, IM, QD, 14–28 days	RCT	94.83%	Acupoint sticking has a significant effect on early threatened miscarriage.	Not reported	[104]

Table 3: Continued.

Approaches	Acupoints	Intervention	Comparison	Study design	Efficacy rate	Outcomes	Adverse events	References
Acupoint sticking	CV 8	CHM (*Semen Cuscutae* 10 g, *Radix Dipsaci* 10 g, *Colla Corii Asini* 6 g, *Ramie root* 10 g, *Cortex Eucommiae* 10 g) placed on CV 8 for 4–6 h per day and combined with comparator	Progesterone, 40 mg, IM, QD, until symptoms disappear, and change to 20 mg, IM, QD, 7 days	RCT	90.0%	The combination can shorten the treatment time, increase serum P level, improve luteal function, prevent subsequent miscarriage, and increase the successful pregnancy rate.	Not reported	[105]
Acupoint sticking	CV 8	CHM (*Cortex Eucommiae* 10 g, *Ramie root* 10 g, *Radix Dipsaci* 10 g, *Semen Cuscutae* 10 g, *Colla Corii Asini* 6 g) placed on CV 8 for 6 h per day and combined with comparator	Progesterone, 20 mg, IM, QD, 7 days	RCT	89.2%	The combination increased the efficacy rate and improved the clinical symptoms of threatened miscarriage.	Not reported	[106]
Acupoint sticking	BL 23, GV4	CHM (*Semen Cuscutae* 10 g, *Radix Dipsaci* 10 g, *Colla Corii Asini* 6 g, *Ramie root* 10 g, *Cortex Eucommiae* 10 g) placed on the acupoints for 4 h per day, combined with Bushen Jianpi decoction∗, PO, BID, and comparator	Progesterone, 20 mg, IM, QD	RCT	93.94%	The combination can improve threatened miscarriage by inhibiting the secretion of INF-γ, promoting the secretion of IL-10, correcting the pathological shift of Th1/Th2 cytokine balance, and increasing the secretion of hCG and P.	Not reported	[107]
Auricular acupuncture	TF5, AT4, CO10, CO15, CO12, AH6a, CO18	The auricular points were changed every 3 days, for 3 weeks of intervention, and combined with dydrogesterone as comparator	Dydrogesterone, 10 mg, PO, BID, until 12 gestational weeks	Prospective study	80.0%	The combination can promote hematoma absorption and regulate immune factors by reducing Th, Th/Ts, and serum CA125 level.	Not reported	[110]

Table 3: Continued.

Approaches	Acupoints	Intervention	Comparison	Study design	Efficacy rate	Outcomes	Adverse events	References
Acupoint injection	ST 36	hCG 1000 IU, injected at ST36 and massaged for 3–5 min, alternating on both sides, QOD, 7–10 days, or until a week after symptoms disappear	hCG 1000 IU, IM, QOD, 7–10 days, or until a week after symptoms disappear	RCT	91.38–91.7%	It increased the levels of hCG, E2, and P; decreased the miscarriage rate and treatment time; reduced the amount of conventional medication; and promoted the development of the fetal sac.	Not reported	[113–115]
Acupoint catgut embedding	BL 17, BL 18, BL 23, BL 20, BL 21, SP 10, SP 8, KI 7	Embedding once every two weeks, 6 times, combined with comparator	Progesterone, 40 mg, IM, QD	RCT	Successful pregnancy rate: 96.0%; spontaneous abortion rate: 16.7%	Embedding reduced the spontaneous abortion rate, improved the successful pregnancy rate, and regulated hormone levels in patients after IVF-ET.	Miscarriage, and premature birth	[117]
Moxibustion	ST 36, PC 6, GV4	Moxibustion was performed at a distance of 3-4 cm from the patient's acupoints to make the patient feel moderate heat, 20–30 min, QD. Combined with dydrogesterone, PO, first dose is 40 mg followed by 10 mg, Q8H, until the symptoms disappear	Progesterone, 40 mg, IM, QD, 14 days	RCT	Successful pregnancy rate: 87.5%	The combinations can increase hCG and P, improve symptoms, and increase the successful pregnancy rate.	Not reported	[118, 119]

RCT: randomized control trial; CHM: Chinese herbal medicine; hCG: human chorionic gonadotropin; E2: estradiol; P: progesterone; QOD: quaque omni die; Q8H: quaque 8 hours; QD: quaque die; BID: bis in die; PO: per os; IM: intramuscular; IVF-ET: in vitro fertilization-embryo transfer. ∗Bushen Jianpi decoction composition: *Radix Codonopsis* 18 g, *Radix Astragali Preparata* 18 g, *Herba Taxilli* 15 g, *Semen Cuscutae* 15 g, *Cortex Eucommiae* 15 g, *Radix Dipsaci* 15 g, *Radix Paeoniae Alba* 15 g, *Rhizoma Atractylodis Macrocephalae* 12 g, *Radix Scutellariae* 12 g, *Cyperus rotundus* 12 g, *Radix Glycyrrhizae Preparata* 6 g.

reported that different styles of acupuncture treatment had benefits for threatened miscarriage, such as auricular point acupuncture, acupoint injection, acupoint sticking, and catgut embedding [11, 100]. Acupuncture might be a safe therapeutic approach for threatened miscarriage, but the current studies are of poor quality and are usually written in Chinese, and thus further research is required to explore whether acupuncture can reduce the incidence of miscarriage.

3.2. Different Styles of Acupuncture in the Treatment of Threatened Miscarriage

3.2.1. Acupoint Sticking. Acupoint sticking is a noninvasive therapy based on TCM theory in which pastes of various medicinal extract mixtures are placed on the skin at specific acupoints or at the diseased sites. This achieves effects not only through the activities of the drugs, but also by activating acupoints and meridians. Acupoint sticking is generally applied in common chronic diseases, particularly for pain relief [101]. The approach is acceptable for patients and is flexible and safe, and thus a large number of clinical practitioners use the intervention to treat women with threatened miscarriage in China. A meta-analysis investigated the efficacy and safety of different styles of acupuncture in threatened miscarriage and demonstrated that acupoint sticking therapy is the best acupuncture approach for this common pregnancy complication [102]. Generally, acupoint sticking with pastes made from modified CHM formulas combined with oral administration of Chinese herbal decoctions and/or progesterone can reduce the miscarriage rate, and the effectiveness of such combinations has been shown to be better than conventional medicine alone [103–107] (Table 3). However, the efficacy and safety of these treatments are in urgent need of confirmation in future studies.

3.2.2. Auricular Acupuncture. Auricular acupuncture is a therapy based on the stimulation of specific points on the ear. Auricular acupoint therapy is applied for situations of pain, inflammatory diseases, functional disorders, and endocrine and metabolic disorders by regulating reticular formation and by regulating the sympathetic and parasympathetic nervous systems [108]. This intervention appears to have analogous effects on sedation as opioids and can reduce pain and anxiety [109]. Auricular acupuncture is usually applied as an adjuvant with conventional approaches for relieving pregnancy-related pain and complications. The acupoints on the ear might regulate pregnancy and nourish fetal developing by achieving chronic shallow acupressure. TCM practitioners reported that a combination of auricular acupuncture and dydrogesterone could promote hematoma absorption in women with threatened miscarriage in early pregnancy complicated with subchorionic hematoma, was more effective than dydrogesterone alone, and could regulate immune factors over a 3-week course of treatment [110] (Table 3).

3.2.3. Acupoint Injection. Acupoint injection, which is also called pharmacoacupuncture, aqua acupuncture, water acupuncture, or herbal acupuncture, is widely used in East Asia and is based on the same meridian theory of acupuncture. Acupoint injection therapy is the combination of acupuncture and medications and has been demonstrated to benefit patients with nonspecific chronic low back pain [111]. The injection of sterile solutions of Chinese herbal extracts or western medicines into acupoint locations leads to synergistic therapeutic effects of acupuncture, medicine, and meridian activation. The drugs are absorbed through the subcutaneous tissues and capillary vessels, while the needles give positive stimulation at local acupoints, which may promote local blood circulation, elevate metabolic ability, and ameliorate the pathology while promoting inflammation resolution [112]. Many studies have found that acupoint injection therapy has a significant effect on threatened miscarriage compared to western medicine alone in reducing early pregnancy loss. The most common therapy is injection of 1000 IU of hCG into the Zusanli acupoint (ST 36) every other day [102, 113–115] (Table 3). This method is safe and reliable, with little side effects, and it treats diseases through the combined action of drugs and acupoints. More well-designed studies are needed to determine the efficacy and safety of acupoint injection therapy for threatened miscarriage.

3.2.4. Acupoint Catgut Embedding. Acupoint catgut embedding refers to embedding absorbable catgut sutures into certain acupoints, and the continuous stimulation of acupoints is believed to cure diseases and strengthen the body. Catgut embedding at acupoints evolved from needle embedding at acupoints by replacing needles with catgut, and this not only has effects similar to standard acupuncture, but also has effects due to the prolonged stimulation time, which can reach 2 weeks or even longer. The approach showed a tendency for equal effects compared to other kinds of acupuncture for reducing abdominal obesity, but the approach had fewer reported adverse events [116]. Acupoint catgut embedding has also been indicated to prevent miscarriage in patients with threatened miscarriage. In a randomized control trial, the patients who had experienced in vitro fertilization-embryo transfer were treated with catgut embedding once every two weeks until 12 weeks of gestation. The early miscarriage rate was 16.7%, and the pregnancy rate was 96.0%; these were significantly lower and higher, respectively, compared to those who received progesterone [117] (Table 3). It thus seems that acupoint catgut embedding is beneficial for threatened miscarriage and is worth further investigation.

3.2.5. Moxibustion. Moxibustion consists in burning the leaves of Chinese mugwort (*Artemisia vulgaris*) close to the skin to induce heat at certain acupoints. The intention is to warm up and invigorate the flow of Qi while also eliminating various pathogenic influences on the body. This therapy is considered safe and has no side effects, making it a viable

solution for pregnant women looking for a remedy for pregnancy-related symptoms. Two randomized, controlled studies in China looked at the combination of daily moxibustion and dydrogesterone compared to progesterone intramuscular injection or dydrogesterone oral administration. The moxa was placed over the acupoints Zusanli (ST36), Neiguan (P6), and Mingmen (GV4), and the combination approach was superior to progesterone or dydrogesterone alone in decreasing the miscarriage rate in women with threatened miscarriage [118, 119] (Table 3). In contrast, it has been reported that moxibustion can stimulate estrogen and prostaglandin production and increase fetal activity and uterine contractions in order to reduce noncephalic presentations at birth [120]. Thus, the safety and efficacy of moxibustion in the treatment of threatened miscarriage are still a matter of debate.

4. Nutritional Supplements in Threatened Miscarriage

Nutritional supplements, also known as dietary supplements, are used as an auxiliary way of supplying amino acids, trace elements, vitamins, and minerals for maintaining maternal health and fetal development. Dietary habits constitute important risk factors for potentially harmful nutritional deficiencies in pregnant women, and inadequate diet and nutritional supplementation during pregnancy can damage placental function and increase the risk of miscarriage and other pregnancy complications [24, 121, 122]. Nutritional supplements include essential nutrients to maintain human health, and vitamins and trace elements can directly affect the growth and development of the fetus. Mineral elements and vitamins can maintain the activity of the internal environment and various bioactive substances, and they participate in the body's energy transfer and metabolic regulation. Therefore, it is of great significance to give nutritional supplements before and during pregnancy because adequate nutritional support for pregnant women is essential for maintaining their own health and for supporting fetal development, growth, and future outcomes.

Oxidative stress can also lead to increased risk of threatened miscarriage, spontaneous abortion, recurrent pregnancy loss, and preeclampsia [123, 124]. Antioxidant supplementation may be effective in controlling the production of ROS and combating damage caused by free radicals, and it continues to be explored as a potential strategy for overcoming pregnancy disorders associated with miscarriage [124]. Natural antioxidant compounds alone or in combination with other antioxidants or micronutrients during pregnancy may have the potential to prevent pregnancy loss and other complications [123]. Nutritional supplements and antioxidant intake are usually within the dietary reference intake range, which includes the effects of food and fortified foods, and only a few minor adverse events, such as gastrointestinal reactions, have been shown to occur [125]. Here, we review studies on the efficacy and safety of nutritional supplements for women with threatened miscarriages.

4.1. Essential Trace Elements. Essential trace elements are involved in various biochemical pathways, and they play a crucial role in maternal health and fetal growth and development during pregnancy [126, 127]. Alterations in the concentrations or homeostasis of these micronutrients during pregnancy appear to be closely linked to various disorders and adverse pregnancy outcomes like miscarriage, preterm delivery, stillbirth, intrauterine growth restriction, fetal malformations, and premature rupture of membranes. Trace elements have been shown to facilitate various vital biochemical reactions by acting as cofactors for many enzymes and by stabilizing the structures of enzymes and proteins, and they are significant for all levels of cellular functions [128, 129]. It may be necessary to supplement essential trace elements in women with threatened miscarriage [126, 130].

Zinc (Zn) is one of the most important essential trace elements in humans, and it participates in multiple biological functions including protein synthesis, cellular division, and nucleic acid metabolism [131]. Zn deficiency during pregnancy gives rise to the risk of pregnancy loss, preeclampsia, placental abruption, preterm birth, low birth weight, birth defects, circulatory disorders, immune response impairments, and psychological disorders [132–135]. Women with threatened miscarriage have been shown to have 35.7% lower Zn levels than healthy pregnant women [126, 136], and low serum Zn levels during the early weeks of pregnancy have been shown to lead to miscarriage and fetal congenital malformations by reducing cell proliferation and protein synthesis, which is associated with increased cellular oxidative damage and apoptosis [126, 137]. Also, it was shown that women with serum Zn levels below 10.5 μmol/l had a miscarriage rate of 23.5%, and adding supplements reduced the rate to 2% [138]. However, studies of the effects of Zn supplementation during pregnancy have shown inconsistent results, possibly in part because of the challenges in establishing the baseline Zn status in different populations [139]. Zn supplementation at 30 mg daily did not seem to confer any benefit on infants' mental development among poor women in Bangladesh [140]. Zn supplementation might be prudent for women with poor gastrointestinal function, but the evidence was limited for beneficial effects of general Zn supplementation during pregnancy, and such treatment should be considered with caution.

Copper (Cu) as a trace element plays an important role in the maturation of hematopoietic cells in normal pregnancy and embryogenesis and in fetal and postnatal growth, and low plasma Cu levels have been found in spontaneous, threatened, and missed miscarriages during the first trimester of pregnancy [141]. Women with threatened miscarriage had 47.0% lower Cu levels than women with healthy pregnancies, and approximately 30% failed to reach term due to Cu deficiency [136, 142, 143]. There is a direct and positive correlation between Zn and Cu levels in women with threatened miscarriage, and there is a significant negative correlation between Cu and the ratio of Cu to Zn in women with a history of spontaneous abortion. Both Zn and Cu have a positive role in pregnancy outcomes, and optimum levels of Zn and Cu might be able to reduce the

occurrence of spontaneous miscarriage [133, 136]. Women who are Cu deficient usually take supplements of 1 mg of Cu daily and 30 mg of Zn separately for 14 days in order to normalize decreased SOD function [140]. In contrast, some studies found maternal serum Cu levels to be increased in threatened miscarriage compared with healthy pregnancy due to the increase of ceruloplasmin as a result of elevated levels of estrogen [126, 144–146]. Thus, there is a debate as to whether women with threatened miscarriage should be treated with Cu supplements.

Iron (Fe) is a component of hemoglobin and myoglobin, and it supports maternal erythropoietic expansion and fetal growth and development during pregnancy. It is also involved in the transport, storage, and use of oxygen [147]. Fe deficiency is associated with increased oxidative stress, placental and fetal hypoxia, and reduced immunity during pregnancy. Previous studies found that serum Fe levels were lower in cases of threatened miscarriage, and the serum Fe level might be an important diagnostic and prognostic parameter [126, 148]. Pregnancy loss is associated with profound changes in maternal Fe metabolism [149], and maternal Fe deficiency, which is common in pregnant women [150], negatively impacts the mother's health and fetal development and increases the risk of prenatal and postnatal complications. Foods rich in Fe include red meat, shellfish, eggs, beans, and leafy green vegetables, and high Fe intake with higher Fe bioavailability is needed by pregnant women to prevent adverse pregnancy outcomes and to meet the needs of the fetus. Pregnant women should routinely receive Fe supplements tailored according to serum ferritin levels. The International Nutritional Anemia Consultative Group recommends oral ferrous iron supplementation at 60 mg/day during pregnancy to prevent Fe-deficiency anemia. Depending on the severity of anemia, international guidelines recommend elemental ferrous iron at 100–200 mg daily or 60 mg twice daily as the first-line treatment [151–153]. Prolonged-released ferrous sulfate (ferrous sulfate–polymeric complex) has the lowest incidence of adverse events of all the available supplements, and this has positive implications for compliance [121]. Moreover, the metabolism of essential trace elements is closely related. For example, Fe deficiency results in an increase in liver Cu levels [126], high intakes of supplemental Fe or the presence of any gastrointestinal disease can interfere with Zn absorption, and Zn and selenium supplementation need to be given along with Fe during pregnancy [154]. The available evidence suggests that Fe supplements are needed for pregnant women, but overtreatment should be avoided.

Magnesium (Mg) and manganese (Mn) are additional essential trace elements for metabolic regulation, and serum Mg and Mn levels have been reported to be lower in women with threatened miscarriage compared to those with a healthy pregnancy [126]. Mg deficiency is associated with hypertension, preeclampsia, placental dysfunction, and premature labor in pregnant women, but Mg levels have not been shown to be different between pathological pregnancies and healthy controls [155]. Furthermore, some studies have reported that Mn concentrations are increased in women with a history of miscarriage [156]. There is still limited evidence for the use of Mg and Mn supplements for the treatment of threatened miscarriage.

4.2. Vitamin Supplements. Vitamins are essential nutrients to maintain metabolism, physical growth, and development and to prevent disease. Nearly 30% of pregnant women suffer from a vitamin deficiency, and about 75% of these would show a deficit of at least one vitamin in the absence of prophylaxis [157]. Vitamin supplementation during pregnancy may prevent adverse pregnancy outcomes and reduce the risk of pregnancy loss [24], but there are still contradicting findings in the research on vitamin supplements and the risk of miscarriage. Insufficient vitamin intake is related to an increased risk of miscarriage, and the intake of multivitamins with Fe and folic acid may decrease the risk of stillbirth, but single vitamin supplements either before or during early pregnancy have not been shown to reduce the miscarriage rate [24, 158, 159].

Vitamin A is a crucial micronutrient for pregnant women and their fetuses, and vitamin A can reduce the risk of anemia, infection, and night blindness in pregnant women [157, 160, 161]. Vitamin A deficiency is prevalent in developing countries, and it impairs Fe status and reduces resistance to infections [157]. In a systematic Cochrane review, there was no positive effect on the total risk of fetal loss, on early or late miscarriage, or on stillbirth in women who received vitamin A with any other combination [24]. Vitamin A supplements enhance infant birth weight and growth in HIV-infected women [157]. The recommended upper limit for vitamin A supplements is 3000 IU/day, but when used in excess during the first trimester of pregnancy, such levels can have teratogenic effects on the first 60 days following conception [160]. However, there is no evidence of vitamin A supplements leading to congenital malformations or other adverse effects [24]. Further research is needed on the dose and duration of vitamin A supplements during pregnancy, especially its use for threatened miscarriage.

Vitamin B complex plays a crucial role in maternal health and fetal development. Vitamin B facilitates the metabolism of homocysteine, and vitamins B6 and B12 determine the homocysteine concentration in the blood, while disturbances in maternal and fetal homocysteine metabolism may result in miscarriage, and hyperhomocysteinemia is considered a risk factor for recurrent miscarriage [162, 163]. Vitamin B6 deficiency is associated with miscarriage, preeclampsia, gestational carbohydrate intolerance, hyperemesis gravidarum, and neurological diseases in infants [157]. Supplementation with vitamin B6 has been shown to reduce the chance of miscarriage by 50% and to prevent stress from affecting fetal growth [164, 165]. Vitamin B6 has also been indicated to improve insulin resistance and to reduce oxidative DNA damage and C-reactive protein, and it may act similarly on progesterone and reduce prolactin in order to prevent miscarriage [166, 167]. Vitamin B12 deficiency is associated with recurrent early pregnancy loss, and the miscarriage risk increases by 3.8-fold with every quartile of severity in vitamin B12 deficiency [168, 169]. Vitamin B12 supplementation has been shown to

lead to successful pregnancy in 80% of women with vitamin B12 deficiency [170]. Deficiency in the conversion of vitamin B3 into nicotinamide adenine dinucleotide causes congenital malformations and miscarriages in mouse models, and vitamin B3 supplementation has been shown to prevent miscarriages and birth defects [171, 172]. However, recent reviews of the literature indicate that there is insufficient evidence for the benefits and harms of routine vitamin B supplementation in humans for threatened miscarriage.

Folate, also known as vitamin B9 and folacin, is one of the B vitamins. Folate deficiency may lead to congenital malformations, anemia, spontaneous abortions, preeclampsia, intrauterine growth restriction, and placental abruption. Clinicians commonly recommend that patients start or continue to take prenatal vitamins with folic acid supplementation in order to prevent neural tube defects and preeclampsia. A daily supplemental dose of 400 μg/day of folic acid is recommended when planning pregnancy, starting from 2 months before to 3 months after conception [121]. Women at high risk for folate deficiency should receive supplemental folic acid at 4-5 mg/day [173–177]. Evidence demonstrates that low dietary folate is associated with high risk of miscarriage [178], but the risk of early or late miscarriage is not reduced with folic acid supplementation alone or with other combinations according to a meta-analysis, and there appears to be no difference between women receiving folic acid and those who do not in terms of congenital malformations and stillbirths [24].

Vitamin C is an essential nutrient involved in the repair of tissue and the enzymatic production of certain neurotransmitters, and it is vital for both maternal and fetal health. Foods high in vitamin C include citrus fruits, tomatoes, and broccoli, and a balanced diet that is high in Fe and vitamin C is beneficial for a healthy pregnancy. However, vitamin C levels tend to be low in women who have a miscarriage, and supplementation of vitamin C may reduce the risk of miscarriage [179]. Further, vitamin C supplements increase maternal progesterone levels, improve psychological disorders, and may be of benefit to prevent miscarriage and decrease the development of preeclampsia [180, 181]. Nevertheless, there is insufficient data to support the role of vitamin C supplementation alone or combined with vitamin E in reducing total fetal loss, the risk of early or late miscarriage, the risk of stillbirth, or the risk of congenital malformations or adverse events [24]. Supplementation with vitamin C may be beneficial for threatened miscarriage, but overdosing should be avoided [157].

Vitamin D mainly regulates the amount of calcium and phosphate needed to keep bones, teeth, and muscles functioning properly. Vitamin D requirements are increased during pregnancy in order to adapt to the heightened physiological demands in the mother, including driving the formation of the fetal skeleton and maintaining an environment that is tolerant of paternal and fetal tissues and their associated alloantigens [182, 183]. It is estimated that 20%–60% of pregnant women in the UK, 10%–40% in the USA, and 30%–50% in Australia, India, and Saudi Arabia have a vitamin D deficiency [184–188]. Vitamin D deficiency may increase the risk of threatened miscarriage, and it is also related to adverse outcomes during pregnancy. Several observational studies demonstrated that serum 25-hydroxyvitamin D (25(OH)D) levels in women with threatened miscarriage tend to be lower than those in women with normal pregnancy, suggesting that low serum levels of vitamin D can be considered a risk factor for threatened miscarriage [189–191]. Global reports suggest that 40%–98% of pregnant women have 25(OH)D levels below 50 nmol/L and 15%–84% have levels below 25 nmol/L [192, 193]. Importantly, vitamin D-binding protein (VDBP) levels are often low in maternal serum, and such deficiencies are associated with miscarriage and adverse pregnancy outcomes including preeclampsia, preterm birth, and fetal growth restriction [194]. VDBP is expressed at low levels in the placenta and decidua in spontaneous miscarriages, and it might serve as a potential biomarker for miscarriages and has implications in the pathophysiology of spontaneous miscarriage [195]. Supplementation with vitamin D seems to be beneficial for threatened miscarriage [157], and a randomized double-blinded study showed that supplementation with vitamin D3 (400 IU/day) led to a decreased incidence of miscarriage and serum IL-23 levels in women with unexplained recurrent spontaneous abortion [196]. Vitamin D supplementation can reduce inflammation and is useful as immunotherapy to prevent miscarriage by downregulating IL-2, IFN-gamma, and TNF-alpha gene transcription [197, 198]. The daily upper safe limit for vitamin D has been set at 4000 IU, and the recommended dose of vitamin D is 1000–2000 IU, which can be supplemented daily in the second and third trimesters without fear of toxicity or teratogenicity. However, no safety data are available for this dose during the first trimester [199].

Vitamin E is important for the proper functioning of many organs, and it is an antioxidant that helps to inhibit processes that damage cells. Low vitamin E levels may be linked to greater miscarriage risk [200, 201]. Vitamin E in combination with aspirin is effective in improving uterine artery blood flow in women with recurrent miscarriage and thus prevents pregnancy loss and other complications [158]. A Hungarian case-control study reported that vitamin E supplements at a dose 450 mg daily are frequently used for the prevention of threatened miscarriage and lead to a nearly one-third reduction in preterm births in pregnant women, although internationally this method is no longer recommended [202]. The use of vitamin E, although generally considered "healthy," may be harmful to the pregnancy outcome by disrupting the physiological oxidative gestational state and is consequently not recommended to prevent preeclampsia [157]. In a placebo-controlled, double-blind trial, women diagnosed with chronic hypertension or who had a prior history of preeclampsia who received daily doses of vitamin E (400 IU) and vitamin C (1000 mg) had an increased risk of premature rupture of membranes [203], while another study did not confirm the teratogenic effects of the relatively high-dose vitamin E intake in pregnant women [204]. Further studies are needed on the effect and safety of vitamin E for threatened miscarriage and during early pregnancy.

Multivitamin supplements are widely used by pregnant women. Multivitamins together with iron and folic acid are commonly recommended to improve birth outcomes and reduce the risk of miscarriage, and Cochrane reviews have shown that a multivitamin plus iron and folic acid can decrease the risk of stillbirth but with no significant effect on the overall risk of fetal loss or miscarriage compared with placebo, folic acid, or vitamin A alone [24, 122]. In all other analyses of the effect of multivitamin supplementation on total fetal loss or early or late miscarriage, there were no differences between groups, including multivitamins versus control, multivitamins with vitamin E versus multivitamins without vitamin E or control, and multivitamins with Fe and folic acid versus Fe and folic acid alone. Only a few trials found that multivitamins with or without vitamin A can reduce total fetal loss; however, these findings should be interpreted with caution due to small sample sizes [24]. In addition, studies have suggested that the use of multivitamin supplements within the range of the dietary reference intakes does not result in excess intake and does not increase mortality, and only minor adverse effects such as gastrointestinal symptoms have been reported with multivitamin supplements [125, 205].

4.3. Other Antioxidants. Oxidative stress has been recognized as one of the main mediators of female infertility by causing various reproductive pathologies such as polycystic ovary syndrome (PCOS), preeclampsia, miscarriage, and unexplained infertility [123]. When oxidative stress develops too early in pregnancy, it can impair placental development and/or enhance syncytiotrophoblastic degeneration, culminating in pregnancy loss [206]. Nowadays, concerned women prefer dietary supplements with antioxidant properties over synthetic drugs as a natural way to lessen the oxidative stress and enhance their fertility and to prevent early pregnancy loss, with the idea of replacing depleted antioxidant stores to combat an overwhelmingly oxidative environment. However, a meta-analysis of relevant studies found no supporting evidence for any beneficial effects of antioxidant supplementation [207]. Thus, caution must be still used in the application of antioxidants during pregnancy. The most commonly used antioxidants for pregnant women are vitamins, alpha-lipoic acid (ALA), and N-acetylcysteine (NAC), and future randomized controlled clinical trials in humans based on current animal or in vitro studies are necessary to elucidate the precise mechanisms through which oxidative stress affects female reproduction.

