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Guest Editors: Xingjiang Xiong, Chun-Tao Che, Francesca Borrelli, Kamal D. Moudgil, and Giuseppe Caminiti

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Editorial Board

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Editorial

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In December 2011, 5 years ago, the *Nature* released a special issue on "Traditional Asian Medicine" (http://www.nature.com/nature/outlook/asian_medicine/) [1]. This issue marked a renaissance of interest in the field of complementary and alternative medicine. Three years later, the *Science* published its special issues on "The Art and Science of Traditional Medicine. Part 1: TCM Today—A Case for Integration; Part 2: Multidisciplinary Approaches for Studying Traditional Medicine; and Part 3: The Global Impact of Traditional Medicine." This convergence is not occasional. From the new WHO traditional medicine strategy (2014–2023) to the application of systems biology in studying traditional medicine, a new clinical trend of integrating traditional medicine into modern health care has been gradually formed [2].

Traditional Asian medicine (TAM), a system of ancient medical practice including traditional Chinese medicine, Kampo, traditional Korean medicine, and Ayurveda, is widely used in Asian countries [3]. It is gaining increasing popularity in western countries [4]. TAM classic herbal formula, one of the important therapeutic modalities of TAM, has both unique theories and rich experience in the past thousands of years and is still being widely applied in modern clinic. It is defined as a formula that has been recorded in ancient classic medical books with fixed herbal drug composition, definite curative effect, and fewer adverse reactions for certain diseases.

From the past to the present, TAM classic herbal formula has made countless contribution to well-being and public illness (cardiovascular diseases, diabetes, etc.) [5]. It has provided new strategies for the treatment of many complex, refractory illnesses. Currently, more and more evidences showing the efficacy from clinic provide us further understanding of the biological functions and potential mechanisms of TAM formula, including Maxingshigan-Yinqiaosan for H1N1 influenza virus [6], Gegen qinlian decoction for type 2 diabetes mellitus [7], Liujunzi decoction for chronic dyspepsia [8], and Hemp Seed pill for functional constipation [9]. On the other hand, integrative medicine, which combined traditional medicine with conventional western medicine treatment, is not only an innovative medical model in clinical practice, but also the bridge for traditional medicine toward the medical sciences [10]. Previous studies have also identified that TAM classic herbal formula as

adjunctive therapy could exhibit synergetic effect with each other [11–13]. Additionally, due to the limitations of "more investments, less drugs" challenge in drug discovery and development, scientists have turned their attention to the natural herbal medicines [14–16]. It is a current trend that TAM classic herbal formula has provided new promising drugs and candidates recently.

However, there are still numerous limitations in the current use and research of TAM classic herbal formula. Due to the difficulties in retrieving and learning original literatures in Chinese, lacking high level of evidence for clinical recommendation and the data of toxicology, adverse effects, potential herb-drug interaction, and pharmacological mechanism, quite a number of TAM classic herbal formulas are still recognized as a "mystery" to the science world. Accordingly, the role of these classic herbal formulas still needs more scientific and clinical data to verify their effectiveness and safety.

This special issue aims to summarize the current clinical and experimental progress of promising TAM classic herbal formulas and their active ingredients for various diseases. Altogether, 34 papers were gathered for publication, out of which 11 articles were accepted. The original research papers as well as comprehensive review articles on TAM classic herbal formula cover a wide range of topics, including clinical trial, systematic review, experimental study, and perspective.

One clinical trial addressed the clinical indications of two TAM classic herbal formulas including Danggui Shaoyao powder (tokishakuyakusan) and Guizhi Fuling pill (keishibukuryogan) for patients with dysmenorrhea. The paper titled "The difference between the Two representative Kampo Formulas for Treating Dysmenorrhea: An Observational Study" proposed models for predicting the use of these two formulas. The indications of Danggui Shaoyao powder included lightheadedness, BMI < 18.5, and a weak abdomen. The indications of Guizhi Fuling pill included tendency to sweat, heat intolerance, leg numbness, a cold sensation in the lower back, a strong abdomen, and paraumbilical tenderness and resistance. Four systematic reviews and meta-analyses summarized the efficacy and safety of some TAM classic herbal formulas, including Liuwei Dihuang pills for diabetic nephropathy, Huangqi jianzhong decoction for chronic gastritis, and other Chinese herbal formulas for breast cancer. Five experimental studies explored the pharmacological mechanism of TAM classic herbal formula and some active ingredients either in vivo or in vitro, including Sheng Mai powder on right ventricular dysfunction during chronic intermittent hypoxia, Yi Gong powder on iron homeostasis in acute inflammation, and other herbs and formulas for diabetes and tumor. Additionally, the role of *Nigella sativa* and its active constituents in learning and memory was also reviewed.

Recently, tremendous support and encouragement for research and development of TAM classic herbal formulas has been made either in policy or funding by the Chinese government and other Asian countries. Although skepticism and mysteriousness are still labeled by some physicians and patients, significant progress focusing on the effectiveness and safety of partial classic herbal formulas has been achieved. Unlike the classic pattern of "from bench to bedside" in translational medicine, it represents a new innovative research mode of "from bedside to bench to bedside." This special issue presented the updated knowledge of partially used TAM classic herbal formulas. Accordingly, we hope that more evidence-based approaches on studying TAM classic herbal formula will be provided to lifting the mysterious veil.

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Research Article

Antitumor Effect of Zhihuang Fuzheng Soft Capsules on Tumor-Bearing Mice

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Chinese medicines (CMs) have been shown to have some advantages in preventing and controlling tumors. In this study, we investigated the antitumor effect of ZFSC by establishing a mouse model of HT-1080, A-549, and HCT-8 tumors. The result showed that tumor volumes of HT-1080 tumor-bearing nude mice in ZFSC low, medium, and high dose groups were lower significantly compared to the model group, and the high dose ZFSC showed the best antitumor effect. Tumor volumes of A-549 tumor-bearing nude mice in ZFSC low, medium, and high dose groups were lower significantly compared to the model group and showed a good dose-response relationship. There was no significant effect on human colon cancer, although inhibition trends disappeared in the bar chart. In order to verify the immunomodulatory effect of ZFSC, ELISA was used to analyze serums IL-2, TNF-α, and IFN in spleens. The results showed that ZFSC could enhance the immune function of tumor-bearing mice. ZFSC reduced IFN-γ and TNF-α content in the serum of HT-1080 tumor-bearing mice and inhibit PD1 and PDL1 and suggested that the antitumor mechanism of ZFSC on human fibrosarcoma could be attributed to inhibition of the PDL1/PD1 pathway.

1. Introduction

Some traditional Chinese prescriptions have been identified as effective antitumor drugs in patients with cancer. Among the existing antitumor drugs, Chinese medicines (CMs) have many advantages, such as multitarget, multichannel, wide antitumor spectrum, low toxicity and side effects, long survival time, pain reduction, and high quality of life.

CMs have been shown to have some advantages in preventing and controlling tumors [1]. For example, artemisinin can selectively induce apoptosis in pancreatic tumor cells [2]. Furthermore, Fuzheng Yiliu Decoction inhibited HepG2 cells *in vitro* [3]. Finally, Jianpi Jiedu Recipe was found to affect reversion of P-glycoprotein-mediated multidrug resistance through the COX-2 pathway in colorectal cancer [4].

In tumors, the expression of programmed death ligand 1 (PDL1) on the cell surface interacts with its receptor, programmed death 1 (PD1) on T cells, which leads to the apoptosis of tumor antigen-specific T cells. This is the main mechanism of PDL1/PD1 signal-mediated tumor immune escape [5]. PDL1 is expressed in many tumor-associated antigen presenting cells (APCs) and tumor cells and can inhibit T cell proliferation. Negative regulation of PDL1/PD1 could reduce release of T cells inflammatory factors such as IL-2, IFN-γ, and TNF-α [6]. Blocking PDL1/PD1 signaling could be used in immunotherapy of human tumors. Iwai et al. found that an anti-PDL1 mAb could inhibit local tumor growth in PDL1-P815 tumor-bearing mice with an effective remission rate [7]. Hence, PDL1/PD1 signaling pathway is expected to become a new strategy for tumor immunotherapy.

Zhihuang Fuzheng Soft Capsule (ZFSC) is composed of *Ganoderma* spore oil, *Ganoderma* extract, *Phellinus igniarius* extract, and *Panax notoginseng saponins*. *Ganoderma* and *Phellinus igniarius* are CMs that may enhance the immune system to help fight cancer [8, 9]. *Panax notoginseng saponin* is CM that may be called Huoxue Quyu. In this study, we investigated the antitumor effects of ZFSC.

2. Methods

2.1. Component and Production Process of Zhihuang Fuzheng Soft Capsules. Zhihuang Fuzheng Soft Capsules (ZFSC) were provided by the Third Hospital of Beijing Armed Police

Corps (Beijing, China). ZFSC is composed of *Ganoderma* spore oil, *Ganoderma* extract, *Phellinus igniarius* extract, and *Panax notoginseng saponins*. According to the clinical dosage of 3.0 g/60 kg/day, the equivalent dosage for mice was set at 550 mg/kg/day. In this study, we used 275, 550, and 1100 mg/kg/day.

2.2. Positive Drug. Zhenqi Fuzheng Particles (positive control drugs) were produced by Gansu Fuzheng Pharmaceutical Technology Co., Ltd. Zhenqi Fuzheng Particles may enhance the immune system to help fight cancer. According to the clinical dosage of 30 g/60 kg/day, the equivalent dosage of mice was set at 5.5 g/kg/day.

2.3. Experimental Animals. Male and female BALB/c-nude mice (SPF-class, 18–20 g) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals of China Academy of Traditional Chinese Medicine.

2.4. Cell Lines Culture and Reagents. Human fibrosarcoma HT-1080 cells, human lung adenocarcinoma A-549 cells, and human colon cancer HCT-8 cells were purchased from the Peking Union Cell Line Resource Center. HT-1080 cells were cultured in Minimum Essential Medium (MEM, HyClone, USA) containing 10% (v/v) fetal bovine serum (FBS, Gibco, USA). A-549 and HCT-8 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, HyClone, USA) containing 10% (v/v) fetal bovine serum (FBS, Gibco, USA). All cells were cultured in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

2.5. Model of Tumor-Bearing Mice. Cell lines were digested with Trypsin-EDTA (0.25%, Gibco, USA), the supernatant was discarded after centrifugation (1000 rpm), and the cells were resuspended in culture medium. Cells were diluted to approximately 1.5×10^7 cells/mL, and 0.4 mL HT-1080, A-549, or HCT-8 cell suspension was injected into the right armpit of each nude mouse (three mice for each tumor). When the average size of the tumors was 1000 mm^3, they were homogenized in physiological saline (0.25 g/mL) and reinjected into three more mice. Tumors were passaged at least three times *in vivo*.

The fourth generation of tumors was injected into 40 nude mice, and mice were randomly divided into five groups: model control group, positive drug control group, 275 mg/kg ZFSC group, 550 mg/kg ZFSC group, and 1100 mg/kg ZFSC group. Mice were given treatments orally for 2 weeks starting 1 day after inoculation. The model control group was given distilled water.

2.6. Measurement of Tumor Volume. Tumor growth was observed daily and tumor size was measured once every other day with calipers as soon as the visible tumor appeared. Tumor volume was calculated with the following formula:

$$\text{Tumor volume (TV)} = \frac{1}{2} \times ab^2. \quad (1)$$

In this formula, "a" represents the longest diameter of tumor and "b" represents the shortest diameter of tumor.

2.7. Measurement of Tumor Inhibition Rate. After the last administration, blood was collected from the eye vein and centrifuged to obtain serums. Tumors were weighted and spleens were removed and stored at −80°C until use. The tumor inhibition rate was calculated with the following formula:

$$\text{Tumor inhibition rate (\%)} = \frac{\text{tumor weight (model)} - \text{tumor weight (drug)}}{\text{tumor weight (model)}} \times 100\%. \quad (2)$$

2.8. ELISA. IL-2, IFN-γ, and TNF-α content in serum were analyzed with ELISA kits according to the manufacturer's instructions. Spleens homogenates were prepared with phosphate buffer solution (PBS, HyClone, USA), and CD4/CD8 value, NK cell activity, and PD1 and PDL1 content in spleens were analyzed with ELISA according to the manufacturer's instructions. The double-antibody sandwich method was used to analyze the NK cell activity. Briefly, a microplate was coated with a mouse NK antibody, and then spleen cell homogenates and HRP-labeled NK cell antibodies were successively added to the microplate. Finally, the substrate 3,3′,5,5′-Tetramethylbenzidine (TMB) was added to the microplate and a change in color was observed. Absorbance (OD value) was determined at 450 nm. The depth and yellow color were positively correlated with NK cell activity.

2.9. Statistical Analysis. All data are expressed as the mean ± standard deviation (SD). ANOVA was used for the determination of statistical significance ($p < 0.05$). For the pairwise comparison of groups, the LSD test (homogeneity of variance) or Dunnett's $t3$ test (heterogeneity of variance) was applied.

3. Results

3.1. Inhibitory Effect of ZFSC on Human Fibrosarcoma, Human Lung Adenocarcinoma, and Human Colon Cancer. ZFSC could significantly inhibit the growth of human fibrosarcoma and human lung adenocarcinoma cells ($p < 0.05$), but not human colon cancer cells ($p > 0.05$). Dosages of 275 mg/kg ZFSC, 550 mg/kg ZFSC, and 1100 mg/kg ZFSC could significantly inhibit human fibrosarcoma from the 8th to the 12th day, while the inhibition of 1100 mg/kg ZFSC could continue to the 14th day. The tumor inhibition rates of the three dosages were 36.3%, 20.4%, and 40.2%, respectively (Table 1, Figure 1). The inhibitory effects of 1100 mg/kg ZFSC on human lung adenocarcinoma were observed on the 9th and 13th day, the inhibitory effects of 550 mg/kg ZFSC on human lung adenocarcinoma were observed on the 11th and 13th day, and the inhibitory effects of 275 mg/kg ZFSC on human lung adenocarcinoma were observed on the 13th day. The tumor inhibition rates of the three dosages were

Table 1: Inhibitory effect of ZFSC on the growth of human fibrosarcoma.

Groups	Dose (mg/kg)	Number of animals	Tumor value (mm^3)					Tumor inhibition rate (%)
			The 6th day	The 8th day	The 10th day	The 12th day	The 14th day	
Model	—	8	58.96 ± 56.48	261.84 ± 71.78	827.13 ± 71.78	1777.54 ± 345.03	2738.46 ± 794.41	—
Positive drug	5500	8	24.89 ± 14.87	153.03 ± 82.35**	564.50 ± 151.06**	1650.73 ± 583.19	2736.53 ± 816.29	−3.6
ZFSC	275	8	42.36 ± 18.35	102.21 ± 49.28**	420.92 ± 159.71**	1034.22 ± 357.48**	1632.26 ± 566.55	36.3
	550	8	23.67 ± 12.29	117.76 ± 49.53**	505.40 ± 167.00**	1249.96 ± 355.70*	1899.87 ± 494.55	20.4
	1100	8	33.91 ± 17.82	104.48 ± 60.49**	305.81 ± 135.94**	1005.37 ± 247.63**	1430.07 ± 260.28*	40.2

Notes: * $p < 0.05$ and ** $p < 0.01$, compared with the model group.

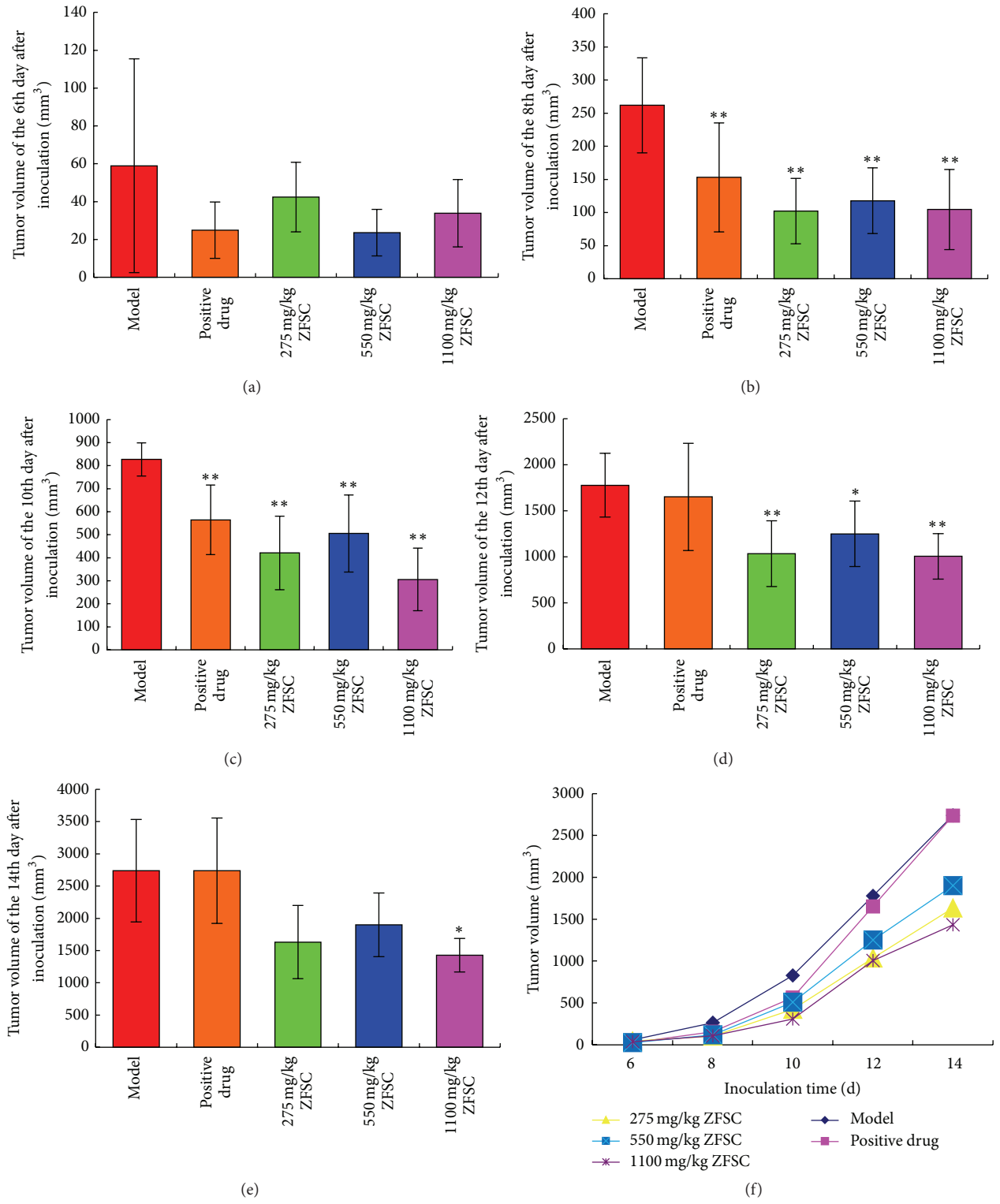

FIGURE 1: Tumors value of HT-1080 tumor-bearing mice. Notes: (a)–(e), respectively, present the tumors value of the 6th, 8th, 10th, 12th, and 14th day after inoculation, 275 mg/kg ZFSC, 550 mg/kg ZFSC, and 1100 mg/kg ZFSC could significantly inhibit human fibrosarcoma from the 8th day to the 12th day after inoculation, but only 1100 mg/kg ZFSC could significantly inhibit human fibrosarcoma at the 14th day after inoculation, and the result revealed that 1100 mg/kg ZFSC has the best inhibitory effect on human fibrosarcoma although 275 mg/kg ZFSC and 550 mg/kg ZFSC also play a role. (f) presents tumors value-time curve of each group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $n = 8$.

15.0%, 24.0%, and 28.4% (Table 2, Figure 2). There were no significant effects of 275 mg/kg ZFSC, 550 mg/kg ZFSC, or 1100 mg/kg ZFSC on human colon cancer within 15 days. The tumor inhibition rates of the three dosages were 11.8%, 17.6%, and 15.6%, respectively (Table 3, Figure 3).

Positive drug (Zhenqi Fuzheng Particles) may enhance the immune system to help fight cancer. In this study, compared with the positive control group, the inhibitory effects of ZFSC on human fibrosarcoma, human lung adenocarcinoma, and human colon cancer were better. The tumor inhibition rates of ZFSC were better compared to positive drug on the three tumors.

These results indicate that the inhibition rate of the 1100 mg/kg ZFSC dosage was the best and the antitumor effect of ZFSC on human fibrosarcoma was better than that on human lung adenocarcinoma.

3.2. Effect of ZFSC on IL-2, TNF-α, and IFN-γ Serum Content. Serum was obtained and analyzed with IL-2, TNF-α, and IFN-γ by ELISA. IL-2 content was not altered by ZFSC in HT-1080 tumor-bearing mice ($p > 0.05$), but IFN-γ content was significantly lower in the 1100 mg/kg and 550 mg/kg ZFSC groups compared with the control group ($p < 0.05$). TNF-α content was significantly lower in the 1100 mg/kg ZFSC groups compared with the control group ($p < 0.05$) (Table 4). The serum of A-549 tumor-bearing mice had significantly higher IL-2 content in each ZFSC group and IFN-γ content was significantly lower in the 275 mg/kg and 550 mg/kg ZFSC groups compared with the control group ($p < 0.05$). The TNF-α content was not significantly altered by ZFSC ($p > 0.05$) (Table 5).

3.3. Effect of ZFSC on CD4/CD8 Value, NK Cell Activity, and PD1 and PDL1 Spleen Content. Spleens were homogenized and CD4/CD8 value, NK cell activity, PD1 and PDL1 contents were analyzed with ELISA. The CD4/CD8 value and NK cell activity were not affected by ZFSC in HT-1080 tumor-bearing mice ($p > 0.05$), but PD1 and PDL1 content were significantly lower compared with the control group ($p < 0.05$) (Table 6). Therefore, the anti-HT-1080 tumor mechanism of ZFSC could be attributed to the inhibition of the PDL1/PD1 pathway. The CD4/CD8 value, NK cell activity, and PD1 and PDL1 contents in spleens of A-549 tumor-bearing mice were not changed by ZFSC administration (Table 7).

4. Discussion

Surgery, radiation therapy, and chemotherapy are the methods commonly used to treat cancer in Western medicine. Although these therapies are constantly improving, they often include side effects, such as weight loss, nausea, vomiting, hair loss, and immune dysfunction. Therefore, comprehensive treatment plays an important role in tumor treatment, and CM is a significant part of comprehensive tumor treatment. CM can decrease the toxicity of chemotherapy, radiotherapy, and target therapy, enhance the antitumor effects of these therapies, alleviate clinical symptoms, stabilize tumor size, strengthen the body constitution, increase survival, relieve complications, and regulate the immune system [10].

ZFSC is composed of *Ganoderma* spore oil, *Ganoderma* extract, *Phellinus igniarius* extract, and *Panax notoginseng saponins* and is used as an adjuvant drug in tumor therapy. ZFSC is able to inhibit tumor growth and enhance immune function in tumor patients. In this study, we investigated the antitumor effect of ZFSC by establishing a mouse model of HT-1080, A-549, and HCT-8 tumors. Tumor growth was observed daily and tumor size was measured every other day as soon as the visible tumor appeared. Throughout the observation period, tumor volumes of HT-1080 tumor-bearing nude mice in ZFSC low, medium, and high dose groups were lower significantly compared to the model group, and the high dose ZFSC showed the best antitumor effect. Meanwhile, tumor inhibition rates of ZFSC low, medium, and high dose groups were 36.3%, 20.4%, and 40.2%, respectively, which also verified the above result. Tumor volumes of A-549 tumor-bearing nude mice in ZFSC low, medium, and high dose groups were lower significantly compared to the model group and showed a good dose-response relationship. Tumor inhibition rates of ZFSC low, medium, and high dose groups were 15.0%, 24.0%, and 28.4%, respectively, which also confirmed this point. There was no significant effect on human colon cancer, although inhibition trends disappeared in the bar chart. All the above results illustrated that ZFSC have very good role in inhibition of human fibrosarcoma and human lung adenocarcinoma growth, which is likely related to favorable immunomodulatory effects of ZFSC.

In order to verify the immunomodulatory effect of ZFSC, ELISA was used to analyze serum IL-2, TNF-α, and IFN-γ and CD4/CD8 value, NK cell activity, and PD1 and PDL1 in spleens. The results showed that ZFSC could enhance the immune function of tumor-bearing mice. ZFSC reduced IFN-γ and TNF-α content in the serum of HT-1080 tumor-bearing mice and inhibit PD1 and PDL1. PDL1 is expressed in many tumor-associated antigen presenting cells (APCs) and tumor cells and can inhibit T cell proliferation. Negative regulation of PDL1/PD1 could reduce release of IFN-γ and TNF-α [6]. Apoptosis of tumor antigen-specific T cells is the main mechanism of PDL1/PD1 signal-mediated tumor immune escape. Blocking PDL1/PD1 signaling could be used in immunotherapy of human tumors. Therefore, the PDL1/PD1 signaling pathway is expected to become a new strategy for tumor immunotherapy. In this study, we found that the PD1 and PDL1 levels in spleens of HT-1080 tumor-bearing mice were significantly lower than those in the control group; meanwhile IFN-γ and TNF-α level in the serum of HT-1080 tumor-bearing mice were also significantly lower than those in the control group, which suggested that the antitumor mechanism of ZFSC on human fibrosarcoma could be attributed to inhibition of the PDL1/PD1 pathway, which could provide potential value to study the antitumor mechanism of ZFSC.

Table 2: Inhibitory effect of ZFSC on the growth of human lung adenocarcinoma.

Groups	Dose (mg/kg)	Number of animals	Tumor value (mm^3)					Tumor inhibition rate (%)
			The 7th day	The 9th day	The 11th day	The 13th day	The 15th day	
Model	—	8	308.24 ± 224.52	648.62 ± 342.61	811.49 ± 418.33	1675.41 ± 770.92	2023.00 ± 917.61	—
Positive drug	5500	8	99.14 ± 59.73	274.79 ± 198.91	517.96 ± 255.54	1166.48 ± 535.88	1787.60 ± 718.99	11.8
ZFSC	275	8	242.54 ± 214.09	628.08 ± 733.08	647.32 ± 443.00	944.05 ± 473.25*	1594.16 ± 614.72	15.0
	550	8	88.04 ± 79.69	279.67 ± 294.85	359.92 ± 325.71*	759.22 ± 496.06**	1366.88 ± 827.85	24.0
	1100	8	88.99 ± 84.81	217.70 ± 168.75*	623.39 ± 508.89	679.60 ± 294.20**	1297.82 ± 491.20	28.4

Notes: * $p < 0.05$ and ** $p < 0.01$, compared with the model group.

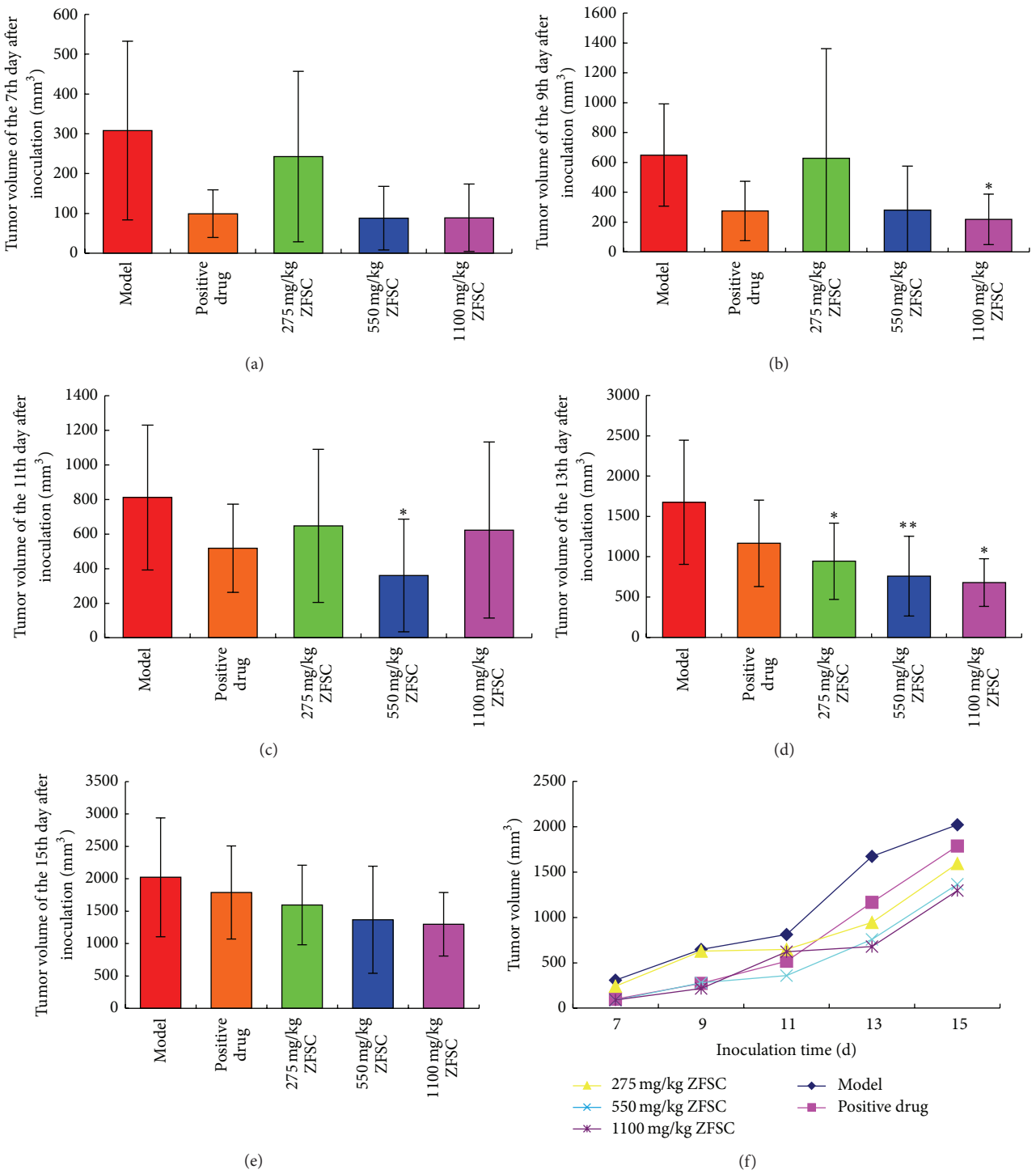

FIGURE 2: Tumors value of A-549 tumor-bearing mice. Notes: (a)–(e), respectively, present the tumors value of the 7th, 9th, 11th, 13th, and 15th day after inoculation, the significant inhibitory effect of 1100 mg/kg ZFSC on human lung adenocarcinoma appeared momently at the 9th day and the 13th day after inoculation, from the 11th day to the 13th day after inoculation 550 mg/kg ZFSC showed significant inhibitory effect on human lung adenocarcinoma, and at the 13th day after inoculation 275 mg/kg ZFSC also revealed significant inhibitory effect on human lung adenocarcinoma. The results showed that 1100 mg/kg ZFSC has better inhibitory effect on human fibrosarcoma. (f) presents tumors value-time curve of each group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $n = 8$.

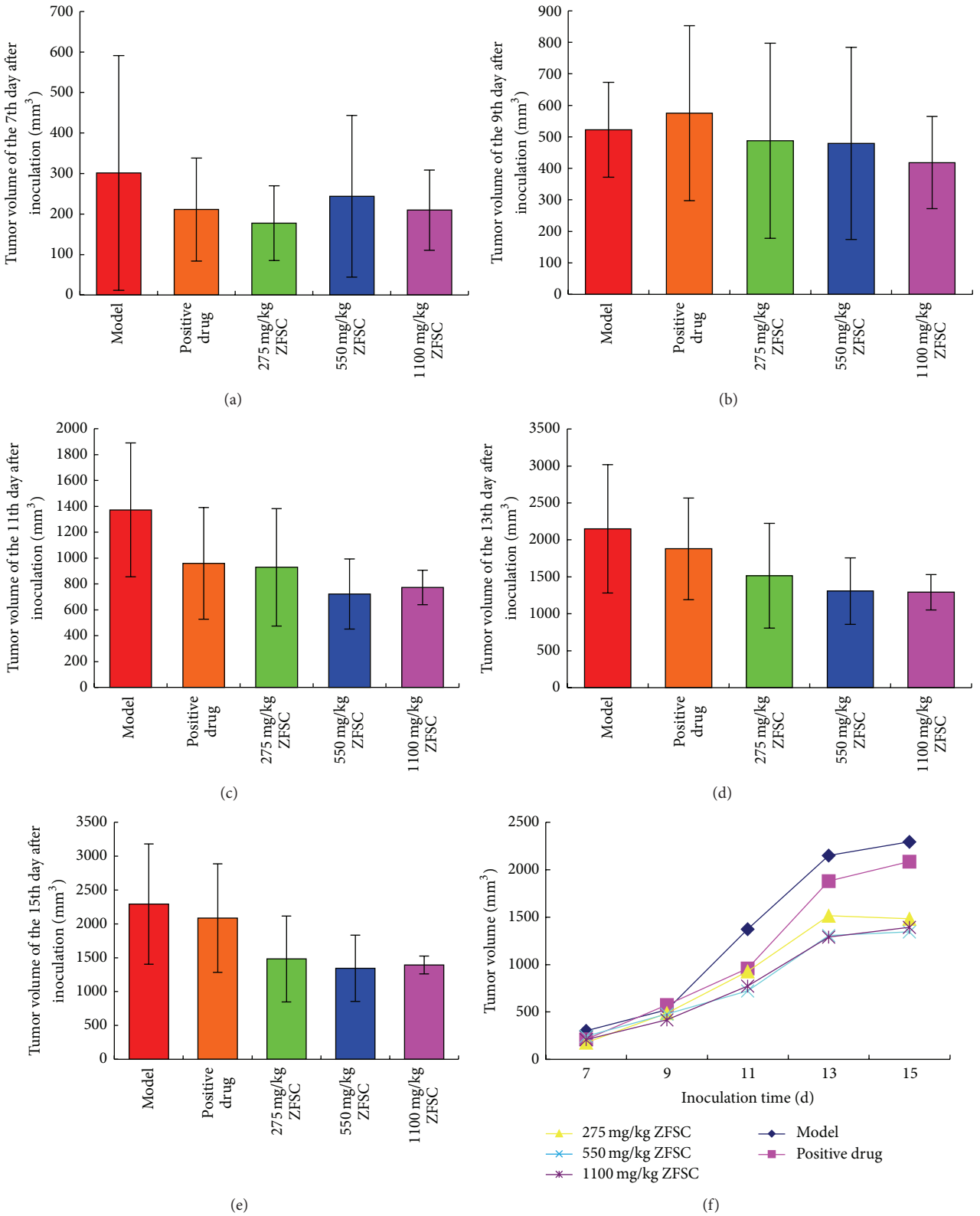

Figure 3: Tumors value of HCT-8 tumor-bearing mice. Notes: (a)–(e), respectively, present the tumors value of the 7th, 9th, 11th, 13th, and 15th day after inoculation; there were no significant effects of 275 mg/kg ZFSC, 550 mg/kg ZFSC, and 1100 mg/kg ZFSC on human colon cancer, although inhibition trends disappeared in the bar chart. (f) presents tumors value-time curve of each group. $n = 8$.

Table 3: Inhibitory effect of ZFSC on the growth of human colon cancer.

Groups	Dose (mg/kg)	Number of animals	Tumor value (mm^3)					Tumor inhibition rate (%)
			The 7th day	The 9th day	The 11th day	The 13th day	The 15th day	
Model	—	8	301.36 ± 289.54	522.50 ± 150.89	1372.86 ± 517.19	2149.74 ± 870.37	2293.42 ± 888.17	—
Positive drug	5500	8	211.20 ± 127.09	575.12 ± 277.36	959.37 ± 431.97	1880.34 ± 687.14	2086.27 ± 800.51	4.9
ZFSC	275	8	177.40 ± 92.09	487.62 ± 309.79	928.94 ± 453.68	1513.82 ± 708.16	1482.41 ± 633.38	11.8
	550	8	243.77 ± 199.55	479.30 ± 304.67	721.97 ± 271.65	1306.55 ± 448.31	1344.31 ± 491.20	17.6
	1100	8	209.68 ± 99.12	418.46 ± 146.06	773.76 ± 133.49	1292.05 ± 239.29	1392.16 ± 131.78	15.6

Table 4: Regulation of ZFSC on IL-2, TNF-α, and IFN-γ in serum of HT-1080 tumor-bearing mice.

Groups	Dose (mg/kg)	Number of animals	IL-2 (ng/L)	IFN-γ (ng/L)	TNF-α (ng/L)
Model	—	8	690.3 ± 26.7	808.4 ± 27.2	1167.5 ± 124.3
Positive drug	5500	8	704.6 ± 38.4	795.9 ± 64.2	1206.1 ± 99.8
ZFSC	275	8	737.1 ± 71.5	782.1 ± 53.1	1136.3 ± 49.8
	550	8	732.8 ± 28.9	639.0 ± 53.7**	1071.5 ± 165.6
	1100	8	737.8 ± 47.9	634.0 ± 58.7**	1023.8 ± 158.1*

Notes: * $p < 0.05$ and ** $p < 0.01$, compared with the model group.

Table 5: Regulation of ZFSC on IL-2, TNF-α, and IFN-γ in serum of A-549 tumor-bearing mice.

Groups	Dose (mg/kg)	Number of animals	IL-2 (ng/L)	IFN-γ (ng/L)	TNF-α (ng/L)
Model	—	8	709.6 ± 29.6	653.4 ± 59.9	993.6 ± 137.0
Positive drug	5500	8	755.3 ± 41.9*	635.9 ± 67.5	1014.1 ± 109.6
ZFSC	275	8	769.4 ± 39.8*	584.4 ± 24.3*	935.2 ± 34.9
	550	8	786.5 ± 40.9**	592.1 ± 43.2*	895.9 ± 70.0
	1100	8	879.6 ± 56.6**	619.0 ± 56.8	979.4 ± 99.6

Notes: * $p < 0.05$ and ** $p < 0.01$, compared with the model group.

Table 6: Effect of ZFSC on CD4/CD8 value, NK cell activity, and PD1 and PDL1 in HT-1080 tumor-bearing mice.

Groups	Dose (mg/kg)	Number of animals	CD4/CD8	NK (nmol/L)	PD1 (pg/mL)	PDL1 (ng/L)
Model	—	8	0.087 ± 0.006	116.2 ± 12.8	82.0 ± 12.6	43.3 ± 8.8
Positive drug	5500	8	0.086 ± 0.009	117.8 ± 12.3	71.2 ± 9.0	47.2 ± 8.1
ZFSC	275	8	0.093 ± 0.011	100.4 ± 11.6*	61.4 ± 9.8**	36.0 ± 5.8
	550	8	0.092 ± 0.007	100.8 ± 24.0	69.0 ± 20.2	37.5 ± 11.1
	1100	8	0.096 ± 0.010	100.8 ± 12.9	66.1 ± 12.1*	33.1 ± 5.0*

Notes: * $p < 0.05$ and ** $p < 0.01$, compared with the model group.

Table 7: Effect of ZFSC on CD4/CD8 value, NK cell activity, and PD1 and PDL1 content in A-549 tumor-bearing mice.

Groups	Dose (mg/kg)	Number of animals	CD4/CD8	NK (nmol/L)	PD1 (pg/mL)	PDL1 (ng/L)
Model	—	8	0.089 ± 0.006	79.7 ± 9.4	57.0 ± 5.4	37.6 ± 2.5
Positive drug	5500	8	0.094 ± 0.009	86.7 ± 13.7	59.9 ± 4.6	37.1 ± 2.6
ZFSC	275	8	0.089 ± 0.005	82.1 ± 8.8	60.6 ± 8.7	37.3 ± 3.0
	550	8	0.087 ± 0.010	86.2 ± 11.6	65.8 ± 10.4	36.6 ± 4.2
	1100	8	0.087 ± 0.010	84.6 ± 6.7	65.0 ± 5.9	38.4 ± 3.3

Competing Interests

The authors declare that they have no competing interests.

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Review Article

Herbal Medicine for Hot Flushes Induced by Endocrine Therapy in Women with Breast Cancer: A Systematic Review and Meta-Analysis

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Objective. This systematic review was conducted to evaluate the clinical effectiveness and safety of herbal medicine (HM) as an alternative management for hot flushes induced by endocrine therapy in breast cancer patients. *Methods.* Key English and Chinese language databases were searched from inception to July 2015. Randomized Controlled Trials (RCTs) evaluating the effects of HM on hot flushes induced by endocrine therapy in women with breast cancer were retrieved. We conducted data collection and analysis in accordance with the Cochrane Handbook for Systematic Reviews of Interventions. Statistical analysis was performed with the software (Review Manager 5.3). *Results.* 19 articles were selected from the articles retrieved, and 5 articles met the inclusion criteria for analysis. Some included individual studies showed that HM can relieve hot flushes as well as other menopausal symptoms induced by endocrine therapy among women with breast cancer and improve the quality of life. There are minor side effects related to HM which are well tolerated. *Conclusion.* Given the small number of included studies and relatively poor methodological quality, there is insufficient evidence to draw positive conclusions regarding the objective benefit of HM. Additional high quality studies are needed with more rigorous methodological approach to answer this question.