ALA is a natural antioxidant synthesized by plants and animals [208], and it has been shown to prevent miscarriage by reducing the levels of proinflammatory cytokines, such as TNF-alpha, IL-1 beta, IL-6, IL-8, IL-17, and INF-gamma, and by inducing anti-inflammatory IL-10 release. In addition, ALA can induce vascular endothelial growth factor to stimulate tissue epithelialization, collagen deposition, and smooth muscle actin fibrogenesis in order to resolve subchorionic hematoma in threatened miscarriage [209–212]. ALA is able to reduce ROS levels and increase the total antioxidant capacity in cultured preantral mouse follicles [213]. In randomized controlled trials, ALA supplementation alone at a dose of 10 mg or 600 mg or combined with vaginal progesterone could prevent pregnancy loss; reduce the symptoms of vaginal bleeding, abdominal pain, and uterine contractions; and promote chorionic hematoma reabsorption in pregnant women with threatened miscarriage compared to pregnant women without intervention or pregnant women treated with progesterone alone [214, 215]. However, there was no statistical difference in the improvement of symptoms of threatened miscarriage, and the sample size was small. In order to further confirm the role of ALA in improving threatened miscarriage in pregnant women and to verify its safety, well-designed clinical studies with larger sample sizes should be conducted [208, 209, 216].

NAC is a commonly used mucolytic drug, and it not only increases cellular antioxidant levels, but also improves insulin receptor activity in human erythrocytes and regulates the secretion of insulin in response to glucose uptake [217]. The supplement can prevent endothelial damage and biological effects caused by oxidants in non-insulin-dependent adult diabetics by preventing ischemia, inhibiting phospholipid metabolism, and promoting inflammatory cytokine release and protease activity [218]. NAC might have potential benefit for threatened miscarriage, and some studies have shown that NAC significantly improves the pregnancy rate and ovulation rate compared with placebo [218, 219]. However, some studies have shown that there is no significant difference in miscarriage rate between NAC supplementation and placebo in PCOS patients [218]. In addition, in a randomized controlled trial of PCOS patients with clomiphene citrate resistance, the live birth rate was higher after treatment with a combination of metformin and clomiphene citrate compared to treatment with NAC [220]. NAC was suggested to reduce fetal loss in a PCOS rat model; however, it was shown that supplementation with NAC could induce miscarriage in control pregnant rats [221]. Considering the poor quality of existing studies and the lack of studies assessing miscarriage rates, well-designed randomized controlled trials for threatened miscarriage are needed.

Omega-3 is a fatty acid that may have potential for preventing miscarriage. In a prospective study, omega-3 combined with aspirin improved the uterine artery blood flow velocity in women with recurrent miscarriage [222]. However, the literature on the relationship between human serum omega-3 concentration and reproduction is limited specifically to infertile people, and whether it can prevent miscarriage is still controversial [223, 224]. Therefore, more research is needed to clarify the role of omega-3 and how it can be used more effectively.

At present, there are few studies focusing on the role of nutritional supplements as treatments for threatened miscarriage. Overall, current studies indicate that deficiencies in several trace elements (Zn, Cu, Fe, Mg, and Mn) and vitamins (A, B, C, D, E, and folate) are associated with high risk of pregnancy loss. In particular, low levels of Zn, Cu, Fe, and vitamin D may result in threatened miscarriage. Thus, proper supplementation with Zn, Cu, Fe, vitamin D, vitamin E, and ALA may have efficacy as treatments for threatened

Table 4: The efficiency and safety of various CAM approaches in the treatment of threatened miscarriage.

CAM therapies	Efficiency	Safety and risk
CHM	CHM may improve endometrial function, inhibit uterine contraction, regulate immune response, and promote the development of the embryo. It is considered beneficial for preventing and treating miscarriage with fewer adverse events compared to current conventional western medicine by being used alone or in combination with western medicines.	Most clinical trials have reported no adverse events or significant risks, but systematic reviews have concluded that no data are available to demonstrate the safety of CHM for the mother or the infant with current publications. In addition, animal studies indicate that the frequently used CHMs have reproductive toxicity for threatened miscarriage, including fetal resorption, growth restriction, and congenital malformations.
Acupuncture and moxibustion	Acupuncture, acupoint sticking, auricular acupoint, acupoint injection, acupoint catgut embedding, and moxibustion are recognized as nonpharmacological approaches, which can maintain pregnancy, and enhance the effects with the CHM or western medicines. Most studies show that acupuncture is safe and effective without side effects.	The safety of acupuncture in pregnancy is reasonably well accepted, but there is still a lack of high-quality scientific research to validate the safety and effectiveness of acupuncture and moxibustion for threatened miscarriage. There remains a debate regarding the needling points that are historically considered to be forbidden during pregnancy.
Nutritional supplements	Nutritional supplements are used as an auxiliary way of supplying amino acids, trace elements, vitamins, and minerals for maintaining maternal health and fetal development. Proper supplementation with Zn, Cu, Fe, vitamin D, vitamin E, and ALA may have efficacy as treatments for threatened miscarriage and to prevent further complications and is convenient to supplement.	Most of studies are of low quality and controversial because of their small sample sizes, poor and inconsistent reporting of methods, excessive confounding factors, and lack of reporting of clinically relevant outcomes such as live birth and adverse events.
Psychological interventions	Psychological interventions may help women to regulate their emotions and promote psychosocial well-being during the current pregnancy or after a miscarriage, and this can be beneficial for the offspring.	There is little evidence to suggest that psychological interventions are beneficial in improving psychological morbidity to prevent threatened miscarriage.

CAM: complementary and alternative medicine; CHM: Chinese herbal medicine; Zn: zinc; Cu: copper; Fe: iron; ALA: alpha-lipoic acid.

miscarriage and in preventing further complications. Although many studies have sought to clarify the positive effects of nutritional supplements such as trace elements, vitamins, and antioxidants on threatened miscarriage, most of them are of low quality and controversial because of their small sample sizes, poor and inconsistent reporting of methods, excessive confounding factors, and lack of reporting of clinically relevant outcomes such as live birth and adverse events. Therefore, it is necessary to carefully design more high-quality studies to clarify the specific effects of nutritional supplements on threatened miscarriage, as well as their safety and tolerance, so as to better provide care for pregnant women. Moreover, there is currently insufficient evidence to determine the effects of different combinations of nutritional supplements on threatened miscarriage.

5. Psychological Interventions and Other CAM Therapies in Threatened Miscarriage

Of all clinically recognized pregnancies, a quarter to a third experience a threatened miscarriage. Miscarriage is often a physically and psychologically traumatic event for these women and their families, and women may suffer psychological morbidities such as stress, anxiety, depression, and grief as a reaction to miscarriage [225]. The psychological distress can last between one and three years after a miscarriage and may affect the woman's quality of life and subsequent attempts to become pregnant [226]. Additionally, psychological distress can impact the fetus and result in preterm birth or low birth weight, and it can result in long-term consequences for the child such as deficits in cognitive functioning and an increase in negative behavior [227, 228]. Despite the potential of follow-up care or emotional support for negative outcomes, more than half of women do not receive psychological interventions at the time of miscarriage. [229], thus there is a need to find ways to mitigate psychological distress in order to prevent miscarriage.

CAM approaches may help women to regulate their emotions during the current pregnancy or after a miscarriage, which can be beneficial for the offspring, and it is reported that approximately 40% of women with a history of miscarriage have used a CAM approach [8]. Psychological interventions or supportive care can help women who have had a miscarriage to overcome their grief and can promote psychosocial well-being, and in a cross-sectional survey distributed to pregnant women residing in the USA, the most frequently reported complementary approaches used by pregnant women with a history of miscarriage were prayer (22.3%), yoga (15%), massage (14.5%), chiropractic treatment (13%), and meditation (11.4%) [8, 230]. Similarly, massage, yoga, and relaxation are the most commonly reported complementary approaches used by pregnant women in Australia [231], while homeopathy, acupuncture, and phytotherapy are the most frequently used during pregnancy in Germany [232]. However, there is little evidence to suggest that CAM therapies are beneficial in improving psychological morbidity to prevent threatened miscarriage, although complementary approaches may upregulate dopamine levels and decrease stress signaling hormones that in

turn control mood [233, 234]. Future research in this area is needed to establish the effectiveness of complementary approaches in pregnant women and to provide evidence for strategies that healthcare providers can use when treating their patients.

6. Summary

We have reviewed the primary literature focusing on the research into the application of CAM in women with threatened miscarriage, with an emphasis on the efficacy and safety of the treatments. In general, CHM, acupuncture and moxibustion, nutritional supplements, and psychological interventions are the most commonly used to prevent pregnancy loss or complications, and they have proven beneficial for the treatment of threatened miscarriage. We briefly summarize the efficacy and safety of various CAM approaches in the treatment of threatened miscarriage (Table 4). Commonly, the CAM therapies are combined with luteal support medications such as progesterone and/or hCG for threatened miscarriage. It is suggested that pregnancy loss had a deficiency of progesterone and hCG, and low levels of maternal serum progesterone or hCG in early pregnancy may be adverse prognostic factors for threatened miscarriage [12, 13, 235]. Hence, supplementation with progesterone or hCG may prevent miscarriage and maintain pregnancy; however, the evidence remains conflicting. Although the meta-analysis indicated that progesterone may reduce the rate of spontaneous miscarriage but has little effect in the preterm birth, and hCG may have no significant effect in women with threatened miscarriage, the evidence is insufficient and of poor quality [12, 13, 236]. Currently, luteal support medications are still main management for threatened miscarriage in clinic. Moreover, majority of data show that the combination of CAM and luteal support medications may increase maternal circulation progesterone and hCG levels, which seems to be more beneficial than that medication alone for maintaining pregnancy and promoting psychosocial well-being in women with threatened miscarriage. However, whether the use of CAMs is main or adjuvant therapy is still unsure. Therefore, not only CAM approaches but also luteal support drugs need further research with good-quality evidence to determine the effects in threatened miscarriage.

However, issues of safety and risks with the use of CAM during early pregnancy require further studies. At present, there is little evidence to indicate whether CHM, acupuncture, and supplements are harmful for fetal development or whether they induce pregnancy loss, premature delivery, or stillbirth. It would be beneficial to conduct large-scale, randomized clinical trials in the future to determine the efficacy and safety of CAM, to help substantiate its therapeutic effects, and to identify possible adverse events. As the Chinese idiom goes "take the essence and discard the dregs," we need further investigations to develop effective and safe CAM therapy regimes during pregnancy to support both the mother and her baby.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Min Hu contributed to the original draft and takes responsibility for the integrity of the final manuscript. Lingjing Lu, Yu Zhou, and Min Hu collected the references and drafted the manuscript. Juan Li and Hongxia Ma revised the manuscript. All authors have read and approved the final version of the manuscript.

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Review Article

The Effects of Traditional Chinese Medicine-Associated Complementary and Alternative Medicine on Women with Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is a touchy clinical and public health problem worldwide, which adversely affects women's health and health-related comorbidities for lifetime, and represents a tremendous burden for both the family of the patient and for society. According to the diagnostic criteria used and the population studied, the prevalence rate of PCOS is between 6% and 21%. However, current conventional modern medicines for PCOS are only moderately effective at controlling the signs and symptoms, while they are not thoroughly able to prevent complications. Therefore, many patients have turned to complementary and alternative medical (CAM) treatments. CAM use is wide spread among patients with PCOS, and more than 70% of patients use CAM at one point during their diseases. The patients' primary motivations include dissatisfaction with available medications, perceive higher risk of drug side effects and crushing health burden and economic costs, desire for symptom relief, pursuit of shortening the course of disease, and the belief that CAM therapy is in accordance with the patients' values and beliefs. At present, several CAM methods have been used in women with PCOS, which has achieved obvious effects. However, biologically plausible mechanisms of the action of traditional Chinese medicine- (TCM-) associated CAM for PCOS have not been systematically reviewed. This review briefly summarizes the current progress of the impact of herbal medicine on the outcomes of PCOS and introduces the mechanisms.

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common and complex endocrinopathy occurring in reproductive aged women. According to the diagnostic criteria used and the population studied, the prevalence rate of PCOS is between 6% and 21% [1]. PCOS is characterized by chronic anovulation, hyperandrogenism (HA), and metabolism disorder leading to symptoms of menstrual dysfunction, subfertility, hirsutism, acne, and obesity [2]. Women with PCOS tend to have other abnormalities, including impaired glucose tolerance, type 2 diabetes mellitus, dyslipidemia, increased prevalence of hypertension, and possibly increased risk of metabolic syndrome and cardiovascular morbidity [3]. The aetiology of PCOS remains poorly understood, but it is believed to result from a complex relationship with metabolic, endocrine, genetic, behavioral, and environmental factors. Different possible theories were reported in various studies, which included detectable insulin resistance (IR), HA, and neuroendocrine gonadotropin secretion disorders, or a combination of these factors [4]. PCOS as a whole is a touchy clinical and public health problem worldwide, which adversely affects women's health and health-related comorbidities lifetime, and represents a

tremendous burden for both the family of the patient and for society. The annual cost to the National Health Service modelled the population dynamics of PCOS, healthcare costs, and quality of life for PCOS patients in the UK from 2014 to 2039 and has been estimated to be more than £237 million [5].

About 70% of people around the worldwide are interested in the prevention and treatment of diseases by complementary and alternative medicine (CAM) although there is insufficient scientific assessment evidence to prove its safety and effectiveness [6]. The National Center for Complementary and Integrative Health (NCCIH), formerly known as the National Center for Complementary and Alternative Medicine (NCCAM), defines CAM as a group of diverse medical and health-care systems, practices, and products that are not presently considered to be part of conventional modern medicine. NCCIH classifies CAM into three more types: (1) natural products (consist of herbs, vitamins, minerals, probiotics, and dietary supplements); (2) mind and body practices (include a wide variety of procedures and techniques such as yoga, acupuncture, Tai chi or qi gong, massage therapy, meditation, spinal manipulation, hypnotherapy, and relaxation techniques), and (3) other complementary health approaches [7] (other complementary health approaches cover those treatments such as traditional Chinese medicine (TCM), naturopathy, and homeopathy as well as functional medicine).

Patients with PCOS rank among the higher users of CAM. Why so many people choose CAM? Their primary motivations include dissatisfaction with available medications, perceive a higher risk of drug side effects and crushing health burden and economic costs, desire for symptom relief, pursuit of shortening the course of disease, and the belief that CAM therapy is in accordance with the patients' values and beliefs. Sometimes, the patients who resort to CAM usually use it as a "alternative" therapy, but there are moments the CAM are used to incorporate with conventional modern medicine on behalf of treating and controlling the primary disease or complications as much as possible to achieve better efficacy. Given the wide application of CAM in women with PCOS, particularly the remarkable efficacy of CAM therapy in clinical randomized controlled trials of PCOS in recent years. The purpose of this narrative review is to provide a broad overview of the types of TCM-associated CAM therapies most commonly be used for PCOS and to enable patients and practitioners who are either interested in pursuing or already employing CAM to know more about the relevant research progress and mechanism.

2. The Utilization of Herbal Medicine in Women with PCOS

Herbal medicine (HM) has been used in eastern Asian countries for thousands of years, and it is one of the key components of CAM. One particular form of HM, herbal formula, involves the use of some herbs in a formula to improve a set of problems or a syndrome. Herbal formula and single herb were reportedly used by over 70% of women with PCOS and are a commonly encountered condition among traditional Chinese doctors [8, 9]. Many studies showed that both herbal formula and single herb are helpful for fertility, menstrual health, and other aspects of PCOS. In view of the use of HM in PCOS are becoming popular in research and clinical practice, we summarized the outcomes of clinical and the mechanisms as follows.

2.1. The Utilization of Herbal Formula in Women with PCOS

2.1.1. Infertility and Ovulatory Dysfunction. As the treatment of infertility has attracted much attention from PCOS patients, numerous clinical studies of herbal formula have been designed, especially, quite prevalent in China. Herbal formula had positive adjunct effects with clomiphene citrate (CC) in the management of ovulation induction for PCOS-related subfertility [10–17]. The results of Chen et al. show that the prevalence of dominant follicle, ovulation rate, and clinical pregnancy rate in the combination group was significantly superior than those in the control group ($P < 0.05$) [18]. Consistent with these data, Zhao et al. also found the same discovery in 120 subjects undergoing inducing ovulation in women with PCOS; there were significantly a higher pregnancy rate (29.0% vs 15.6%) and lower early abortion rate (12.9% vs 23.5%) in the cotreatment group than that in the CC group [19], since CC is a selective estrogen modulator known to negatively impact endometrial development. Previous studies have shown that cotreatment with herbal formula significantly increased the endometrial thickness and pregnant rate for infertility treatment of PCOS [13, 20, 21]. Furthermore, adjuvant treatment of herbal formula also plays a positive role in improving the morphology and reserve capacity of the ovary, particularly ovarian volume and basal antral follicle count (AFC) [22–26]. Ru et al. applied to CC with herbal formula or without herbal formula in 180 PCOS patients and found that endometrial thickness, ovulation function, and clinical pregnancy rate were remarkable improved ($P < 0.05$) [12]. Moreover, Danhuang Quyu capsule, Tiangui capsule, and Dingkun pill all have outstanding advantages in improving pregnancy rate and enhancing the sensitivity to ovulation-inducing drugs [13, 27]. Wei et al. carried out a prospective clinical study and reported higher single follicle rate, mature follicle rate, and clinical pregnancy rate among those subjects who received Dingkun pill and CC compared with subjects in the control group (58.0% vs. 38.0%; 98.0% vs. 78.0%; 42.0% vs. 18.0%; $P < 0.05$) [13]. Recently, letrozole has been considered as an alternative to CC and has been used in ovulating and nonovulating infertile women with PCOS, especially for patients who are against CC. With regard to cycle ovulation rate and pregnancy rate, as well as the total effective rate of intervention, the results of the present studies reveal that herbal formula conjunction with letrozole is more effective than letrozole monotherapy in the treatment of PCOS [28, 29]. In a randomized controlled trial (RCT), Yuan et al. evaluated the effectiveness and efficacy of letrozole combined with HM prescription in CC-resistant infertile women with PCOS. The data exerted a significantly

higher ovulation rate, clinical pregnancy rate, endometrial thickness, and a dramatically regulated hormonal status over 6 treatment cycles in the combination group [14]. In addition, others argue that Chinese herbal formula (CHF) appears to be at least as effective as CC in ovulation induction with some potential advantages over CC [30–32]. Li in a 3-menstrual cycle human study found that CHF improved pregnancy outcome compared with CC among 120 infertile PCOS patients [30]. In agreement with Li's findings, Zhou et al. also demonstrated the benefits of CHF on ovulation rate and pregnancy rate, ovarian volume, and endometrial thickness [31].

CAM treatments are commonly used as adjunctive therapy for women when they undergo in vitro fertilization (IVF) treatment, and herbal formula is widely chosen. There are positive effects on reproductive outcomes for those PCOS patients undergoing ovulation when adjuvant treatment with CHF at different time points of IVF treatment [33, 34]. Zhang et al. randomized 98 cases of PCOS patients who would undergo in vitro fertilization and embryo transfer (IVF-ET) to the CHF group and control group; CHF group received 3 months treatment with herbal formula before IVF-ET; control group did not receive any treatment before IVF-ET; and the results presented better higher quality embryo rate, implantation rate, and pregnancy rate in the CHF group than in the control group [33]. Meanwhile, using Cangfu Congxian decoction, before 2 months of IVF-ET, could significantly increase oocyte retrieval number, high-quality embryos, and 2 PN fertilization rate [34]. Adjuvant drug of CHF could improve patients' sensitivity to medicine of superovulation when the patient received controlled ovarian hyperstimulation [35–37]. The results of Lian confirmed that, in the CHF group, patients took herbal formula until human chorionic gonadotropin (HCG) injection day and achieved a higher increment in the oocytes number and clinical pregnancy rate compared with the placebo group undergoing IVF-ET [37].

Although there are hundreds of positive results referring to herbal formulas for infertility treatment in women with PCOS, there are still negative results. Zhu et al. discovered the negative results in 54 subjects undergoing IVF-ET; they found that treatment with herbal medicine compound could not dramatically increase the numbers of fertilized oocytes, high-quality embryos, and the pregnant chance ($P > 0.05$) [38]. However, some other studies have demonstrated that although herbal formula could not change ovulation rate and pregnancy rate, herbal formula could improve the level of estradiol (E_2), luteinizing hormone (LH), and testosterone (T) and increase the blood flow of ovary [39, 40]. The possible reasons for these different findings might be concerned with differences in the study design and compound prescription composition.

2.1.2. Hormonal Status and Menstruation Cycle. With the continuous exploration of the treatment of PCOS, herbal formula has been a potential treatment for irregular menstruation and hormonal imbalance in PCOS. A pilot study conducted recently in UK found herbal formula could statistically improve menstrual rates; meanwhile, liver and kidney function and adverse events data were largely normal [17]. Additionally, as HM and Western medicine (WM) have different mechanisms of action, the combination of treatment was more effective in the regulation of menstrual cycle and hormonal status [15, 16, 24, 41]. Chen provided that Chinese medicine cycle therapy combined with metformin had beneficial effects for women with oligomenorrhea, amenorrhea, and sex hormone in PCOS [42]. Zhang conducted a randomized controlled prospective study; the results confirmed that the combination group achieved a significant reduction in T, LH, and E_2 levels and the scores for Chinese medical syndromes ($P < 0.05$) [43]. Li and Yuan applied to an adjuvant treatment with CHF or without CHF in PCOS patients and found that hormonal serum, anti-Mullerian hormone (AMH), and Chinese medical syndromes in the adjuvant treatment group were better than those in the control group [44]. However, because the composition of Chinese herbal formulas is different, the influence on the outcomes is not completely the same. The results of Wang Ping supported the fact that, despite no significant difference in serum of E_2, T, follicle stimulating hormone (FSH), and ovarian volume, CHF would be clinically useful, especially in patients with LH, LH/FSH, and basal AFC disturbances in PCOS [45].

Moreover, herbal formula could cause hormonal changes and modify HA with little sideeffects [46–49]. Liu et al. randomized 120 cases of PCOS-HA women to the CHF group and control group; after 3 months treatment, the results showed significant reduction in T, free androgen index (FAI), dihydrotestosterone (DHT), and dehydroepiandrosterone sulfate (DHEAS) levels and increased the level of sex hormone binding globulin (SHBG) and FSH in the CHF group than in the control group ($P < 0.01$); and CHF had better effects for modifying Rosenfield and hirsutism ($P < 0.01$), promoting recovery of menstrual and spontaneous ovulation ($P < 0.05$) [50].

Herbal formula can improve reproductive dysfunctions, but at the same time, it has beneficial effects on balancing hormone status and menstrual frequency of PCOS that is also very intriguing [15, 16, 41, 42, 51]. Susan et al. conducted a RCT of 122 patients; the results found that herbal formula adjuvant group recorded a reduction in oligomenorrhoea and other significant improvements such as pregnancy rates and quality of life compared with controls ($P < 0.01$) in these overweight patients with PCOS [52]. In one trial by Zhang et al., 291 women were identified as PCOS and underwent metformin, Diane-35, or Tiangui capsule. After 3 months intervention, women who received Tiangui capsule had a higher menstrual rate and ovulation rate and lower ovarian volume serum and T level than the metformin group; the reduction of HA was similar to who received Tiangui capsule or Diane-35. This result was similar to another result that was noted by Huang [53], who used CHF to treat PCOS women. Furthermore, the long-term effect of herbal formula was superior than that of Diane-35 after consecutive 6 months. The hirsute, acne score, and hormonal-related parameters of the herbal formula group were still significantly lower than those of the control group at the end of 6

months and 3 months of withdrawal ($P < 0.05$), while those in the control group increased.

2.1.3. Metabolic Dysfunction and Emotional Disturbance. Some studies showed herbal formula displayed the similar or superior effect on ameliorating glucolipid metabolism alterations compared with WM in women with PCOS [54–56]. A randomized, double-blind, placebo-controlled trial focused on the impact of Kuntai capsule in subjects with PCOS found out a significant decrease in total cholesterol (TC), triglyceride (TG), and low-density lipoprotein level, as well as insulin and homeostasis model assessment of insulin resistance (HOMA-IR) in the CHF group than in the control group, while insulin sensitivity index (ISI) and high-density lipoprotein cholesterol (HDL-C) increased markedly. The results implied that Kuntai capsule resembled an insulin sensitizing agent in therapeutic effect [55]. The study of Wei Feng also showed that CHF was similar to metformin in improving IR [57]. The herbal formula for Bushen Quyu Huatan has marvelous effect on controlling metabolic parameters of PCOS [58, 59]. Cangfu Daotan decoction a classic prescription commonly used for the treatment of PCOS, which is effective in regulating insulin, glucose, and lipid metabolism, has no obvious adverse effects [43, 60–62]. Many studies have reported that herbal formula intensified the metabolic effects of conventional drugs and maybe a newer therapeutic option for the same purpose [26, 63–65]. During Li's study, 126 participants were advised to undergo conventional intervention, or adding HMs, there was significant improving in glycated hemoglobin (HbA1c), fasting insulin (FINS), HOMA-IR, TC, and TG parameters between the combination group and the conventional treatment group [44].

The effects of different herbal formulas on regulating abnormal metabolism are different. Some clinical trials reported controversial findings of diverse herbal formulas treatment about waist hip rate (WHR), body weight (BW) and body mass index (BMI), lipid profiles, and glucose homeostasis parameters of PCOS patients [62–66]. And some studies showed that herbal formulas in PCOS patients led to WHR reduction and BW loss [27, 66]. Administration of Tiangui capsule, Guizhi Fuling capsule, and Wenjing Shexue recipe led to a substantial decrease in BW, BMI, and other anthropometric indices [27, 39, 53], whereas the findings of Lian were not exactly the same to the above, and Lian found sequential CHF could intensify amelioration in insulin (INS) and ISI level but no changes were observed in fasting blood glucose (FBG) and WHR and BMI outcomes in IR women with PCOS [67, 68]. Fewer adverse events were found in adjuvant treatments of CHF than that in the conventional treatments [24, 43, 60, 61]. The similar discovery was found by Li, they asserted that cotherapy with Bailing capsule could conspicuously regulate metabolic parameters compared with Diane-35 alone, and the cotherapy could protect renal function with no negative effect on liver function [69]. Adjuvant therapy with herbal formula may not only help to ameliorate the glucose and lipid metabolism disorder but also to indirectly improve fertility and hormonal condition in women with PCOS [56, 70–73]. Teng suggested the optional use of herbal formula as an adjuvant therapy for infertility to modify lipid disturbances and then remarkably increase the embryo implantation rate in 227 dyslipidemia women with PCOS undergoing IVF [74].

Furthermore, PCOS is a common endocrine disorder with psychological and emotional disturbance throughout the life course of affected women. Herbal formulas appear to improve symptoms of anxiety, depression, and quality of life in PCOS patients and may enhance the overall effects [15, 75, 76]. Fu conducted a randomized controlled prospective study, which also confirmed this point; the results confirmed that the herbal formula decreased the anxiety and depression scale scores (self-rating anxiety scale and self-rating depression scale) and improved the hormonal status causing considerable alterations in ovulation and pregnancy rate [77]. We have listed some clinical trials in Table 1.

2.1.4. The Mechanism of Herbal Formula Action in Women with PCOS. Regarding the potential mechanisms of herbal formula effect on PCOS, the following points deserve careful consideration. The first point is that herbal formula can be effective to improve IR and glucolipid disorders. Herbal formula improving IR is related to interventions on all fronts of the insulin signaling pathway mainly including insulin receptor, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt), glucose transporter, glycogen synthase kinase 3, mitogen-activated protein kinase (MAPK), and AMP-activated protein kinase (AMPK) as revealed by both in vitro and in vivo studies [37, 43, 44, 78–83]. Considerable herbal formulas, for instance, Cangfu Daotan decoction, Liuwei Dihuang pill, and Heqi san, result in promoted insulin sensitivity through modulation of diverse physiological and cell signaling pathways [78, 80, 82]. The effects of herbal formula are not only confined to boosting insulin sensitivity but also can be beneficial for alleviating lipid abnormality. The previous studies suggested that CHF could significantly change in glycemic index, insulin sensitivity, TG, and HDL-C, exerting protective effects against metabolic disorder [55, 64, 84, 85]. Visfatin (VF), leptin (LEP), and adiponectin (APN) are closely related to obesity [86, 87]. The imbalance of adipose cytokines such as LEP and APN secreted by adipose tissue in obese patients will also aggravate the IR of PCOS. Some experts have found that herbal formulas also can decrease serum levels of VF and leptin LEP while increase APN level, improving glucolipid metabolism and wellbeing [71, 88].

The second point is that herbal formula can enhance the endometrial receptivity (ER), which improves fertility. The major target organ of estrogen and progesterone (P) is endometrium. The estrogen and P supplied by the ovary can facilitate the proliferation and differentiation of endometrial cells, strengthening the ability of endometrium to accept embryos. Studies shown CHF can increase the levels of estrogen and P and the expressions of their receptors [13, 21, 89]. Endometrial thickness and endometrial morphology are also important parameters affecting the success

Table 1: Summary of randomized studies of the effect of herbal formula on PCOS outcomes.

Authors (year)	Sample size	Composition	Interventions	Duration of study	Results	AEs	References
Mo et al., 2019	148	Bushen Quyu Huatan decoction	Treatment arm: CHF + clomiphene citrate Control arm: clomiphene citrate capsules	3 cycles	Cure (18) Markedly improved (30) Moderately improved (9) Ineffective (17) Overall efficacy: 77.02% Pregnancy rate level increased; PSV and PI levels of ovarian artery increased; RI level of ovarian artery decreased; LH, LH/FSH, and T levels decreased	Nausea (T: 3, C: 1); abdominal distension (T: 3, C: 2); stomach ache (T: 2, C: 3); blurred vision (T: 1, C: 1); no SAE	[10]
Luo et al., 2018	100	Yangyin Shugan capsule	Treatment arm: CHF + clomiphene citrate Control arm: clomiphene citrate	3 menstrual cycles	Cure (12) Markedly improved (20) Moderately improved (6) Ineffective (12) Overall efficacy: 76.00% Pregnancy rate level increased; FSH and E_2 levels increased; LH level decreased; PI and RI levels increased	Nausea and vomiting (T: 2, C: 0); diarrhea (T: 1, C: 0); abdominal distension (T: 0, C: 4); stomach ache (T: 0, C: 2); blurred vision (C: 1); no SAE	[11]
Ru et al., 2013	180	Kidney-supplementing blood-quickening liver-clearing formula	Treatment arm: CHF + clomiphene Control arm: clomiphene	3 menstrual cycles	Cure (61) Moderately improved (22) Ineffective (7) Overall efficacy: 92.2% Ovulation rate and pregnancy rate levels increased; endometrial thickness level increased; LH and T levels decreased; FSH level increased	NS	[12]

Table 1: Continued.

Authors (year)	Sample size	Composition	Interventions	Duration of study	Results	AEs	References
Chen et al., 2017	150	Zuogui soothing liver decoction	Treatment arm: CHF + clomiphene Control arm: clomiphene	6 months	Cure (44) Markedly improved (3) Moderately improved (21) Ineffective (7) Overall efficacy: 90.67% Ovulation rate, pregnancy rate, and dominant follicular rate levels increased; LH, FSH, and T levels decreased	Intrauterine membrane thinning (T: 6, C: 8); OHSS (T: 2, C: 3); no SAE	[18]
Ding et al., 2014	355	Cangfu Daotan decoction	Treatment I arm: clomiphene + HMG + HCG Treatment II arm: CHF + clomiphene + HMG + HCG Control arm: no intervention	3 cycles	Pregnancy rate level increased; endometrial thickness level increased; PI and RI levels decreased; HOMA-IR level decreased; UCP2 level increased (treatment II)	NS	[21]
Wang et al., 2016	110	Bailing Tiaogan decoction	Treatment arm: CHF + ethinylestradiol cyproterone acetate + clomiphene Control arm: ethinylestradiol cyprogesterone + clomiphene	4 menstrual cycles	Pregnancy rate and endometrial thickness levels increased; ovarian volume level decreased; TCM symptom scores level decreased; FFA and CRP levels decreased; β-EP level increased	NS	[22]
Zhang et al., 2014	291	Tiangui capsule	A arm: Tiangui capsule B arm: metformin C arm: Diane-35	3 months	Markedly improved (47) Moderately improved (35) Ineffective (28) Overall efficacy: 74.55% HOMA-IR level decreased; weight level decreased; TC level decreased; FSH and T levels decreased; ovary volume level decreased	Mild in B arm: numbness, nausea, diarrhea; mild in C arm: the lower limbs are swollen and the weight gain is obvious; no SAE	[27]

Table 1: Continued.

Authors (year)	Sample size	Composition	Interventions	Duration of study	Results	AEs	References
Li et al., 2017	120	Bushen Huoxue Cupailuan decoction	Treatment arm: CHF Control arm: clomiphene citrate	3–6 menstrual cycles	Pregnancy rate level increased; the number of follicles, endometrial thickness, and ovarian volume levels increased; INS level decreased; FSH and E_2 levels increased; LH, PRL, and T levels decreased Cure (12) Markedly improved (16) Moderately improved (28) Ineffective (18) Overall efficacy: 75.68%	NS	[30]
Yu et al., 2019	175	Yushi Qinggan recipe	Treatment arm: CHF Control arm: Diane-35	3 months	Cycle ovulation rate and pregnancy rate levels increased; 0.5hINS, 1hINS, 2hINS, and IAUC levels decreased; LH, LH/FSH, T, FT, SHBG, and DHEAS levels decreased; endometrial thickness, blood flow of maximum uterine artery velocity increased	Irritable and nausea (T: 0, C: 2)	[32]
Zhang, 2011	98	Invigorating kidney and removing phlegm fang	Treatment arm: CHF Control arm: no intervention	3 months	Pregnancy rate, high-quality embryo rate, and embryo implantation rate levels increased; FINS level decreased; T and E_2 levels decreased	OHSS (T: 3, C: 6); no SAE	[33]

Table 1: Continued.

Authors (year)	Sample size	Composition	Interventions	Duration of study	Results	AEs	References
Qian et al., 2015	120	Tonifying kidney and removing blood stasis decoction	Treatment arm: CHF + clomiphene Control arm: clomiphene	6 menstrual cycles	Cure (31) Moderately improved (20) Ineffective (9) Overall efficacy: 85.0% Ovulation rate and pregnancy rate levels increased; BBT double-phase rate level increased; LH, FSH, and LH/FSH levels decreased	AEs: none	[41]
Zhang, 2015	90	Qingre Yangyin recipe	Chinese herbs arm: CHF Western medicine arm: metformin Combined arm: CHF + metformin	3 months	BBT double-phase rate level increased; BMI level decreased; FPG, 2hGLU, FINS, 2hINS, HOMA-IR, and leptin levels decreased; APN level increased; LH, PRL, T, and E_2 levels decreased (combined arm)	NS	[43]
Liu et al., 2018	120	Dihuang wan combined with Xianggui Erchen tang	Treatment arm: CHF Control arm: ethinylestradiol and cyproterone acetate	3 menstrual cycles	Markedly improved (33) Moderately improved (22) Ineffective (5) Overall efficacy: 76.67% Menstruation recovery rate, ovulation recovery rate, and BBT double-phase rate levels increased; BMI and WHR levels decreased; IGF-1, LP, and HOMA-IR levels decreased; T, DHT, DHEAS, FAI, LH, LH/FSH, and PRL levels decreased; SHBG, FSH, and APN levels increased; ovary volume level decreased; Rosenfield and hairy score levels decreased	NS	[50]

Table 1: Continued.

Authors (year)	Sample size	Composition	Interventions	Duration of study	Results	AEs	References
Liang et al., 2019	100	Kuntai capsule	Treatment arm: CHF Control arm: placebo	6 months	BMI and WHR levels decreased; FPG, 2hGLU, FINS, 2hINS, and HOMA-IR levels decreased; ISI level increased; TC, TG, and LDL-C levels decreased; HDL-C level improved; LH, LH/FSH, and T levels decreased	Mild adverse events (T: 3, C: 8); no SAE	[55]
Chen et al., 2017	114	Invigorating kidney and dispersing stagnated liver qi and promoting blood tablet	Chinese herbs arm: CHF Western medicine arm: metformin Combined arm: CHF + metformin	3 months	Cure (19) Moderately improved (18) Ineffective (2) Overall efficacy: 94.87% BMI, WHR, and central obesity rate levels decreased; TC, TG, and LDL-C levels decreased; HDL-C level increased	NS	[56]
Wei et al., 2014	124	Qiangshen table combined with Longlu capsule	Treatment arm: CHF Control arm: metformin	6 months	Markedly improved (48) Moderately improved (22) Ineffective (3) Overall efficacy: 95.89% BMI, FINS, HOMA-IR, and visfatin levels decreased	AEs: none	[57]
Lu et al., 2015	121	Bushen Huatan fang	Treatment arm: CHF Control arm: metformin	3 menstrual cycles	BMI, waist circumference, and hip circumference levels decreased; 1hGLU and HOMA levels decreased; CHO, LDL-C, and ApoB levels decreased; SHBG level increased; acanthosis nigricans scores level decreased	AEs: none	[58]

Table 1: Continued.

Authors (year)	Sample size	Composition	Interventions	Duration of study	Results	AEs	References
Chen et al., 2016	93	Quyu Huatan decoction	Treatment arm: CHF Control arm: metformin	3 menstrual cycles	Cure (10) Markedly improved (19) Moderately improved (13) Ineffective (5) Overall efficacy: 89.4% BMI level decreased; FPG and INS levels decreased; TG and TC levels decreased; HDL level increased; FSH, LH, and T levels decreased	NS	[59]
Deng et al., 2019	117	Dingkun pill	A arm: Diane-35 B arm: CHF C arm: CHF + Diane-35	3 months	1hGLU and HOMA-IR levels decreased; QUICKI level increased	AEs: none	[63]
Hua et al., 2003	107	Yishen Jianpi Yangxue Jianpi Tongli therapy fang	Treatment arm: CHF Control arm: clomiphene	6 months	Cure (39) Moderately improved (29) Ineffective (8) Overall efficacy: 89.5% Pregnancy rate level increased; BMI and F-G scores levels decreased; 1hGLU and 2hGLU levels decreased; LH and T levels decreased	AEs: none	[70]
Ma et al., 2017	147	Bushen Tiaochong decoction	Treatment arm: CHF + Diane-35 + letrozole Control arm: Diane-35 + letrozole	3 menstrual cycles	Markedly improved (31) Moderately improved (35) Ineffective (7) Overall efficacy: 90.41% Endometrial thickness level increased; BMI, LEP, and VF levels decreased; APN level increased; LH, FSH, and LH/FSH levels decreased; E_2 level increased; PI and RI levels decreased	Nausea (T: 5, C: 4); headache (T: 0, C: 1); no SAE	[71]

Table 1: Continued.

Authors (year)	Sample size	Composition	Interventions	Duration of study	Results	AEs	References
Zhang, 2017	113	Tiaojing Huoxue decoction	Treatment arm: CHF + ethinylestradiol cyproterone acetate Control arm: ethinylestradiol cyproterone acetate	3 months	Markedly improved (31) Moderately improved (18) Ineffective (8) Overall efficacy: 85.96% HOMA-IR and LP levels decreased; T and LH levels decreased; TNF-α and IL-8 levels decreased; IGF-1 level increased	NS	[72]
Cao et al., 2014	105	Kuntai formula	Treatment arm: CHF Control arm: Diane-35	3 months	Markedly improved (21) Moderately improved (22) Ineffective (5) Overall efficacy: 89.6% Ovulation rate and pregnancy rate levels increased; BMI level decreased; LH, T, and leptin levels decreased; SHBG level increased Embryo implantation rate level increased; TC, TG, and LDL-C levels decreased; HDL-C level increased; WHR level increased; T level decreased (C arm)	NS	[73]
Teng et al., 2019	227	YiShen JianPi decoction	A arm: lifestyle modification B arm: lifestyle modification + metformin C arm: lifestyle modification + CHF	3 months	Cure (6) Moderately improved (32) Ineffective (7) Overall efficacy: 84.4% FSH and E_2 levels increased; LH, PRL, and T levels decreased; SDS and SAS score levels decreased	NS	[74]
Wang, 2011	90	Shugan Tiaojing formula	Treatment arm: CHF Control arm: CHF + Diane-35 + clomiphene	3 menstrual cycles		NS	[75]

of embryo implantation. Many mechanisms have verified that the defective condition of ER can be improved by regulating endometrial thickness and endometrial morphology, when undergoing treatment with herbal formula. At the same time, herbal formula treatments also are beneficial to reduce the volume of ovary and the number of ovarian cysts [12, 44, 45, 89].

Du reported that herbal formula therapy had positive effective in endometrial thickness, type A endometrium, and menstruation recovery of infertility patient with PCOS and further found higher pregnancy rates (52.63% versus 28.07%) in the combination group than conventional pharmacological therapy [90]. With endometrial microcirculation disturbance, the implantation rate is low. Herbal formula can modify the endometrial microcirculation-related indicators such as resistance index and hemodynamic index and increase endometrial blood supply, and as a result, the endometrial receptivity was improved to provide good environment for embryo implantation and improve the pregnancy rate [71, 88]. Notably, kidney herbs nourish could increase endometrial growth by promoting circulation of blood. In addition, both clinical trials and animal experiments have been certified that HF could regulate the expression of molecular biologicals, such as integrin ($\alpha v\beta 3$), vascular endothelial growth factor (VEGF), and uncoupling protein 2 (UCP2), which are closely related to ER [21, 91, 92]. The previous study showed that using herbal formula as an adjuvant therapy has remarkable improvement effect on pulsatility index (PI) and resistance index (RI), endometrial thickness, and the expression of HOXA10 in endometria while further found higher pregnancy rates (42.5% versus 22.5%; $P < 0.05$) in the combination group compared with the control group [93]. And the animal experiment also verified these benefits that herbal formula could facilitate embryo implantation and litter size by promoting endometrium and the expression of HOXA10 [94].

The third point is that herbal formula may modulate the secretion of gonadotropin and sexual hormone via hypothalamus-pituitary ovary axis and is subsequently related to the promotion of follicular maturation and the success of ovulation [45, 50, 95]. PCOS increases the gonadotropin-releasing hormone (GnRH) pulse rate, raises the LH and lowers the FSH, and elevates LH/FSH ratio which aggravates the secretion of androgen and restrains the form of dominant follicle, thus resulting in the state of PCOM and impairing ovulation. Some HF ameliorated sex hormones by markedly upregulating FSH level and downregulating LH and T levels as well as improving circulating E_2 concentration, which could promote follicular development and induce ovulation in PCOS rats [96]. The ovarian hormones estrogen, P, and T play the role in the menstrual cycle and reproductive function. Some clinical researches have exhibited that herbal formula has beneficial effects on improving menstrual cyclicity and increasing rate of ovulation by affecting plasma levels of LH, FSH, E_2, P, and T [24, 41, 42, 96]. Research evidences also have shown herbal formula application positively affecting the ovarian architecture by increasing the number of corpus luteum and decreasing cystic and atretic follicles [97, 98].

The fourth point is that herbal formula may exhibit better antiandrogenic effect by regulating steroidogenic enzymes and steroid receptors and gonadotropin receptors. Ovary is the major source of androgen biosynthesis. In PCOS, theca cells or granule cells overexpress mRNA encoding enzymes involved in steroidogenesis, including steroidogenic acute regulatory protein (StAR), CYP11, CYP17, and CYP19, while their overexpression could cause ovarian hormone synthesis disturbance. Studies have reported that herbal formula can reinstate the balance of androgen and estrogen by rescuing the suppressed expression of LHR, FSHR, and aromatase, thus leading to improved serum E_2 and T concentration, the changes in cystic morphologic of ovaries, and the attenuation of the disordered estrous cycle [96, 99–102]. Many CHFs play important roles on ovarian function by regulating hormone synthesis, which regulated the expression of enzymes involved in androgen synthesis, and these changes paralleled the changes in hormone level both in vivo and in vitro [99, 103, 104]. Animal studies have confirmed that Liuwei Dihuang pill and Mahuang Tang both can promote follicular development and induce ovulation and improve the ovarian polycystic pathogenesis by modulating the dysregulation of steroid hormones and steroidogenic enzymes [80, 96]. Moreover, some researchers considered that herbal formula may play a vital function in treatment by regulating some useful cytokines' expression, such as insulin-like growth factor (IGF), VEGF, tumor necrosis factor (TNF), and inflammatory cytokines expression, thereby alleviating the symptoms of PCOS [91, 92, 105].

The last point is that herbal formula can alleviate emotional distress by relieving chronic stress, which is in connection with activity of sympathetic nervous system. Adverse mental and psychological state can affect the activity of sympathetic nervous system and disturb the release of noradrenaline (NE) and nerve growth factor, thereby affecting follicular development. Recent studies suggested that adjuvant herbal formula could assist PCOS patient better, handle psychological and mood states, as well as increase the success rate of pregnancy when they received infertility treatment [15, 77]. Sun et al. found follicle development abnormality, endocrine disturbance, increased NE level, and activation of locus coeruleus in PCOS model rat, and suggested that the beneficial role of Xiaoyaosan was correlated with the regulation of the sympathetic nerve activity [106].

2.2. The Utilization of Single Herb in Women with PCOS

2.2.1. The Effects of Single Herb for PCOS. Single herbs use in PCOS is increasing worldwide as RCT evidence is emerging. Simple composition having no major adverse effects has made single herb medicine treatment a valuable option. Recent studies provided growing evidence that single herb medicine as CAM can help to improve health outcome and can manage the complications in women with PCOS. Many studies have revealed that single herb medicine may be a promising potent therapy in the treatment of clinical and laboratory symptoms of PCOS, including infertility,

menstrual cyclicity, hormonal irregularities, IR, dyslipidemia, and anthropometric indices [102, 107]. Some single herbs (e.g., *Salvia officinalis*, *Cimicifuga racemosa*, and *Coptidis rhizome*) combined with conventional ovulation induction drugs (e.g., letrozole and clomiphene citrate) have many superiorities, such as ameliorated ovulation, thickened uterine wall thickness, increased pregnancy rates and live birth rate, as well as regulated menstruation [84, 108–111]. Two clinical trials, in China, both found that treatment with tanshinone (*Salvia officinalis*) before promoting ovulation could increase ovulation and pregnant rate [112, 113]. *Cimicifuga racemosa* might affect infertility for PCOS not only using alone but also in combination with other medications [109, 110, 113]. *Cimicifuga racemosa* plus CC also could increase clinical pregnancy rates [109, 110]. Additionally, *Cimicifuga racemosa* used alone resulted in a significant reduction in the LH level and LH/FSH ratio as well as increase in endometrial thickness, which had similar advantages to CC in the regulation of ovulation induction [109]. Some studies have found that BBR can not only improve metabolism but also has a positive effect on reproductive endocrine and reproductive outcome in patients with PCOS [114, 115]. Therefore, single Chinese herb may be popularized as a new inducing agent with good ovulatory rates and fewer sideeffects. There are still some controversies about the benefits from BBR (*Coptidis rhizome*) on fertility of PCOS. Several researches pointed out that BBR, as an assisted drug, has a positive effect on ovulation or pregnancy outcome [116]. However, a RCT showed adding BBR to letrozole did not promote fecundity in PCOS [117]. Single herb medicines also play a remarkable role in balancing menstrual pattern and the hormone status [110, 118–121]. Kort et al. investigated the effect of cinnamon on menstrual cyclicity in PCOS. After 6 months of intervention, menstrual cyclicity and menstrual flow significantly improved in cinnamon group, whereas parameters in the placebo group did not [118]. *Vitex agnus* was found to have the same and even better effect to oral contraceptives in menstrual regularity for PCOS with less adverse effects [122, 123]. It seems that fenugreek seeds are effective in the regulation of menstrual cyclicity and had promising effects on fertility [111].

Curcuma, Pueraria, and cinnamon have been explored to have strong anti-hyperinsulinemia, anti-hyperlipidemia, and antioxidant properties. Besides adjusted oxidative stress, Curcuma had remarkable decreased effects on BMI, FBG, serum insulin, TC, and low-density lipoprotein cholesterol (LDL-C) [84, 124, 125]. Using Curcuma along with metformin could cause a significant reduction in WHR and HOMA-IR in addition to a remarkable advance in glucose disposal rate (GDR) [84, 85, 125]. Several surveys of *Salvia officinalis* and Pueraria demonstrated their efficacy in the regulation of metabolic parameters and menstrual cycles [126–130]. Amini et al. also showed that the consumption of *Salvia officinalis* extract could lead to a statistically decrease in BW, waist circumference (WC), and diastolic blood pressure level [130]. The results also showed significant improvements in glycemic control and insulin sensitivity due to the levels of insulin, FBG and HOMA-IR were markedly decreased, and the quantitative insulin sensitivity check index (QUICKI) was observably increased in the *Salvia officinalis* group. A survey reported the advantageous impact of Pueraria on normalizing the menstrual cycle, IR markers, and serum level of triglyceride when used for 12 weeks [131]. Cinnamon and Curcuma have similar therapeutic effects on glucolipid profile, such as reducing FBG, IR, TC, and TG and enhancing HDL-C level [132–134]. However, there still are different opposite opinions, and some experts suggest that administration of cinnamon may not improve IR and serum TG level [118, 134]. The inconsistent effect of single herb treatment for PCOS may due to the differences in sample size, duration of use, dosage, and different drug forms. We have listed some studies' results in Table 2.

2.2.2. The Mechanisms of Single Herbs for PCOS. The main mechanisms of the effectiveness of single herbs (extract) in PCOS are not yet fully understood. Nevertheless, the following points deserve careful consideration. The first point is that single herb may improve the menstrual cycle, ovulation, and fertility by regulating the secretion of endocrine hormones. Single herb may act directly on the hypothalamus to affect the hypothalamo-pituitary axis and reduce the release of gonadotrophin-releasing hormone, therefore improving the hormonal balance of LH, FSH, and T [133, 135, 136]. Reduction of the LH level increases the sensitivity of ovarian tissue to circulating FSH, contributing to alleviation of the symptoms of excessive androgens, the follicular growth, and ovulation. Also, single herbs have estrogen-like function, which was directly related to improve the implantation rate and pregnancy outcome, and can decrease the serum level of androgens by increasing the level of SHBG [137]. It has been proved that *Vitex agnus* could regulate the menstrual frequency and enhance fertility capacity by inducing the growth in the level of midluteal P, normalizing FSH and LH release, as well as inhibition of type II dopamine receptors [138]. The single herb may have agonistic and/or antagonistic effects on different oestrogenic receptors.

The second point is that single herb can reduce the IR and improve glucose homeostasis parameters of PCOS by affecting the insulin signaling pathways, which manage insulin-stimulated glucose uptake and glycogen synthesis [139, 140]. The animal studies have displayed that the extract isolated from the single herb, including flavonoids, polyphenols, diterpenoids, and triterpenoids, can potentiate glycogen synthesis increase, stimulate glucose uptake, and increase insulin sensitivity by activating insulin receptors and stimulating autophosphorylation of the insulin receptors [139, 141]. Polyphenol polymers, for instance, can exert better hypoglycemic effects by reinforcing insulin signaling at the postreceptor level and stimulating PI3K activity, and consequently, the activation of this pathway could cause the translocation of glucose transporter (GLUT) receptors, finally advancing glucose utilization by facilitating intracellular glucose transport and increasing glycogen synthesis.

The third point is the hypoglycemic effect of single herb may be related with stimulation of insulin synthesis and

Table 2: Summary of the effect of randomized studies of single herb on PCOS outcomes.

Authors (year)	Herbal medicine Scientific name	Extract and dosage form	Interventions	Study duration	Results	AEs	References
Jamilian et al., 2020	Rhizoma Curcumae	Curcumin capsule	Treatment arm: curcumin Control arm: placebo	12 weeks	BMI and weight levels decreased; FPG, FINS, and HOMA-IR levels decreased; QUICKI level increased; TC and LDL-C levels decreased; HDL-C level increased; PPAR-g and LDLR gene expression levels increased	NS	[84]
Wu et al., 2019	Rhizoma Curcumae	Curcuma water decoction	Treatment arm: curcuma + metformin Control arm: metformin	3 months	BMI and WHR levels decreased; TC, TG, and LDL-C levels decreased; HDL-C level increased; FAI and LH/FSH levels decreased; FPG, 2hGLU, FINS, 2hINS, HbA1c, and HOMA-IR levels decreased	Gastrointestinal complications, dizziness, pruritus, and edema (T: 10, C: 13)	[85]
Wang et al., 2011	Rhizoma Coptidis	Berberine tablet	Treatment arm: berberine + metformin Control arm: metformin	3 months	Ovulation rate level increased; BMI, FIN, and HOMA-IR levels decreased; LH, LH/FSH, and T levels decreased	NS	[108]
Kamel, 2013	Rhizoma Cimicifugae	Klimadynon	Treatment arm: klimadynon Control arm: clomiphene	3 months	LH and FSH/LH levels decreased; P level increased; endometrial thickness level increased	Hyperstimulation (T: 1, C: 2)	[109]
Bashtian et al., 2013	Semen Trigonellae	Fenugreek seed extract as capsule	Treatment arm: fenugreek + metformin Control arm: placebo + metformin	8 weeks	Menstrual cyclicity improved; polycystic-appearing ovaries decreased	AEs: none	[111]
Wang et al., 2016	Radix Salviae	Tanshinone capsule	Treatment arm: tanshinone + letrozole Control arm: placebo + letrozole	3 months	Ovulation rate and pregnancy rate levels increased; BMI level decreased; TC and TG levels decreased; LH, FSH, and T levels decreased	NS	[112]

Table 2: Continued.

Authors (year)	Herbal medicine Scientific name	Extract and dosage form	Interventions	Study duration	Results	AEs	References
Li et al., 2017	Rhizoma Coptidis	Berberine capsule	Treatment arm: berberine Control arm: metformin	3 months	BMI, HOMA-IR, FPG, FINS, and 2hINS levels decreased; TC, TG, and LDL-C levels decreased; LH, LH/FSH, and T levels decreased	Gastrointestinal complications (T: 0, C: 1)	[114]
Li et al., 2015	Rhizoma Coptidis	Berberine	Treatment arm: berberine + dydrogesterone Control arm: dydrogesterone	3 months	HOMA-IR level decreased; TC and TG levels decreased; LH, FSH, T, DHEA, and A levels decreased	NS	[115]
An et al., 2014	Rhizoma Coptidis	Berberine tablet	BBR arm: berberine MET arm: metformin Placebo arm: placebo	3 months	Live birth rate level increased; BMI and WHR levels decreased; TC and LDL-C levels decreased (BBR arm)	Nausea (MET: 12, BBR: 9, placebo: 4), OHSS (MET: 2, BBR: 2, Placebo: 6), no SAE	[116]
Wu et al., 2016	Rhizoma Coptidis	Berberine capsule	Letrozole arm: letrozole + berberine placebo Berberine arm: berberine + letrozole placebo Combination arm: letrozole + berberine	6 months	Ovulation rate level mild Increased (combination arm); BMI and waist circumference levels decreased (berberine arm)	Mild in three arms: gastrointestinal complications in three groups; no SAE	[117]
Kort et al., 2014	Cortex Cinnamomi	Cinnamon capsule	Treatment arm: cinnamon Control arm: placebo	6 months	Menstrual cyclicity improved	NS	[118]
Sohaei et al., 2019	Rhizoma Curcumae	Curcumin	Treatment arm: curcumin + metformin Control arm: placebo + metformin	6 weeks	FINS and HOMA-IR levels decreased; QUICKI level increased	Gastrointestinal complications (T: 3, C: 0)	[124]
Heshmati et al., 2020	Rhizoma Curcumae	Curcumin capsule	Treatment: curcumin Control arm: placebo	3 months	GPx level increased; SIRT1 and PGC-1a gene expression levels increased	AEs: none	[125]
Su et al., 2015	Radix Salviae	Tanshinone capsule	Treatment arm: tanshinone Control arm: placebo	3 months	1hGLU and 2hGLU levels decreased; serum glucose clearance rate level increased	NS	[126]
Zhang et al., 2015	Radix Salviae	Tanshinone capsule	Treatment arm: tanshinone Control arm: placebo	3 months	TC and TG levels decreased; HDL-C level increased; T level decreased	AEs: none	[127]

Table 2: Continued.