1. Introduction

As a result of increased screening and improved treatment, more women are becoming long-term survivors with breast cancer. This increasing survivorship has increased demand to improve quality of life (QoL). Endocrine therapy, is a widely used strategy in improving breast cancer survival [1]. Hot flushes are the most frequently reported adverse effects (AEs) associated with endocrine therapy in breast cancer. The symptoms are sometimes so frequent and severe that they interfere with daily activities and decrease QoL of breast cancer survivors [2, 3]. Hot flushes are described as a sudden episode of a sensation of internal heat often preceded by chills and redness of face and upper body and are often accompanied by profuse sweating and dizziness [4]. The incidence of hot flushes reported was approximately 39.9%–54% with tamoxifen, 37% with initial aromatase inhibitors (AIs) therapy, and 42%–48% with sequential AI therapy following 2-3 years of tamoxifen [5–8]. Hot flushes are most commonly experienced in the first year after commencing adjuvant therapy and gradually reduce over time following breast cancer treatment, particularly following the cessation of tamoxifen [9]. The exact etiology of hot flushes is unknown. The sudden decline in oestrogen levels [10] has one reason, but there is not sufficient evidence to explain their occurrence in breast cancer patients with endocrine

therapy. It may be also related to chemotherapy-induced ovarian disruption and another is the natural aging process which is similar to that experienced by healthy women [11].

There are many pharmacological therapies that currently treat hot flushes. It is reported that hormone replacement therapy (HRT) is considered to be effective for hot flushes; however it raised significant concern when it is used on women with breast cancer [12, 13]. Animal and in vitro models have shown that progestational agents may increase or accelerate breast cancer development; this is not thought to be an appropriate intervention [14]. Tibolone was confirmed to create a significant reduction in hot flushes and improvement of QoL in breast cancer patients but at the cost of increased risk of breast cancer recurrence [15]. In addition to clonidine and the anticonvulsant gabapentin, several selective serotonin reuptake inhibitors (SSRIs), such as venlafaxine, paroxetine, fluoxetine, and citalopram, were reported to alleviate hot flushes [16–21]. Unfortunately, using those agents is limited by their AEs profile, which includes dry mouth, decreased appetite, nausea, constipation, and drowsiness, and contributed to increased rate of participant dropout in study [22]. Furthermore, aforementioned antidepressant drugs used in conjunction with tamoxifen could affect anticancer efficacy of endocrine therapy and increase the risk of recurrence of breast cancer due to inhibition of cytochrome P450 2D6 (CYP2D6) and by reducing the active metabolite of tamoxifen-endoxifen plasma concentrations [23–25]. As a popular complementary and alternative medicine, vitamin E was reported to decrease frequency of hot flushes. This was statistically but not clinically significant [26, 27].

HMs are widely used for reduction of various AEs related to chemotherapy, radiotherapy, and endocrine therapy in breast cancer patients. HMs include herbs, herbal materials, herbal preparations, and finished herbal products, which contain parts of plants, plant materials, or combinations as active ingredients [28]. Traditional use of HMs refers to the long historical use of these medicines [28]. Their use is well established and widely acknowledged to be safe and effective. There are numerous studies investigating HMs for hot flushes and menopausal symptoms in normal women. Studies on some extracts with the isoflavones from HM were found to significantly reduce mean daily hot flush frequency compared with placebo [29]. However, there are other HM studies that failed to demonstrate a significant improvement in hot flushes symptoms [30, 31]. There are other HMs used in many countries that have potential effectiveness in decreasing hot flushes or menopausal symptoms, such as hop (*Humulus lupulus*) [32], linseed or flaxseed [33], maritime pine bark extract [34], maca (*Lepidium meyenii*) [35], and *Hypericum perforatum* L. (St. John's Wort) [36]. While these are effective in reducing hot flushes in women without breast cancer, there is no evidence that the aforementioned HMs are safe and effective treatment of hot flushes induced by endocrine therapy in breast cancer. As for Chinese herbal formulations, a RCT in a non-breast cancer setting conducted by a Dutch research team [37] used Zhi Bai Di Huang Wan versus HRT and placebo to treat menopausal symptoms. It was concluded that the formula was more effective in reducing the amount of hot flushes compared to placebo.

There is growing interest in HM for hot flushes induced by endocrine therapy in women with breast cancer; this has led to an increased number of clinical trials being performed in this area. However, specific evidence-based recommendations on the use of these herbs in breast cancer patients cannot be made. There are some reviews that have explored the effect and safety of HM for vasomotor symptoms including hot flushes and accompanying symptoms in women with early breast cancer. There are also a number of studies that have explored the effect and safety of soy and some food products [38]. Some reviews, regarding the efficacy of herbal treatments for hot flushes, have not been conducted in women with breast cancer and many are not specifically related to endocrine therapy [39]. Some reviews have had a focus on the safety of herbal medicinal products in women with breast cancer but not the effect of herbal medicines on hot flushes [40–42]. Therefore, we conducted this systematic review to determine whether HM is effective and safe for reducing hot flushes and vasomotor symptoms, induced by endocrine therapy in patients with breast cancer, and to identify the limitations of existing studies as a guide for future clinical research in this area. If shown to be effective, HM can offer an alternative intervention to those patients.

2. Methods

2.1. Search Strategy. The search of the scientific literature was performed in the English language databases including MEDLINE, PubMed, EMBASE, PsycINFO, CINAHL, and the Cochrane Central Register of Control Trials (CENTRAL) electronic databases. The search for scientific literature was also performed in Chinese language databases including China National Knowledge Infrastructure (CNKI) and Wanfang databases. We used search strategies with the following medical subject headings (MeSH): "Chinese herbal medicine", or "traditional Chinese medicine", or "herbal medicine", or "plants medicine", or "kampo medicine", and "hot flushes", or "hot flashes", or "menopausal symptoms", or "vasomotor symptoms", in combination with "breast cancer". There are limited articles included in the search MeSH as "endocrine therapy". Therefore we selected articles related to endocrine therapy after completion of the above search strategy (date last searched: 24 July 2015). Figure 1 shows the whole flowchart of article search.

2.2. Selection Criteria. We included only RCTs that tested the effectiveness of HM for hot flushes induced by endocrine therapy in female breast cancer patients. The inclusion criteria were controlled studies where women were diagnosed with breast cancer and were treated with endocrine therapy. We included any types of HM interventions managing hot flushes. Detailed preparations may be single herbs, extract ingredient from one or several herbs, and Chinese herbal formulae such as standardised formula and tailored formula. Control interventions may include placebo, conventional therapy (e.g., HRT), western medicine therapy (e.g., venlafaxine), acupuncture or other complementary therapies (e.g., yoga), and no intervention for hot flushes or other HM

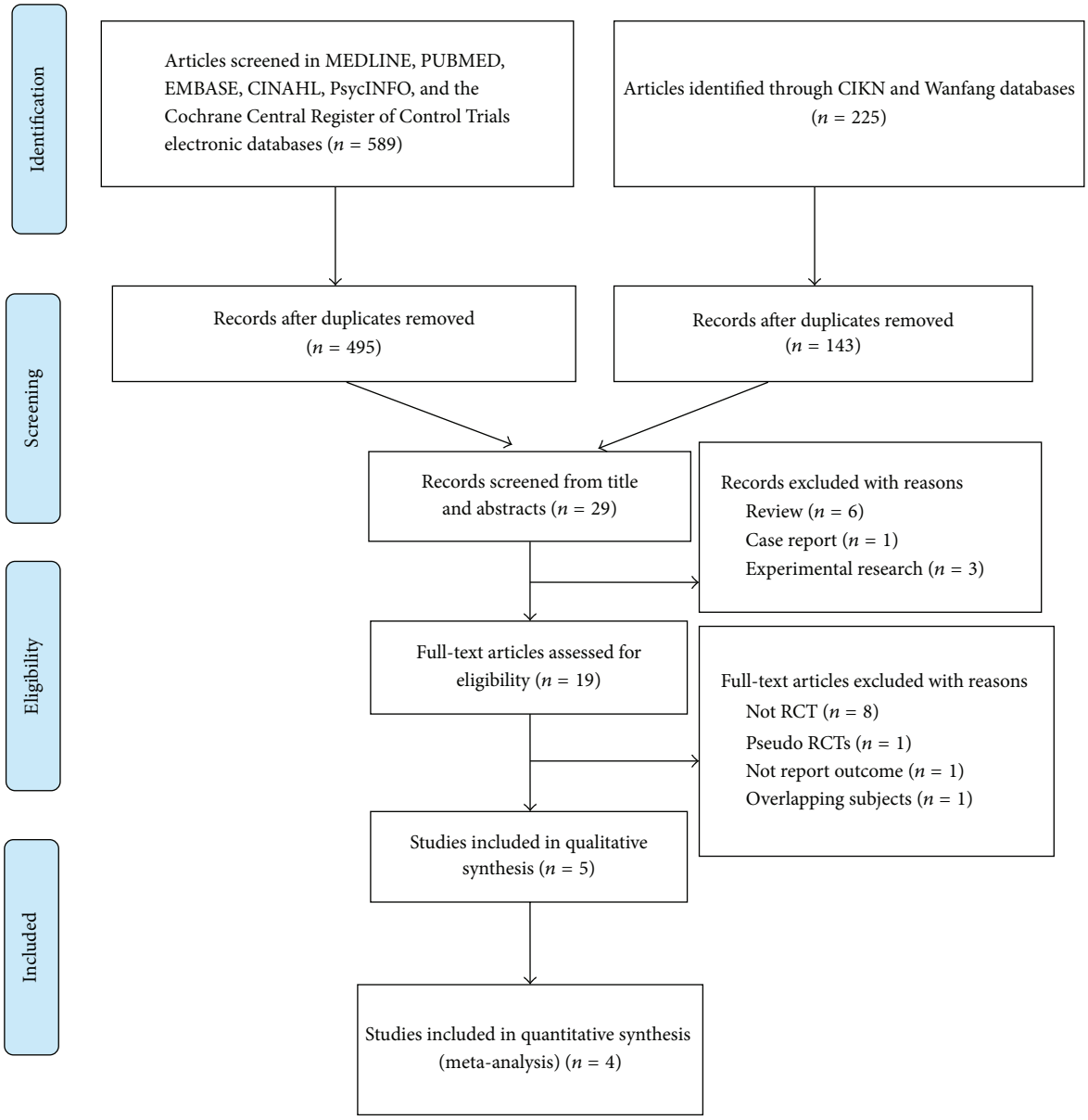

Figure 1: Flowchart of article search.

but not for hot flushes. Quasi-RCTs, case reports, studies including fewer than 10 participants, experimental studies, incomplete reports studies, and review studies were excluded. We also excluded dietary products such as soy products. Two reviewers (Yuanqing Li and Luke McPherson) independently screened articles to identify those that met the study criteria. Yuanqing Li screened title and abstract of articles found in the search and discarded trials that were clearly ineligible. Two authors (Yuanqing Li and Xiaoshu Zhu) assessed whether the trials met the inclusion criteria, with disagreements resolved by discussion. When articles contained insufficient information to make a decision about eligibility, we attempted to contact authors of the original reports to obtain further details.

2.3. Data Collection and Analysis. We conducted data collection and analysis in accordance with the Cochrane Hand book for Systematic Reviews of Interventions [48]. Two review authors (Yuanqing Li and Xiaoshu Zhu) independently extracted data using a form designed by the review authors for this purpose. For each included trial, we collected information regarding the location of the trial, methods of the trial, risk of bias, participants (age range and eligibility criteria), type of interventions, and effect of interventions. We assessed risk of bias using The Cochrane Collaboration's "Risk of Bias" tool. For each study, the seven domain-based criteria were as follows: random sequence generation, quality of allocation concealment, blinding of participants and personnel, blinding of outcome assessors, completeness of

Figure 2: Figure to demonstrate the bias with eligible studies as noted by authors. For each study, the presence (+) and absence (−) of a characteristic are recorded. If the characteristic was not clear in the trial, then it was marked as uncertain (?).

outcome data, risk of selective outcome reporting, and other potential bias. The review authors assessed each domain as at high, low, or unclear risk of bias. The following comparisons were included: ① HM versus no treatment; ② HM versus placebo; ③ HM versus active medications; ④ HM versus HT; ⑤ HM versus other complementary therapy.

2.4. Statistical Analysis. Statistical analysis was performed with the software (Review Manager 5.3) for meta-analysis. In studies that reported the exact same outcomes of continuous data, the mean difference (MD) was calculated between treatment groups. If similar outcomes were reported on different scales, the standardized mean difference (SMD) was calculated. We combined data from included studies using fixed-effects models. We presented 95% confidence intervals (CI) for all estimates. Dichotomous outcomes were analysed as per woman randomized (e.g., number of women with an adverse effect/total number of women randomized). Statistical heterogeneity among studies was evaluated using the Cochran's Q and I^2 statistics [49]. Heterogeneity was considered present for $P < 0.05$ or $I^2 \geqq 50\%$.

3. Results

3.1. Study Characteristics. A total of 814 articles were retrieved from electronic databases, 275 from PubMed, 252 from EMBASE, 36 from MEDLINE EBSCO, 17 from CINAHL EBSCO, 6 from PsycINFO EBSCO, 3 from the Cochrane Library, 143 from CNKI, and 82 from Wanfang databases. After checking duplicates, 638 articles remained. Articles not related to endocrine therapy were excluded upon review of the title and abstracts. This process left 19 articles that were eligible for the inclusion criteria. Because we did not use "endocrine therapy" as a MeSH, the difference of article number between initial search and reviewed search is significant. 7 articles that were not RTCs were further excluded after full texts were reviewed. One study was considered as Pseudo RCT. One study did not have the required information to calculate results. One article was excluded due to overlapping subjects. One study was excluded pertaining to low quality after assessment [50]. Therefore the final analysis involved five articles [43–47]. According to the bias judgement of Review Manager analysis (Figure 2), one study was high quality [46] and other studies were moderate bias risk. Only one study [46] in the included studies has a double-blind design.

The number of participants varies from 60 to 136. The total number of participants included in the studies for this analysis amounts to 397. Of the five studies, two studies evaluated HM in comparison to no treatment [43, 45], one study compared the effects of HM with placebo [46], and two studies examined the effects of HM versus HM [44, 47]. Table 1 presents basic characteristics of all five trials that compared HM preparations (monotherapy or combination therapies) with placebo (the authors, published year, age span, sample size, outcomes, and intervention method).

Table 1: Characteristics of 5 articles.

Author (year)	Country	n	Age	Comparison and sample size	Endocrine therapy	Intervention (usage, dosage, and duration)	Treatment duration	Outcomes and measurement	Design	Results
Hernández Muñoz and Pluchino 2003 [43]	Venezuela	136	35–52	(i) Intervention: *Cimicifuga racemosa* ($n = 90$) (ii) Control: no treatment group ($n = 46$)	TAM	(i) CR BNO 1055 (one tablet corresponds to 20 mg of herbal drug *Cimicifuga racemosa*), one tablet twice daily (bid), 12 months (ii) No treatment	12 months	(i) Frequency & intensity of hot flushes measured by a diary and menopausal symptoms (ii) Questionnaire (iii) AEs measured by a self-report of events	Two-armed, randomised, and open study	(i) Frequency and severity of hot flushes were reduced after intervention (ii) Minor AEs events were reported
Jiang et al. 2012 [44]	China	60	46.60 ± 5.51 versus 44.57 ± 6.99	(i) Intervention: CHM Yishen Tiaogan Decoction ($n = 30$) (ii) Control: CHM Gengnianan ($n = 30$)	Not mentioned	(i) kidney-reinforcing and liver-regulating formula bid (containing Shendi, Shanyao, Shanyurou, Danpi, Baihe, Baishao, Yujin, Foshou, Fuxiaomai, Muli, Nvzhenzi, Gancao, and Hanliancao) (ii) Gengnian An capsule 0.3 g tid (containing Shenshudi, Zexie, Maidong, Yuanshen, Fuxiaomai, Danpi, Fuling, Zhenzhumu, Xianmao, Wuweizi, Cishi, Shouwuteng, Gouteng, and Zhishouwu)	8 weeks	(i) Overall menopausal symptoms scores measured by reduction rate of Kupperman Index (KI) scores (ii) Successful rate (iii) Quality of life (QoL) measured by Karnofsky scores (KPS) (iv) Hormone profile measured by hormone levels in serum (v) AEs measured by full blood counts and liver and renal functions in serum and ECG	Two-armed, randomised, and open study	(i) The main clinical symptoms were ameliorated after treatment, especially (ii) The hectic fever and sweating and irritability
Li and Zuo 2009 [45]	China	64	35.23 ± 5.33 versus 36.86 ± 4.98	(i) Intervention: CHM Zhibo Dihuang Wan ($n = 32$) (ii) Control: no treatment ($n = 32$)	TAM	(i) Zhibai Dihuang Wan (containing Zhimu, Huangbai, Dihuang, Shanyurou, Danpi, Fuling, Zexie, and Shanyao) 8 wan (3 g) tid (ii) No treatment	2 months	(i) Overall menopausal symptoms scores measured by reduction rate of KI (ii) Hormone profile measured by E2 and FSH in serum (iii) Endometrium thickness measured by ultrasound scan (iv) AEs measured by liver and renal functions, urine routine test, and full blood counts	Randomised single-blind study	(i) Improvements on flush, perspiration, insomnia, fatigue, and irritation, without obvious side effects

Table 1: Continued.

Author (year)	Country	n	Age	Comparison and sample size	Endocrine therapy	Intervention (usage, dosage, and duration)	Treatment duration	Outcomes and measurement	Design	Results
Sun et al. 2009 [46]	China	73	45.9 ± 5.1 versus 46.4 ± 4.1	(i) Intervention: CHM Shugan Liangxue Decoction (n = 37) (ii) Control: placebo (n = 36)	TAM	(i) Shugan-Liangxue compound (containing Chaihu, Danpi, Baiwei, Baishao, Wuweizi, etc.) 100 mL daily (ii) Placebo (Shanzha, bitter flavor)	3 weeks	(i) Frequency and severity of hot flushes measured by KI (ii) Sleep quality measured by a self-reported diary (iii) AEs measured by a self-report, full blood counts, liver and renal function, and hormone levels	Double-blind, randomised placebo-controlled study	(i) Effective in alleviating hot flashes and mi proving the condition of sleep
Zhang et al. 2000 [47]	China	60	35–70	(i) Intervention: CHM Ruxian 1# (n = 30) (ii) Control: CHM Ruxian 2# (n = 30)	TAM	(i) Formula based on principle of regulating liver Qi and tonifying kidney Yin, combined with formula based on principle of clearing heat and toxin (ii) Formula based on principle of clearing heat and toxin	30 days	(i) Hot flushes and other menopausal symptoms measured by indefinable tool (ii) QoL measured by KPS	Randomised, parallel study	(i) Symptoms such as flush, insomnia, night sweat, palpitation, depression, and heat sensation in the chest, palms, and soles were improved and QoL changed significantly

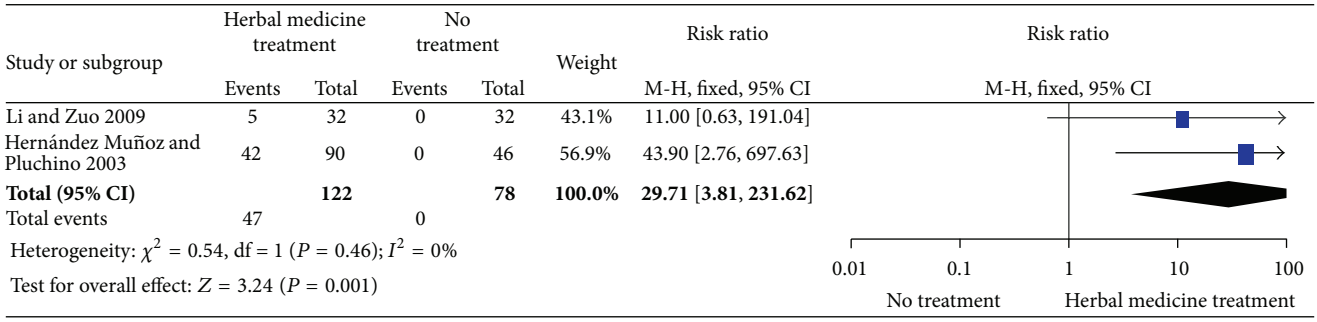

Figure 3: Effect of HM and no treatment on risk ratio in those free of menopausal symptoms. Forest plot includes only two studies.

3.2. Summary Analysis of the Included Studies. There are a total of five studies being analysed. The outcomes of analysis for the primary and secondary endpoints only involved the following 3 categories.

3.2.1. HM versus No Treatment. This analysis includes two studies, of which the results are concerned with the issue of overall scores of menopausal symptoms.

In the study by Li and Zuo [45], the change of overall scores of menopausal symptoms showed significant difference in the Kupperman Index (KI), which is used worldwide to investigate menopausal symptoms including hot flushes. Compared with control group, MD is 13.36 [95% CI 9.37–17.35]. After 2 months the scale of KI decreased from an initial 43.41 ± 7.369 to 30.43 ± 8.905 in the treatment group and from 43.82 ± 7.222 to 43.79 ± 7.32 in the control group ($P < 0.01$). The baselines showed no significant differences.