Authors (year)	Herbal medicine Scientific name	Extract and dosage form	Interventions	Study duration	Results	AEs	References
Wu et al., 2016	Radix Salviae	Tanshinone capsule	Treatment arm: tanshinone + Diane-35 Control arm: Diane-35	3 months	TC, TG, and LDL-C levels decreased; HDL-C level increased; LH and TSH levels decreased; GH level increased; ACTH, β-EP, Cor, and UFC levels decreased	NS	[128]
Li et al., 2017	Radix Puerariae	Puerarin tablet	Obesity treatment arm: puerarin + Diane-35 + metformin Thin treatment arm: puerarin + Diane-35 + metformin Obesity control arm: Diane-35 + metformin	3 months	Menstrual cyclicity improved; BMI level decreased; HOMA-IS level decreased; TC and TG levels decreased; HDL-C level increased; SOD level increased; T level decreased; (obesity treatment arm)	NS	[131]
Khan et al., 2018	Cortex Cinnamomi	Darchini capsule	Treatment arm: darchini Control arm: metformin	60 days	Menstrual cyclicity improved; ovarian size level decreased; FT level decreased	Epigastric burning and belching (T: 1, C: 1); no SAE	[132]
Wang et al., 2007	Cortex Cinnamomi	Cinnamon extract as capsule	Treatment arm: cinnamon Control arm: placebo	8 weeks	FPG and HOMA-IR levels decreased; QUICKI and matsuda insulin resistance index levels increased	NS	[136]

secretion from the beta-pancreatic cells of Langerhans [142]. There also is a cognition that single herb could affect the activity of digestive enzymes, such as pancreatic and intestinal lipase, alpha-amylase, and pyruvate kinase, which are involved in the digestion and absorption of glucose and lipid [143]. Lastly, the single herb may modulate the expression of genes closely related to cellular glucose absorption and metabolism [144].

3. The Utilization of Acupuncture Combined with Herbal Medicine

Acupuncture involves the insertion and retaining of very fine needles into specific anatomical points and has been used in eastern Asian countries to regulate the women's health disorders for many centuries. The major theory of acupuncture is based on Chinese medical theory which believes that there is a kind of energy flow through the body; when the energy flow is represented as a balance of Yin and Yang or Qi and Xue, the body is disease-free. In view of the high security of acupuncture treatment, transient adverse effects including pain, bleeding, redness, and hematomas are uncommon. Acupuncture therapy is now accepted, worldwide, as a kind of therapy of CAM. As early as two 2500 years ago, doctors are often used in combination with acupuncture and Chinese medicinal herbs together to treat gynaecological diseases in China. The Handbook of Prescription for Emergencies describe that "Those who apply acupuncture without medicine or medicine without acupuncture are especially not good doctors. Those who know both medicine and acupuncture are good doctors." More importantly, herbal medicine combined with acupuncture has advantages over each alone.

A RCT trial was delivered in hospital of China in oligomenorrhea women with PCOS, and 88 patients were randomized (1 : 1) to abdominal acupuncture-medicine group or abdominal acupuncture for 3 months. During the 3 months of intervention, menstrual cycles were more frequent and menstrual blood volume was more increased in patients receiving acupuncture and Bushen Huoxue decoction compared with patients receiving acupuncture ($P < 0.05$) [145]. Acupuncture therapy adjuvant to HM could affect reproductive endocrinology and medium- to long-term functional outcome [146, 147]. Yin et al. not only found statistically significant improvements in menstrual rates but also found the BW and AMH were significantly lower;

however, the ovulation and pregnancy rates were higher in the acupuncture-herbal medicine group than the control group in 120 infertility women with PCOS [147].

Electroacupuncture (EA) is considered to be an effective alternative to conventional needle acupuncture, is valuable in patients with PCOS, is a therapy where fine needles are placed in the skin and underly muscle tissue at specific areas of the body, and then input current flow in the near human body bioelectricity. Yu and Liao randomized 67 cases of obese patients of PCOS to the combination group and control group, combination group was treated with EA and Tiankui capsule, and control group was only treated with EA [148]. Results showed that BMI, WHR, BW, and FINS decreased and ISI and APN were higher in the combination group than that in the control group ($P < 0.01$). Su et al. also found the similar results; moreover, there were significantly improvement of LH, LH/FSH, T, and leptin in this study [149]. The combination treatment could better alleviate the symptoms and endocrine indices, insulin sensitivity, leptin, and APN levels, to improve the quality of life of women with PCOS. Overall, in recent ten years, many RCTs have proved that the combination of acupuncture and herbal medicine has positive impact on reproductive dysfunction, endocrine disorder, and abnormal glucolipid metabolism in PCOS [145, 146, 148–150]. The possible mechanisms of action of acupuncture for PCOS are as follows: (i) lower high sympathetic nerve activity and regulate parasympathetic and sympathetic activity; (ii) regulate the central nervous system through the hypothalamus-pituitary-ovary (HPO) axis and hypothalamus-pituitary-adrenal (HPA) axis; (iii) modulate the metabolic system by modulating expression of genes related to IR, obesity, and sympathetic activity in skeletal muscle and adipose tissue [151–153]. The animal study confirmed that combination of acupuncture and medicinal herb significantly enhanced curative effects by improving the absorption of salvianolic acid B which were extracted from the Chinese medicine formula in the PCOS rat mode [154]. The research of Yu revealed that the mechanism of combination would be EA plus herbal medicine can restore the equilibrium between Yin and Yang, as well as Qi and blood; recover kidney function, the chong and conception vessels, and the uterus; and improve endocrine function and ovarian microcirculation [148].

4. Dietetic Therapy and Its Mechanism of Action in PCOS

Dietetic therapy is one of the key components of TCM, according to traditional theory of TCM, and food is considered as medication. As food and medication are the same in terms of nature, origin, taste, and function, they are equally important in preventing and curing diseases [155]. Moreover, food is considered as tonic, which can treat various deficiencies including "Yin," "Yang," "Qi," and "Xue," and it can help individuals smooth body mechanisms and facilitate rehabilitation. Dietetic therapy insists that different foods have different effects on "Yin" and "Yang," "Qi" and "Xue," and "Zang Fu" organs. Fitting dietetic therapy can nourish "Zang Fu" organs and promote the rehabilitation; oppositely, unfitting diet will lead to the imbalance of "Yin Yang" and "Qi Xue," even the unhealthy condition of "Zang Fu" organs. Dietetic therapy has played an important role in auxiliary treatment for PCOS. Tea, soup, and porridge are most common forms of TCM dietetic therapy. Dietetic therapies are quite suitable for PCOS patients, particularly in women who intend to be conceptive. Both meat soup and rice porridge add some herbals such as Radix Astragali Seu Hedysari, Rhizoma Polygonati Odorati, Rhizoma Dioscoreae, and Radix Ophiopogonis, to promote microcirculation and reproductive endocrine function of the ovary [156, 157].

All these foods have therapeutic effects, and these are what the doctor provided as therapy to patient, according to individual's health condition and physical problems. The food can nourish kidney, strengthen spleen, regulate Qi flow, and generate blood. Modern pharmacological research discovers that the adjunct ingredients Radix Astragali Seu Hedysari, Radix Ginseng, and Radix Ophiopogonis contain polysaccharide fractions which have antioxidant, antidiabetic, hypolipidemic, and immunomodulatory activities [158]. Many herbs, meanwhile, are rich in diosgenin which contains some synthetic materials necessary for steroid hormones, and these herbs have a similar effect as sex hormones and can promote gonadal. Recently, tea as a natural herbal medicine also has been widely considered in treating PCOS and has been intensely researched. Green tea, a commonly consumed beverage in Asia, has been found to exert beneficial effects on the endocrine system, on glucose and lipid metabolic. The latest findings of Tehrani et al. show that green tea consumption has a significant effect on overweight and obese women with PCOS, leading to weight loss and a drop in fasting insulin and free testosterone level [159]. Other studies also found that the weight, BMI, waist, and hip circumference in the green tea group were markedly declined [160, 161]. In a prospective, double-blind, placebo-controlled RCT, Grant found that spearmint herbal tea has significant antiandrogen effects, and it may be a natural and helpful treatment for hirsutism in PCOS [162]. Marjoram tea showed beneficial effects on the hormonal indicator of PCOS women, improving insulin sensitivity and reducing the levels of adrenal androgens [163]. However, when the doses of green tea were inadequate, different study had different results about glucose and lipid profile [164]. We have listed some clinical trials in Table 3.

In general, the major potential mechanisms in the support of PCOS women's health by tea involve the following [165, 166]: (i) antioxidant properties, anti-hyperlipidemic, and anti-diabetic activity; (ii) reducing carbohydrate absorption by inhibition of various digestive enzymes; (iii) increased glucose uptake in skeletal muscle, while decreased glucose uptake in adipose tissue.

5. Moxibustion on Patients with PCOS

Moxibustion is a noninvasive CAM therapy which has the advantage of good clinical efficacy and no toxic effects. It is characterized by the use of moxa as burning material directly or indirectly at acupoints. TCM theory considers that

Table 3: Summary of the effect of randomized studies of dietetic therapy on PCOS outcomes.

Authors (year)	Design	Form and composition	Interventions	Duration of study	Results	References
Liu et al., 2017	RCT	POT: Radix Astragali seu Hedysari + Rhizoma Polygonati + Rhizoma Dioscoreae + Herba Dendrobii + Radix Morindae Officinalis + equal	Treatment arm: oral warm nest pot + letrozole Control arm: letrozole	3 months	Markedly improved (24) Moderately improved (8) Ineffective (5) Overall efficacy: 87.18% Ovulation rate level increased; menstrual cyclicity improved	[156]
Liu et al., 2019	RCT	Porridge and Tea: (1) Poria Rhizoma Dioscoreae Porridge: Poria Rhizoma + Dioscoreae + rice (2) Pericarpium Citri Reticulatae Fructus Hordei Germinatus Porridge: Pericarpium Citri Reticulatae + Fructus Hordei Germinatus + rice (3) Fructus Lycii Rhizoma Dioscoreae Porridge: Fructus Lycii + Rhizoma Dioscoreae + rice (4) Fructus Citri Sarcodactylis Pericarpium Citri Reticulatae tea: Fructus Citri Sarcodactylis + Pericarpium Citri Reticulatae + tea (5) Flos Rosae Rugosae Fructus Citri Sarcodactylis tea: Flos Rosae Rugosae + Fructus Citri Sarcodactylis + tea	Treatment arm: dietotherapy + acupoint embedding therapy Control arm: acupoint embedding therapy	3 months	Markedly improved (17) Moderately improved (22) Ineffective (4) Overall efficacy: 90.7%	[157]
Tehrani et al., 2017	RCT/ double-blind	Tea: green tea	Treatment arm: tea Control arm: placebo	12 weeks	Weight and fasting insulin levels decreased; FT levels decreased	[159]
Farhadian et al., 2020	RCT/ double-blind	Tea: green tea	Green tea arm: tea Metformin arm: metformin Control arm: placebo	3 months	BMI, weight, waist circumference, and hip circumference levels decreased	[160]
Mombaini et al., 2017	RCT/ double-blind	Tea: green tea	Treatment arm: tea Control arm: placebo	45 days	BMI, weight, body fat, waist circumference, and hip circumference levels decreased	[161]
Grant, 2010	RCT/ double-blind	Tea: spearmint tea	Treatment arm: tea Control arm: placebo herbal tea	1 month	FT and TT levels decreased; FSH and LH levels increased; hirsutism scored level decreased	[162]
Haj-Husein et al., 2016	RCT/ double-blind	Tea: marjoram tea	Treatment arm: tea Control arm: placebo	1 month	HOMA-IR level decreased; DHEA-S level decreased	[163]
Chan et al., 2006	RCT/ double-blind	Tea: Lung Chen tea	Treatment arm: tea Control arm: placebo	3 months	Weight level decreased; TC level decreased	[164]

moxibustion therapy can not only dredge meridians and regulate qi-blood but also has a dual effect of tonification and purgation. It has been used to prevent and cure various diseases for more than 2000 years. Recent clinical and animal studies have testified that moxibustion therapy could alleviate the symptoms and/or pathology of PCOS.

In the clinical setting, moxibustion has been used to combine with oral herbal medicine (OHM) or WM in

treating PCOS, especially in East Asia. Combination treatment of moxibustion is beneficial to enhance the therapeutic effect of infertility in PCOS. Both moxibustion plus OHM and moxibustion combined with OHM plus WM can get significantly a higher ovulation rate and pregnant rate than that of WM alone [167–170]. In a clinical trial, 187 infertility women with PCOS were randomized to receive CC, CC plus moxibustion, or CC plus HCG. Combination group of moxibustion had more dominant follicles compared to the CC group ($P < 0.05$) and achieves a similar effect of ovulation induction compared to the HCG group ($P > 0.05$) [171]. Findings implicated that moxibustion treatment can improve the therapeutic effects of conventional WMs including letrozole, clomiphene citrate, oral contraceptives, and metformin [167–169, 172]. With regard to the parameters of reproductive hormone, the combination group of moxibustion was associated with significantly lower levels of LH, FSH, and T [169, 170, 173]. Furthermore, adjuvant treatment of moxibustion offers advantages over WM alone in the improvement of efficacy and safety of drugs, significantly reducing the occurrence of luteinized unruptured follicle syndrome (LUFS) [171, 174]. Hence, moxibustion combined with OHM, which could normalize the sex hormones, may be useful for the PCOS women who plan to get pregnant. The therapeutic effect of moxibustion may be related with a series of physiological responses to heat stimulation generated by burning moxa from patients' and chemical stimulation from the pharmaceutical components of moxa [175]. Pharmacological research displays that the moxa, which is rich in flavonoids and polysaccharides, has strong antioxidant activity. Modern scientific research found that moxibustion exerted significantly anti-inflammatory effect, and it also can ameliorate the body immunity by regulating immune factors and immune cells [176–178]. Moxibustion on governor vessel can effectively adjust the biased state of constitution of people with yang deficiency constitution and observably elevates the levels of immunoglobulin M (IgM), immunoglobulin G (IgG), serum supple C3, and serum supple C4 [179, 180]. These are the potential mechanisms of moxibustion therapy.

6. Summary

At present, the majority of conventional western drugs contain single active ingredients which are active against a single biological target. However, because of the complexity of the human body, the western drug treatment might seem rather simplistic and limited for PCOS. And then, more and more women with PCOS turn to CAM to treat ovulatory and menstrual dysfunction, hormonal imbalance, insulin resistance, and other mental and psychological problems. Among the four kinds of complementary and alternative therapies for PCOS discussed in this review, herbal formula and single herbal are the most commonly used main therapies of PCOS. Moreover, there are combination therapies of acupuncture and herbal medicine, dietetic therapies, and moxibustion therapies which were used for the treatment of PCOS. All of these therapies of CAM can contribute to improve the symptoms of PCOS women in different degrees. A great deal of RCT data are available for HM; however, owing to the lack of blinding in most of the studies, it is likely that the results have a tendency to bias. High-quality designs are desperately needed to assess the efficacy of herbal medicine-associated CAM for PCOS. Additionally, novel and innovative therapies of CAM such as combinational methods are needed in treating PCOS. Importantly, herbal medicine-associated CAM therapy as a promising therapy for the patients with PCOS is worthy of further research in the near future.

Conflicts of Interest

The authors declare that there are no conflicts of interest in this work.

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Review Article

The Complementary and Alternative Medicine for Polycystic Ovary Syndrome: A Review of Clinical Application and Mechanism

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As a reproductive endocrine disease, polycystic ovary syndrome (PCOS) has influenced billions of women during childbearing age worldwide. Owing to its complex etiology and ambiguous pathogenesis, there is still not a specific method to cure it. Clinical treatments, such as hormone therapy and surgical treatment, have side effects. Therefore, it is essential and urgent to seek alternative treatment to solve these problems. The satisfactory efficacy of complementary and alternative medicine (CAM), such as traditional Chinese medicine (TCM), immunotherapy, medicinal foods, vitamin therapy, diet therapy, psychotherapy, spa, and oxygen therapy, in treating PCOS, has aroused an increasing number of medical workers' concern and gradually become the mainstream. This paper reviews the application of CAM in the treatment of PCOS, especially from the perspective of TCM. Meanwhile, the limitations of the literature about CAM in the treatment of PCOS are mentioned and analyzed as well.

1. Introduction

Polycystic ovary syndrome (PCOS) is a reproductive endocrine disease characterized by menstrual disorder, infertility, and obesity, usually accompanied by insulin resistance and metabolic disorders [1]. Due to various reasons such as bad eating habits and unprecedented psychological and social pressure, its incidence has gradually increased as high as 5%–10% [2, 3]. The pathogenesis of PCOS is relatively complex, which may be closely related to genetic factors, endocrine, and specific environment [4]. It is usually diagnosed with the Rotterdam diagnostic criteria. According to the pathophysiology and clinical characteristics of PCOS, the treatment of PCOS includes exercise to lose weight, decrease androgen, and improve insulin resistance (IR), oral contraceptives to regulate the menstrual cycle, and ovulation induction. These conventional treatments, however, have problems such as adverse reactions, low compliance, poor efficacy, and even contraindications [5]. Therefore, it is urgent to find a more effective treatment for PCOS.

Complementary and alternative medicine (CAM), an alternative therapy independent of Western medicine, has been extensively used in health care systems around the world. Numerous studies have indicated that using CAM including acupuncture, Chinese herbal medicine, Tai Chi, yoga, and Qigong can also effectively treat PCOS with fewer adverse reactions. Based on this, the purpose of this review is to provide more accurate evidence for the treatment of PCOS by CAM and to analyze its underlying mechanisms.

2. Acupuncture

Acupuncture, an indispensable part of CAM, has been used in China for more than 3000 years. With the modernization of TCM, more and more Western countries have begun to

apply it for treating and preventing diseases. Due to its superiorities, namely, ease of operation, economical and satisfactory effect, etc., acupuncture has gradually been used in the treatment of PCOS. According to literature searched at the database PubMed and CNKI, we concluded that after thousands of years of development, acupuncture has not only been limited to its original model but has gradually evolved into different forms such as acupuncture, electroacupuncture, warm acupuncture, acupoint embedding, and auricular points [6, 7]. At present, there are different mechanisms of acupuncture for PCOS summarized through considerable clinical and animal trials such as the fact that it can regulate the function of the hypothalamus-pituitary-ovarian axis (HPOA) and the metabolism, promote ovulation, and improve insulin resistance (IR) and endometrial receptivity (ER) [8, 9].

2.1. Acupuncture. Acupuncture refers to using the needle inserted into the patient's specific area of the body at a certain angle to cure diseases through the manipulations such as twisting, lifting, and thrusting [10]. Several studies have proven that acupuncture is beneficial in treating PCOS. A review summarized from limited evidence-based studies showed that acupuncture can be effective in improving PCOS-related symptoms, including the induction of ovulation and restoration of menstruation [11]. An animal experiment conducted by Zhang showed that acupuncture could significantly reduce the number of cystic expanded follicles and the rate of cystic expanded follicles, increase the corpus luteum, and significantly improve the ovarian morphology [12]. In addition, other studies have shown that acupuncture can reduce the incidence of PCOS by changing the distribution of specific intestinal flora, increasing the content of beneficial bacteria, and maintaining the balance between the internal and external environment of the patients [13]. Ee designed a protocol to verify their hypothesis that the mechanism of acupuncture for obese PCOS patients is improving IR [14].

Wu et al. [15] randomized 112 obese PCOS patients into the treatment group and control group. The treatment group was administrated with metformin combined with acupuncture Xiawan (CV10), Zhongwan (CV12), Guanyuan (CV4), Qihai (CV6), Tianshu (ST25), Shuidao (ST28), and bilateral Liangmen (ST21), while the control group was merely given metformin. After 3 months, they found that, in obese PCOS patients, acupuncture can effectively improve the ovarian function by regulating the luteinizing hormone (LH), the follicle-stimulating hormone (FSH), and the testosterone (T) and reducing LH/FSH, leptin (LEP), and obesity. Its mechanism may be related to the reduction of LEP [16]. Jin et al. [17] summarized 52 articles on the rules of acupuncture in PCOS patients and concluded that the most frequently used acupoints were Sanyinjiao (SP6), CV4, Zigong (EX-CA), Zhongji (CV3), and CV6. Ren [18] conducted a systematic review and meta-analysis on the treatment of PCOS with acupuncture and concluded that acupuncture can reduce HOMA-IR and BMI, promote ovulation, increase pregnancy rate, etc. while ensuring the safety of the treatment. However, due to issues such as the quality of the literature, further researches are needed to prove its effectiveness. Based on the data analysis, Yu et al. [19] outlined the rules of acupoint-selection for PCOS (Table 1)

2.2. Electroacupuncture (EA). With the increasing application of acupuncture in Western countries, EA, a derivative of acupuncture, is gradually being used in the treatment of PCOS. EA refers to the combination of electric current with acupuncture to achieve the purpose of strengthening the stimulus [20]. At present, the mechanism of EA on PCOS has not been clarified. Xu G conducted an animal experiment showing that the number of immature follicles, the FSH, the LH/FSH, and the serum anti-Müllerian hormone (AMH) were decreased by EA stimulated on CV4, SP6, and ST36 [21]. Similarly, some studies have shown that EA can correct the imbalance of FSH and AMH in PCOS granulosa cells by inhibiting the excessive secretion of AMH, thus improving hyperandrogenemia and follicular development stagnation [22]. Peng et al. concluded that EA can improve IR, mitochondrial dysfunction, and ER by enhancing autophagy in PCOS-like rats [23].

Budihastuti UR randomized 44 PCOS patients into a control group and an EA group. The EA group was treated for 15 minutes per time, twice a week, for six consecutive weeks. The results show that EA can promote the growth of oocytes in PCOS patients [24]. Li [25] conducted a study on 62 PCOS patients undergoing IVF-ET and found that EA can improve the quality of oocytes. The mechanism may be as follows: EA acts on HPOA to regulate the secretion of gonadotropin-releasing hormone or increase the sensitivity of the pituitary to gonadotropin, indirectly regulate the secretion of FSH, and then improve the quality of follicles and embryos. Cui [26] also reached the same conclusion in the RCT and proposed that its mechanism may be related to the changes of the ovarian microenvironment.

2.3. Acupoint Catgut Embedding. Acupoint catgut embedding refers to using needles and absorbable catgut to stimulate meridians, balance yin, and yang, reconcile qi, and blood to achieve the purpose of curing diseases [27]. Acupoint catgut embedding not only has a function similar to acupuncture but can also stimulate continuous and spontaneous. The superiority of it lies in its remarkable and stable efficacy in treating disease with fewer adverse reactions. Numerous researches have confirmed the effectiveness and have elaborated the mechanism of acupoint catgut embedding in PCOS. Lin [28] conducted an animal experiment to explore the mechanism of acupoint catgut embedding in PCOS. They found that acupoint catgut embedding can significantly improve IR and increase the expression of MiR-125b in PCOS rats' ovaries. MiR-125b can reduce the high expression of ERK1 and ERK2, downregulate the abnormal activation of the MAPK/ERK pathway, restore the balance between proliferation and apoptosis of granulosa cells, and reduce the excessive synthesis of androgen by theca cells. It may be the epigenetic posttranscriptional mechanism of

Table 1: The rules of acupoint-selection of acupuncture for PCOS.

Meridian	Acupoints
Ren meridian	Guan yuan (CV4), zhong ji (CV3), qi hai (CV6), zhong wan (CV12), shui fen (CV9), qu gu (CV2), dan zhong (CV17)
Spleen meridian of foot-Taiyin	San yin jiao (SP6), xue hai (SP10), yin ling quan (SP9), da heng (SP15), di ji (SP8)
Stomach meridian of foot-Yangming	zu san li (ST36), tian shu (ST25), gui lai (ST29), feng long (ST40), hua rou men (ST24), shui dao (ST28)
Bladder meridian of foot-Taiyang	Shen shu (BL23), pi shu (BL20), gan shu (BL18), ge shu (BL17), ci liao (BL32)
Extra points	Zi gong (EX-CA1), luan chao (TF2)
Kidney meridian of foot-Shaoyin	Tai xi (KI3), da he (KI12), heng gu (KI11), fu liu (KI7)
Liver meridian of foot-Jueyin	Tai chong (LR3), xing jian (LR2)
Du meridian	Bai hui (GV20), ming men (GV4), da zhui (GV14)
Large intestine meridian of hand-Taiyang	he gu (LI4)
Pericardium meridian of hand-Jueyin	Nei guan (PC6)
Gallbladder meridian of foot-Shaoyang	Dai mai (GB 26), xia xi (GB43)

acupoint catgut embedding in improve IR and reproductive endocrine in PCOS. Wang [29] randomly divided 60 obese PCOS patients into acupoint catgut embedding group and acupuncture group. After treatment, hyperandrogenemia was significantly improved, and the total effective rates were 54% and 42%, respectively. There was no significant difference between the two groups, which indicates that acupoint catgut embedding has the same effect as acupuncture. Because of its time-saving and low-cost characteristics, it is worthy of further promotion in clinical practice. Yu et al. [30] analyzed the published literature and concluded that the top 10 acupoints used in acupoint catgut embedding for PCOS were ST25, CV4, ST40, SP6, GB26, BL23, CV12, ST36, CV6, and SP9. Due to the heterogeneity of the small sample size and low quality of literature, more clinical trials are needed to verify its efficacy to promote the clinical application.

2.4. Other Acupuncture Therapies

2.4.1. Warming Acupuncture. Warming acupuncture refers to the process of keeping the needle, twisting the moxa mass around the needle handle to ignite, and transferring heat into the acupoints through the needle. Warming acupuncture is also a combination of acupuncture and moxibustion. Lin et al. [31] randomized 58 PCOS patients into a control group (using acupuncture treatment) and a treatment group (using warming acupuncture). After 3 sessions of treatment, the effective rates of the two groups were 66.67% and 44.43%, respectively. Xu's clinical trials also reached the same conclusion that warming acupuncture can treat PCOS effectively. The results showed that after treatment, the observation group was better than the control group in terms of ovarian volume, follicle number, ovulation number, pregnancy rate, etc. ($P < 0.05$) [32]. Gu [33] divided 62 PCOS patients into the obese group and nonobese group according to BMI. Both groups were treated with warming acupuncture for 3 cycles. The results show that warming acupuncture can effectively reduce T, LH, and LH/FSH, increase SOD activity, reduce MDA level, and improve oxidative stress (OS) in PCOS patients. Through comparison, we found that warming acupuncture improves the OS level of obese PCOS patients more significantly than nonobese PCOS patients.

2.4.2. Auricular Points. When problems occur in certain parts of the body, it will react in the corresponding of the auricle. These parts refer to the auricular point. Auricular point is mainly used to treat various diseases by stimulating auricular point, which has been used as adjuvant therapy for a long time in China. The main methods of stimulating auricular point are acupuncture, embedding needle, bloodletting, auricular point sticking, magnetic therapy, massage, etc. [34]. Gao [35] randomized 60 patients with PCOS infertility into a treatment group (30 participants) and a control group (30 participants). The treatment group was given auricular points, and the control group was given oral Chinese herb medicine (CHM). The total effective rate of these two groups was 53.3% and 46.7%, respectively. There was no difference between the two groups. It shows that auricular points for PCOS infertility have an equal effect of CHM. Studies have also reported that the mechanism of auricular points in PCOS is that it can regulate the functions of the anterior pituitary and ovaries [36]. Compared with other CAMs, the auricular point is easy to operate and inexpensive, so it has a high acceptance rate and is worthy of promotion.

3. Chinese Herbal Medicine

As a part of CAM, CHM has saved thousands of Chinese people in the development of China. It started with Shen Nong, a medical scientist in ancient China. After thousands of years' exploration and practice, CHM has gradually become a major part of China's medical system. The clinical efficacy of CHM is remarkable, especially for gynecological diseases such as dysmenorrhea, infertility, and PCOS [37]. The following content mainly focuses on the clinical efficacy and mechanism of TCM monomer and TCM compound in PCOS [38, 39].

3.1. Chinese Herbal Monomer Extracts

3.1.1. Berberine. Berberine is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids. It can be found in plants such as *Berberis*, *Berberis aquifolium* (Oregon grape), and *Berberis vulgaris* (barberry) [40, 41]. Several studies have already reported about berberine on PCOS and summarized its mechanism including lowering blood glucose, improving IR, decreasing androgen, and affecting lipid metabolism. Meanwhile, a meta-analysis drew a conclusion that berberine showed a promising prospect in treating PCOS-IR [42].

Animal experiments showed that berberine could reduce blood glucose and improve IR by activating the PI3K/AKT pathway and inhibiting the upregulation of the MAPK pathway and glucose transporter 4 (GLUT4) [43, 44]. Zhang et al. [45] found that berberine could decrease the level of IR in PCOS-like rats by improving GLUT4, which can regulate both the PI3K/AKT and MAPK pathways. Another animal experiment conducted by Feng L et al. also confirmed that berberine could effectively decrease the blood supply and angiogenesis of ovarian tissue and improve the morphology of ovarian tissue in PCOS rats [46]. Zhang et al. [47] made a study on 20 rats to observe the mechanism of berberine on PCOS. The study confirmed that berberine could improve abnormal glucose and lipid metabolism in PCOS-like rats and regulate the level of serum steroid hormones. Berberine plays a vital role in the treatment of PCOS-related reproductive disorders. Studies about the mechanism of berberine pointed out that it could improve the gene level, upregulate the expression of ovarian CYP19a1 gene and uterine Glut4 gene, and downregulate the expression of ovarian CYP17a1 gene.

3.1.2. Cryptotanshinone. Cryptotanshinone is extracted from danshen root, a Chinese herb. Studies have shown that it can significantly improve the abnormal glucose and lipid metabolism and hyperandrogenemia and can reduce the inhibitory effect of PI3K inhibitor LY294002 on the PI3K pathway in cerebral cortex nerve tissue, thereby protecting the activity of nerve cells, which may be the mechanism of cryptotanshinone in treating PCOS [48].

An animal experiment conducted by Kuang HY showed that cryptotanshinone could significantly reduce the capability of PCOS mice to modulate glucose levels in response to varying diets; meanwhile, it could increase the phosphorylation level of key molecules in the PI3K signaling pathway of ovarian granulosa cells that reduced the ovarian volume of PCOS rats. The expression levels of steroid hormone synthase STAR and CYP17 in rat granulosa cells can improve hyperandrogenism [49]. Another rat experiment showed that cryptotanshinone improved abnormal glucose and lipid metabolism in PCOS rats and reduced LH and T levels to restore the estrous cycle and ovulation of rats [50]. Studies have suggested that the effect of cryptotanshinone on PCOS may be achieved by downregulating the expression of the CYP17 gene and IR [51]. Wu et al. [52] randomized 72 PCOS patients into the observation group (36 cases) and the control group (36 cases). The observation group took Diane-35 combined with a Tanshinone capsule, while the control group was given Diane-35 merely. After 3 months, TG, TC, LDL-C, TSH, LH, ACTH, β-EP, Cor, and UFC were all improved. It is concluded that the Tanshinone capsule could improve the lipid metabolism of PCOS patients and regulate the function of the HPOA.

3.1.3. Other Chinese Medicine Monomers. The total flavonoids of Cuscuta are extracted from the herbal medicine Cuscuta. Studies have shown that the total flavonoids of Cuscuta can reduce the ovarian index of PCOS-like rats and alleviate the proliferation; meanwhile, it can also restore the HPOA function by regulating the secretion of estrogen and androgen and inhibiting the expression of ovarian apoptotic proteins [53, 54]. Glycyrrhetinic acid, a main component of the traditional Chinese medicine licorice, can treat PCOS due to its anti-inflammatory, analgesic, antiallergic, etc. properties. Studies have indicated that the mechanism of glycyrrhetinic acid in treating PCOS lies in the fact that it can enhance insulin sensitivity and reduce testosterone in PCOS patients. In addition, glycyrrhetinic acid can increase the expression of AMPK mRNA in porcine ovarian granulosa cells and reduce the androgen secretion capacity of insulin-resistant granulosa cells and the level of CYP17 mRNA [55, 56]. Silymarin is extracted from the dried fruits of the Compositae plant *Silybum marianum*. Studies have found that both glycyrrhetinic acid and silibinin can increase the expression level of AMPK mRNA and reduce the androgen secretion capacity and CYP17 mRNA of insulin-resistant granule cells [57].

In summary, considerable trials have verified the effectiveness of Chinese medicine monomers on PCOS and concluded that its mechanism may be related to improving IR, reducing androgen level, regulating lipid metabolism, etc. However, the existing studies on PCOS by Chinese medicine monomers still lack persuasiveness due to some inevitable factors. Therefore, more high-quality research studies are needed to add weight to its efficacy in PCOS.

3.2. Chinese Herbal Formulas. Compared with other CAMs, formulas are the most commonly used in China. However, compared with the remarkable efficacy of formulas, the explanation of its mechanism lacks unified evidence. Therefore, the existing mechanisms of formulas in treating PCOS are still not convincing and need to be confirmed by further studies. A review about formulas in treating PCOS showed that several herbs were beneficial in improving menstrual and ovulatory dysfunctions, obesity, IR, lipid metabolism dysfunction, and androgen excess-related conditions [58]. Based on one study, the treatment of PCOS with formulas may correct the abnormal secretion of endocrine hormones and improve glucose and lipid metabolism, etc., thereby improving ovarian morphology and pregnancy outcome [59]. The usual clinical formulas mainly include Cangfu daotan Decoction, Xiaoyao San, Jiawei Xiaoyao San, and Danggui Shaoyao San. The composition of

these formulas is listed in Table 2. The following content describes the treatment of PCOS individually [60].

3.2.1. Cangfu Daotan Decoction (CDD). Cangfu Daotan decoction (CDD) is used for PCOS patients, especially the patients who are the type of stagnation of phlegm and dampness. According to a rat trial conducted by Yi about CDD on obesity-type PCOS, the result showed that CDD decreased the serum levels of TCHO, TG, LDL-c, LH, T, IL-1β, IL-6, and TNF-α and increased the levels of HDL-c, FSH, and E2 in a dose-dependent manner. Meanwhile, CDD could induce the expression of OATP2B1 and OATP3A1 in ovarian and uterine tissues. Therefore, CDD could improve pregnancy outcomes [61]. Zhang and Wu [62] analyzed that the mechanism of CDD in the treatment of PCOS is to improve IR. Fan and Ling [63] randomly assigned 60 cases of obese PCOS infertility patients into two groups each of 30 cases. Both groups were given oral letrozole and progesterone, and the treatment group was given CDD on this basis. The results showed that the pregnancy rates in the treatment group and control group were 53.33% and 26.67%, respectively, with a significant difference ($P < 0.05$). In addition, it also has obvious advantages in increasing the diameter of HCG follicles and the thickness of the endometrium.

3.2.2. Xiao Yao San (XYS). Xiao Yao San (XYS) is a traditional Chinese formula that is widely used for treating gynecological disease, especially for patients with liver constraint type PCOS. The results of animal experiments by Sun et al. showed that the mechanism of XYS treatment of PCOS may ameliorate chronic unpredictable mild stress-induced irregular estrous cycles and follicles development abnormalities, a decrease of estradiol and progesterone level as well as the increase of luteinizing hormone in serum, reduce cystic follicles formation and the apoptosis and autophagy of granulosa cells, and attenuate the increase in dopamine beta-hydroxylase and c-fos level in locus coeruleus, the noradrenaline level in serum and ovarian tissue, and the expression of beta 2 adrenergic receptor in ovarian tissue. Besides, XYS alleviated the reduction of phosphorylation of ribosomal protein S6 kinase polypeptide I and protein kinase B, as well as the increase of microtubule-associated protein light chain 3-I to microtubule-associated protein light chain 3-II conversion both in vivo and in vitro [64].

3.2.3. Jiawei Xiao Yao San (JWXYS). Jiawei Xiaoyao San (JWXYS), also called Danzhi Xiaoyao San, is composed of 10 herbs, which is the most common herbal formula used for PCOS [65]. Wang [66] made an RCT to observe the effectiveness of JWXYS. 128 patients with PCOS were randomly assigned into two groups with 64 cases in each group. The control group received Ethinylestradiol cyproterone, and the treatment group was combined with JWXYS based on it. The results showed that, compared with the control group, the E2, LH, and endometrial thickness can be improved significantly in the treatment group. An animal experiment reached the same conclusion either.

3.2.4. Danggui Shaoyao San (DSS). Danggui Shaoyao San (DSS), created by Zhang Zhongjing, has been a common Chinese herbal formula, which was used since the Han dynasty. Jia and Nie [67] randomized 90 PCOS patients into the treatment group (47 cases) and the control group (43 cases). DSS was prescribed to treatment group patients, while the control group was given gonadotropin. After 6 months, the total effective rate was 85.1% and 55.8%, respectively. The results of an RCT made by Chen and Xue [68] showed that DSS could improve the sex hormones (LH, FSH) and adjust the menstrual cycle.

3.2.5. Other Chinese Herbal Formulas. In addition to the commonly used Chinese herbal formula for treating PCOS mentioned above, some other formulas have been reported to be effective in PCOS. A rat study demonstrated that the underlying mechanism of Guizhi Fuling Wan in improving IR in PCOS-like rats is to regulate intestinal flora to control inflammation [69]. Heqi San, a traditional Chinese herbal formula, has been reported to regulate hormone levels in PCOS patients with metabolic disease, which may be an alternative application for treating PCOS. Zhao et al. [70] concluded through animal experiments that the beneficial effects of Heqi San on PCOS include altering serum hormone levels, recovering ovary morphological lesions, and improving IR, which are mediated through the PI3K/AKT pathway. Besides, Bushen Culuan Decoction (CCBCD) could effectively treat infertility patients with PCOS, according to a protocol of systematic review [71].

4. Dietary Supplements

4.1. Vitamin A. Vitamin A is a fat-soluble vitamin, also called retinol. Vitamin A-derived metabolites, such as retinoic acid and retinol, contribute to antioxidant activity and steroid metabolism, promote nuclear maturation of oocytes, and inhibit cell apoptosis [72–74]. A study [75] has shown that genes related to retinoic acid synthesis are expressed differently in intermembranous cells isolated from PCOS patients. A study made by Wickenheisser JK [76] showed that the use of retinol derivatives in the endometrial cells of PCOM and healthy women found that all intermembranous cells treated with trans-retinol increased dehydroepiandrosterone expression. Meanwhile, obesity and abnormal glucose metabolism are associated with retinol-binding protein 4 (rbp4) in overweight PCOS patients [77]. Another study reported that rbp4 was expressed in PCOS subcutaneous and omental adipocytes, and the rbp4 gene was upregulated, leading to changes in gonads and adrenal steroids [78].

4.2. Vitamin B. At present, many studies have found that vitamin B6, vitamin B12, and folic acid, which are related to homocysteine, are expressed at higher levels in PCOS

Table 2: Ingredients of formula.

Formula	Composition
Cangfu Daotan Decoction (CDD)	White atractylodes rhizome (12 g), cyperus (12 g), pinellia rhizome (6 g), citrus (9 g), medicated leaven (6 g), bile arisaema (6 g), poria (9 g), bitter orange (12 g), fresh ginger (6 g), licorice root (6 g)
Xiao Yao San (XYS)	Licorice root (15 g), Chinese angelica (30 g), poria (30 g), tree peony bark (30 g), white atractylodes rhizome (30 g), bupleurum (30 g)
Jiawei Xiao Yao San (JWXYS)	Chinese angelica (3 g), white peony root (3 g), poria (3 g), white atractylodes rhizome (3 g), bupleurum (3 g), tree peony bark (1.5 g), gardenia (1.5 g), licorice root (1.5 g)
Danggui Shaoyao San (DSS)	Chinese angelica (10 g), white peony root (30 g), Sichuan lovage root (15 g), white atractylodes rhizome (15 g), poria (15 g), water plantain rhizome (15 g)

patients. The reason is that homocysteine is a sulfhydryl amino acid, mainly derived from dietary methionine. Studies have pointed out that elevated HCY will increase the risk of long-term complications such as cardiovascular and reproductive symptoms in patients with PCOS [79, 80]. Folic acid, vitamin B6, and vitamin B12 play an important role in regulating homocysteine. It has been reported that in the pathophysiological study of PCOS, IR and HCY are positively correlated [81, 82]. Kaya et al. [83] confirmed that the increase in IR, obesity, and HCY levels in PCOS patients is related to the decrease of vitamin B12. Regular exercises can reduce the concentration of HCY [84]. Metformin, a sensitizer for IR, is a commonly used medicine for the treatment of PCOS. Metformin has a good effect on regulating IR, and it will reduce the levels of vitamin B12 and folic acid in the body during the treatment [85]. The elevated homocysteine levels increase the risk of cardiovascular disease in patients with PCOS.

Inositol, one of the B vitamins, is a water-soluble vitamin. Like choline, it is a lipophilic vitamin. An experiment conducted by Minozzi et al. suggested that combined contraceptives and inositol may be more effective than OCP alone in controlling the endocrine, metabolism, and clinical manifestations of patients with PCOS and may reduce insulin levels and insulin resistance [86]. Another study carried out by Genazzani et al. demonstrated that after 2 weeks of MYO administration in patients with PCOS, plasma LH, PRL, T, insulin, and LH/FSH levels were significantly reduced. All subjects with amenorrhea and oligomenorrhea resumed their menstrual cycle [87].

4.3. Vitamin D. Vitamin D, apart from the most well-known nonskeletal functions, has a potential role in glucose homeostasis which is connected with the secretion of insulin by pancreatic beta cells, IR in different tissues, and its influence on systemic inflammation [88]. Krul-Poel et al. [89] have confirmed that vitamin D is a significant and independent factor predicting IR, and vitamin D status is closely related to the metabolic disorder of PCOS. Other research studies showed that women with PCOS often lack vitamin D, and its concentration was lower in patients with abdominal obesity. In overweight/obese people with PCOS, vitamin D is related to fasting blood glucose and HOMA [90]. Vitamin D supplementation can reduce abnormally elevated serum AMH levels, and the reduction of serum AMH levels may improve follicular formation by reducing androgens in the ovaries of women with PCOS and increasing the sensitivity of follicles to FSH [91]. However, there are some controversial reports. After vitamin D treatment for 11 patients with PCOS for 3 weeks, there has a good effect on IR, but there is no significant change in the levels of dehydroepiandrosterone, total testosterone, free testosterone, and androstenedione [92]. Meanwhile, a recent study showed that [93] simultaneous vitamin D supplementation and low-calorie diet did not change androgen levels in overweight and obese women with PCOS, but the menstrual frequency was significantly improved.

4.4. Vitamin E. Vitamin E as a lipid-soluble substance with nonenzymatic antioxidant properties, also known as tocopherol, which can effectively reverse the adverse influence by oxidative stress brought to the reproductive system and endocrine system, is widely used in the field of reproductive medicine [94, 95]. Vitamin E plays a vital role in the entire reproductive process. It can antagonize the oxidative stress caused by oxygen free radicals and antioxidation imbalance and regulate the normal physiological functions of the reproductive system [96]. The combined treatment of magnesium and vitamin E for PCOS for 12 weeks has benefits for hirsutism, serum hs-CRP, plasma NO, and TAC levels [97]. Extensive research papers have proven that the treatment of vitamin E in patients with PCOS has a significant effect [98, 99]. It can significantly reduce TG and serum total cholesterol and improve IR, T, and free testosterone index in patients with PCOS. In addition, new evidence confirms that vitamin E can improve the endometrial thickness of patients with unexplained infertility but does not support the hypothesis that vitamin E can increase the ovulation rate and pregnancy rate in PCOS [100].

5. Tai Chi, Yoga, and Qigong

Tai Chi, a unique Chinese exercise, combines physical activity and breathing organically, allowing people to enjoy a pleasant mood while exercising. The key point of Tai Chi is concentration and slow movements. Therefore, it is suitable for the elderly with a weak constitution and chronic diseases [101]. In 2012, NEJM published an article on the treatment of Parkinson's with Tai Chi. The results confirmed that Tai Chi can alleviate the balance disorder of patients with mild to moderate Parkinson's disease, improve functional ability, and reduce falls [102]. Tai Chi can maintain the harmony of qi and blood and regulate the balance of yin and yang, which can significantly reduce BMI; risk factors reduce

cardiovascular disease and improve psychological health [103–105]. Studies have shown that Tai Chi is effective for long-term complications such as obesity, cardiovascular disease, diabetes, and psychological diseases caused by PCOS [106, 107]. Meng [108] summarized that Tai Chi can promote the metabolism of cells and tissues, increase the body's utilization of glucose, the responsiveness of target cells, and the body's tolerance to glucose, prevent the composition of HbA1c, and accelerate the combination of hemoglobin and oxygen to further control blood glucose, thereby reducing the levels of FBG, HbA1c, and 2hPBG. Paul-Labrador et al. [109] believed that Tai Chi can inhibit the activation of sympathetic nerves to improve the neutral-mediated vasoconstriction of blood glucose control and reduce the glucose transport and uptake of skeletal muscle, while Tai Chi can reduce IR [110].

Yoga, a form of holistic mind-body medicine developed thousands of years ago, is a low-impact exercise that can help people maintain balance among physical, psychological, mental, sentimental, and spiritual aspects of life [111]. As a branch of CAM, it is used to treat many different diseases, such as hypertension, asthma, low back pain, arthritis and pain, stress management, and PCOS [112]. A study has shown that yoga can help control endocrine function and relieve symptoms of PCOS [113]. Compared with aerobic exercise, yoga will not cause any harm to the female reproductive system, at the same time, with the low-cost and feasible operation, so it is very promising to reduce the risk of PCOS through yoga [114]. An RCT conducted by Nidhi et al. found that yoga was more effective than conventional physical exercises in improving glucose, lipid, and insulin values, including IR values, in adolescent girls with PCOS [115]. Meanwhile, Patel et al. confirmed through RCT that regular yoga practice could reduce serum androgen in PCOS patients, which is a useful complementary therapy [112]. In addition, Ram Nidhi et al. found that a 12-week holistic yoga program was significantly better than a physical exercise program in reducing anxiety symptoms for adolescents with PCOS [116]. Ratnakumari et al. also reached a similar conclusion that yoga is efficient in bringing about beneficial changes in polycystic ovarian morphology and speculated that a longer intervention might require to regulate menstruation [117].

Although Qigong existed for thousands of years, it has never been widely adopted. In the early 1950s, Qigong became popular after Liu Guizhen advocated it. Medical Qigong, a specific form of Qigong, may have the effects of decreasing blood glucose, triglycerides, total cholesterol, body weight, BMI, and IR in type 2 diabetes in empirical and randomized controlled pilot studies [118]. An RCT conducted by Liu X has proved that after intervention by Qigong, the weight, waist circumference, leg strength, and IR improved which indicated weight reduction in the control of diabetes. Besides, a meta-analysis by Meng suggested that Qigong can improve the blood glucose of type 2 diabetes patients and benefit the management of it [119]. Owing to the fact that PCOS has the risk of obesity and cardiovascular disease, etc., we speculate that Qigong also has a certain effect in the treatment of PCOS [120].

6. Meditation Stress Reduction

Patients with PCOS are troubled by obesity, hirsutism, acne, irregular menstruation, and infertility, which affects their psychological health, physical condition, and quality of life to varying degrees [121]. The risk of anxiety and depression was significantly increased in patients with PCOS. Relevant research showed that more than 60% of PCOS patients were diagnosed with at least one kind of mental disorder [122]. The severity of depression, anxiety, obsessive-compulsive disorder, and somatization disorder measured by different scales in PCOS patients was higher than that in women without PCOS [123].

Emotional depression activates the neuroendocrine stress response system and simultaneously alters the immune function, leading to changes in brain structure [124, 125] and activity [126, 127]. Many people use meditation to relieve stress and treat stress-related diseases. Meditative stress reduction (MBSR) can help patients reduce emotional symptoms (e.g., anxiety, depression, and stress) and improve physical symptoms (e.g., pain) to a certain extent [128]. In addition, MBSR can reduce blood pressure, blood glucose, and inflammation [129–131]. These changes were due to improved autonomic nervous system and HPOA function after MBSR treatment [127, 132, 133]. Through MBSR treatment, PCOS patients' risk of diabetes and cardiovascular disease will eventually be reduced. Takahashi et al. [134] conducted a study on quantitatively analyzed changes in psychophysiological parameters during Zen meditation. Their findings can be an important clue to the efficient use of meditation as a therapeutic procedure. Since most of the current trials on meditation in the treatment of PCOS are small-scale, nonrandomized studies, more large-scale trials are needed to prove its efficacy on PCOS [135].

7. Conclusions

PCOS is a reproductive endocrine disease closely related to infertility, obesity, diabetes, cardiovascular disease, and other metabolic diseases, accompanied by different degrees of psychological disorders. CAM for PCOS mainly includes acupuncture, Chinese medicine, diet and nutrition, Tai Chi, yoga, Qigong, and meditation. Through these therapies, the incidence of cardiovascular disease and diabetes can be reduced, and anxiety and depression can be relieved as well, therefore improving the quality of life. Although CAM has been used in some countries and regions, the globalization of CAM has been hindered due to the lack of more clear research on its safety and mechanism. The specific obstacles include the following: (1) there is a lack of a larger sample size and more structured methods to evaluate the safety and pharmacological mechanism of TCM; (2) there is not enough convincing evidence to support the efficacy or safety of dietary supplements; (3) there is a lack of verification of Taijiquan, yoga, meditation, and other interventions; (4) the molecular mechanism of some CAM therapies remains to be further studied and confirmed [136]. In the future, we need larger samples and RCTs to confirm the efficacy and safety of

CAM in the treatment of PCOS to provide a new method for PCOS.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Li-Yan Jia, Jia-Xing Feng, and Juan-Li Li contributed equally to this work.

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Research Article

Network Pharmacology Strategy to Investigate the Pharmacological Mechanism of Siwu Decoction on Primary Dysmenorrhea and Molecular Docking Verification

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Objective. To study the pharmacological mechanisms of Siwu decoction (SWD) on primary dysmenorrhea (PDM) and verify with molecular docking. *Methods*. The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) was utilized to acquire the active compounds and their corresponding target genes. The GeneCards database was utilized in the search for target genes that were associated with PDM. The intersection genes from the active target genes of SWD and those associated with PDM represented the active target genes of SWD that act on PDM. The Gene Ontology (GO) function enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were both carried out by RGUI 3.6.1 and Cytoscape 3.6.0 software. Cytoscape was also utilized for creating a compound-target network, and a protein-protein interaction (PPI) network was created through the STRING database. Molecular docking simulations of the macromolecular protein target receptors and their corresponding compounds were performed using AutoDockTool 1.5.6 and AutoDock Vina software. *Results*. We identified 14 active compounds as well as 97 active target genes of SWD by using the TCMSP. We compared the 97 active target genes of SWD to the 299 target genes related to PDM, and 23 active target genes for SWD that act on PDM which correlated with 11 active compounds were detected. The compound-target network as well as the PPI network were created, in addition to selecting the most essential compounds and their targets in order to create a key compound-target network. The most essential compounds were kaempferol, beta-sitosterol, stigmasterol, and myricanone. The key targets were AKT1, PTGS2, ESR1, AHR, CASP3, and PGR. Lastly, molecular docking was used to confirm binding of the target with its corresponding compound. *Conclusion*. The pharmacological mechanisms of SWD that act on PDM were investigated, and the active compounds in the SWD for treating PDM were further verified.

1. Introduction

Primary dysmenorrhea (PDM) is defined as dysmenorrhea resulting from nonpelvic organic lesions [1]. The clinical manifestations of PDM include lower abdominal spasmodic pain during menstrual periods accompanied by headache, nausea, vomiting, and lumbar and leg pain [2].

Oral contraceptives and nonsteroidal anti-inflammatory drugs (NSAIDs) have been prescribed as first choice of treatment in PDM [3, 4]. However, these drugs have adverse effects, and their long-term effects are less than optimal. Lately, there have been studies in which Chinese herbal medicine (CHM) was given as PDM therapy, and the results showed that certain curative effect was achieved [5–7].

The Siwu decoction (SWD), first used during the Tang Dynasty as the basic prescription and the first prescription in the treatment of gynecological diseases, primarily consists of baishao, chuanxiong, danggui, and shudihuang, with the

Latin names Radix Paeoniae Alba (RPA), Rhizoma Chuanxiong (RC), Radix Angelicae Sinensis (RAS), and Radix Rehmanniae Preparata (RRP), respectively [8, 9].

SWD is used for tonifying blood, activating blood, regulation of menstruation, and alleviating pain. A meta-analysis and systematic review reported the effects of SWD as treatment for PDM where beneficial and potentially safer in comparison to standard therapy [10].

In the present study, a network pharmacology approach was used to identify the pharmacological mechanism of SWD on PDM; in addition, we performed molecular docking for verification. We first identified both the active compounds of the four herbs as well as the target genes in SWD. Then, the active target genes of SWD and the target genes related to PDM were used to detect the active target genes of SWD that act on PDM. Gene Ontology (GO) function enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were both carried out. Subsequently, we constructed the compound-target network and the protein-protein interaction (PPI) network, and the most essential compounds and targets were screen out. Finally, we used these to create a key compound-target network, followed by performing molecular docking verification. Figure 1 illustrates the flowchart of our study.

2. Materials and Methods

2.1. Screening the Active Compounds in SWD. Data on the compounds that can be found in the four herbs (RPA, RC, RAS, and RRP) were acquired from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com/tcmsp.php) [11]. TCMSP is considered as a one-of-a-kind platform of systems pharmacology of CHM that includes details regarding the association between different diseases, medicine, and their targets. The oral bioavailability (OB) was preset at ≥30%, in addition to drug-likeness (DL) at ≥0.18, as filter benchmarks during the screening for compounds regarded as active. OB indicates the corresponding quantity and pace at which the body absorbs the drugs into the bloodstream. DL is defined as the equivalence between compounds and previously identified drugs [12].

2.2. Screening the Target Genes of Active Compounds. Data on the genes that are targeted by the active compounds were also retrieved from the TCMSP. In the search format "homo sapiens" was chosen, and we imported the target genes into the Universal Protein Resource (UniProt) knowledge base, an extensive and high-quality online source that contains information regarding annotation data and protein sequences (http://www.uniprot.org/) [13]. Then, the human official gene symbols of these target genes were identified, and they were also considered as the proportion of target genes of SWD that are active.

2.3. Identification of PDM-Related Target Genes and Acquisition of Active Target Genes of SWD with Effects on PDM. "Primary dysmenorrhea" was the key word that was used for searching the GeneCards database (https://www.genecards.org/) [14], a database providing comprehensive data on the predicted and annotated type of human genes. The PDM-related target genes were searched and acquired. Then, the intersecting genes derived from the active target genes of SWD and the PDM-related target genes were designated as the active target genes of SWD which have effects on PDM.

2.4. GO Function Enrichment and KEGG Pathway Enrichment Analysis. The RGUI 3.6.1 as well as org.Hs.eg.db packages were used for retrieval of the entrezIDs of the active target genes. The clusterProfiler package, RGUI, and Cytoscape 3.6.0 software with the plugin apps, CluePedia and ClueGO, were employed to carry out the GO function enrichment analysis as well as analysis of the KEGG pathway enrichment. The GO function enrichment consisted of the analysis of the biological process (BP), molecular function (MF), and cellular component (CC) [15–18].

2.5. Development of the Compound-Target Network and Selection of Key Compounds. Cytoscape software with the incorporated NetworkAnalyzer tool function was used to develop as well as evaluate a network of compounds and targets [16]. The nodes depict the target genes and compounds, while the edges depict the correlations that exist between them. Based on the degree of the relationship between the target genes and compounds, the key compounds in the SWD that acted on PDM were selected.

2.6. Development of PPI Network and Designation of the Key Targets. A PPI network consisting of the SWD that acts on PDM was constructed after introducing the active target genes into the STRING database, which is used for the discovery of functional interactions within genome-wide experimental datasets (https://string-db.org/) [19]. We created a PPI network by setting the type of species being researched as "homo sapiens" and the interaction score at a minimum value of 0.4. Then, we used the PPI network to make a topology analysis, and Cytoscape and its Network Analyzer tool were used to determine the exact degree values of every target gene in order to select the key targets of SWD that act on PDM.