In the study by Hernández Muñoz and Pluchino [43], evaluation of menopausal symptoms was assessed with frequency of hot flushes episodes combined with sweating and sleep disturbance. Hot flushes were considered severe when five or more sudden episodes of heat are experienced during the day, accompanied by sweating, sleep disturbances, and feeling of irritation and anxiety. Less than five episodes of heat with discrete sweating were classified as moderate hot flushes. The difference between values of numbers of hot flushes was not significant at 6 months, either for severe or for moderate hot flushes (5–9% decline; $P = 0.71$), but they were significantly different at the end of the study (after 12 months). Among the 90 study participants included in the intervention group 46.7% were free of hot flushes while none were free among the usual-care group. Severe symptoms cases were 24.4% versus 73.9% with odds ratio (OR) 0.11 [0.05, 0.26].

The dichotomous data in these two studies assessing those free of menopausal symptoms were suitable for meta-analysis. There was a difference between HM group and the no treatment group, in favour of the HM group (RR 29.71; 95% CI 3.81–231.62; 2 RCTs, 200 women), with no heterogeneity ($I^2 = 0\%$), Figure 3.

Adverse Events. There were no significant AEs in one study [45]. In another study [43], eleven minor AEs occurred: seven in the usual-care group and four in the intervention group. No serious events were reported. One study [45] reported that there was no significant difference between two groups in hormone levels with OR 1.02 [95% CI 0.46–2.24]. This study also compared the thickness of endometrium, which showed increase in both groups, but there was no significant difference ($P = 0.14$) with MD 0.80 [95% CI 0.57, 1.03].

3.2.2. HM versus Placebo. Only one study was included. The study of Sun et al. [46] reported decrease of the frequency of hot flushes in two groups; OR for traditional Chinese medicine (TCM) called Shugan-Liangxue Compound versus placebo was 3.12 [95% CI 1.13, 8.60]. In TCM group, 15.2% were free of hot flushes and 42.4% showed no change, while no participant was free of hot flushes and 69.7% showed no change in placebo group. The difference between values was significant ($P = 0.012$).

Sleeping behaviour was studied in this publication. The ratio of insomnia improvement RR was 2.69 [95% CI 1.00, 7.28] ($P = 0.05$).

Adverse Events. There were no significant side effects in all participants.

3.2.3. HM versus HM. In this profile two studies used different scales for evaluating symptoms, which made a quantitative comparison to hot flushes impossible.

In the study by Jiang et al. [44], a significant reduction of 1.47 points in overall score of KI for menopausal symptoms was observed in TCM group (kidney-reinforcing and liver-regulating formula) with 95% CI = 0.9–2.05 ($P < 0.01$). In this study, TCM symptoms score also was observed with a significant reduction of 2.27 points with 95% CI = 1.39–3.15 ($P < 0.01$).

In the study by Zhang et al. [47], the incidence of hot flushes was evaluated. This value in observation group decreased from 66.7% to 33.3%, while it decreased from 70.0% to 60.0% in control group. OR was 0.33 [95% CI 0.12, 0.96]. Study also evaluated other menopausal symptoms for one month, there was significant improvement in the subscales including night sweat ($P = 0.0017$), insomnia ($P = 0.029$), irregular menstruation ($P = 0.0017$), palpitation ($P = 0.003$), and heat sensation in chest, palms, and soles ($P = 0.004$).

Both studies showed positive effect on QoL. The pooled RR estimate in QoL showed significant improvement in

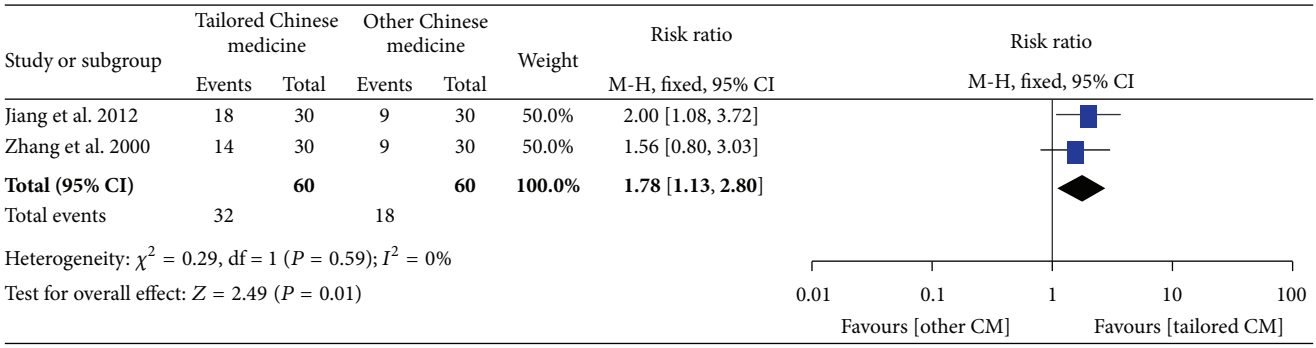

Figure 4: Effect of HM and no treatment in QoL. Forest plot includes only two studies.

the tailored TCM group which means formulas were prescribed guided by TCM discipline. RR estimate was 1.78 [95% CI 1.13–2.80], which suggests that tailored TCM group showed a great improvement of QoL than other TCM groups. There was no significant heterogeneity with $\chi^2 = 0.29$ (df =1, $P = 0.59$) and $I^2 = 0\%$. These results are depicted in Figure 4.

Adverse Events. In the studies by Jiang et al. [44], the articles reported that the changes of hormones level were not significantly different between the two groups.

4. Discussion

This review summarizes the evidence from RCTs of mono- or combined use of HM for hot flushes induced by endocrine therapy in women with breast cancer. It is the first meta-analysis to review HM alone in treatment of hot flushes induced by endocrine therapy and first to evaluate the methodological quality of existing RCTs on this crucial issue.

Based on the existing data, there is no clear evidence of the benefit of HM in treatment of hot flushes induced by endocrine therapy in women with breast cancer, although some individual studies showed slight improvements. However, there is a beneficial effect of HM on menopausal symptoms and improvement of QoL to some extent. This review focused on hot flushes and menopausal symptoms; both individual studies and the main meta-analysis in HM versus no treatment profile showed a favourable result. This result was also found in the individual study in HM versus placebo. We also found that TCM was more effective when guided by a Chinese medical discipline. Therefore, reasonable HM may be considered as an alternative treatment in treating hot flushes induced by endocrine therapy among women with breast cancer, especially for whose worried about the adverse effects from HRT or other nonhormonal therapies. Nevertheless, this assumption needs to be supported by more extensive, high quality, and transparent studies with an appropriate number of subjects. More randomized, double-blind, multicenter clinical trials that are designed with rigorous methodology are required to draw firm conclusions.

The strength of this systematic and meta-analysis review is the investigation of a uniform population. All participants in this review are patients suffering from breast cancer. Patients with hot flushes induced by endocrine therapy were included, while menopausal symptoms induced by chemotherapy or radiotherapy were excluded. Only studies without obvious risk of bias were included in this review. There were many other studies of HMs and TCM formulas related to hot flushes or menopausal symptoms. We also excluded studies about soy isoflavone products which were considered as food and health products.

There are some potential limitations of the meta-analysis results. The results showed slight improvement on the total effect of menopausal symptoms and hot flushes as well as other simultaneous symptoms, while the overall effect should be interpreted very carefully because the analyses were based on a small number of included studies. In those five included studies, there are no multicenter and large size trials. The total sample size is only 397 which is too small for a meta-analysis. The main reasons for the limited number of articles included in this review are the following: (1) Clinical trials on hot flushes related to endocrine therapy are still insufficient due to failure to report adverse effects. (2) Given the small number of eligible trials, we excluded studies with biased evaluation and low quality literature. On the other hand, varying methodological quality of individual trials and lack of standardized measurement of hot flushes symptom scores in all trials make difficult it to conduct a meta-analysis. In those five studies, three studies used KI but no hot flushes diary. The results only showed the overall score of menopausal symptoms. One study used hot flushes diary and assessed vasomotor symptoms. Results only reported total effective rates of vasomotor symptoms but were not specifically tailored to the effect of hot flushes. One study used hot flushes diary to evaluate frequency and severity of hot flushes and disturbance of sleep, but there are no adequate mean difference (MD) variable extracted from data. Therefore, there is little heterogeneity among studies in different profiles, making the comparison of the studies difficult or impossible. Thirdly, there were no profound reports on the effect on hot flushes, as well as night sweat which is the main accompanying symptoms. The lack of transparency and deficient information made the interpretation of studies difficult. In addition, the period of observation and follow-up was too short to assess advantage and disadvantage of HM. Although the observation period in one study was for

12 months, it was within 3 months in other four studies. It is important to note that all included studies reported a favorable effect in hot flushes, but only one study reported minor AE. Reports of AE were too brief from study to study and usually relied on self-reported symptoms experienced in the course of the trial. For long-term adverse effects the reports were limited by too short an observation and follow-up period.

We should also consider the effects of phytoestrogen. Some herbs were reported to act by enhancing oestrogen production or have oestrogen-like effects, such as Dang Gui [51] and Ren Shen (Radix Panax ginseng) [52]. It is considered that phytoestrogens may stimulate breast cancer and decrease the antitumor effects of tamoxifen. As the literature is conflicting and the safety of phytoestrogens in breast cancer patients is unknown, it has been suggested that high-dose phytoestrogen supplement should not be recommended to these women [53]. As a result, data on AEs are not definite enough for us to draw any conclusions and safety needs to be assessed particularly regarding potential herb-drug and herb-herb interactions and long-term AEs.

In this review, we have not included other language databases especially Japanese and Korean databases, so we considered it was possible that some studies related to HM for hot flushes induced by endocrine therapy in patients with breast cancer have not been searched and included. However, for reducing the potential bias during analysis, every step in conducting this review has been done by two authors individually; if there is some difference we discussed the problem to reach a consensus. Furthermore, we need to think of potential sources of heterogeneity including preparation type (mono or multiple, a standard herbal formula, or tailored formula) and dosage.

In conclusion, based on the overall results of the available studies, we could not confirm the positive effects of HM on hot flushes and quality of life induced by endocrine therapy in women with breast cancer. We need more studies with higher quality data to assess the effects on hot flushes and other menopausal symptoms, over the long-term, as well as a more comprehensive evaluation of adverse effects associated with HM.

Disclosure

The authors alone are responsible for the content and writing of this paper.

Competing Interests

The authors report no conflict of interests.

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Research Article

Jiang Tang Xiao Ke Granule, a Classic Chinese Herbal Formula, Improves the Effect of Metformin on Lipid and Glucose Metabolism in Diabetic Mice

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In the present study, the hypoglycemic, hypolipidemic, and antioxidative effects of metformin (MET) combined with Jiang Tang Xiao Ke (JTXK) granule derived from the "Di Huang Tang" were evaluated in mice with type 2 diabetes mellitus (DM) induced by high-fat diet/streptozotocin. DM mice were orally treated with MET (0.19 g/kg) either alone or combined with different doses (1.75, 3.5, or 7 g/kg) of JTXK for 4 weeks. Results showed that the serum and hepatic glucose, lipids, and oxidative stress levels were elevated in DM mice, when compared with the normal mice. MET treatment decreased FBG and serum glucagon levels of DM mice. Combination treatment with MET and JTXK 3.5 g/kg increased the hypoglycemia and insulin sensitivity at 4 weeks when compared with the DM mice treated with MET alone. However, neither MET nor MET/JTXK treatment could completely reverse the hyperglycemia in DM mice. JTXK enhanced the serum triglyceride (TG) and hepatic lipid-lowering effect of MET in a dose-dependent manner in DM mice. JTXK 1.75 and 3.5 g/kg improved the hepatoprotective effect of MET in DM mice. Synergistic effect of combination treatment with MET and JTXK on antioxidant stress was also found in DM mice compared with MET alone.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease with decreased glucose transport into muscle and fat cells and increased hepatic glucose output resulting from dysfunction in insulin secretion or resistance to its activity [1, 2]. DM characterized by chronic hyperglycemia and multiple complications affects millions of individuals worldwide nowadays [3]. One of the most common complications of DM is nonalcoholic fatty liver disease (NAFLD), a risk factor for the development of type 2 diabetes [4, 5]. NAFLD is considered as the hepatic manifestation of the metabolic syndrome caused by abnormal accumulation of triglyceride (TG) inside the hepatocytes [6, 7]. NAFLD induced lipotoxicity is particularly germane to the liver and can lead to apoptosis referred to as lipoapoptosis [8, 9], nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [5, 10]. In the recent past, both experimental and clinical data have demonstrated that oxidative stress is involved in DM and DM secondary complications such as NAFLD and NASH [11–13]. Oxidative stress can lead to mitochondrial dysfunction, endoplasmic reticulum stress, and insulin resistance, all of which could lead to DM ultimately [14]. Oxidative stress and diminished antioxidants within the liver are the key features of NAFLD/NASH while insulin resistance is largely responsible for the development of NAFLD/NASH, which causes hepatic steatosis [15].

Currently, there are 4 kinds of available drugs for the treatment of DM, including α-glucosidase inhibitors, biguanides, sulphonylureas, and thiazolidinediones [16]. However, some of these antidiabetic agents are noted to have adverse side effects such as gastrointestinal disturbances and hypoglycemia [17]. Metformin (MET) is a currently available oral antidiabetic/hypoglycemic agent to deal with patients with type 2 diabetes. It can lower blood glucose and positively

affect lipid profiles associated with few clinically deleterious adverse events [18]. More than two thousand years ago, herbal remedies had been widely used by traditional Chinese medicine (TCM), a major oriental healthcare system, practitioners for the prevention, and treatment of various diseases in China [19–21]. Up to now, the herbal remedies have shown universal adjustment in the treatment of DM by lowering blood glucose [22] and mitigating its related complications such as lipid metabolic disorders [23, 24], including NAFLD associated with lipid metabolism [25, 26]. TCM have been attracting more and more attentions for their complementary therapeutic effects to western medicine [27, 28] and overall adjustment. It has been demonstrated that several medicinal plants recorded in traditional Chinese pharmacopeia have antidiabetic effect. For example, *Coptis chinensis* and *Astragalus membranaceus* were found to improve insulin resistance for Type 2 DM [29–31].

For more than 30 years, professor Gao has been probed to use medicinal plants for treating DM. Based on the clinical experience, laboratory research, and classic herbal formula, he has created Jiang Tang Xiao Ke (JTXK) granule which lowered blood glucose and improved insulin resistance in patient and/or animals with DM [32, 33]. In this study, we aimed to assess the antidiabetic and hypolipidemic effect of MET combined with JTXK granule on glucose and lipid profiles, as well as the oxidative stress parameters in both the serum and liver tissues in DM mice.

2. Materials and Methods

2.1. Preparation Procedure of JTXK Granule. Chinese herbal medicine *Radix Rehmanniae* (Di Huang), *Radix Salviae Miltiorrhizae* (Dan Shen), *Fructus Corni* (Shan Yu Rou), *Panax Ginseng* (Ren Shen), and *Rhizoma Coptidis* (Huang Lian) at the proportion of 3 : 3 : 1 : 1 : 1 were used to prepare JTXK granule. The raw herbs were purchased from Beijing Tong Ren Tang medicinal materials Co., Ltd., Beijing, China, and authenticated by Professor Chun-Sheng Liu in the Beijing University of Chinese Medicine. 4.5 kg of dried tuber of *Radix Rehmanniae* and *Radix Salviae Miltiorrhizae* was boiled thrice with 12 volumes of distilled water for 1 hour. The pooled extract was filtered and concentrated by rotary evaporator at 40°C until the relative density reached 1.15. 1.5 kg of dried *Fructus Corni*, *Panax Ginseng*, and *Rhizoma Coptidis* was extracted thrice with 12 volumes of 60% (v/v, in H_2O) ethanol under reflux after soaking for half an hour. The pooled ethanolic extract was filtered and concentrated by abovementioned method. Every gram (wet weight) of ethanolic extract was equivalent to 1 g of dried herbs. JTXK granule was prepared using the mixture of both aqueous extract and ethanolic extract and stored at 4°C until use [32]. One gram of JTXK was equivalent to 5 g of crude herbs. Fingerprinting of JTXK granule was shown in Figure 1.

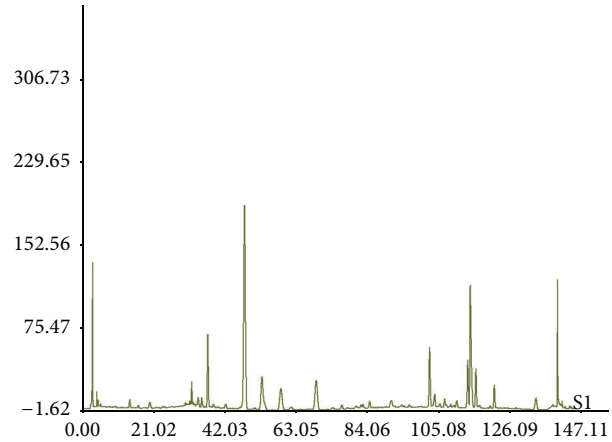

Figure 1: Fingerprinting of JTXK granule.

2.2. Chemicals and Regents. MET was purchased from Tianjin Yabao pharmacy Co., Ltd. (Tianjin, China). Streptozotocin (STZ, Cat. number SLBB7526V) was obtained from Sigma Aldrich Chemical Co., Ltd. (St. Louis, USA). STZ was dissolved into 0.1 mol/L and pH 4.5 sodium citrate hydrochloric acid buffer when needed. Blood glucose kit and triglyceride (TG) kit were obtained from Beijing Leadman Biochemical Co., Ltd. (Beijing, China). Assay kits for total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), superoxide dismutase (SOD), malondialdehyde (MDA), and total glutathione (GSH) were obtained from Zhongsheng Beikong Biotechnology and Science Inc. (Beijing, China). Insulin ELISA assay kit and glucagon kit were obtained from Beijing North Biotechnology Research Institute (Beijing, China).

2.3. Animals and Treatment. Crl:CD1 (Institute of Cancer Research) outbred male mice, weighing 42±3 g, were supplied by Vital River Lab Animal Co., Ltd. (Beijing, China). Animals were kept on a 12 h light/dark cycle and at 24 ± 2°C, with the humidity of 55 ± 5%. Mice were allowed to adapt to the environment for one week before experiment. All animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee of Beijing University of Chinese Medicine, China.

2.4. Experimental Design

2.4.1. Diabetic Mouse Model. Six mice were randomly assigned to normal control group and given standard diet, and the other 40 mice were fed with high-fat diet (HFD) containing 20% sucrose (w/w), 2.5% cholesterol (w/w), 10% lard (w/w), and 0.3% sodium cholic acid (w/w) in standard feed, which was provided by Ke'ao Xieli Feed Co., Ltd. (Beijing, China). Mice were fed with HFD for 4 weeks and intraperitoneally injected with STZ 100 mg/kg to induce DM. The mice with fasting blood glucose (FBG) $\geq$ 11.1 mmol·L^{-1} were confirmed as having diabetes at 72 h after STZ treatment [34].

2.4.2. DM Mice Treatment. According to the FBG level and weight of each mouse, DM mice were randomly divided into 5 groups of 6 animals in each: (1) drug-untreated DM mice; (2) DM mice treated with MET 0.19 g/kg; (3), (4), and (5) DM mice treated with MET 0.19 g/kg plus JTXK granules 1.75, 3.5, and 7 g/kg, respectively. Both MET and JTXK granule suspended in water were administered by gavage for

Table 1: Effect of MET combined with JTXK on serum FBG levels in DM mice.

Groups	Dose (g/kg)	FBG (mmol/L)		
		Before treatment	2 weeks of treatment	4 weeks of treatment
Normal	—	5.98 ± 0.69	6.03 ± 0.78	5.95 ± 0.60
DM	—	27.13 ± 2.31**	26.78 ± 3.05**	26.34 ± 3.17**
MET	0.19	28.70 ± 2.90**	21.92 ± 4.56**	15.97 ± 2.46**##
MET/JTXK	0.19/1.75	26.52 ± 3.65**	20.03 ± 3.01**#	15.53 ± 1.87**##
	0.19/3.5	28.65 ± 4.84**	16.97 ± 3.01**##	11.12 ± 3.13**##ΔΔ
	0.19/7	27.78 ± 2.05**	16.53 ± 4.12**#	13.50 ± 3.24**##

* $p < 0.05$ and ** $p < 0.01$ versus the normal group; # $p < 0.05$ and ## $p < 0.01$ versus the drug-untreated DM group; ΔΔ $p < 0.01$ versus the MET alone group.

4 weeks. Weekly, body weights were recorded for all groups. After fasting for 12 h, mice were sacrificed under light ether anesthesia. Whole blood samples obtained from the abdominal aorta were centrifuged for 15 min at 3,000 rpm/min to obtain the serum. Serum samples were stored at −20°C until biochemical analyses. Liver were dissected out of mice, and hepatic weight and hepatic index (liver weight/body weight × 100) were measured and then washed in ice-cold 0.9% NaCl solution for biochemical and histopathological analysis.

2.5. Oral Glucose Tolerance Test (OGTT). Mice were fasted overnight. The next morning, glucose 2 g/kg was gavaged into the fasten mice. Glucose levels of blood sample from tail vein of mice with a tail-incision technique were estimated by using glucometer at 0, 30, 60, and 120 min.

2.6. Blood/Serum Analysis. At 0, 2, and 4 weeks after the drug administration, the FBG from the tail vein were monitored by glucometer (Johnson & Johnson). The fasting serum insulin (FINS) and glucagon levels were determined according to the instruction of kits. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA) contents, and superoxide dismutase (SOD) activity were measured with the relevant kits and methods according to the manufacturer's protocols.

2.7. Liver Biochemical Analysis. Liver were cut into small pieces and homogenized on ice with corresponding buffer (1 : 9, w/v). The homogenates were centrifuged at 3000 rpm for 15 min at 4°C and the supernatants were used to determine the TG, TC, HDL-C, LDL-C, and MDA contents and SOD and glutathione (GSH) activities according to the manufacturer's protocols of different commercial kits. Protein of the liver homogenate was estimated by BCA protein quantitative analysis kit.

2.8. Liver Histological Evaluation. Liver were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4-5 μm by rotary microtome, stained with hematoxylin-eosin, and examined by laboratory microscopy (Olympus, Tokyo, Japan) to assess the histopathological changes.

2.9. Statistical Analysis. All values are expressed as means ± SD of the mean. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS (version 17.0) statistical analysis program, and then differences among means were analyzed using Dunnett's multiple comparisons test or post hoc analysis. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Effect of MET Combined with JTXK on Serum Glucose Levels in DM Mice. In this study, the FBG levels in DM mice were approximately 5-fold higher than that in normal mice. Compared with the DM mice, the FBG values in MET (0.19 g/kg) treatment alone had a trend of reduction (by 18.15%) after 2 weeks of treatment but did not reach statistical difference ($p > 0.05$). Significant difference ($p < 0.01$) was observed at 4 weeks after MET treatment. However, the same dose of MET combined with JTXK 1.75, 3.5, and 7 g/kg lowered FBG levels by 25.21, 36.63, and 37.81%, respectively, at 2 weeks after MET/JTXK treatment, when compared with DM group mice, but had no statistical differences ($p > 0.05$) in comparison with mice treated with MET alone. The FBG levels were lowered by 41.04, 57.78, and 48.75%, respectively, in the mice treated with same dose of MET combined with JTXK 1.75, 3.5, and 7 g/kg at 4 weeks after treatment, when compared with the untreated DM mice. Compared with MET alone group, MET combined with JTXK 3.5 g/kg treatment can significantly reduce the level of FBG in DM mice (Table 1).

3.2. Effect of MET Combined with JTXK on Serum OGTT Levels in DM Mice. OGTT is an efficient way to assess the insulin secretion induced by glucose taken and glycemic control. After 4-week treatment with MET and MET/JTXK, DM mice were gavaged with glucose (2 g/kg body weight). Both normal and untreated DM mice received the same values of vehicle. Blood glucose levels were measured from the tail vein at 0, 30, 60, and 120 min after glucose treatment. Results showed that blood glucose was significantly decreased (up to 57.57%) in the drug-treated DM mice compared with the drug-untreated DM mice at 120 min after glucose taken. However, MET combined with JTXK 3.5 g/kg, but not 1.75 and 7 g/kg, increased the MET-induced hypoglycemia (by 38.37%) compared with MET alone (Table 2).

Table 2: Effect of MET combined with JTXK on OGTT levels in DM mice.

Groups	Dose (g/kg)	Blood glucose (mmol/L) after oral glucose gavage			
		0 min	30 min	60 min	120 min
Normal	—	6.13 ± 0.65	13.03 ± 0.60	10.17 ± 1.92	7.17 ± 0.76
DM	—	26.90 ± 1.22**	33.30 ± 0.00**	32.33 ± 1.00**	30.40 ± 1.25**
MET	0.19	17.67 ± 1.10$^{**\#\#}$	31.57 ± 1.55**	27.30 ± 1.40$^{**\#}$	20.93 ± 1.26$^{**\#\#}$
MET/JTXK	0.19/1.75	17.97 ± 2.21$^{**\#\#}$	30.90 ± 2.12**	28.97 ± 2.10**	20.23 ± 2.46$^{**\#\#}$
	0.19/3.5	11.40 ± 3.32$^{*\#\#\Delta\Delta}$	27.90 ± 4.07**	24.80 ± 4.44$^{**\#\#}$	12.90 ± 2.36$^{**\#\#\Delta\Delta}$
	0.19/7	12.60 ± 3.41$^{**\#\#\Delta}$	29.90 ± 2.95**	26.60 ± 2.51$^{**\#}$	21.10 ± 2.70$^{**\#\#}$

$^{*}p < 0.05$ and $^{**}p < 0.01$ versus the normal group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus drug-untreated DM group; $^{\Delta}p < 0.05$ and $^{\Delta\Delta}p < 0.01$ versus the MET alone group.

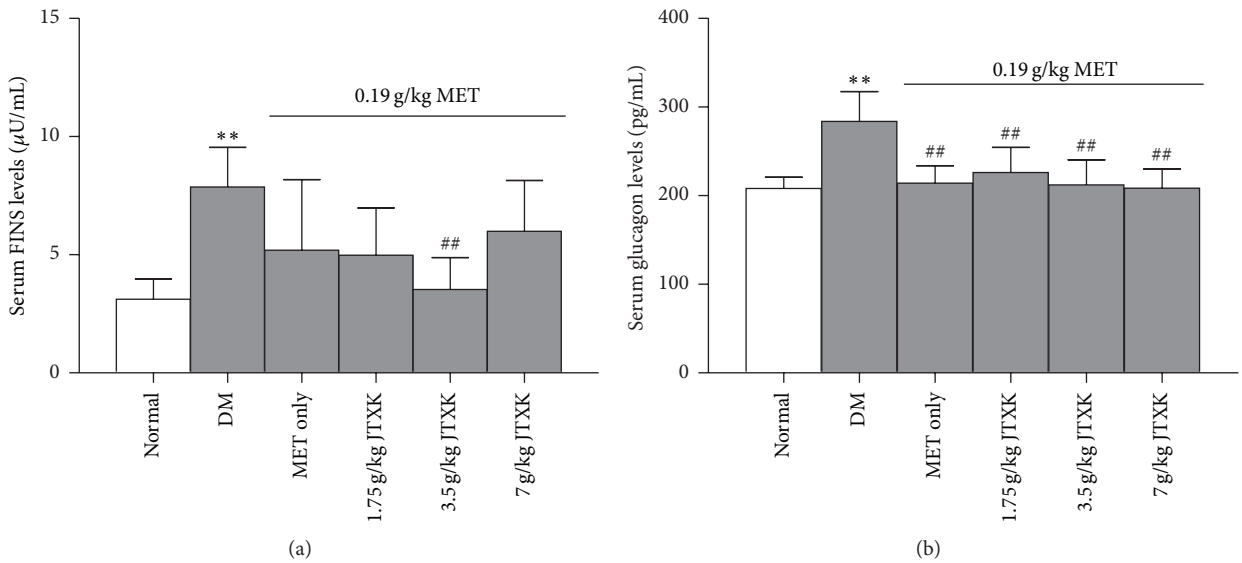

Figure 2: Effect of MET combined with JTXK on serum FINS and glucagon levels in DM mice. $^{**}p < 0.01$ versus the normal group; $^{\#\#}p < 0.01$ versus the DM group.

3.3. Effect of MET Combined with JTXK on Serum FINS and Glucagon Levels in DM Mice. After 4-week treatment, the serum FINS and glucagon levels were determined. Results showed that both serum FINS and glucagon levels of DM mice were much higher (by 60.36 and 26.70%, resp.) than those of normal mice. Although MET treatment alone did not reduced the FINS levels, combination of MET and JTXK 3.5 g/kg treatment significantly lowered FINS levels (up to 55.15%), when compared with the drug-untreated DM mice. A lower level of serum glucagon was found in the DM mice treated with MET, which was not affected by the combination of JTXK (Figure 2).

3.4. Effect of MET Combined with JTXK on Serum Lipids in DM Mice. The serum TG, TC, and LDL levels in DM mice were markedly increased by 434, 235, and 316%, respectively, compared with the normal mice. However, serum HDL levels were decreased by 43.83% in DM mice. MET treatment for 4 weeks markedly reduced serum TG and TC levels (by 38.69 and 36.61%, resp.), but it increased serum HDL level (by 57.99%), when compared with the drug-untreated DM mice. JTXK treatment enhanced the TG-, TC-, and LDL-lowering effect of MET (up to 38.57, 27.22, and 15.01%, resp.) in a dose-dependent manner. The combination of MET and JTXK 1.75 g/kg elevated the serum HDL level by 25.90% compared with the MET alone (Table 3).

3.5. Effect of MET Combined with JTXK on Hepatic Lipids in DM Mice. Figure 3 showed that DM mice developed a significant increase in hepatic TG (up to 367%), TC (up to 154%), and LDL (up to 157%) levels and a marked decrease in HDL (by 38.23%) level in liver homogenate when compared with those of normal mice. Treatment with MET significantly reduced hepatic TC and LDL contents (by 23.85 and 35.98%, resp.), but it increased HDL contents (by 41.87%) in DM mice. JTXK enhanced the effect of hepatic TG-, TC-, and LDL-lowering effect by MET (up to 70.82, 21.91, and 24.85%, resp.) in a dose-dependent manner. Nevertheless JTXK did not affect the alteration of MET on hepatic HDL contents.

3.6. Effect of MET Combined with JTXK on Hepatic Function and Mass in DM Mice. As shown in Table 4, a significant

Table 3: Effect of MET combined with JTXK on serum lipids in DM mice.

Groups	Dose (g/kg)	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Normal	—	0.64 ± 0.10	2.65 ± 0.16	4.70 ± 0.31	0.25 ± 0.03
DM	—	3.42 ± 1.06*	8.87 ± 1.06**	2.64 ± 0.20**	1.02 ± 0.30*
MET	0.19	2.10 ± 0.30**	5.62 ± 0.66$^{**\#\#}$	4.17 ± 0.66$^{\#}$	0.80 ± 0.18**
MET/JTXK	0.19/1.75	1.84 ± 0.18**	5.25 ± 1.00$^{**\#\#}$	5.25 ± 0.77$^{\#\#}$	0.74 ± 0.20*
	0.19/3.5	1.68 ± 0.23**	4.84 ± 0.70$^{**\#\#}$	4.48 ± 0.34$^{\#\#}$	0.72 ± 0.12**
	0.19/7	1.29 ± 0.45$^{\#}$	4.09 ± 0.90$^{**\#\#\Delta\Delta}$	4.79 ± 1.17	0.68 ± 0.08**

$^{*}p < 0.05$ and $^{**}p < 0.01$ versus the normal group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus the drug-untreated DM group; $^{\Delta\Delta}p < 0.01$ versus the MET alone group.

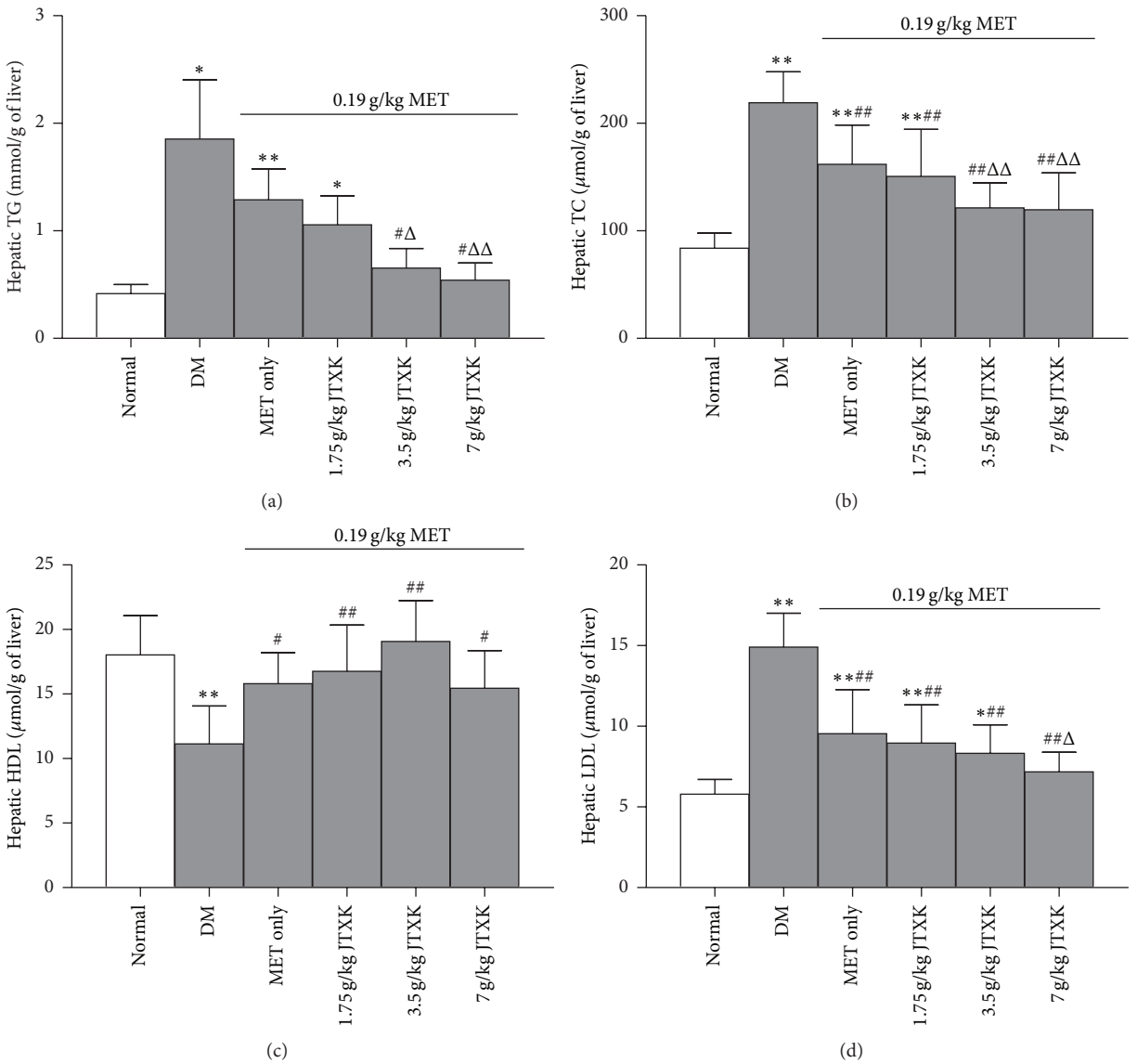

Figure 3: Effect of MET combined with JTXK on hepatic lipids in DM mice. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus the normal group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus the drug-untreated DM group; $^{\Delta}p < 0.05$ and $^{\Delta\Delta}p < 0.01$ versus the MET alone group.

Table 4: Effect of MET combined with JTXK on hepatic function and mass in DM mice.

Groups	Dose (g/kg)	ALT activity (U/L)	AST activity (U/L)	Hepatic weight (g)	Hepatic index (%)
Normal	—	28.33 ± 4.93	23.87 ± 4.06	2.18 ± 0.20	5.02 ± 0.45
DM	—	92.73 ± 13.64**	77.61 ± 5.33**	4.22 ± 0.45**	9.77 ± 0.80**
MET	0.19	61.47 ± 9.39$^{**\#}$	48.37 ± 5.32$^{**\#\#}$	3.27 ± 0.42$^{**\#\#}$	8.01 ± 0.89$^{**\#\#}$
MET/JTXK	0.19/1.75	48.50 ± 7.34$^{**\#\#}$	42.93 ± 7.00$^{**\#\#}$	2.94 ± 0.44$^{**\#\#}$	7.69 ± 1.18$^{**\#\#}$
	0.19/3.5	41.80 ± 5.81$^{**\#\#\Delta}$	36.47 ± 3.40$^{**\#\#\Delta\Delta}$	2.44 ± 0.40$^{\#\#\Delta\Delta}$	6.59 ± 1.08$^{**\#\#\Delta\Delta}$
	0.19/7	78.70 ± 6.85**	69.07 ± 7.81$^{**\#\Delta\Delta}$	2.36 ± 0.32$^{\#\#\Delta\Delta}$	6.27 ± 1.01$^{\#\#\Delta\Delta}$

$^{*}p < 0.05$ and $^{**}p < 0.01$ versus the normal group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus the drug-untreated DM group; $^{\Delta}p < 0.05$ and $^{\Delta\Delta}p < 0.01$ versus the MET alone group.

Table 5: Effect of MET combined with JTXK on body weight in DM mice.

Groups	Dose (g/kg)	Body weight (g) after treatment with drugs				
		Week 0	Week 1	Week 2	Week 3	Week 4
Normal	—	41.50 ± 1.58	42.22 ± 1.80	42.33 ± 1.93	43.42 ± 2.04	43.45 ± 2.09
DM	—	42.92 ± 2.01	43.13 ± 0.82	43.13 ± 0.97	44.53 ± 1.12	43.15 ± 1.55
MET	0.19	41.18 ± 1.80	41.83 ± 1.60	40.93 ± 2.35$^{\#}$	41.58 ± 2.69$^{\#}$	40.88 ± 2.57$^{\#}$
MET/JTXK	0.19/1.75	40.93 ± 1.38	39.68 ± 1.88$^{\#\#\Delta}$	38.52 ± 2.21$^{\#\#\Delta}$	38.65 ± 1.62$^{**\#\#\Delta}$	38.32 ± 2.01$^{**\#\#\Delta}$
	0.19/3.5	42.02 ± 1.67	40.25 ± 1.59$^{\#\#}$	37.87 ± 1.54$^{**\#\#\Delta\Delta}$	36.93 ± 1.59$^{**\#\#\Delta\Delta}$	37.12 ± 0.86$^{**\#\#\Delta\Delta}$
	0.19/7	41.32 ± 1.65	38.85 ± 2.15$^{\#\#\Delta\Delta}$	38.30 ± 1.53$^{**\#\#\Delta}$	38.87 ± 2.00$^{**\#\#\Delta}$	37.80 ± 1.68$^{**\#\#\Delta\Delta}$

$^{*}p < 0.05$ and $^{**}p < 0.01$ versus the week 0; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus the drug-untreated DM group.; $^{\Delta}p < 0.05$ and $^{\Delta\Delta}p < 0.01$ versus the MET alone group.

increase in serum ALT and AST (by 227 and 225%, resp.) levels was observed in DM mice, when compared with those of normal mice. Treatment with MET for 4 weeks decreased the serum ALT (by 33.71%) and AST (by 37.68%) activities in DM mice. Treating DM mice with MET plus JTXK (1.75 and 3.5 g/kg) significantly reduced ALT (up to 31.99%) and AST (up to 24.60%) activities, when compared with MET alone. However, MET combined with JTXK 7 g/kg raised the serum ALT and AST levels (by 28.03 and 42.80%, resp.) in comparison with the DM mice treated with MET alone. Hepatomegaly was found in DM mice. MET treatment lowered the hepatic mass by 22.51% in DM mice compared with the untreated DM mice. JTXK treatment dose-dependently extended the hepatic mass-lowering effect of MET.

3.7. Effect of MET Combined with JTXK on Oxidative Stress in DM Mice. Serum (Figure 4(a)) and hepatic (Figure 4(c)) MDA levels were markedly elevated by 103% and 58.11%, respectively, in DM mice. However, serum SOD (Figure 4(b)), hepatic SOD (Figure 4(d)), and hepatic GSH (Figure 4(e)) levels were significantly reduced by 62.46, 42.44, and 35.08%, respectively, in DM mice, when compared with the normal animals. MET treatment decreased serum and hepatic MDA (by 32.28 and 14.77%, resp.) and increased serum and hepatic SOD (by 46.93 and 29.32%, resp.) and hepatic GSH (by 23.74%) compared with drug-untreated DM mice. MET combined with JTXK 3.5 g/kg was most effective in reducing serum and hepatic MDA levels (by 25.10 and 23.79%, resp.) and increasing serum SOD (by 43.09%), hepatic SOD (by 28.58%), and hepatic GSH (by 24.07%) contents, when compared with MET alone.

3.8. Effect of MET Combined with JTXK on Hepatic Histology in DM Mice. Light microscopic findings showed that there was no abnormal cell structure in the liver sections of normal mice (Figure 5(a)). Compared with normal liver, the liver architecture of DM mice showed an increased number of lipid droplets associated with hepatocytes hypertrophy, lymphocytes infiltration, sinusoidal space dilation, and microvascular steatosis (Figure 5(b)) in the mice fed with HFD. MET treatment showed a protective effect against DM induced liver injury, which was expressed as decreased sinusoidal space dilation and lymphocytes (Figure 5(c)). MET combined with JTXK (1.75, 3.5, and 7 g/kg) significantly reversed hepatotoxicity and hepatic steatosis caused by DM status in a dose-dependent manner (Figures 5(d), 5(e), and 5(f)).