2.7. Verification of Molecular Docking. Molecular docking was utilized in order to confirm the binding of target and its corresponding compound. Data on the construction of the small-molecule compounds was acquired via the PubChem Database (https://pubchem.ncbi.nlm.nih.gov/), and the macromolecular protein target receptors were retrieved via the RCSB PDB database (http://www.rcsb.org/). Molecular docking simulations of macromolecular protein target receptors and their corresponding compounds were performed using AutoDockTool 1.5.6 and AutoDock Vina software [20].

2.8. Statistical Analysis. A part of the statistical analysis was simultaneously conducted with the biotechnology add-ons of the software and platforms that have been disclosed in

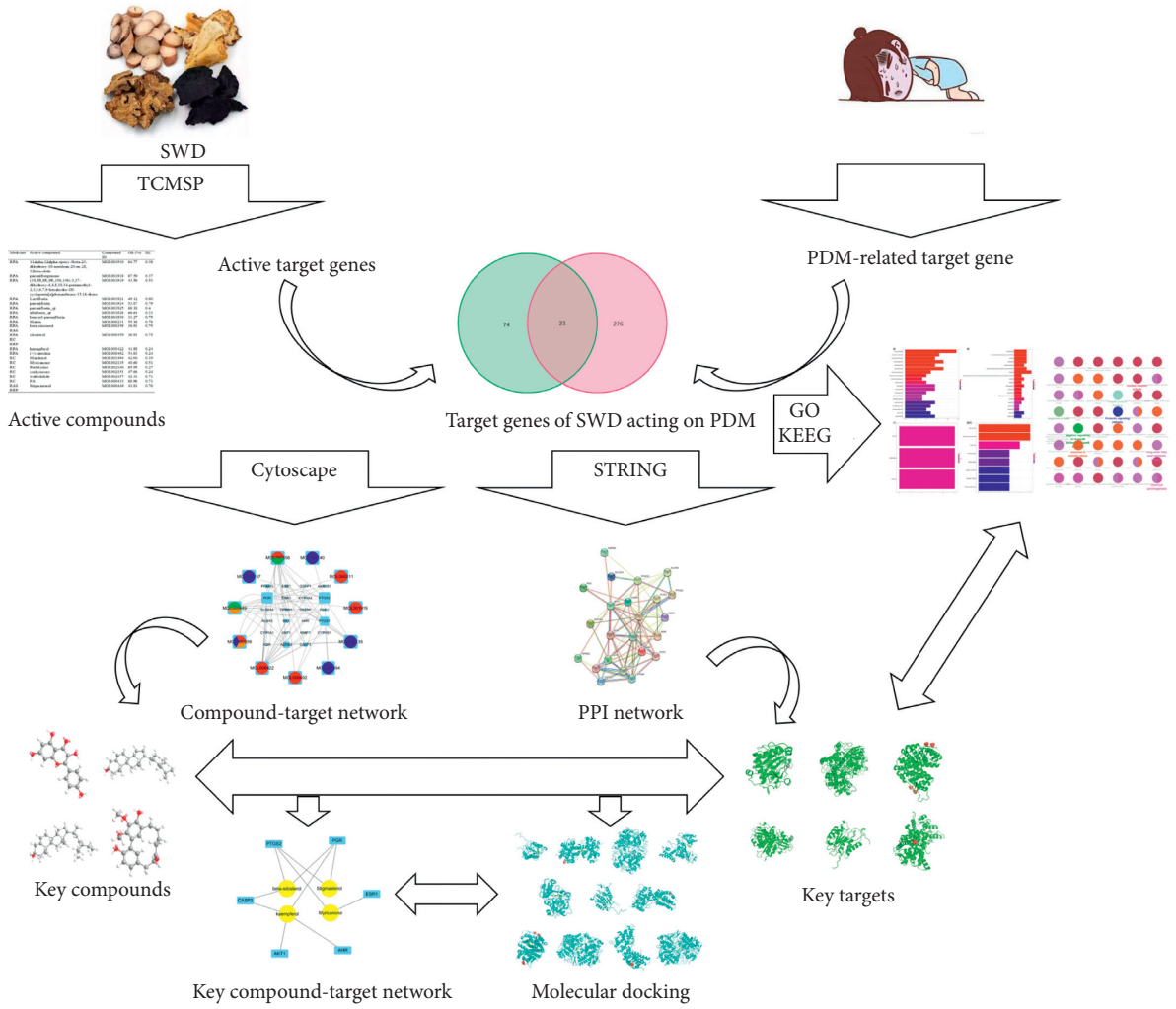

Figure 1: Flowchart of the present study.

previous sections. In the GO function and KEGG pathway enrichment, an adjusted P (adj. P) value was used and adj. $P < 0.05$ was perceived as statistically significant.

3. Results

3.1. Screened Active Compounds of SWD. A total number of 86 compounds were obtained from RPA, 189 compounds from RC, 125 compounds from RAS, and 76 compounds from RRP using the TCMSP (Supplementary Files 1A–1D). By adjusting the filter criteria to OB ≥30% as well as DL ≥0.18, the following number of active compounds was selected: 13 from RPA, 7 from RC, 2 from RAS, and 2 from RRP. Lastly, 20 active compounds of SWD were verified with the exclusion of duplications. Table 1 contains the general data on the active compounds in the SWD.

3.2. Screening Active Target Genes of SWD. Data on the target genes related to the 20 active compounds have been retrieved from the TCMSP as well, in which 6 compounds (MOL001910, MOL001921, MOL001925, MOL001928, MOL001930, and MOL002151) did not have targets (Supplementary File 2A). Then, we screened equivalent symbols depicting genes in the UniProt knowledge base (Supplementary File 2B). Finally, a total of 97 active target genes related to 14 active compounds of SWD were detected (Supplementary File 2C).

3.3. Attained PDM-Related Target Genes and Identified Active Target Genes of SWD that Act on PDM. "Primary dysmenorrhea" was the key word that was used to perform a search in the GeneCards database, which resulted in the identification of 299 PDM-related target genes (Supplementary File 3A and 3B).

A comparison was conducted between the previously identified 97 active target genes of SWD and the 299 PDM-related target genes, leading to the identification of 23 active target genes of SWD that act on PDM (Figure 2 and Table 2).

3.4. The GO Function Enrichment and KEGG Pathway Enrichment Analysis. The entrez IDs of the active target genes of SWD that act on PDM were retrieved through RGUI as well as org.Hs.eg.db (Table 2). Thereafter, we conducted the

Table 1: General data on the active compounds in the SWD.

Medicine	Active compound	Compound ID	OB (%)	DL
RPA	11α,12α-Epoxy-3beta-23-dihydroxy-30-norolean-20-en-28, 12beta-olide	MOL001910	64.77	0.38
RPA	Paeoniflorgenone	MOL001918	87.59	0.37
RPA	(3S,5R,8R,9R,10S,14S)-3,17-Dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	MOL001919	43.56	0.53
RPA	Lactiflorin	MOL001921	49.12	0.80
RPA	Paeoniflorin	MOL001924	53.87	0.79
RPA	Paeoniflorin_qt	MOL001925	68.18	0.4
RPA	Albiflorin_qt	MOL001928	66.64	0.33
RPA	Benzoyl paeoniflorin	MOL001930	31.27	0.75
RPA	Mairin	MOL000211	55.38	0.78
RPA	Beta-sitosterol	MOL000358	36.91	0.75
RAS				
RPA	Sitosterol	MOL000359	36.91	0.75
RC				
RRP				
RPA	Kaempferol	MOL000422	41.88	0.24
RPA	(+)-Catechin	MOL000492	54.83	0.24
RC	Mandenol	MOL001494	42.00	0.19
RC	Myricanone	MOL002135	40.60	0.51
RC	Perlolyrine	MOL002140	65.95	0.27
RC	Senkyunone	MOL002151	47.66	0.24
RC	Wallichilide	MOL002157	42.31	0.71
RC	FA	MOL000433	68.96	0.71
RAS	Stigmasterol	MOL000449	43.83	0.76
RRP				

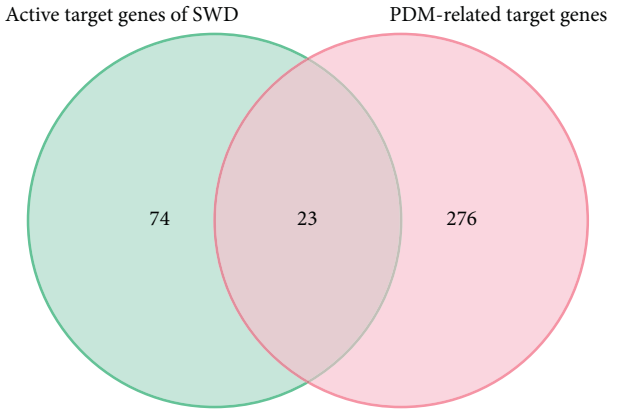

Figure 2: Active target genes of SWD that act on PDM. The 97 active target genes of SWD were compared with the 299 PDM-related target genes, and 23 active target genes of SWD acting on PDM were identified.

Table 2: Gene symbols and entrezIDs of the active target genes.

Gene symbol	EntrezID
PGR	5241
PTGS1	5742
PTGS2	5743
ADRB2	154
OPRM1	4988
BAX	581
CASP3	836
PPARG	5468
AKT1	207
MMP1	4312
CYP3A4	1576
CYP1A1	1543
CYP1B1	1545
ALOX5	240
GSTP1	2950
AHR	196
SLC2A4	6517
GSTM1	2944
ESR1	2099
KDR	3791
ESR2	2100
AKR1B1	231
PLAU	5328

GO function enrichment and KEGG pathway enrichment analyses with the RGUI and clusterProfiler package.

The GO function enrichment analysis of BP indicated the following: active target genes of SWD that act on PDM have been enriched significantly as reaction to toxic substances, the cellular response to xenobiotic stimulus, the metabolic processes of long-chain fatty acids, fatty acids, and unsaturated fatty acids, the response to xenobiotic stimulus, the xenobiotic metabolic process, the maternal process related to female pregnancy, the long-chain fatty acid biosynthetic process, the response to osmotic stress, and other processes (Supplementary File 4A). The 20 most enriched GO BP are visualized in Figure 3(a) and ranked according to their adj. P values.

The GO function enrichment analysis of MF indicated the following: the active target genes of SWD that act on

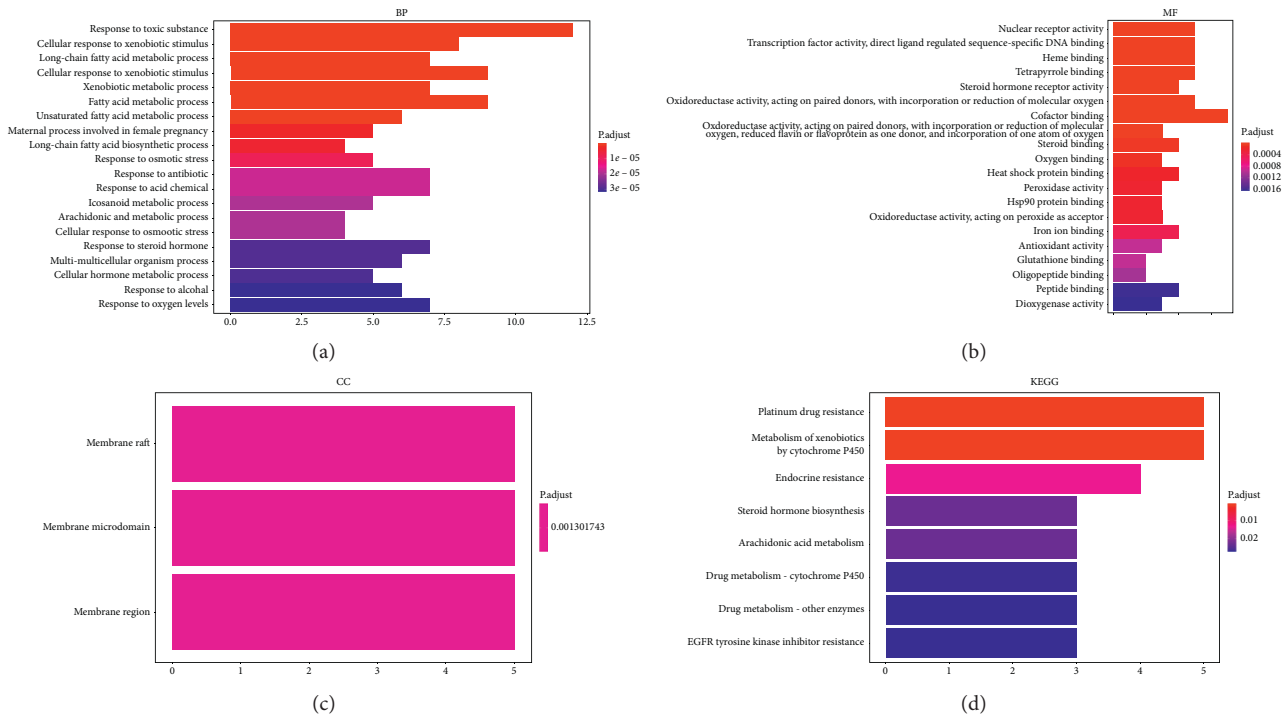

Figure 3: The GO function and KEGG pathway enrichments. (a) Enriched BP functions of active target genes; (b) enriched MF activities of active target genes; (c) enriched CC regions of active target genes; and (d) KEGG pathway enrichments.

PDM have been enriched significantly in nuclear receptor activities, transcription factor activities/direct ligand regulated sequence-specific DNA binding, heme binding, tetrapyrrole binding, steroid hormone receptor activities, oxidoreductase activities/acting on paired donors/with incorporation or reduction of molecular oxygen, cofactor binding, oxidoreductase activities/acting on paired donors/with incorporation or reduction of molecular oxygen/reduced flavin or flavoprotein as one donor/and incorporation of one atom of oxygen, steroid binding, oxygen binding, and other functions (Supplementary File 4B). The 20 most enriched GO MFs are visualized in Figure 3(b) and ranked according to their adj. P values.

The GO function enrichment analysis of CC indicated the following: the active target genes of SWD that act on PDM have been enriched significantly in membrane raft, membrane microdomain, and membrane region (Supplementary File 4C). The three most enriched GO CCs are visualized in Figure 3(c) and ranked according to their adj. P values.

The KEGG pathway enrichment analysis has shown that active target genes of SWD that act on PDM were enriched significantly in platinum drug resistance, xenobiotics metabolism induced by cytochrome P450, endocrine resistance, steroid hormone biosynthesis, arachidonic acid metabolism, metabolism of drugs by cytochrome P450, metabolism of drugs by other enzymes, and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance (Supplementary File 4D). The eight most enriched KEGG pathways are visualized in Figure 3(d) and ranked according to their adj P values; in addition, the KEGG pathways are shown in Supplementary File 5.

By using ClueGO and CluePedia plugin apps, found in Cytoscape software, the GO function enrichment as well as KEGG pathway enrichment were visualized extra naturally (Figure 4).

3.5. Constructed Compound-Target Network and Selection of Key Compounds. We utilized Cytoscape software to create a compound-target network and the Network Analyzer tool to perform the analysis. As MOL001918, MOL001924, and MOL000433 had no correspondences to an overlapping target gene, the 23 overlapping active target genes correlated with the following 11 active compounds: 6 from RPA, 5 from RC, 2 from RAS, and 2 from RRP (Supplementary File 6A). There were 34 nodes (11 compound nodes as well as 23 target gene nodes) and 62 edges in total identified in the network (Supplementary File 6B, Figure 5).

The top four compounds ranked according to their degree within the network were MOL000422 (kaempferol), MOL000358 (beta-sitosterol), MOL000449 (stigmasterol), and MOL002135 (myricanone), which can be regarded as the essential compounds in SWD that act on PDM. General information of the essential compounds can be found in Table 3, and the 2D structure of these compounds acquired from the PubChem Database is shown in Figure 6.

3.6. Construction the PPI Network and Selecting Key Targets. The PPI network was obtained by importing a total of 23 overlapping active target genes into the STRING database. After setting the interaction score to a minimum value of 0.40, 23 target proteins with interactions were identified in

Figure 4: GO function enrichment and KEGG pathway enrichment visualized by ClueGO.

the network as well as 90 edges that depict the various interactions between different proteins (Supplementary File 7 and Figure 7).

The target genes that were ranked as top six in the network according to degree were selected and regarded as the key targets of SWD that act on PDM (Table 4).

3.7. Constructed Key Compound-Target Network. After introducing the key compounds (small-molecule compounds) and key targets (macromolecular protein target receptors) and their relationships to Cytoscape 3.6.0 software, a key compound-target network could be constructed (Supplementary File 8, Figure 8).

3.8. Verification Using Molecular Docking. Data on the 3D compositions of the small-molecule compounds were acquired through the PubChem Database and that of the macromolecular protein target receptors through the RCSB PDB database. Then, AutoDockTool and AutoDock Vina software were used to perform simulations of the molecular docking of potential targets and their designated compounds. Finally, we performed molecular docking to verify the binding of target and its designated component. The simulations of the molecular docking of AKT1-kaempferol are shown in Figure 9, and those of CASP3-beta-sitosterol are shown in Figure 10. The molecular docking simulations of all of the key targets and key compounds are shown in Supplementary File 9.

4. Discussion

PDM is characterized by cold coagulation, qi stagnation, blood stasis, dampness and heat, and a shortage of blood and qi according to the Traditional Chinese Medicine theory, which considers a blood and qi disorder as the primary etiology of PDM and accentuates that patients should be treated in accordance to these aspects [21, 22]. According to

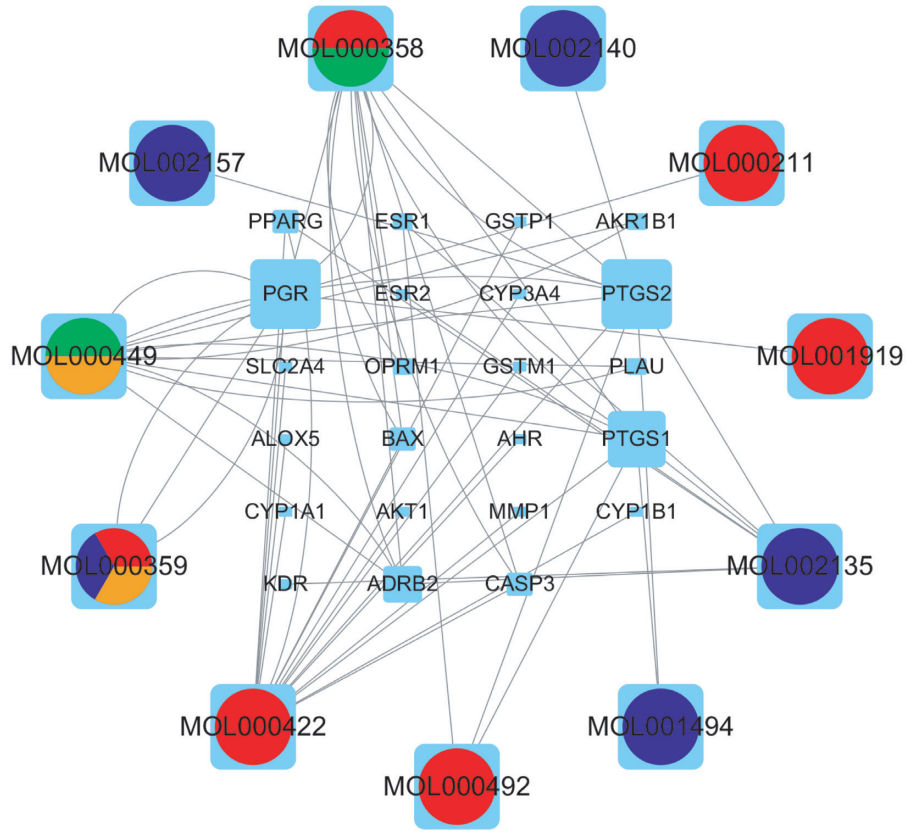

Figure 5: Compound-target network. There were 34 nodes (11 compound nodes and 23 target gene nodes) and 62 edges in the network. Circles represent active compounds (red represent compounds from RPA, bule represents compounds from RC, green represents compounds from RAS, and yellow represent compounds from RRP), rectangles represent the active target genes, and the edges represent links between the nodes.

Table 3: Key compounds in SWD that act on PDM.

Compound name	PubChem CID	Molecular formula	Degree	Medicine
Kaempferol	5280863	$C_{15}H_{10}O_6$	17	RPA
Beta-sitosterol	222284	$C_{29}H_{50}O$	14	RPA RAS
Stigmasterol	5280794	$C_{29}H_{48}O$	12	RAS RRP
Myricanone	161748	$C_{21}H_{24}O_5$	7	RC

modern medicine, the pathogenesis of PDM is primarily related to prostaglandin, vasopressin, oxytocin, estradiol, and endothelin, and it is closely related to immune function and endocrine dysfunction [23, 24].

SWD has been considered as the basic prescription and the first prescription in the treatment of gynecological diseases, which consists of RPA, RC, RAS, and RRP. RPA tonifies the blood to collect yin and calms the liver to relieve pain. RC moves qi to relieve pain. RAS tonifies and harmonizes the blood and regulates menstruation to relieve pain. Finally, RRP tonifies the blood and nourishes yin [8, 9].

At present, there is a wide application of network pharmacology in research related to CHM. It supports the methodology of researching network-based as well as multicomponent therapies, which is compatible with the multipathway, multitarget, and multicomponent features of CHM [25, 26].

In this study, 23 active target genes of SWD that act on PDM were detected. The GO BP function enrichment analysis of SWD as PDM treatment were significantly enriched as reaction to numerous processes as described in the results section and shown in Supplementary File 4A and Figure 3(a). The GO analysis of MF showed that SWD as PDM treatment involved many different activities as described previously in the results section and Supplementary File 4B and Figure 3(b). The results of the GO CC for SWD as PDM treatment are shown in Supplementary File 4C and Figure 3(c). These results indicated that these enrichments

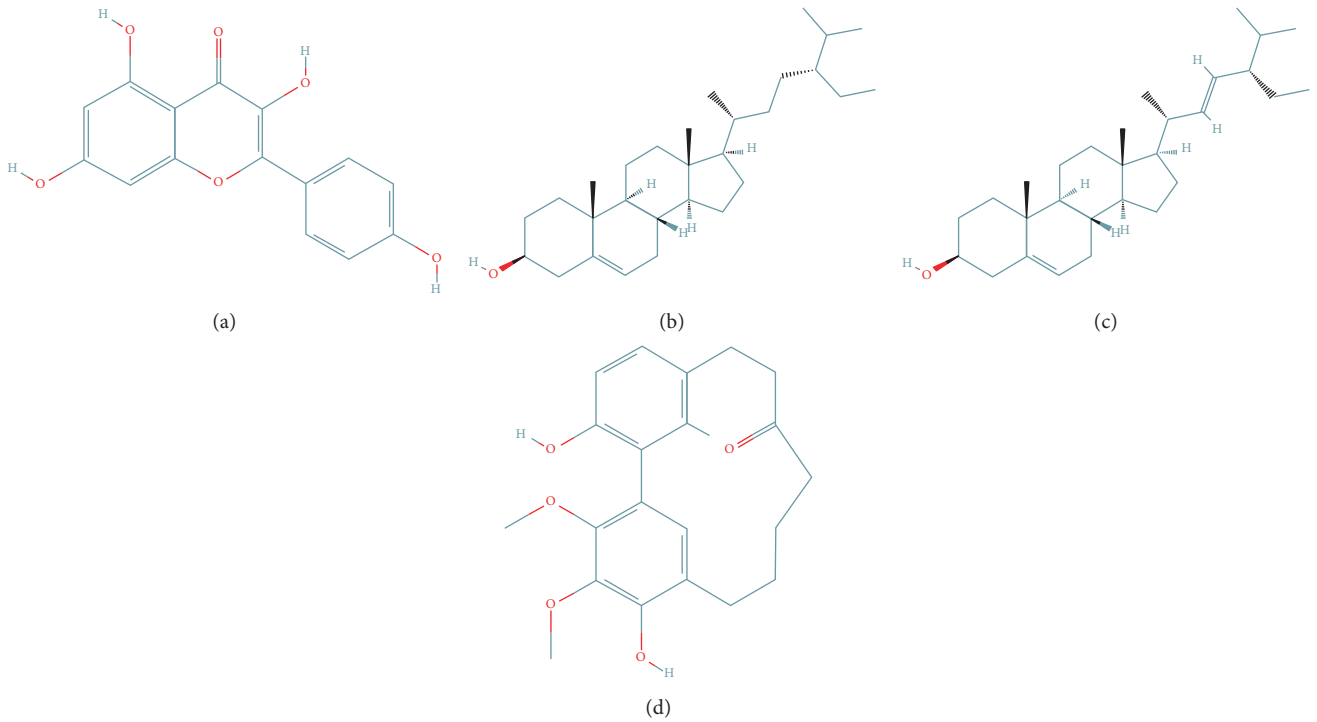

Figure 6: 2D structure of the key compounds. (a) Kaempferol; (b) beta-sitosterol; (c) stigmasterol; (d) myricanone.

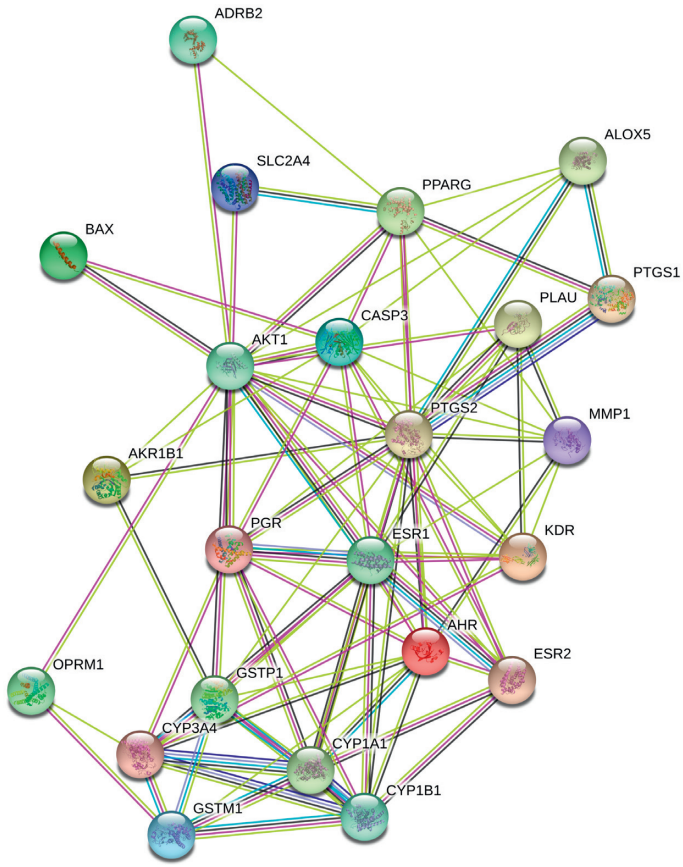

Figure 7: The PPI network of the SWD acting on PDM. In the network, 23 target proteins had an interaction and 90 edges represented the interactions between the proteins, when lowest interaction score was set to 0.40.

Table 4: Key targets of SWD that act on PDM.