3.9. Effect of MET Combined with JTXK on Body Weight in DM Mice. There was no difference in body weight between normal mice and DM mice. Treatment with MET alone had relatively little effect in lowering the body weight of DM mice. However, treatment with MET combined with JTXK lowered body weight during the period of medication compared with the MET treatment alone (Table 5).

4. Discussion

Currently, type 2 diabetes mellitus (T2DM) patients make up about 90% of all patients with DM [35, 36]. Present observations indicate that diabetes can be a driving force for NAFLD in terms of inflammation and oxidative stress [37, 38]. STZ is the most commonly used diabetogenic agent to establish diabetes animal model by destroying pancreatic β cells

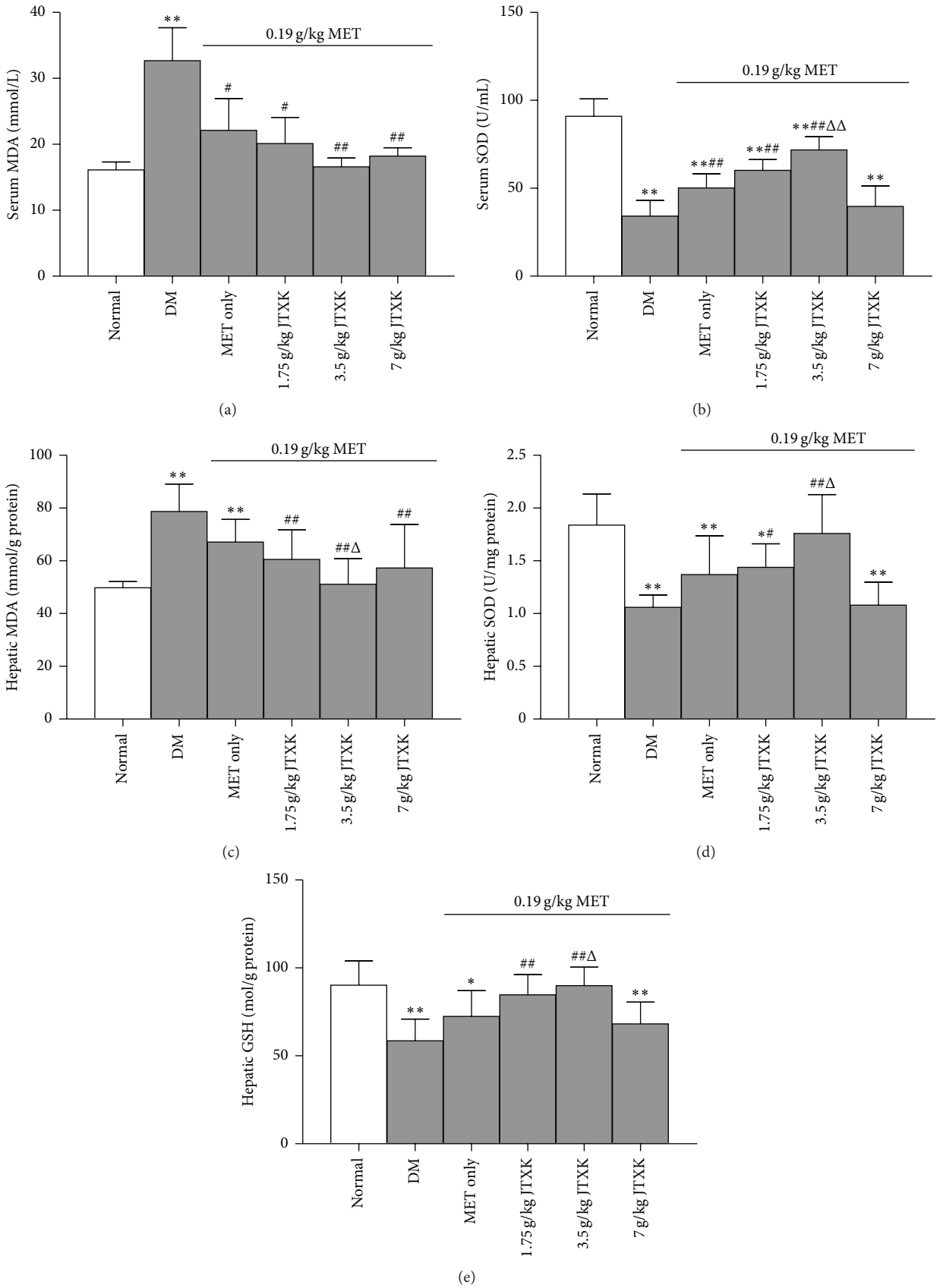

FIGURE 4: Effect of MET combined with JTXK on oxidative stress in DM mice. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus the normal group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus the drug-untreated DM group; $^{\Delta}p < 0.05$ and $^{\Delta\Delta}p < 0.01$ versus the MET alone group.

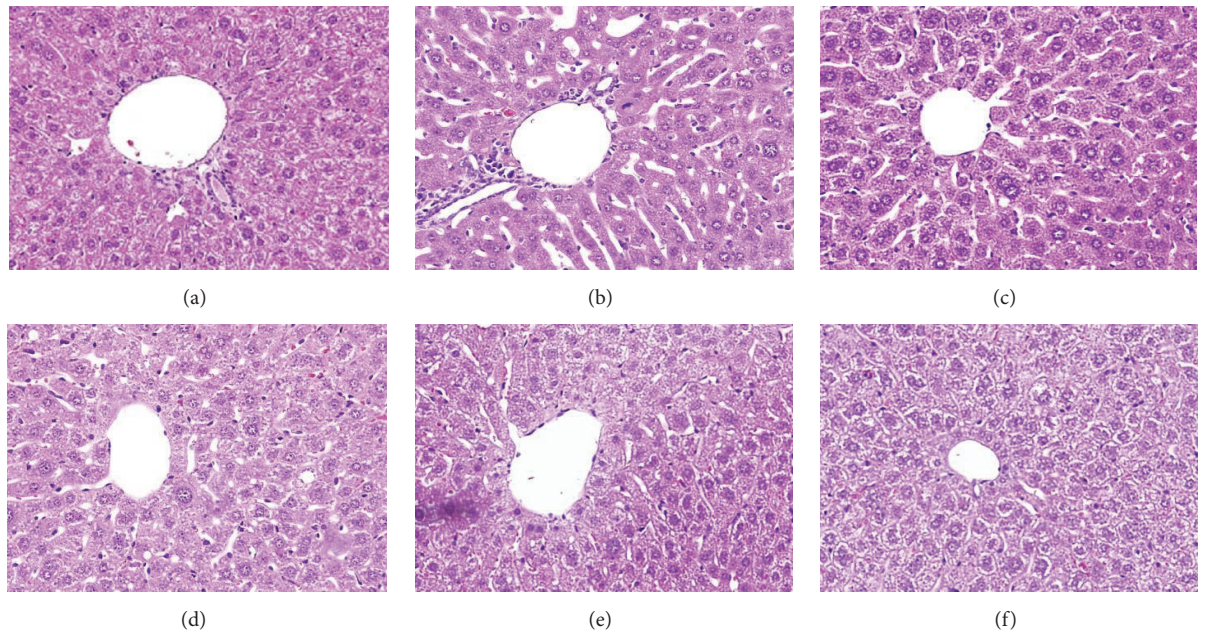

Figure 5: Effect of MET combined with JTXK on hepatic histology in DM mice: (a) normal mice; (b) drug-untreated DM mice; (c) DM mice treated with MET 0.19 g/kg; (d), (e), and (f) DM mice treated with MET 0.19 g/kg plus JTXK 1.75, 3.5, and 7 g/kg, respectively. Hematoxylin and Eosin (H&E) Staining (×20).

selectively, which can lead to insulin resistance and oxidative stress systemically [39–42]. HFD also associates with insulin resistance and adipocyte dysfunction; the high prevalence of NAFLD and insulin resistance among obese individuals reflect this relationship between HFD and NAFLD [43–45]. In this way, HFD fed animals with exposure to low dose of STZ are commonly used in scientific research for DM models. In the present study, DM mice model induced by HFD/STZ showed stable fasting hyperglycemia associated with impairment of glucose tolerance and elevation in FINS and glucagon, which indicated the glucose metabolism disorders. Meanwhile, metabolic abnormality of lipid such as hyperlipidemia and hepatic steatosis, liver injury, and oxidative stress status in both blood and liver were also observed in DM mice. All these results implied that DM associated with fatty liver in human was successfully mimicked by a mouse model induced by HFD intake and STZ injection.

Liver plays a vital role in the regulation of systemic glucose and lipid metabolism. In the setting of DM conditions, target organs such as liver, adipose tissue, and muscle tissue show insensitivity to the stimuli of insulin. At the same time, glucose utilization is reduced and adipose tissue lipolysis is enhanced, contributing to excessive systemic glucose and lipid levels. These changes are accompanied by compensative increased insulin secretion and hepatocellular energy alterations. Systemic lipids entered liver and increased hepatic fatty acid β-oxidation. When the mount of lipid exceeds the oxidative capacity, it will be deposited in the liver, referred to a condition as steatosis. As steatosis progresses, liver lobule and portal area are infiltrated with inflammatory cells and hepatocytes are filled with lipid droplets, resulting in steatohepatitis.

T2DM is a progressive disease which required lifestyle modifications and pharmacological interventions such as oral antihyperglycaemic agents and insulin injection [46–50]. However, some patients will not achieve their ideal glycaemic control until two oral antihyperglycaemic agents are administered. For some patients, sustained diabetes control is not achieved even after taking two agents in severe insulin resistance [51–54]. JTXK can improve the function of the liver, spleen, and kidney organ system and dispel pathogenic factors according to the theory of TCM. In our previous study, JTXK is effective in improving lipid metabolism, reducing DM symptoms and complications through regulating the activation of adenosine monophosphate activated protein kinase (AMPK), which is a regulator of energy metabolism and a key mechanism that brings about a wide range of metabolic benefits [55, 56]. Phosphorylated AMPK blocks SREBP1c, a transcription factor controlling enzymes involved in the fatty acid synthesis, contributes to lipid metabolism [55]. Currently, FBG, FINS, and OGTT levels are clinical parameters for definitely diagnosing DM. Here, they were used as the indicators for evaluating the effectiveness of the MET and JTXK plus MET antidiabetic activity in DM mice. Treating mice with combination with MET and JTXK significantly reduced FBG, FINS, and glucagon levels and partly restored glucose tolerance compared with MET alone. These findings suggested that combination treatment with MET and JTXK (3.5 g/kg) effectively reversed the disturbance of glucose and lipid metabolism and oxidative stress status in DM mice. It is well known that MET, an insulin sensitizer, inhibits hepatic glucose production via decreasing gluconeogenesis, increasing glycogenolysis, and diverting fatty acids

from TG to mitochondrial beta oxidation [57]. In the present study, it was found that JTXK treatment could improve the antidiabetic effect of MET. It means that JTXK affects MET-induced glucose and lipid metabolism through the same pathways. Further studies are needed.

It is well established that serum aminotransferase activity is widely adopted as sensitive biomarker of liver damage in both clinic and animal experiment. In the present study, increased serum ALT and AST activities and decreased sinusoidal space dilation and lymphocytes in liver histology are commonly regarded as signs of credible hepatic injury. Treatment with MET and JTXK altered serum and hepatic lipid contents and ameliorated hepatic injury in DM mice. Hepatic lipid-lowering effect of MET alone and combined with JTXK was shown in a dose-dependent manner in both serum and liver. Some studies have reported that administration of MET resulted in improving aminotransferase levels, while others have not found this effect [58–62]. In the current study, it was found MET treatment alone lowered the serum ALT and AST activities, which was enhanced by combination with JTXK 3.5 g/kg, in DM mice. However, large dose of JTXK (7 g/kg) eliminated ALT- and AST-lowering effect of MET.

Oxidative stress plays a key role in the development of DM and chronic complications in DM [63]. DM may induce hepatic MDA formation and lipid peroxidation, which can affect the fluidity and permeability of hepatocytes membrane and lead to cellular damage [64]. The reduced activities of antioxidative enzymes (SOD, GSH, etc.) also indicated the insufficient ability against oxidative stress. In the present study, treatment with MET and JTXK improved the antioxidant ability and restored their activities near to normal group. Combination therapy was more effective than given MET alone, except large dose of JTXK (7 g/kg) combination.

In conclusion, the current study revealed that JTXK, a Chinese herbal medicine formula, treatment for 4 weeks could promote the improvement of MET on the serum and liver glucose and lipid metabolism, as well as insulin sensitivity in HFD and low dose STZ induced DM mice. The hepatoprotective activity and antioxidative activity of MET against DM were accelerated by the combination with JTXK. Combination therapy with proper dose of JTXK (classic Chinese herbal formula) and MET (chemical drug) may represent a good strategy for the management of the patient with DM.

Competing Interests

The authors declared no competing interests with respect to the authorship and/or publication of this paper.

Authors' Contributions

Yi Zhang and Hong An contributed equally to the work.

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Research Article

Effect of Catnip Charcoal on the *In Vivo* Pharmacokinetics of the Main Alkaloids of Rhizoma Coptidis

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This study aims to explore the effect of catnip *Nepeta cataria* (CNC) charcoal on the pharmacokinetics of the main alkaloids of Rhizoma Coptidis *in vivo*. Twenty-four rabbits were randomly divided into four groups and given oral administration of an aqueous extract of Rhizoma Coptidis (RCAE), RCAE plus CNC, RCAE plus activated carbon (AC), or distilled water, respectively. Plasma samples were collected after administration. The concentrations of berberine, coptisine, palmatine, and epiberberine in plasma were measured by high-performance liquid chromatography (HPLC). The pharmacokinetics data were calculated using pharmacokinetic DAS 2.0 software. The results showed that the area under the concentration-time curve (AUC) of berberine increased, while the AUC of coptisine, palmatine, and epiberberine decreased in the rabbits that received RCAE plus CNC. Meanwhile, the AUC of berberine, coptisine, palmatine, and epiberberine decreased in the group given RCAE plus AC. The difference of main pharmacokinetics parameters among the four groups was significant ($P < 0.05$). This study showed that CNC improved the bioavailability of berberine in comparison to AC and prolonged its release in comparison to RCAE alone. However, it decreased the bioavailability of coptisine, palmatine, and epiberberine. In comparison, AC uniformly declined the bioavailability of berberine, coptisine, palmatine, and epiberberine.

1. Introduction

Herbal charcoals have been used traditionally in Chinese medicine for many years, being one of the most characteristic processing methods of Chinese herbal medicines with the purpose of changing the herbal nature, enhancing the astringency, hemostasis, and antidiarrheal activities, and also reducing toxicity of some herbals [1, 2]. The catnip *Nepeta cataria* (CNC) charcoal is typically made from cut pieces of CNC, which are carbonized until coke-black on a strong fire. Catnip *Nepeta cataria* (CNC) charcoal has been shown to exhibit better effects than the noncharcoal form in the treatment of hematochezia, metrorrhagia, and postpartum anemic fainting [3]. Notably, although in charcoal form, various charcoals of Chinese herbs partially retain the inherent nature of the raw herbal [4].

Pharmacological research has indicated that the charcoal form of Chinese herbal medicines could enhance the astringency, hemostasis, and antidiarrheal activity of herbs due to the absorption and astringency of activated carbon (AC), which is generated during the processing of charcoals [5, 6]. It was unclear, however, whether the carbonized herbs subsequently absorbed the active components of other herbals when used in combination, thus decreasing their therapeutic effects due to nonselective absorption of AC. In addition, Mullins et al. found that AC could accelerate the excretion of other drugs from the body and decrease the bioavailability of some drugs due to the interruption of drug recirculation following reabsorption from the gastrointestinal tract or the promotion of vasoconstriction of the capillaries in the intestinal wall [7]. In summary, no common consensus has been reached with regard to the mechanisms of carbonized Chinese herbal medicines and their effects on other drugs taken concomitantly.

Nepeta cataria has an acrid and bitter taste. From a traditional Chinese medicinal perspective, it is slightly warm in nature and often used to expel pathogenic wind from the body surface. Clinically, it may be used to treat exanthema and as a hemostatic. On the other hand, Rhizoma Coptidis (RC) has been used in traditional Chinese medicine to clear

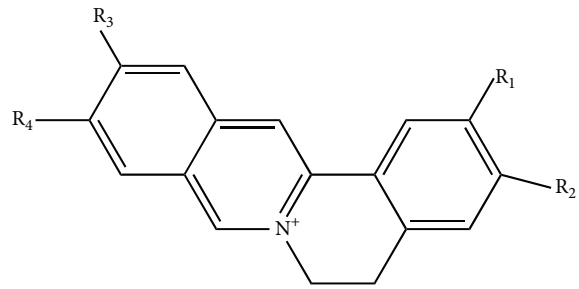

Figure 1: The molecular structure of berberine, epiberberine, coptisine, and palmatine. Note: berberine (R_1-R_2 = –O–CH_3–O–; R_3 = –OCH_3; R_4 = –OCH_3); epiberberine (R_1 = –OCH_3; R_2 = –OCH_3; R_3-R_4 = –O–CH_3–O–); coptisine (R_1-R_2 = –O–CH_3–O–; R_3-R_4 = –O–CH_3–O–); palmatine (R_1 = –OCH_3; R_2 = –OCH_3; R_3 = –OCH_3; R_4 = –OCH_3).

heat, purge intense heat, and dry dampness and it may also be used in detoxification. *Nepeta cataria* and RC have been used together to clear "heat evil," eliminate wind, and relieve liver conditions, as a part of the Jingjielianqiao decoction [8]. The purpose of this study was to clarify the effect of carbonized Chinese herbal medicines on the absorption of other drugs taken concomitantly. As such, to provide a basis for the clinical application of carbonized Chinese herbal medicines, the effect of CNC on the pharmacokinetics of berberine, coptisine, palmatine, and epiberberine, which are the main alkaloids in RC (showed in Figure 1), was investigated.

2. Materials and Methods

2.1. Agilent 1100 Series HPLC System. The Agilent 1100 Series LC consists of Agilent 1100 Series Quaternary Pump (G1311A), Agilent 1100 Series Autosampler (G1313A), Agilent 1100 Series Thermostatted Column Compartment (G1316A), Agilent 1100 Series Vacuum Degasser (G1379A), and Agilent 1100 Series variable wavelength UV detector (G1314A).

2.2. Herbal Medicines and Reagents. RC and CNC were purchased from Hefei Lejia Herbal Pieces Co. Ltd. (Anhui, China) and authenticated by Professor Shunxin Guo (Chinese Academy of Medical Science, Peking Union Medical College Institution of Medicinal Plant Development) in accordance with the *Chinese Pharmacopoeia*, 2010 edition. Medicinal AC was purchased from Sinopharm Chemical Reagent Co. Ltd. (China). Berberine, coptisine, palmatine, and epiberberine were purchased from the National Institutes for Food and Drug Control (China). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile were provided by J. T. Baker Co. Ltd. (USA). Potassium dihydrogen phosphate and sodium lauryl sulfate were obtained from Anaqua Chemicals Supply (USA).

2.3. Animals. Twenty-four clean-grade adult male New Zealand rabbits (scxk (Shandong) 2014-0006) weighing 3.1 ± 0.6 kg were purchased from Jinan Jinfeng Experimental Animal Co. Ltd. Animals were treated humanely according to the National Research Council's guidelines.

2.4. Preparation of Stock Solutions and Herbal Medicines

2.4.1. Preparation of Standardized Solution. Stock solutions were prepared by dissolving the accurately weighed four standard reference compounds in methanol (28 μg/mL for coptisine, 20 μg/mL for epiberberine, 11 μg/mL for palmatine, and 28 μg/mL for berberine).

2.4.2. Preparation of Aqueous Extract of RC (RCAE). Fifty g RC was soaked in 600 mL water for 30 min and then boiled over a strong flame prior to simmering for 30 min and the decoction liquid was collected. The remaining herbal residue was mixed with 300 mL water and boiled for a second time. After filtration, the two filtrates were mixed and concentrated to 500 mL to attain a final concentration of 0.1 gram of raw herb in 1 mL (0.1 g/mL) using a rotary evaporator at 45°C. Then they were divided into three equal parts (a, b, and c): part b was mixed with CNC at the ratio of 0.5 percent, and part c was mixed with AC at the ratio of 0.5 percent.

2.4.3. Preparation of Powdered CNC and AC. CNC and AC were pulverized and sieved using 80-mesh and 120-mesh strainers, respectively. The resulting fine powders were kept for use in this study.

2.5. HPLC Analysis for Charred Nepeta cataria and Nepeta cataria. According to the stipulation of Chinese Pharmacopoeia (2015), the condition of HPLC was set and detecting solution was preparated. A ZOBAX C18 chromatography column (250 mm × 4.6 mm, 5 μm) was used in this study. The mobile phase was a mixture of water (A) and methol (B). The elution of p-menthone was performed using an isocratic method of 80% A. The flow rate was set at 0.8 mL/min. The injection volume was 10 μL, temperature of the column oven was set at T = 25°C during all experiment, and the wavelength for detection was 252 nm. The reference solution of p-menthone was prepared by dissolving the accurately weighed p-menthone in methanol.

2.6. Establishment of HPLC Method for Detecting Four Alkaloids in Rhizoma Coptidis. A ZOBAX C18 (250 mm × 4.6 mm, 5 μm) chromatography column was used in this study. The mobile phase consisted of water containing 0.05 mol/L potassium dihydrogen phosphate (A) and acetonitrile (B) at a ratio of 50 : 50 (v/v). The injection volume was 10 μL, the flow rate was 0.8 mL/min, the column temperature was 25°C, and the wavelength for detection was 345 nm.

2.7. HPLC Method Validation. The specificity was tested by comparison of the plasma sample to blank rabbit plasma and blank plasma spiked with the four analytes using established HPLC methods to observe the interference from endogenous substances contained in the analyte. Six samples were tested for specificity.

Calibration curves were prepared using standard plasma samples with different concentrations of the four analytes, the standard concentrations of berberine were 7.0, 17.5, 35, 70, 100, and 140 ng/mL, the standard concentrations of coptisine were 1.4, 8.75, 17.5, 35, 70, and 140 ng/mL, the standard concentrations of palmatine were 13.5, 27, 56.25,

112.5, 225, and 550 ng/mL, and the standard concentrations of epiberberine were 5, 12.5, 25, 50, 80, and 100 ng/mL. The peak area of the analyte was set as the vertical coordinate and the concentration of the analyte was set along the x-axis.

The precision and accuracy were evaluated by assaying six sample replicates with low, medium, and high concentrations during a single day and measuring six sample replicates with low, medium, and high concentrations once a day for five days. The precision was measured by the relative standard deviation (RSD) and the accuracy was described by the relative error (RE).

The extraction recovery and matrix effects of the four analytes were determined at three levels with six replicates. The extraction recoveries were evaluated by comparing the peak area obtained from the plasma sample spiked before extraction with the plasma sample spiked after extraction. The matrix effect was investigated by comparing the peak area of the analyte added to the preextracted plasma from untreated rats with that of the analyte dissolved in matrix component-free reconstitution solvent.

2.8. Pharmacokinetics Experiments. Twenty-four New Zealand rabbits were randomly divided into four groups, labeled Group A, Group B, Group C, and Group D. Group A received RCAE orally, Group B received RCAE and CNC orally, Group C received RCAE and AC orally, and Group D was administrated distilled water orally. The dosage of Groups A, B, and C was 15 mL/kg. All rabbits were fasted 24 h before administration and had free access to water. Before and immediately after oral treatment, rabbit blood samples (2.0 mL) were obtained from the auricular vein and samples were subsequently taken 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 18, 24, 36, and 72 h after administration. The blood samples were immediately heparinized and centrifuged at 4000 rpm for 10 min at 4°C. Prepared plasma samples were stored at −20°C until analysis. All of the pharmacokinetic parameters were processed by noncompartmental analysis using DAS 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China) and all animal studies were performed according to the *Guide for the Care and Use of Laboratory Animals*.

2.9. Plasma Sample Preparation. Plasma samples (200 μL) were placed in Eppendorf tubes and extracted with 0.8 mL acetonitrile by vortex mixing for 3 min and ultrasonic extraction for 30 min. After centrifugation at 12000 rpm for 10 min, the supernatant (0.8 mL) was pipette-transferred to another Eppendorf tube. Then, the residue was extracted via the same procedure for a second time. The supernatant was combined for each sample and evaporated to dryness under nitrogen at 45°C. The residue was redissolved in 80 μL of the HPLC mobile phase and the solution was filtrated through a microporous filter membrane (0.22 μm) prior to analysis. All samples were measured within a week. Interday precision and accuracy of the assay reached the standard of quantitative analysis, and the standard samples in low, medium, and high concentration of four analyses were measured to assure the accuracy every day.

Table 1: Calibration curves, correlation coefficients (r), and linear ranges of the four analytes in RCAE.

Compound	Calibration curve	r value	Linear range (ng/mL^{-1})
Epiberberine	$y = 178.99x + 2.6353$	0.9992	5.0–100
Coptisine	$y = 508.97x + 3.9844$	0.9990	1.4–140
Palmatine	$y = 368.91x - 5.9279$	0.9950	13.5–550
Berberine	$y = 332.46x - 0.2947$	0.9997	7.0–140

2.10. Statistical Analysis. Data were represented in the mean ± standard deviation of the mean. Comparisons between different groups were carried out by Turkey's test. The level of significance was set at $P < 0.05$. SPSS software (version 19.0, IBM, Inc., USA) was used in statistical analysis.

3. Results

3.1. HPLC Analysis for Charred Nepeta cataria and Nepeta cataria. The retention time of p-menthone was 5.037 min under the stipulated HPLC condition, and the calibration curve was $y = 12135x + 641.5$ ($r = 0.9999$), as shown in Figure 2; (a) showed the HPLC image of p-menthone, (b) showed the HPLC image of charred *Nepeta cataria*, and (c) showed the HPLC image of *Nepeta cataria*. The results of the accuracy, precision, and extraction recovery showed that the extraction recovery was more than 90%, and both the RSD and reproducibility met the measure requirements. The results showed that the content of p-menthone of *Nepeta cataria* was 0.96 mg/g which was higher than 0.08% stipulated in *Chinese Pharmacopoeia* (2015) and the content of p-menthone of charred *Nepeta cataria* was 0.43 mg/g.

3.2. Specificity of the HPLC Method. The retention times of berberine, coptisine, palmatine, and epiberberine were 9.94, 11.10, 13.501, and 14.99 min, respectively. As shown in Figure 3, (a) showed the HPLC trace for the plasma sample after administration of RC, (b) showed the blank plasma spiked with the four analytes, and (c) showed the trace for blank plasma. There was no obvious interference from endogenous substances contained in the analytes according to the HPLC trace of blank rabbit plasma, blank plasma spiked with the four analytes, and the plasma sample after administration.

3.3. Calibration Curves. The standard curves of the four analytes all exhibited good linearity and good coefficients of correlation ($r > 0.993$). The limit of quantitation was appropriate for the quantitative detection of the four analytes in the plasma samples. The linear ranges, regression equations, and correlation coefficients were shown in Table 1.

3.4. Accuracy, Precision, and Extraction Replicates. The results of the specificity of the HPLC method showed that the matrix effect of the plasma taken from rabbits in the control group would not disturb measurement of the four alkaloids. The results of the accuracy, precision, and extraction recovery showed that the extraction recovery was more than 90%, and both the RSD and reproducibility met the measure

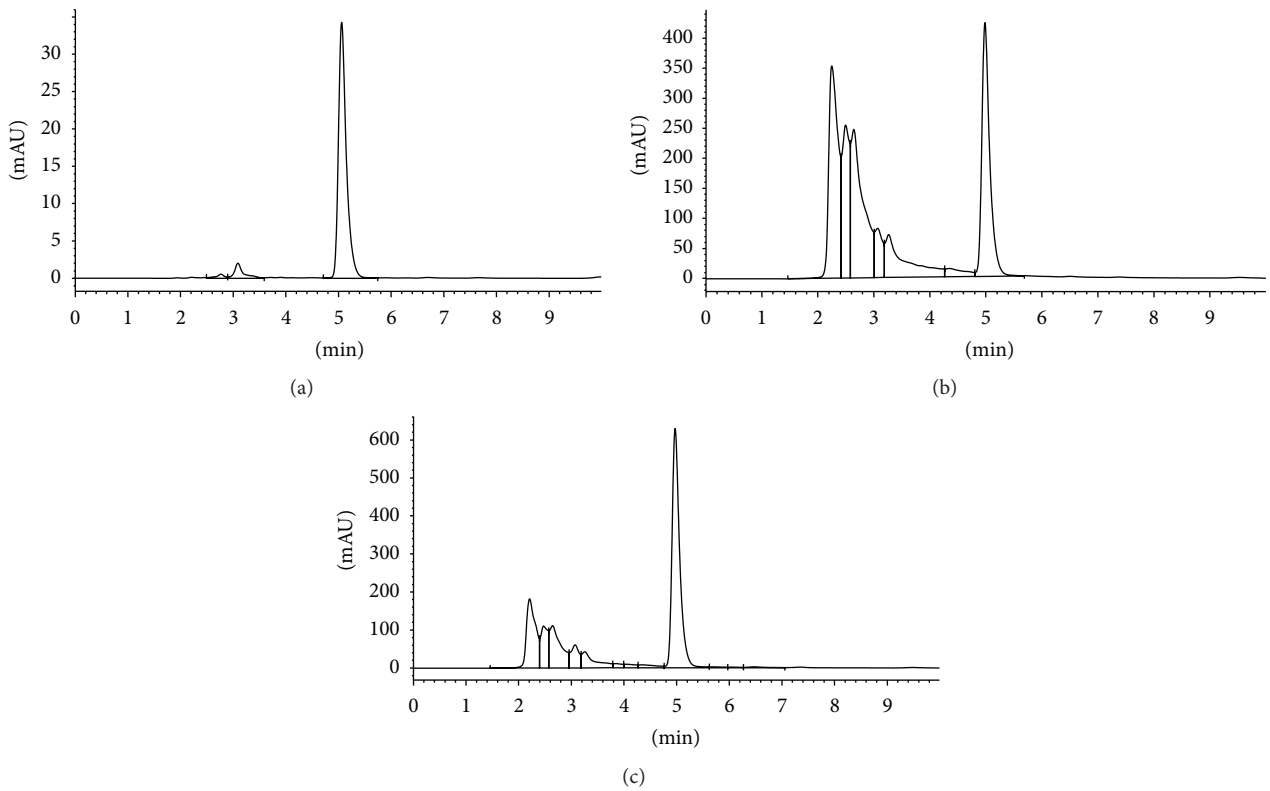

Figure 2: (a) shows the HPLC image of menthone, (b) shows the HPLC image of charred *Nepeta cataria*, and (c) shows the HPLC image of *Nepeta cataria*.

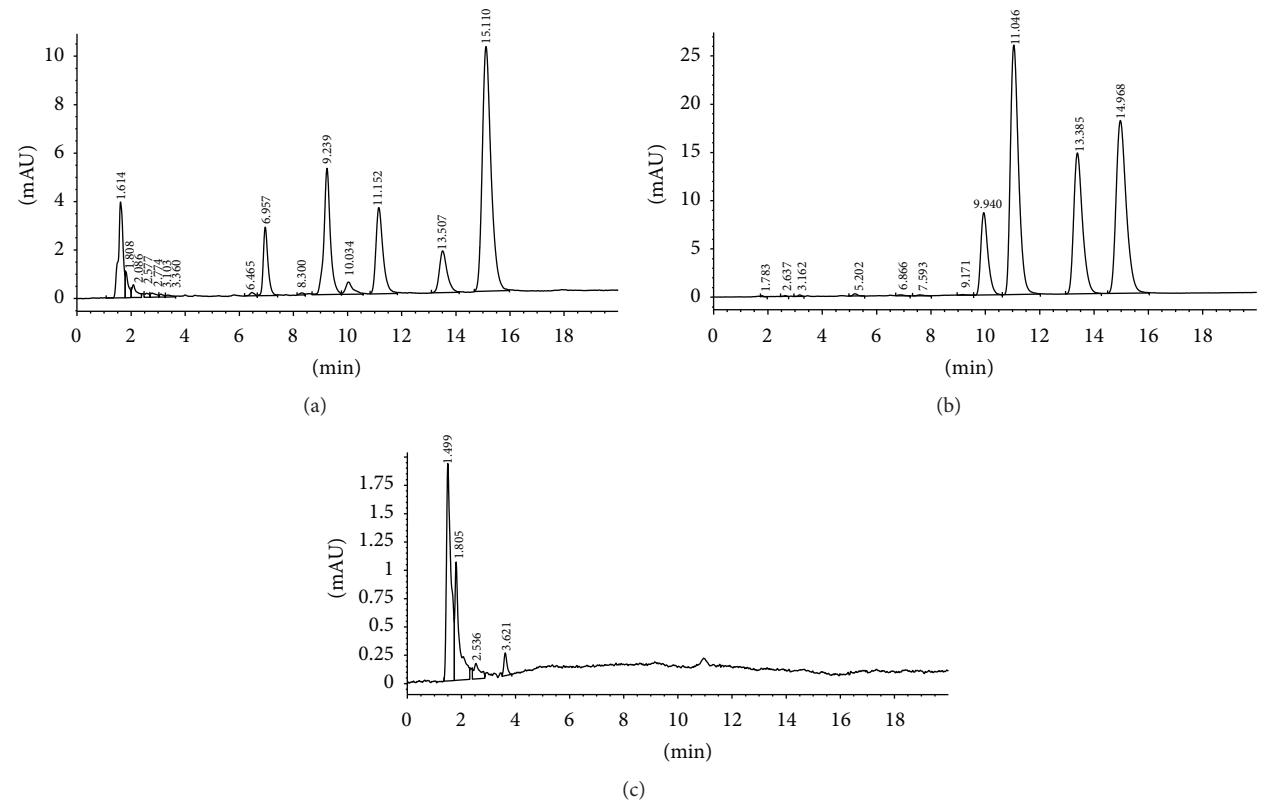

Figure 3: HPLC chromatogram of a plasma sample after administration (a), blank plasma spiked with the four analytes (b), and blank rabbit plasma (c).

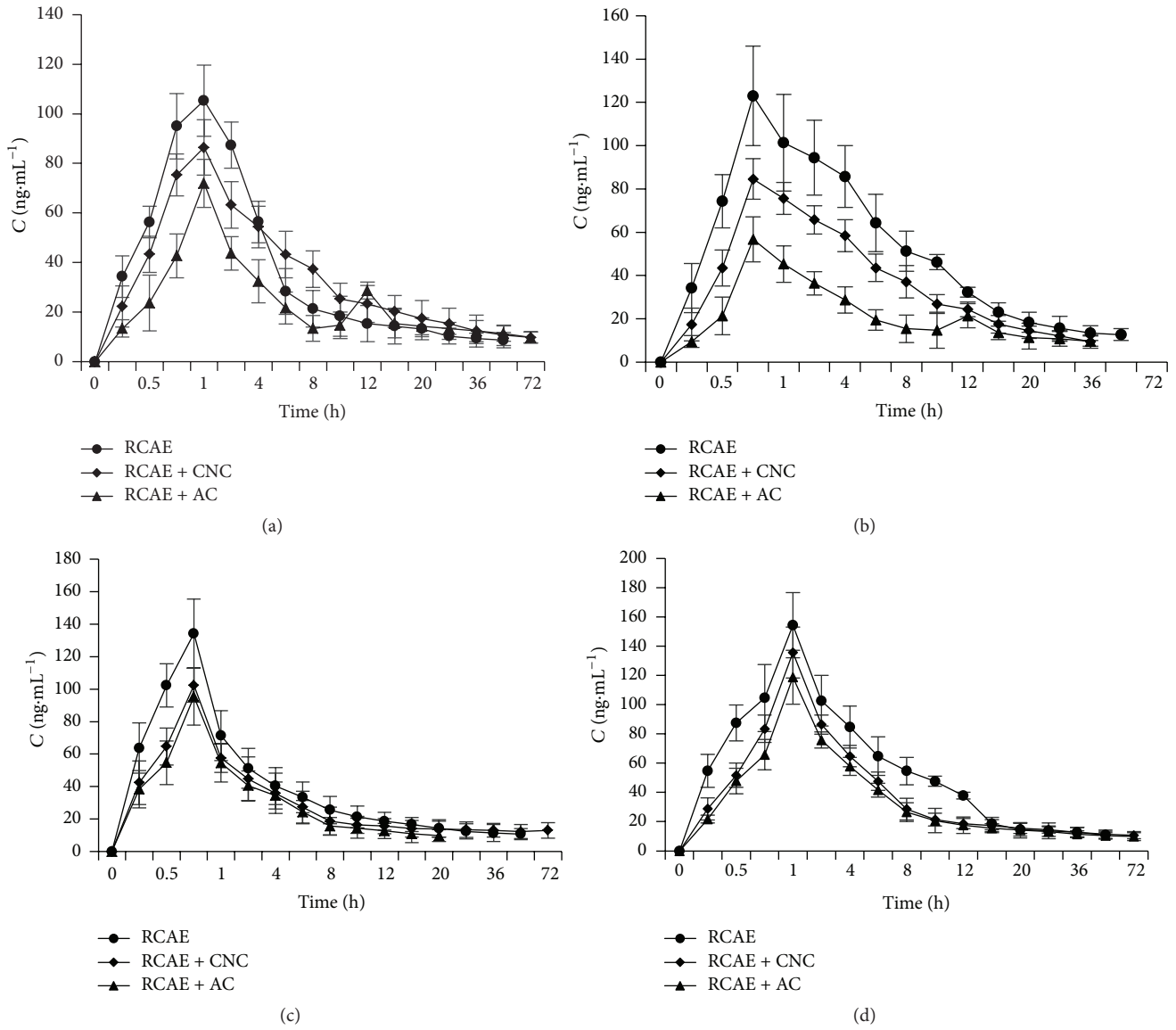

Figure 4: Concentration-time profile in plasma for berberine (a), coptisine (b), palmatine (c), and epiberberine (d).

requirements. All results showed that the HPLC method was reliable (Tables 2 and 3).

3.5. Concentration-Time Profile

3.5.1. Concentration-Time Profile of Berberine in Plasma. Figure 4(a) showed the concentration changes of berberine in plasma extracted from rabbits in Groups A–C. The concentration of plasma berberine in Group A was higher than in Group B from the point of administration to 2 h after administration. However, at 4 h after administration, the concentration in Group B exceeded that of Group A and this trend continued up until 72 h after administration. Meanwhile, the concentration of plasma berberine in Group C was lower than in Groups A and B, although the berberine concentration of Group C did increase again at 10 h after administration, showing a second peak in the concentration profile at 12 h after administration.

3.5.2. Concentration-Time Profile of Coptisine in Plasma. Figure 4(b) showed the concentration changes for coptisine in plasma for Groups A–C, indicating that the concentration in Group B was lower than in Group A during the study, while the concentration in Group C was lower than in Groups A and B. Again, the profile for Group C showed a second peak at 12 h after administration.

3.5.3. Concentration-Time Profile of Palmatine in Plasma. Figure 4(c) showed the concentration changes of plasma palmatine in Groups A–C. As can be seen from the figure, the concentration of plasma palmatine in Group B was lower than in Group A during the study, but the concentration of plasma

Table 2: Extraction replicates of the four analytes.

Compound	Origin amount/ng	Addition amount/ng	Measured amount/ng	Recovery rate (%)	Average recovery rate (%) (RSD%)
Epiberberine	50	25.0	74.3	97.2	98.5 (2.3)
	50	25.0	74.8	99.2	
	50	25.0	75.3	101.2	
	50	25.0	73.8	95.2	
	50	25.0	74.1	96.4	
	50	25.0	75.5	102.0	
Coptisine	35	17.5	52.4	99.4	98.0 (2.04)
	35	17.5	51.8	96.0	
	35	17.5	52.3	98.9	
	35	17.5	51.5	94.3	
	35	17.5	52.8	101.7	
	35	17.5	52.1	97.7	
Palmatine	55	27.5	81.1	94.9	96.2 (1.51)
	55	27.5	81.6	96.7	
	55	27.5	82.1	98.5	
	55	27.5	81.9	97.8	
	55	27.5	80.8	93.8	
	55	27.5	81.3	96.7	
Berberine	35	17.5	51.9	96.5	97.1 (0.98)
	35	17.5	52.1	97.7	
	35	17.5	51.7	95.4	
	35	17.5	52.1	97.7	
	35	17.5	51.9	96.6	
	35	17.5	52.3	98.8	

Table 3: Accuracy and precision of the four analytes in RCAE ($n = 6$).

Compound	Concentration (ng/mL)	Intraday precision			Interday precision		
		Measured amount ($\overline{x} \pm s$)	RSD (%)	RE (%)	Measured amount ($\overline{x} \pm s$)	RSD (%)	RE (%)
Coptisine	2.0	1.98 ± 0.033	0.41	1.0	1.97 ± 0.33	1.61	1.5
	35	34.950 ± 0.122	0.35	0.14	35.017 ± 0.213	0.61	0.048
	110	109.667 ± 0.314	0.28	0.30	109.967 ± 0.829	0.75	0.03
Berberine	10.0	9.932 ± 0.073	0.74	0.68	9.968 ± 0.117	1.17	0.032
	50.0	49.968 ± 0.084	0.17	0.11	50.050 ± 0.139	0.28	0.10
	110.0	109.93 ± 0.125	0.11	0.064	110.110 ± 0.827	0. 75	0.12
Palmatine	16	15.888 ± 0.085	0.53	0.70	15.922 ± 0.164	1.03	0.49
	200	199.682 ± 0.226	0.11	0.17	200.328 ± 0.839	0.42	0.164
	400	397.182 ± 1.594	0.40	0.71	399.945 ± 2.468	0.62	0.013
Epiberberine	6.0	5.96 ± 0.025	0.42	0.68	6.02 ± 0.052	0.87	0.33
	50.0	49.785 ± 0.093	0.19	0.43	50.047 ± 0.592	1.18	0.094
	80.0	79.713 ± 0.294	0.37	0.36	79.823 ± 0.356	0.45	0.22

palmatine in Group C was lower than in Group B. However, the difference between Groups B and C was not significant.