Key target	Entry	Entry name	Protein names	Degree
AKT1	P31749	AKT1_HUMAN	RAC-alpha serine/threonine-protein	17
PTGS2	P35354	PGH2_HUMAN	Prostaglandin G/H synthase 2	16
ESR1	P03372	ESR1_HUMAN	Estrogen receptor	13
AHR	P35869	AHR_HUMAN	Aryl hydrocarbon receptor	12
CASP3	P42574	CASP3_HUMAN	Caspase-3	11
PGR	P06401	PRGR_HUMAN	Progesterone receptor	10

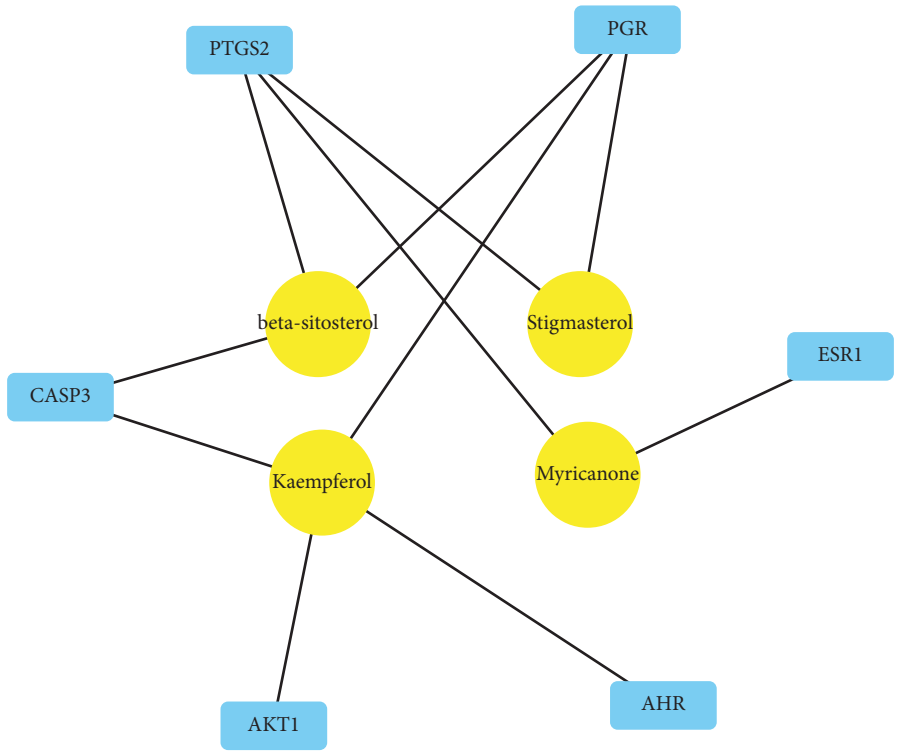

Figure 8: Key compound-target network. Kaempferol, beta-sitosterol, Stigmasterol, and Myricanone are the key compounds considered to be small-molecule compounds, while AKT1, PTGS2, ESR1, AHR, CASP3, and PGR are the key targets considered to be macromolecular protein target receptors.

are closely related to immunity, metabolism, and endocrine activity, which are all strongly associated to the mechanisms of disease in PDM [27–30].

The results of the KEGG enrichment pathway analysis also demonstrated that numerous pathways were strongly associated to the pathological mechanisms of PDM as shown in Supplementary Files 4D and 5 as well as Figure 3(d). These results have clearly demonstrated that SWD can act on PDM via various pathways. These pathways as well as relevant target genes are important and should be further studied.

In the present study, a network of compound-target network was created, followed by the identification of the essential compounds of SWD that act on PDM. The following essential compounds were identified: kaempferol, beta-sitosterol, stigmasterol, and myricanone. Kaempferol can be used to treat numerous acute and chronic inflammation-induced diseases due to its anti-inflammatory properties [31]. Beta-sitosterol has anti-inflammatory, anthelminthic, and antimutagenic activities [32]. Stigmasterol is an organic steroid alcohol that possesses the well-known ability of immune modulation [33]. Myricanone has been reported to have apoptosis-promoting abilities [34]. These studies were related to the regulation of immunity and endocrine function. The SWD action on PDM could be the result of the interaction of these multiple compounds. However, there are few related studies regarding the single compound action on PDM, which requires further investigation.

The PPI network showed that the actions of SWD on PDM were associated with various targets. Of which, the most essential targets were AKT1, PTGS2, ESR1, AHR, CASP3, and PGR. Previous research indicated that the levels of PTGS2 and ESR1 were abnormal in an estradiol benzoate-oxytocin induced dysmenorrhea mice model [9]. It has been shown that the ESR1 gene is statistically significant different in PDM patients and controls [35]. The polymorphisms PGR-CYP17A1-CYP19A1 have been shown to have a gene-gene interaction when there is a risk of developing

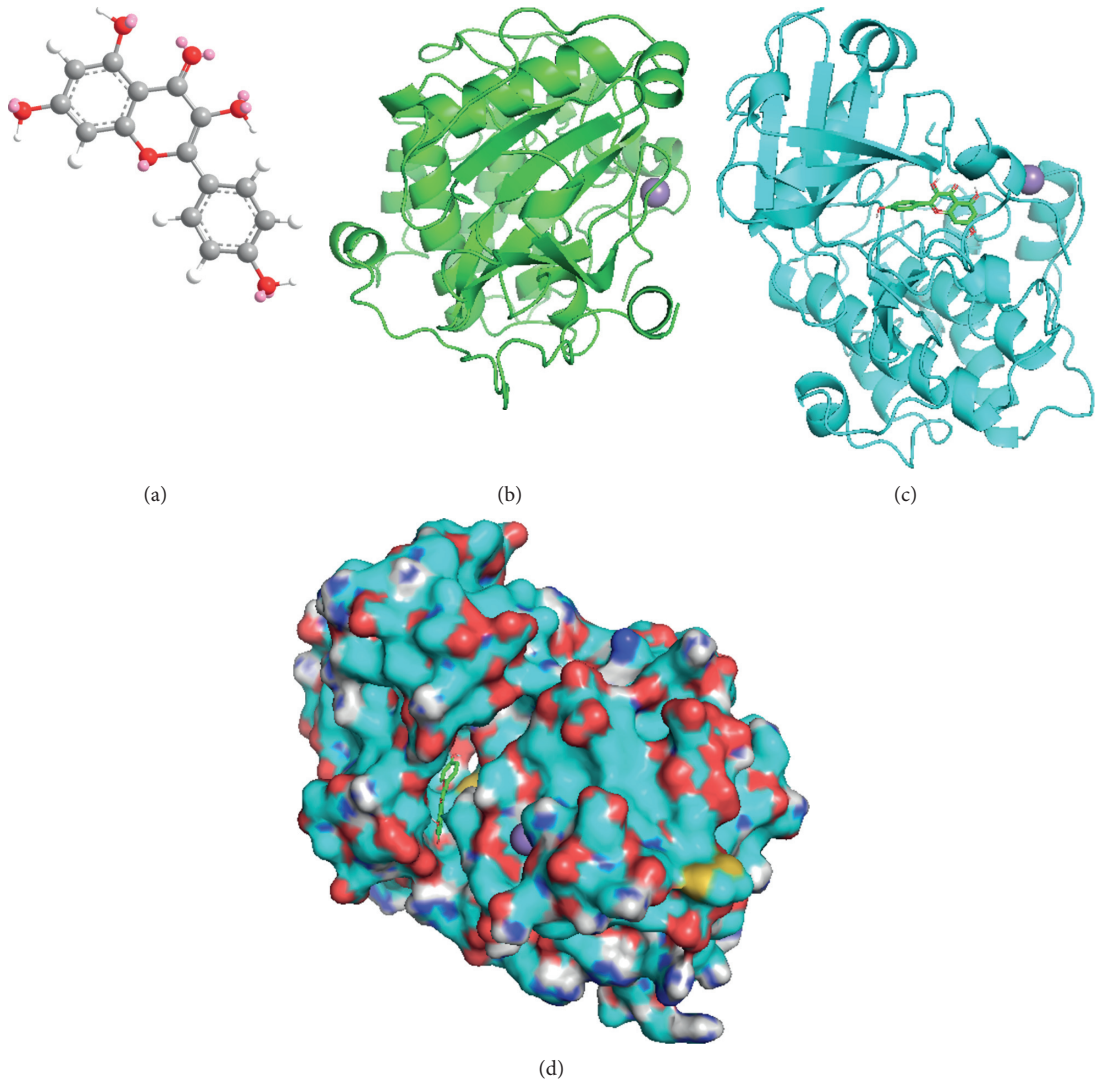

Figure 9: AKT1 kaempferol molecular docking. 3D structures of (a) kaempferol and (b) 3D structures of AKT1; (c) molecular docking simulation; and (d) molecular docking simulation (display protein surface).

endometriosis [36]. The relationship between these genes and PDM, some of which already have relevant studies, can be further studied, and the possible mechanisms can be explored, which have not previously been investigated.

Molecular docking was also conducted to identify specific interactions among key compounds and their predicted protein targets, which could improve the network's accuracy [37]. The preliminary molecular docking results showed kaempferol, beta-sitosterol, stigmasterol, and myricanone, the key active compounds in RPA, RC, RAS, and RRP, had high binding activities with AKT1, PTGS2, ESR1, AHR, CASP3, and PGR gene target proteins. These active compounds may be important foundational materials in SWD for treating PDM through related signaling pathways.

A network pharmacology approach was adopted with the intention of studying the various pharmacological mechanisms of SWD that act on PDM, and the binding of the target and its corresponding compound were verified using molecular docking. However, there are some limitations using these approaches. First, we used the TCMSP database to detect the active compounds of SWD and their corresponding target genes. The criteria for screening the active compounds were fixed, and the target genes associated with PDM were acquired through the GeneCards database. Even though at present these databases are increasingly comprehensive, some compounds and target genes may still have been omitted. Second, the GO function enrichment and KEGG pathway enrichment analyses were undertaken, in addition to the construction of a PPI network to investigate the pathways and target genes of the SWD acting on PDM. These potential target genes and pathways require further study using experimental analyses. Third, preliminary molecular docking verification was only conducted in this study, and research regarding the molecular docking of small-molecule compounds and macromolecular protein target receptors should be further studied.

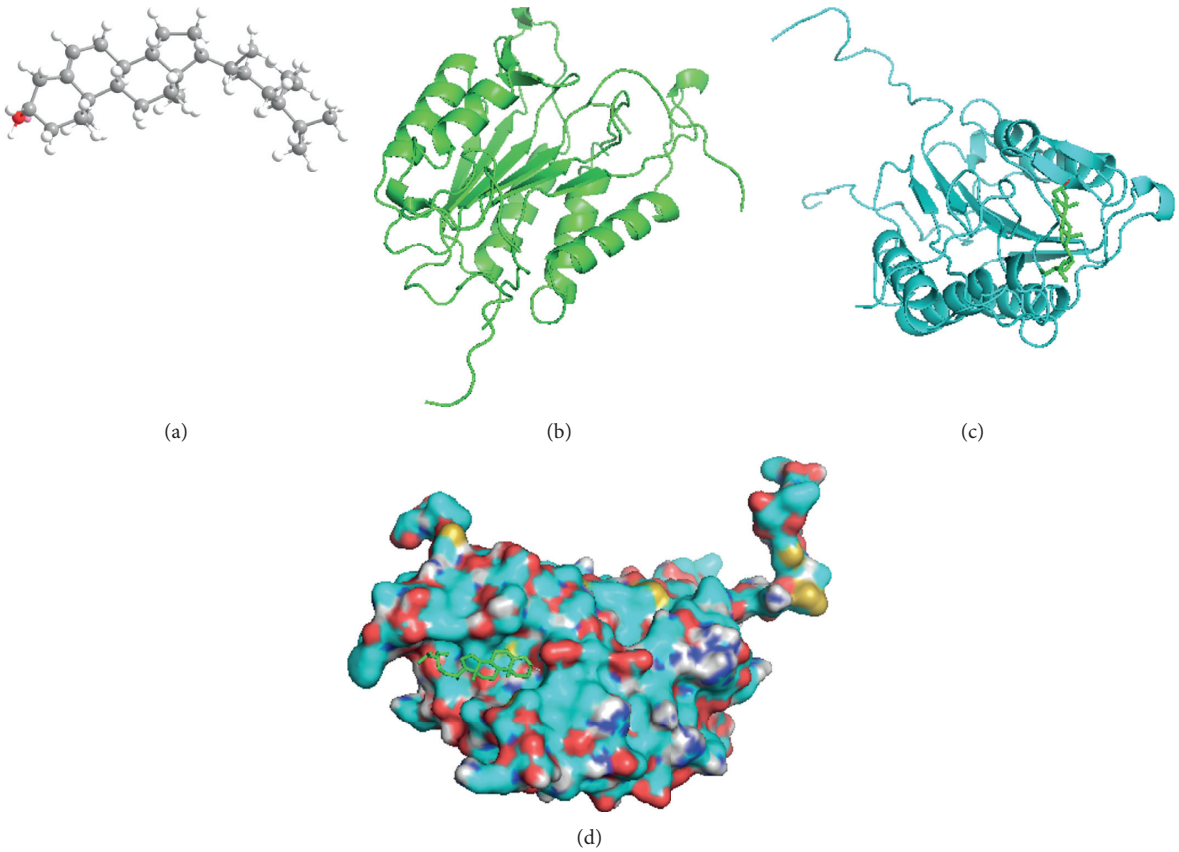

Figure 10: CASP3 beta-sitosterol molecular docking. 3D structures of (a) beta-sitosterol and (b) CASP3; (c) molecular docking simulation; and (d) molecular docking simulation (display protein surface).

5. Conclusion

In conclusion, the interactions among the active compounds, pathways, and target genes of SWD acting on PDM, via the effects of various compounds, targets, and pathways, were investigated in the present study. In addition, molecular docking was conducted lastly to identify specific interactions between key compounds and their predicted target proteins, which indicated these compounds may be an important material foundation in the SWD for treating PDM. In sum, the pharmacological mechanisms of SWD that act on PDM were investigated, and the active compounds in the SWD for treating PDM were further verified.

Data Availability

The data used in this study can be found online as supplementary files (http://www.mediafire.com/file/87m1y65c596wxbt/Supplementary_Files.zip/file).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Dandan Jiang and Xiaoyan Wang created and designed this study and wrote the manuscript. Dandan Jiang and Yufeng Zhang collated and extracted data. Dandan Jiang, Lijun Tian, and Yufeng Zhang conducted analysis of the data. Lijun Tian and Yufeng Zhang supervised and conducted project administration. All the authors have read and authorized the final version of this paper.

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Supplementary Materials

Supplementary files can be found online (). Supplementary File 1: compounds of SWD from TCMSP: (A) 86 compounds of RPA from TCMSP; (B) 189 compounds of RC from TCMSP; (C) 125 compounds of RAS from TCMSP; (D) 76 compounds of RRP from TCMSP. Supplementary File 2: target genes of SWD: (A) corresponding target genes of active compounds; (B) corresponding target gene symbols of active compounds; (C) 97 active target genes of SWD.

Supplementary File 3 PDM-related target genes. (A) PDM-related target genes from GeneCards; (B) 299 PDM-related target genes. Supplementary File 4: GO function enrichment and KEGG pathway enrichment analysis: (A) BP function enrichment analysis; (B) MF function enrichment analysis; (C) CC function enrichment analysis; (D) KEGG pathway enrichment analysis. Supplementary File 5: KEGG pathway. Supplementary File 6: compound-target network: (A) compounds and targets of compound-target network; (B) data of compound-target network. Supplementary File 7: data of PPI network. Supplementary File 8: data of key compound-target network. Supplementary File 9: molecular docking simulations of all the key targets and key compounds. (*Supplementary Materials*)

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Review Article

The Effects of Aromatherapy on Premenstrual Syndrome Symptoms: A Systematic Review and Meta-Analysis of Randomized Clinical Trials

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*Objectives*Premenstrual syndrome (PMS) is a common disturbance among women of childbearing age. Aromatherapy is a commonly used form of complementary and alternative medicine (CAM) to treat PMS. The purpose of this study is to quantify and summarize the effects of aromatherapy on premenstrual syndrome symptoms. *Methods*. PubMed, Scopus, and Cochrane Library databases were searched through relevant search terms until October 2020. The effect sizes were pooled as weighted mean difference (WMD) and 95% confidence interval (CI) using the random effect model. Egger tests and visual inspection of the funnel plot were performed to identify the existence of publication bias. The *I*-squared (I^2) test was applied to measure heterogeneity. *Results*. Eight studies ($n = 8$) were included in this analysis. The quantitative synthesis of evidence found that aromatherapy decreases PMS scores (WMD −13.83; 95% CI (−22.04, −5.63), $I^2 = 94.5\%$), total psychological symptoms of PMS (WMD −3.51; 95% CI (−4.84, −2.18), $I^2 = 82.6\%$), anxiety of PMS (WMD−1.78; 95% CI (−3.17, −0.38), $I^2 = 94.2\%$), depression of PMS (WMD−2.0; 95% CI (−3.65, −0.34), $I^2 = 93.7\%$), and fatigue of PMS (WMD − 1.44; 95% CI (−2.44, −0.44), $I^2 = 89.7\%$) compared to the control group. *Conclusion*. Aromatherapy is an effective tool for the relief of PMS symptoms. Additional randomized controlled clinical trials with different durations and essential oils should be conducted to confirm our findings.

1. Introduction

Premenstrual syndrome (PMS) refers to unpleasant changes in psychological, physical, and behavioral health that occur in the last week of the menstrual cycle and resolves at the beginning of the new menstrual cycle [1]. This syndrome can be quantified by the sum of psychological symptoms (anxiety/tension, depression, confusion, anger/irritability, mood swings, vigor, fear of rejection, lethargy, and sleep disorders), as well as physical symptoms (tenderness of the breasts, bloating, appetite changes, weight gain, headache, aches, abdominal pain, swelling, fatigue, gastrointestinal symptoms, and skin problems) [2]. Although the complete etiology of PMS is unclear, it can be partly attributed to hormonal changes during the menstrual cycle and the subsequent effect on neurotransmitters such as gamma-aminobutyric acid (GABA) and serotonin [2, 3].

PMS affects 20% to 40% of women of childbearing age all over the world [2, 3]. Because PMS can disrupt both the professional and personal lives of women [4], safe and effective treatments are urgently needed. Some medications such as psychotropic medications (e.g., selective serotonin

reuptake inhibitors), hormone treatments (estradiol and progesterone), or nonsteroidal anti-inflammatory drugs (NSAIDs) have been prescribed for treatment of PMS [3]. Due to the side effects and lack of therapeutic response in some patients, women have turned to other therapeutic approaches [5]. Complementary and alternative medicine (CAM) is widely used as a safe, lower cost, alternative solution for coping with common health concerns such as PMS [6, 7].

Aromatherapy is a component of CAM which involves the inhalation of plant extracts as essential oils [8]. Essential or volatile oils are said to stimulate olfactory receptor cells and consequently send messages to the limbic system, the emotional center of the brain [9]. Previous meta-analyses have documented the beneficial effects of aromatherapy for dysmenorrhea [10], depressive symptoms [11], and sleep improvement [12].

One previous review evaluated the effectiveness of aromatherapy in conjunction with Iranian herbal medicines on PMS and primary dysmenorrhea [13]. However, this review focused on Iranian countries and omitted many trials conducted elsewhere in the world. Furthermore, the review focused on primary dysmenorrhea rather than PMS and did not conduct quantitative meta-analysis on PMS specifically. The existing scientific literature includes multiple randomized clinical trials evaluating the effects of aromatherapy on PMS. Some of them identified beneficial effects of aromatherapy on PMS symptoms [14–16]. To our knowledge, there is no meta-analysis evaluating the entirety of the clinical literature on the effects of aromatherapy on PMS symptoms. The purpose of this study is to quantify and summarize the findings of RCTs regarding the effects of aromatherapy on premenstrual syndrome.

2. Method

2.1. Search Strategy. This study was designed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [17].

Databases such as PubMed/Medline, Cochrane Library, and Scopus were searched to recognize relevant published articles until October 2020. The advanced search was performed using a prepared syntax based on Medical Subject Headings (MeSH) and related keywords including ("Aroma" OR "Aromatherapy" OR "Aromatic therapy" OR "Essential oil" OR "Fragrance" OR "Fragrant oil" OR "Scent") AND ("Premenstrual Syndrome" OR "Premenstrual Tension" OR "Premenstrual Dysphoria" OR "Premenstrual Dysphoric Disorder"). To identify all possible studies, the search was conducted without the use of filters. In addition, the reference lists from eligible papers and pertaining review articles were manually inspected to ensure no other articles were missed.

2.2. Eligibility Criteria. Based on the predefined eligibility criteria, two independent investigators (SE and MQ) reviewed the title and abstract of every article for possible inclusion in this study. The inclusion criteria were (1) randomized clinical trials (RCTs) with either parallel or crossover design; (2) studies conducted on females with premenstrual syndrome; (3) studies assessing the effect of aromatherapy on premenstrual symptoms; and (4) studies reporting mean and standard deviation (SD) of symptoms of PMS. Studies which used other methods in addition to aromatherapy were omitted unless the control group also received the additional treatment. This was done to ensure that the aromatherapy treatment was the only difference between the groups.

2.3. Data Extraction. Data extraction was conducted by two separate authors who thoroughly searched each eligible article to extract the following data: author's name, publication year, country, essential oil selection, control group intervention, duration of intervention, duration of the treatment session, the total number of sessions, participants mean age or age range, sample size, study design, outcome assessment tool, and PMS symptom values (mean and standard deviation) before and after the intervention.

2.4. Risk of Bias Assessment. The risk of bias for each study was assessed by two independent examiners using the Cochrane Collaboration tool [18]. This scale evaluates six items: random sequence generation, allocation concealment, blinding (patients, personnel, and outcome assessors), incomplete outcome data, selective reporting, and other sources of bias. Studies were ranked as low risk, ambiguous risk, or high risk of bias based on each item.

2.5. Statistical Method. Data were reported as weighted mean differences (WMDs) with 95% confidence intervals (CIs). The random effects model was used to assess the weighted mean difference between values of PMS symptoms. For studies which did not provide mean change with standard deviations, we calculated these data using the following formula: mean change = final values – baseline values; SD = square root $((\text{SD baseline})^2 + (\text{SD final})^2 - (2R \times \text{SD baseline} \times \text{SD final}))$ [19]. A correlation coefficient equal to 0.9 was used for the R value in the abovementioned formula [19, 20]. To convert standard deviations (SDs) to standard errors (SEs), we used the following formula: SD = SEM × sqrt (n), where n is the number of participants.

Heterogeneity was evaluated using the I^2 index, and I^2 values >50% were considered to be evidence of heterogeneity. Subgroup analyses were conducted based on predefined factors, including sample size, duration of intervention, outcome assessment tool, and study design. Sensitivity analyses were performed to examine the influence of each study on the overall effect size. Potential publication bias was identified by Egger's test and a visual inspection of funnel plots. In the presence of publication bias, Duval and Tweedie's trim and fill method was used to control the analysis for its effects [21]. All statistical analyses were conducted via STATA (Version 12.0, Stata Corp, College Station, TX). Statistical significance was defined as a P value below 0.05.

3. Result

A total of 253 articles were identified via online databases, and no additional articles were found through the additional manual search. A total of 234 articles remained after the elimination of duplicates. The abstracts of these 234 papers were screened, and the full text of 22 studies was evaluated. Of those 22 studies, 14 articles were omitted for the following reasons: 12 studies reported irrelevant outcomes, and 2 studies were review articles. This left a total of 8 studies in this analysis. The flow diagram of the study selection process is shown in Figure 1. Table 1 presents details of eligible studies. These studies represented a total of 295 participants and were published between 2016 and 2020. Six of the studies had parallel design, and the other 2 studies had a crossover design. Studies were conducted in Iran [15, 16, 22, 23], Japan [24, 25], India [26], and Turkey [14]. All of the studies were published in the English language.

Study participants were women who had moderate to severe PMS. Four of the studies measured PMS symptoms with PSST questionnaires [15, 22, 23, 26], two used PMOS [24, 25], one used ACOG [14], and one used the PMS score [16] questionnaire. One of the studies used aromatherapy with massage while the other seven studies used aromatherapy as the exclusive intervention [16]. Aromatherapy treatment time varied from 5 to 35 minutes, and total sessions ranged from 1 to 5. All of the studies used single oil as the intervention. Lavender was used in two studies [14, 25], as was *Citrus aurantium* blossom [15, 23] and rose ($n = 2$) [22, 23]. Yuzu, a Japanese citrus fruit (*Citrus junos Sieb. ex Tanaka*) [24], geranium [16], and clary sage [26] were each used in one study. Three studies used a diffuser to administer the aromatherapy treatment [24–26], three studies used eye pad [15, 22, 23], one study used steam inhalation [14], and one study used massage [16] as the method of administration.

3.1. Assessment of the Risk of Bias. Five studies were categorized as high quality, and the remaining articles were classified as fair, based on six domains of the Cochrane Collaboration tool. Only three studies described the exact method used for randomization, and only two studies reported blinding. The details of quality assessment for articles included in the present systematic review are illustrated in Table 2.

3.2. Meta-Analysis

3.2.1. The Effect of Aromatherapy on Psychological Symptoms of PMS. Seven of the studies examined the impact of aromatherapy on psychological symptoms of PMS [14–16, 22–25]. This meta-analysis found that aromatherapy treatment decreases psychological symptoms of PMS (WMD–3.51; 95% CI (−4.84, −2.18), $I^2 = 82.6\%$), anxiety of PMS (WMD–1.78; 95% CI (−3.17, −0.38), $I^2 = 94.2\%$), and depression of PMS (WMD–2.0; 95% CI (−3.65, −0.34), $I^2 = 93.7\%$) in the intervention group compared to the control group. However, aromatherapy did not produce a significant effect on confusion as a symptom of PMS (WMD–0.65; 95% CI (−1.33, 0.02), $I^2 = 66.5\%$) (Figure 2).

3.2.2. The Effect of Aromatherapy on Physical Symptoms of PMS. All eight studies investigated the impact of aromatherapy treatment on physical symptoms of PMS [14–16, 22–26]. This meta-analysis found that aromatherapy treatment significantly reduces physical symptoms of PMS (WMD–1.28; 95% CI (−2.75, 0.19), $I^2 = 94.6\%$) as well as fatigue from PMS (WMD–1.44; 95% CI (−2.44, −0.44), $I^2 = 89.7\%$) based on the random effects model (Figure 3).

3.2.3. The Effect of Aromatherapy on the Overall Score of PMS. Three of the studies examined the impact of aromatherapy on the overall score of PMS [14, 15, 22]. This meta-analysis found that aromatherapy also decreased the severity of PMS in the intervention group compared to the control group (WMD–13.83; 95% CI (−22.04, −5.63), $I^2 = 94.5\%$) (Figure 4).

3.2.4. Subgroup Analyses. Subgroup analyses were conducted based on predefined factors such as symptom, sample size, duration of treatment, study design, and outcome assessment tool. The duration of treatment and outcome assessment tools were identified as the sources of heterogeneity regarding the psychological symptoms of PMS. However, none of these factors were found to be sources of heterogeneity regarding the physical symptoms of PMS. Furthermore, aromatherapy had a more favorable effect on psychological symptoms of PMS and physical symptoms of PMS in studies with a crossover design, studies which used the ACOG questionnaire for PMS measurement, studies with higher duration, and studies with smaller sample sizes ($P < 0.001$ for all) (Table 3).

3.2.5. Sensitivity Analyses. By omitting each study and reanalyzing the data, we found that none of the studies affect the significance of the summary effect size of aromatherapy on psychological symptoms of PMS (Supplementary Figure 1), physical symptoms of PMS (Supplementary Figure 2), or the overall score of PMS (Supplementary Figure 3).

3.3. Publication Bias. The funnel plot and Egger test ($P = 0.039$) identified publication bias regarding the effect of aromatherapy on psychological symptoms of PMS (Supplementary Figure 4). Therefore, we performed Tweedie's trim and fill to adjust for this bias. This did not change the effect size. The funnel plot visually showed that there was no publication bias regarding physical symptoms of PMS (Supplementary Figure 5) and the overall score of PMS (Supplementary Figure 6). In addition, the Egger test also confirmed the findings regarding physical symptoms of PMS ($P = 0.22$) and the overall score of PMS ($P = 0.38$).

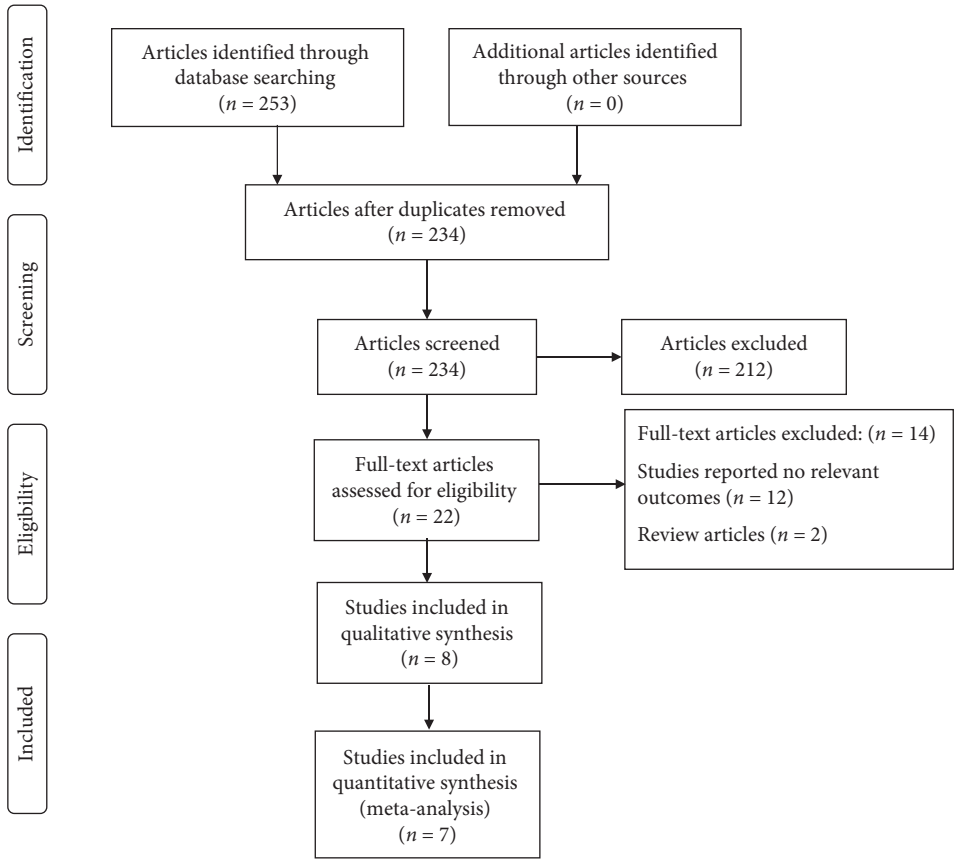

Figure 1: Flow diagram of study selection.

4. Discussion

This meta-analysis investigated the effect of aromatherapy on premenstrual syndrome symptoms in women. This study provides evidence that inhaling essential oils can alleviate the symptoms of PMS whether measured as psychological symptoms, physical symptoms, or total PMS symptom scores.

Psychological symptoms of PMS may occur following the decrease in quantity or function of serotonin, tryptophan, and estrogen level [27, 28]. A prospective, randomized controlled trial conducted on elderly persons with symptoms of depression showed an increase in 5-hydroxytryptamine (serotonin) concentrations in the aromatherapy group (lavender + sweet orange (*Citrus sinensis*) + bergamot (*Citrus bergamia*)) compared to the control group [29]. In a study conducted by Choi et al, daily inhalation of Citrus aurantium essential oil at concentrations of 0.5% for 5 days by postmenopausal women could increase the estrogen level slightly [30].

The present study found that aromatherapy could decrease the severity of psychological symptoms of PMS such as anxiety and depression, as well as fatigue as a physical symptom of PMS and the total score of PMS. Systematic review sand meta-analyses have found that aromatherapy can decrease depressive symptoms [11] and preoperative anxiety [31] in other demographic groups, which is consistent with our findings. In addition, one study found a favorable effect of aromatherapy with essential oils containing 0.5% neroli oil on ICU (intensive care unit) patients [30]. One clinical trial study showed that lavender aromatherapy could significantly decrease the mean scores of fatigue and anxiety of patients undergoing hemodialysis treatment [32]. However, one study with one session of aromatherapy with 5 min exposure did not find a significant effect on depressive symptoms on pregnant woman [33]. The insignificant result may be due to short intervention exposure time in the treatment groups.

4.1. Oil Selection. One important factor to consider when assessing the effectiveness of aromatherapy is that essential oils are plant extracts. Therefore, each batch differs with regard to the chemical volatile compounds contained within the oils used in these studies. The volatile compound of essential oils contains flavonoid and trepan chemicals such as limonene, gamma-terpinene, linalool, and linalyl acetate that have shown to act as anxiolytic antidepressants and contain sedative properties [34, 35]. Limonene, the main odorant of citrus fruits, plays a role in the stimulation of the sympathetic system and subjective alertness [36]. Linalool as a key volatile component of lavender has sedative properties through harnessing glutamate binding [37]. Gamma-Terpinene, another volatile compound found in yuzu, decreases stress by enhancing the dopamine release [38]. Another volatile component (β-caryophyllene) has been found to

Table 1: Characteristics of randomized trials on the effects of aromatherapy on the premenstrual syndrome symptoms included in the meta-analysis.

Reference	Location	Publication year	Subjects and gender	Age, y[1]	Design	Intervention type		Duration (mo)	Outcome assessment tool	Outcomes	Findings	Notes about subjects
						Intervention	Control					
(1) Heydari et al.	Iran	2016	A : 33 C : 33	A : 22.48 ± 1.76 C : 21.84 ± 1.50	Parallel	*Citrus aurantium* blossom essential oil (0.5%) (eye pad)	Odorless sweet almond oil	2 (twice a day for 5 minutes for 5 days)	PSST	(i) Score of psychological symptoms (ii) Score of physical symptoms (iii) Score of social symptoms (iv) Score of PMS	Total score of PMS and psychological symptoms decreased significantly after aromatherapy compared to the control group; however, aromatherapy had not significant effects on physical and social symptoms	66 students with moderate PMS
(2) Heydari et al	Iran	2016	A : 33 C : 31	A: 22.66 ± 3.41 C : 21.84 ± 1.50	Parallel	Essential oils of *Rosa damascena* (4%) (eye pad)	Aromatherapy with 100% sweet almond oil	2 (twice a day for 5 minutes for 5 days)	PSST	(i) Score of psychological symptoms (ii) Score of physical symptoms (iii) Score of social symptoms (iv) Score of PMS	Total score of PMS, psychological, physical, and social symptoms decreased significantly after aromatherapy compared to the control group	64 students with moderate PMS

Table 1: Continued.

Reference	Location	Publication year	Subjects and gender	Age, y[1]	Design	Intervention type		Duration (mo)	Outcome assessment tool	Outcomes	Findings	Notes about subjects
						Intervention	Control					
(3) Heydari et al	Iran	2016	A : 33 C : 33	A: 22.33 ± 2.38 C: 22.33 ± 2.38	—	*Citrus aurantium* blossom essential oil (0.5%) (eye pad) Essential oils of *Rosa damascena* (4%) (eye pad)	Sweet almond oil	2 (twice a day for 5 minutes for 5 days)	PSST	(i) Anxiety/tension (ii) Tearful/increased sensitivity to rejection (iii) Depressed mood/hopelessness (iv) Decreased interest in work activities (v) Decreased interest in home activities and decreased interest in social activities (vi) Difficulty in concentrating (vii) Feeling overwhelmed or out of control (viii) Fatigue/lack of energy (ix) Overeating/food craving (x) Insomnia (xi) Hypersomnia (xii) Physical symptoms: breast tenderness, headaches, joint/muscle pain, bloating, and weight gain (xiii) Symptom interference with work efficiency or productivity (xiv) Symptom interference with relationships with coworkers and friends (xv) Symptom interference with relationships with family (xvi) Symptom interference with social life activities (xvii) Symptom interference with home responsibilities	Psychological symptoms were significant between the three groups except for the variables of anxiety, interest in work activities, and social activities and insomnia; the aromatherapy with *Citrus aurantium* compared to placebo significantly reduced the score of symptoms such as tearful/increased sensitivity to rejection, feeling overwhelmed, or out of control; aromatherapy with *Rosa damascena* compared to placebo significantly reduced the anger/irritability, tearful/increased sensitivity to rejection, depression, hopelessness, difficulty in the concentration, and hypersomnia Rosa damascena had a significant effect on physical variables, such as fatigue and lack of energy, breast tenderness, headache, muscle and joint pain, bloating, and weight gain, but the effect of *Citrus aurantium* was not significant on any of the physical variables Aromatherapy with *Citrus aurantium* had a positive effect only on the relationship between the coworkers and friends, but *Rosa damascena* in addition to effect on this variable had a positive effect on family relationship, social life activities, and home responsibilities	66 students with moderate PMS

Table 1: Continued.

Reference	Location	Publication year	Subjects and gender	Age, y[1]	Design	Intervention type		Duration (mo)	Outcome assessment tool	Outcomes	Findings	Notes about subjects
						Intervention	Control					
(4) Lotfipur-Rafsanjani et al.	Iran	2018	A : 37 C : 38	18–29 yrs	Parallel	Geranium 2% in almond oil + massage (30 min/week)	Sweet almond oil + massage (30 min/week)	2 (30 min/week)	PMS score	(i) Psychological symptoms (ii) Physical symptoms	Aromatherapy massage decreased the PMS physical and mental symptoms significantly compared to the massage therapy	75 students with PMS
(5) Matsumoto et al.	Japan	2016	A : 9 C : 8	20.6 ± 0.2	Crossover	Fragrance from yuzu, a Japanese citrus fruit (*Citrus junos* Sieb. ex Tanaka) (diffuser)	Lavender	1 (35 min)	POMS	(i) Tension and anxiety (ii) Depression and dejection (iii) Anger and hostility (iv) Vigor (v) Fatigue (vi) Confusion (vii) High-frequency power	Tension-anxiety, anger-hostility, and fatigue improved in the aromatherapy group compared to the control group; however, other symptoms did not change significantly	17 women with moderate PMS
(6) Matsumoto et al.	Japan	2016	A : 9 C : 8	21.7 ± 0.8	Crossover	Lavender (diffuser)	Water	1 (35 min)	POMS	(i) Tension and anxiety (ii) Depression and dejection (iii) Anger and hostility (iv) Vigor (v) Fatigue (vi) Confusion (vii) High-frequency power	Depression-dejection and confusion declined significantly in the aromatherapy group; however, other symptoms did not change significantly	17 women with moderate PMS
(7) Uzuncakmak et al.	Turkey	2018	A : 40 C : 37	—	Crossover	Lavender (steam inhalation)	—	3 (5 sessions on average for each cycle)	ACOG	(i) Anxiety (ii) Depressive effect (iii) Fatigue (iv) Nervousness (v) Pain (vi) Appetite change (vii) Sleep-related changes (viii) Swelling (ix) Depressive thought (x) PMS scale	Aromatherapy improved the PMS scale and subdimensions of anxiety, depressive affect, nervousness, pain, bloating, and depressive thought mean scores compared to the control group	87 students with PMS
(8) Geethanjali et al	India	2020	A : 30 C : 30	18–35	Parallel	Clary sage (Salvia sclerae) (diffuser)	Water	1 (20 min)	PSST	(i) High-frequency power	Aromatherapy increased high-frequency power significantly compared to the control group	60 women with PMS

[1]Values of overall ranges and mean ± SDs in each group. A, aromatherapy; ACOG, American College of Obstetricians and Gynecologists; C, control; CI, confidence interval; PMS, premenstrual syndrome; POMS, Profile of Mood State; PSST, premenstrual symptoms screening tool; WMD, weighted mean difference.

Table 2: Cochrane risk of bias assessment for randomized controlled trials on the effect of sesame consumption on diabetic indices in adults.

Reference	Random sequence generation	Allocation concealment	Blinding of participants, personnel, and outcome assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Heydari et al.	L	U	L	L	L	L
Heydari al.	L	L	L	L	L	L
Heydari et al.	L	U	U	L	L	L
Lotfipur-Rafsanjani et al.	L	U	H	L	L	L
Matsumoto et al.	L	U	U	L	L	L
Matsumoto et al.	L	U	U	L	L	L
Uzuncakmak et al.	L	L	H	L	L	L
Geethanjali et al.	L	L	H	U	L	L

H, high risk of bias; L, low risk of bias; U, unclear risk of bias.

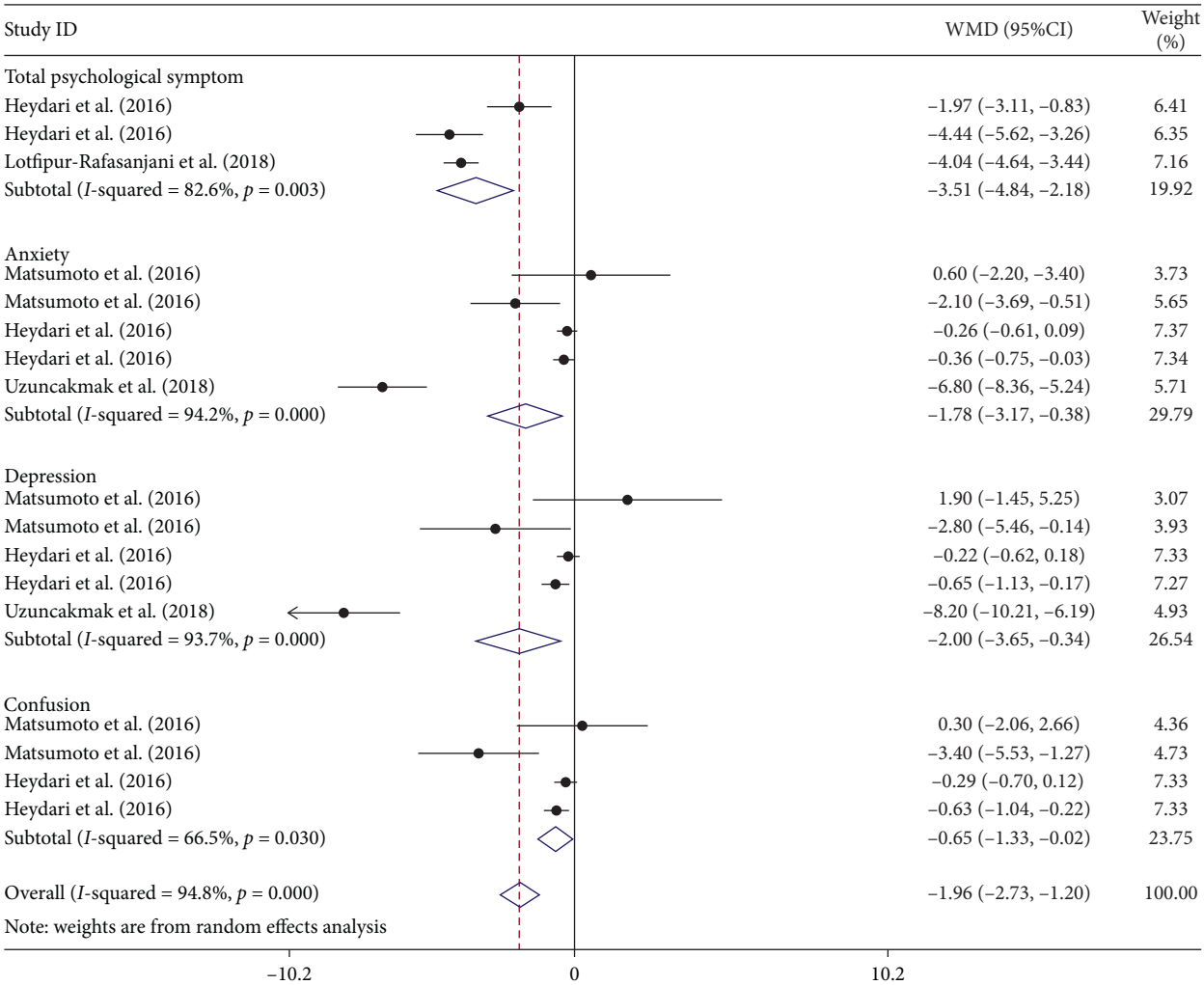

Figure 2: Forest plot showing the effects of aromatherapy on psychological symptoms of PMS (WMDs and 95% CIs) in women with PMS using the random effects model. CI, confidence interval; PMS, premenstrual syndrome; WMD, weighted mean difference.

improve psychological symptoms such as depression and anxiety [39]. A major phytoncide, α-pinene, also has alleviating effects on autonomic stress response to novel environments [40]. Lehrner et al. showed after inhalation of fragrance from orange (*Citrus sinensis*), made up of limonene (88.1%), myrcene (3.77%), and α-pinene (1.19%), female patients had lower anxiety, higher peace, and a more positive mood in a waiting room of dental office [41].

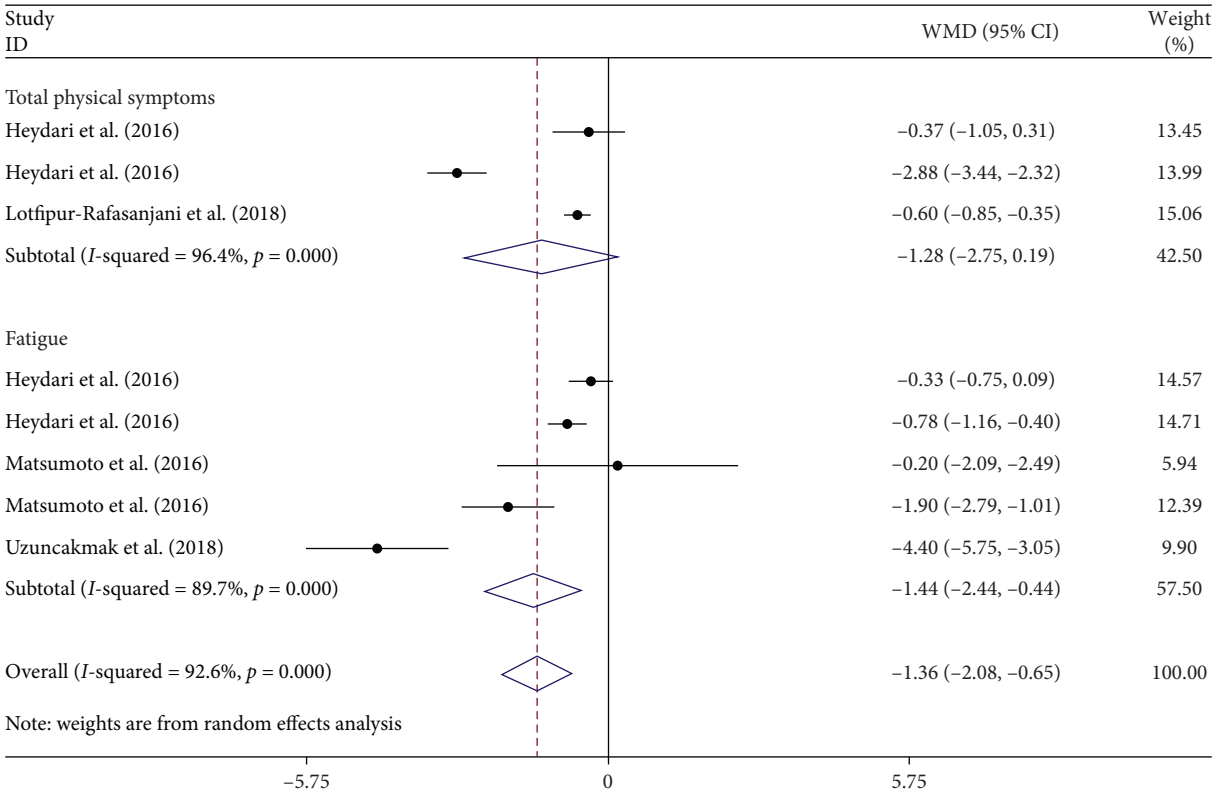

Figure 3: Forest plot showing the effects of aromatherapy on physical symptoms of PMS (WMDs and 95% CIs) in women with PMS using the random effects model. CI, confidence interval; PMS, premenstrual syndrome; WMD, weighted mean difference.

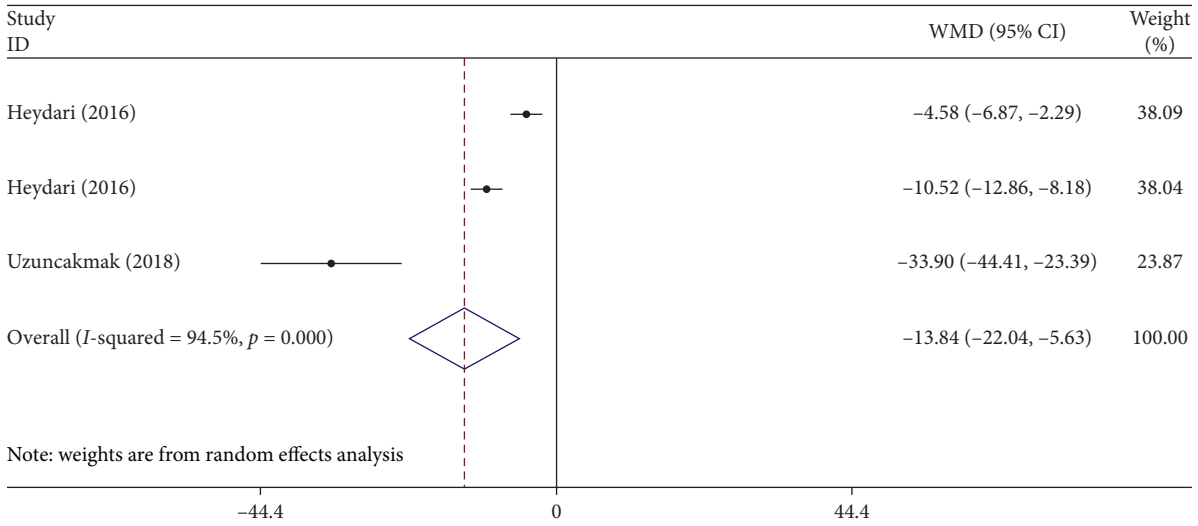

Figure 4: Forest plot showing the effects of aromatherapy on total score of PMS (WMDs and 95% CIs) in women with PMS using the random effects model. CI, confidence interval; PMS, premenstrual syndrome; WMD, weighted mean difference.

One reason for the variation in previous clinical studies on aromatherapy might be the variation in distance between the aroma and the nostrils, which affects the overall dose of inhalation. Most studies in our analysis used a diffuser or eye pads, both of which are effective methods for producing favorable effects [42].

One explanation for aromatherapy's effects on PMS is the way in which these volatile compounds affect the limbic system. The volatile odorant molecules are inhaled through the roof of the nose where cilia enable them to reach the receptor cells in the nose. When these volatile odorant molecules reach these cells, the olfactory bulb and olfactory

Table 3: Pooled estimates of the effects of aromatherapy on premenstrual syndrome symptoms within different subgroups.

	Number of trials		WMD (95% CI)		P value		P heterogeneity		I^2 (%)	
	Psychological symptoms	Physical symptoms	Psychological symptoms	Physical symptoms	Psychological symptoms	Physical symptoms	Psychological symptoms	Physical symptoms	Psychological symptoms	Physical symptoms
Total	17	8	−1.79 (−2.54, −1.04)	−1.27 (−1.96, −0.58)	<0.001	<0.001	<0.001	<0.001	96.2	92.9
PMS symptoms										
Score of psychological symptoms	3	—	−3.73 (−4.22, −3.25)	—	<0.001	—	0.003	—	82.6	—
Anxiety	5	—	−0.51 (−0.77, −0.26)	—	<0.001	—	<0.001	—	94.2	—
Depression	5	—	−0.58 (−0.88, −0.28)	—	<0.001	—	<0.001	—-	93.7	—
Confusion	4	-	−0.50 (−0.78, −0.21)	—	0.001	—	0.03	—	66.5	—
Score of physical symptoms	—	3	—	−0.90 (−1.12, −0.69)	—	<0.001	—	<0.001	—	96.4
Fatigue	—	5	—	−0.82 (−1.08, −0.56)	—	<0.001	—	<0.001	—	89.7
Study design										
Parallel	9	6	−0.73 (−0.88, −0.57)	−0.84 (−1.01, −0.67)	<0.001	<0.001	<0.001	<0.001	95.6	94.3
Crossover	8	2	−3.59 (−4.33, −2.84)	−1.62 (−2.45, −0.79)	<0.001	<0.001	<0.001	0.094	89.9	64.4
Outcome assessment tool										
PSST	8	4	−0.49 (−0.65, −0.32)	−0.95 (−1.18, −0.71)	<0.001	<0.001	<0.001	<0.001	87.3	94.7
POMS	6	2	−1.45 (−2.38, −0.52)	−1.62 (−2.45, −0.79)	<0.001	0.005	0.025	0.094	61.2	64.4
ACOG	2	1	−7.32 (−8.55, −6.09)	−4.40 (−5.74, −3.05)	<0.001	<0.001	0.28	—	14.2	—
PMS score	1	1	−4.04 (−4.63, −3.44)	−0.60 (−0.84, −0.35)	<0.001	<0.001	—	—	—	—
Duration of treatment										
<2 mo	6	2	−1.45 (−2.38, −0.52)	−1.62 (−2.45, −0.79)	0.002	0.005	0.025	0.094	61.2	64.4
=2 mo	9	5	−0.73 (−0.88, −0.57)	−0.78 (−0.95, −0.61)	<0.001	<0.001	<0.001	<0.001	95.6	93.4
>2 mo	2	1	−7.32 (−8.55, −6.09)	−4.40 (−5.74, −3.05)	<0.001	<0.001	0.28	—	14.2	—
Sample size										
<20	6	2	−1.45 (−2.38, −0.52)	−1.62 (−2.45, −0.79)	0.002	<0.001	0.025	0.098	61.2	64.4
≥20	11	6	−0.83 (−0.99, −0.68)	−0.84 (−1.01, −0.67)	<0.001	<0.001	<0.001	<0.001	96.6	95.3

ACOG, American College of Obstetricians and Gynecologists; CI, confidence interval; PMS, premenstrual syndrome; POMS, Profile of Mood State; PSST, premenstrual symptoms screening tool; WMD, weighted mean difference.

tract transmit an electrochemical impulse to the primary olfactory areas in the brain that are contained in the hypothalamus, hippocampus, and the limbic system. These systems are responsible for controlling autonomic homeostasis, managing conscious thought processes, and creating emotional feelings, respectively [37].

4.2. Limitations. The small number of studies that examined symptoms of PMS is a primary limitation of this study. Publication bias in the studies that examined the impact of aromatherapy on PMS scores produces another limitation although publication bias should be interpreted with caution given the small sample size. Furthermore, the heterogeneity among studies is high. The high amount of heterogeneity among studies may be due to the differences in duration of the treatment session, the total number of sessions, frequency of the treatment, forms of essential oils, different volatile compounds, and outcome assessment tool. In addition, three out of the 8 studies included in the systematic review were only found to be of fair quality.

5. Conclusion

The meta-analysis provides evidence that aromatherapy reduces overall symptom scores and both physical and psychological symptoms of PMS. To reproduce these results, a pretest is recommended before using aromatherapy, ensuring that participants have healthy olfactory function and do not experience negative responses to the oils selected. In addition, an increase in inhalation time and a higher number of sessions should be considered for future aromatherapy treatments.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Malihe Tabarrai and Somayeh Es-haghee conceptualized and designed the study. Somayeh Es-haghee, Fatemeh Shabani, and Marzieh Qaraaty drafted the manuscript and collected the data. Mohammad Ali Zareian analyzed the data. Malihe Tabarrai, Fatemeh Shabani, and Somayeh Es-haghee reviewed the protocol for important intellectual contents. Jessie Hawkins, as a native, did English language editing. All authors read and approved the final manuscript.

Supplementary Materials

Supplementary Figure 1: analysis of the influence of aromatherapy on psychological symptoms of PMS. CI, confidence interval; PMS, premenstrual syndrome. Supplementary Figure 2: analysis of the influence of aromatherapy on physical symptoms of PMS. CI, confidence interval; PMS, premenstrual syndrome. Supplementary Figure 3: analysis of the influence of aromatherapy on total score of PMS. CI, confidence interval; PMS, premenstrual syndrome. Supplementary Figure 4: funnel plot for assessing publication bias in the studies reporting the effects of aromatherapy on psychological symptoms of PMS. PMS, premenstrual syndrome; SE, standard error; WMD, weighted mean difference. Supplementary Figure 5: funnel plot for assessing publication bias in the studies reporting the effects of aromatherapy on physical symptoms of PMS. PMS, premenstrual syndrome; SE, standard error; WMD, weighted mean difference. Supplementary Figure 6: funnel plot for assessing publication bias in the studies reporting the effects of aromatherapy on total score of PMS. PMS, premenstrual syndrome; SE, standard error; WMD, weighted mean difference. (*Supplementary Materials*)

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