3.5.4. Concentration-Time Profile of Epiberberine in Plasma. Figure 4(d) showed the concentration changes of epiberberine in plasma for Groups A–C. Akin to the results for palmatine, the concentration of plasma epiberberine in Group B was lower than in Group A during the study, while the concentration of plasma epiberberine in Group C was lower than in Group B. Again, the difference between Groups B and C was not significant.

3.6. Maximum Plasma Concentration, Time to Reach the Maximum Concentration, Area under Curve, and Drug Half-Life. Table 4 and Figures 5 and 6 showed the maximum plasma concentration (C_{max}), the time required to reach

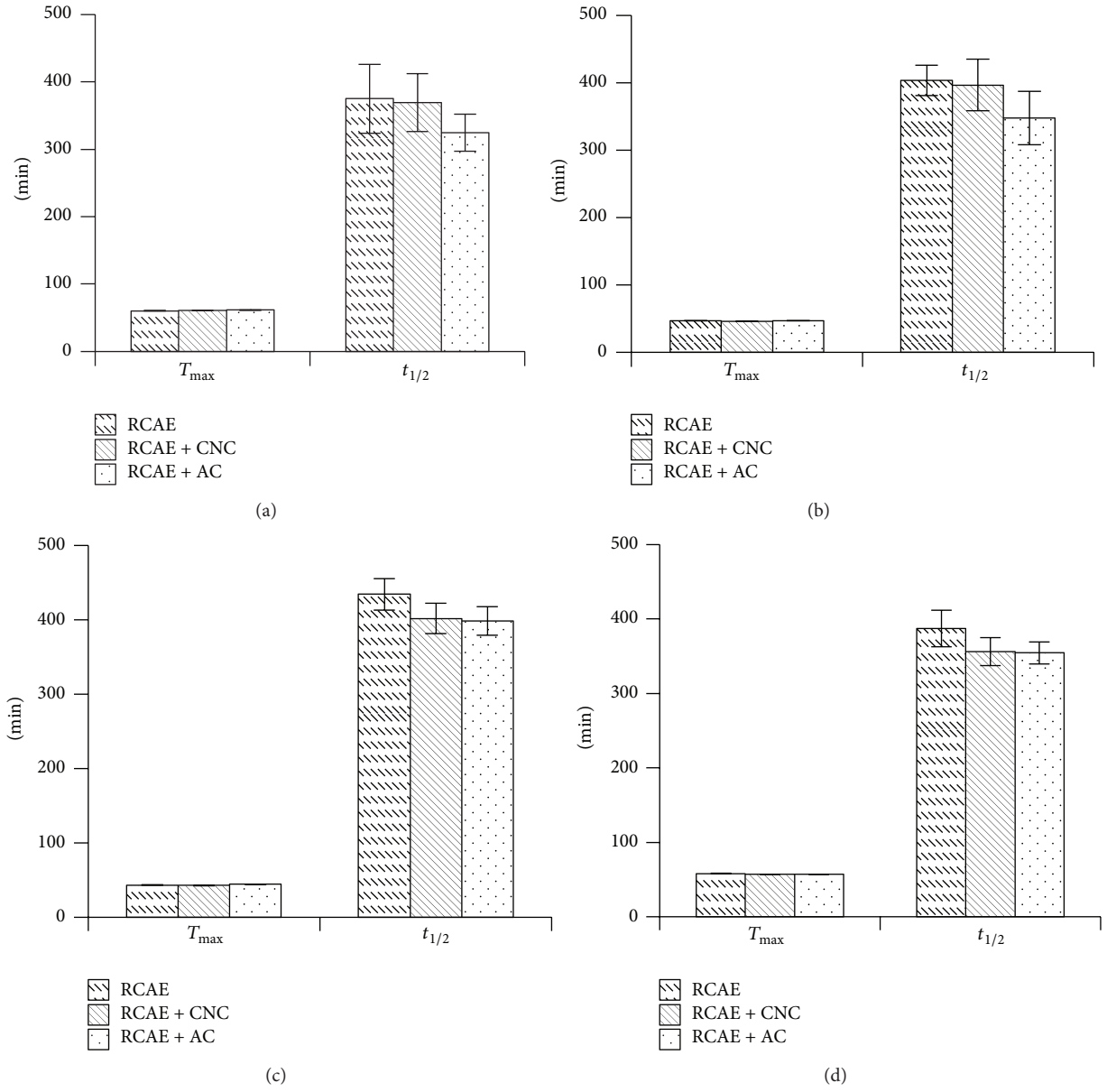

Figure 5: The time required to reach the maximum concentration (T_{max}) and the half-life ($t_{1/2}$) for berberine (a), coptisine (b), palmatine (c), and epiberberine (d).

the maximum concentration (T_{max}), the area under the concentration-time curve (AUC_{0-t}), and the half-life ($t_{1/2}$) for berberine (Figure 5(a)), coptisine (Figure 5(b)), palmatine (Figure 5(c)), and epiberberine (Figure 5(d)).

There was a significant difference with regard to C_{max} of berberine between the groups, whereby Group A > Group B > Group C. However, there was no significant difference with regard to T_{max} among the three groups ($P > 0.05$). $t_{1/2}$ of berberine in Group C was significantly lower than those of Groups A and B. The differences with regard to the AUC_{0-t} among the three groups were significant ($P < 0.05$), whereby Group B > Group A > Group C (Table 4). All of the above results (Figure 3(a)) indicated that CNC enhanced the bioavailability of berberine in comparison to AC, which decreased the bioavailability. The results also suggested that CNC may prolong the release of berberine in comparison to RCAE alone.

There was a significant difference with regard to C_{max} of coptisine among the groups, whereby Group A > Group B > Group C. However, there was no significant difference with regard to T_{max} among the three groups ($P > 0.05$). Meanwhile, $t_{1/2}$ of coptisine for Group C was significantly lower than those of Groups A and B ($P < 0.05$). The difference with regard to the AUC_{0-t} among the three groups was significant ($P < 0.05$), with Group A > Group B > Group C (Table 4). All of the above results (Figure 3(b)) indicated that

Table 4: Area under concentration-time curve (AUC_{0-t}) for the four analytes in Groups A–C.

Group	Berberine	Coptisine	Palmatine	Epiberberine
Group A	8123.2 ± 1734.1[b]	8092.3 ± 1423.7[a]	8674.3 ± 1534.7[a]	8415.1 ± 1434.2[a]
Group B	8432.21 ± 1831.3[a]	7532.4 ± 1231.4[b]	8347.2 ± 1617.4[b]	8117.4 ± 1534.3[b]
Group C	5472.41 ± 1041.7[c]	5317.3 ± 1047.4[c]	8274.7 ± 1537.8[b]	8074.2 ± 1374.5[b]

Note: "a, b, and c" indicated the difference of AUC_{0-t} among three groups.

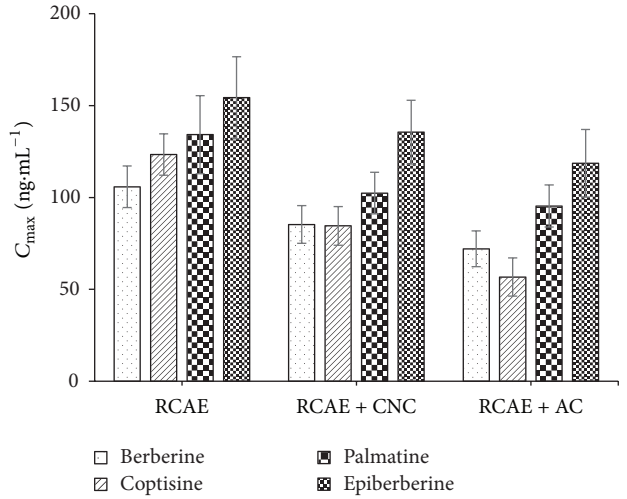

Figure 6: Maximum plasma concentration (C_{max}) for berberine, coptisine, palmatine, and epiberberine.

both CNC and AC decreased the bioavailability of coptisine in comparison to RCAE alone; however, CNC had a less significant effect compared to AC.

The C_{max} of palmatine in Group A was significantly higher than those of Groups B and C ($P < 0.05$), but the difference in C_{max} values between Groups B and C was not significant ($P > 0.05$). The differences with regard to T_{max} and $t_{1/2}$ of palmatine among the three groups were not significant. Meanwhile, the AUC_{0-t} of Group A was higher than Groups B and C, whereby Group A > Group B > Group C (Table 4); however, the differences were not significant. These results (Figure 3(c)) indicated that both CNC and AC may decrease the bioavailability of palmatine in comparison to RCAE alone.

With regard to C_{max} of epiberberine, Group A showed a significantly higher concentration than Groups B and C ($P < 0.05$), but the difference between Groups B and C was not significant ($P > 0.05$). With regard to T_{max} and $t_{1/2}$ of epiberberine, differences between the three groups were not significant. Furthermore, differences in the AUC_{0-t} between the groups were not significant (Table 3). These results indicated that CNC and AC may decrease the concentration of epiberberine in plasma in comparison to RCAE alone, although other parameters appear to be less affected.

4. Discussion

Carbonized herbal medicines have traditionally been used in Chinese medicine, with their use being first recorded 2000 years ago in *Prescriptions for Fifty-Two Diseases*. Recent researches have suggested that carbonized drugs may indeed have clinically relevant, curative effects [9–11]. However, investigation of the mechanism of action for carbonized Chinese herbal medicines has largely fallen behind their clinical applications and has mainly focused on the various chemical components and trace elements contained therein [12–15]. To address this shortfall, in this study the mechanism of carbonized herbal medicines was investigated via the effects of CNC on the pharmacokinetics of berberine, coptisine, palmatine, and epiberberine *in vivo*, which are the main alkaloids contained in RC. The results indicated that orally administered CNC in combination with RCAE enhanced the bioavailability of the alkaloids in comparison to RCAE and AC, and CNC prolonged the release of berberine in comparison to RCAE alone. However, CNC with RCAE resulted in decreased bioavailability of coptisine, palmatine, and epiberberine. The reason why CNC enhanced the bioavailability of some compounds over AC and prolonged the release of berberine may be due to the presence of CNC micropowder, which may adsorb alkaloids, thus prolonging their retention in the small intestine, from where they can be reabsorbed. However, this adsorption capacity may be strong, resulting in the decreased release of some alkaloids from CNC prior to excretion. This mechanism would account for the differences found here between the alkaloids as some may adhere more strongly to the micropowdered CS, hindering their release in the small intestine.

The bioavailability of berberine, coptisine, palmatine, and epiberberine decreased when RCAE was orally administered with AC. This result is in accordance with the accelerated clearance of drugs as a result of AC, with a concomitant decline of bioavailability [16, 17]. This is one of the reasons why AC has been used for the treatment of intoxication as a result of some drugs [7, 18]. However, the reason for the second peak in the concentration-time profiles of berberine and coptisine for Group C (Figure 2) is not well understood. However, similar secondary peaks have been observed in the concentration-time profiles of aconitine, hypaconitine, and mesaconine following administration of prepared Radix Glycyrrhizae and prepared *Aconitum carmichaelii* Debx. [19]. Typically, there are five reasons for a double peak concentration-time profile in pharmacology. Firstly, the drug may arrive at the small intestine in two (or more) batches due to nonuniform gastric emptying. The second reason may be that two different parts of the gastrointestinal tract are involved in drug absorption with different rates. The third possible explanation is due to the enterohepatic cycle. The fourth reason relates to pharmaceutics containing ingredients that delay release or promote fast release, and the fifth and

final reason is due to the liposolubility of the drug distributed throughout the tissue, which may allow release of the drug into the blood again when the component in blood has declined to a certain extent. In this study the double peak concentration-time profile for some alkaloids in Group C may arise mainly as a result of pharmaceutic agents, with the aqueous extract providing fast release while the addition of AC resulted in delayed release.

5. Conclusions

The results of this research have shown that there was a significant difference between the effect of AC and CNC on the transportation of RC alkaloids *in vivo*, whereby AC results in a double peak concentration-time profile for some alkaloids. It was noteworthy that while CNC decreased the bioavailability of RC alkaloids in comparison to RCAE administered alone, it increased their bioavailability in comparison to AC and it prolonged the release of berberine.

Further investigations will be required to elucidate the precise mechanism of action of carbonized Chinese herbal medicines *in vivo*.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Authors' Contributions

Yanfei He and Siyu Chen made equal contribution.

Acknowledgments

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Review Article

Chinese Herbal Medicine as Adjunctive Therapy to Chemotherapy for Breast Cancer: A Systematic Review and Meta-Analysis

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Chinese herbal medicine (CHM) has been increasingly employed during therapy for breast cancer, but its efficacy remains a matter of debate. This systematic review examined randomized controlled trials to provide a critical evaluation of this treatment. The results demonstrated that the combined use of CHM with chemotherapy may improve the immediate tumor response and reduce chemotherapy-associated adverse events. Our findings highlight the poor quality of Chinese studies, and additional well-designed randomized controlled trials addressing the role of CHM are warranted. The lack of molecular-based evidence for CHM and Zheng has resulted in a limited understanding and acceptance of CHM and traditional Chinese medicine in Western countries. We believe that researchers should immediately explore a CHM-based cure, and CHM should be applied to routine care as soon as conclusive data are available.

1. Introduction

Breast cancer is the leading cause of cancer-related deaths in women worldwide [1]. Breast cancer has been one of the fastest growing cancers in China over the past 30 years, with an incidence approaching approximately 96%, which is only slightly lower than that of lung cancer [2].

Chemotherapy plays a key role in the systemic treatment of breast cancer, and it is the cornerstone of therapy for patients who are not candidates for endocrine therapy [3]. Adjuvant chemotherapy can increase the pathological complete response (CR) rate and improve survival in most patients with early stage breast cancer [4–6]. The primary objectives of treatment are palliation and improved survival for the vast majority of metastatic breast cancers, which are incurable.

The chemical agents used in chemotherapy are selectively destructive to malignant tissues, but these agents also damage healthy tissue, which results in adverse side effects that negatively impact compliance with cancer treatment. Therefore, there is a clinical need to find an intervention to manage the adverse side effects of chemotherapy and increase patient tolerance and well-being.

Many breast cancer patients take complementary and alternative medicine (CAM), usually in combination with anticancer treatments, and the global use of CAM continues to increase dramatically [7–9]. The application of CAM in Western countries ranges from 9% to 69% [7, 8, 10–15]. Notably, these studies highlighted the use of traditional Chinese herbal medicine (CHM) for breast cancer.

Traditional Chinese medicine (TCM) was developed thousands of years ago, long before the advent of modern

science. The use of TCM-based CHM for breast cancer has been described in Chinese medical texts for more than 2000 years [16, 17]. CHM, including botanical, animal, and mineral agents, used by TCM physicians aims to control the side effects and toxicities of cancer therapies, which improves the patient's quality of life (QoL), prevents recurrence, and prolongs survival [18].

Most studies on the clinical efficacy of CHM are based on either personal experience or case reports. Therefore, it is difficult to reach evidence-based conclusions. We conducted this systematic review to evaluate the efficacy of CHM as an adjunctive therapy to chemotherapy for the treatment of breast cancer compared with the use of chemotherapy alone because of the high prevalence of breast cancer in women and the frequent use of chemotherapy in women who present to TCM practitioners.

2. Methods

This systematic review was conducted using the PRISMA statement [19]. We developed a protocol prior to conducting the review.

2.1. Databases. Two authors independently searched for publications dated as late as August 30, 2014, in the following electronic databases: MEDLINE via OvidSP, EMBASE via OvidSP (as shown in the list below), CINAHL via OvidSP, AMED via OvidSP, PubMed, CENTRAL via OvidSP, Chinese BioMedical Literature Database (CBM), the Chinese database CNKI, Wan Fang, and VIP. The following terms were used in the search: breast cancer, breast neoplasm, breast tumor, mammary cancer, mammary neoplasm, human mammary carcinomas, chemotherapy, Chinese herbals, Chinese herbal drugs, Chinese medicine, traditional Chinese medicine, CHM, TCM, herbals, Kampo, herbal therapy, complementary medicine, alternative medicine, plants, and botany. A manual review of the reference lists of all accepted papers was also conducted.

Search Strategy for EMBASE via OvidSP to Identify Potential Articles

(1) Clinical trial/
(2) Randomized controlled trial/
(3) Randomization/
(4) Single blind procedure/
(5) Double blind procedure/
(6) Crossover procedure/
(7) Placebo/
(8) Randomized controlled trial$.tw.
(9) Rct.tw.
(10) Random allocation.tw.
(11) Randomly allocated.tw.
(12) Allocated randomly.tw.
(13) (allocated adj2 random).tw.
(14) Single blind$.tw.
(15) Double blind$.tw.
(16) ((treble or triple) adj (blind$)).tw.
(17) Placebo$.tw.
(18) Prospective study/
(19) Or/1–18
(20) Case study/
(21) Case report.tw.
(22) Abstract report/or letter/
(23) Or/20–22
(24) 19 not 23
(25) exp breast neoplasms/
(26) (breast adj5 neoplasm$).ti,ab.
(27) (breast adj5 cancer$).ti,ab.
(28) (breast adj5 tumo$).ti,ab.
(29) (breast adj5 carcinoma$).ti,ab.
(30) (breast adj5 adenocarcinoma$).ti,ab.
(31) (breast adj5 dcis).ti,ab.
(32) (breast adj5 ductsl).ti,ab.
(33) (breast adj5 sarcoma$).ti,ab.
(34) (breast adj5 infiltrating).ti,ab.
(35) (breast adj5 intraductal).ti,ab.
(36) (breast adj5 lobular).ti,ab.
(37) (breast adj5 medullary).ti,ab.
(38) Or/25–37
(39) exp chinese medicine/
(40) exp medicine, east asian traditional/or exp medicine, chinese traditional/or exp medicine, kampo/or exp medicine, korean traditional/or exp medicine, mongolian traditional/
(41) exp complementary medicine/
(42) exp traditional chinese medicine/
(43) Or/39–42
(44) 38 and 43
(45) 24 and 44

2.2. Inclusion Criteria. The inclusion criteria were as follows. (1) *Types of Studies*. There were randomized controlled trials (RCTs) with two arms, without blinding or language restrictions. (2) *Types of Participants*. They were female breast cancer patients who were diagnosed using pathological sections and treated with chemotherapy. (3) *Types of Interventions*. They included chemotherapy protocols of experimental and control groups that were the same or comparable; CHM was used in experimental groups as adjunctive therapy to chemotherapy; placebo or blank controls were eligible; and other treatment was identical in both groups. (4) *Type of Outcome*. The following primary outcomes were included: survival;

immediate tumor response, defined as CR or partial response (PR) using the World Health Organization scale; control of nausea and vomiting; and improvement in myelosuppression. The following secondary outcomes were included: performance status evaluated using the Karnofsky performance score (KPS); immune system response, including percent change in T lymphocytes and natural killer (NK) cell activity; QoL; and other side effects, such as alopecia, chemotherapy-related cardiotoxicity, and cognitive dysfunction.

2.3. Exclusion Criteria. The exclusion criteria were studies of breast cancer patients with other primary cancer; sample size < 30; CHM used in both groups other than experimental medicine and placebo; studies with > 20% withdrawal and/or dropout rates; nonoriginal studies; or duplicate studies.

2.4. Study Selection. Two authors independently screened the trials by first scanning abstracts, titles, and key words to select potential studies based on the inclusion and exclusion criteria. Full articles of the potential studies were obtained for a final determination. If a disagreement occurred, the two authors reviewed the study again, and a third reviewer resolved any disagreement.

2.5. Quality Assessment. Methodological quality was assessed using the 5-point Jadad scale [20]. The Jadad scale includes three domains: randomization, blinding, and withdrawals and dropouts. Only studies with a Jadad score of 3, 4, or 5 were included.

2.6. Assessment of the Risk of Bias. The risk of bias was assessed using the method recommended by the *Cochrane Handbook* [21]. This tool is a domain-based evaluation in which critical assessments are made separately for the following concepts: randomization, blinding, outcome reporting, and other issues.

2.7. Data Management. Two authors independently extracted the following variables: article title; author(s); journal title; year of publication; study design; sample size; sampling and diagnostic procedures; loss to follow-up; exclusions and reasons; baseline characteristics of patients (e.g., age, breast cancer stage); intervention characteristics (e.g., chemotherapy drugs, CHM patterns, CHM type, duration, and dosage); outcome(s); and conclusions. A third reviewer reviewed the extracted data and stored the original data in a secure computer to avoid changes.

2.8. Statistical Analysis. We performed a meta-analysis using RevMan 5.2 only when sufficient and suitable data were obtained. We conducted a narrative synthesis when there were too few clinically homogeneous studies for a meta-analysis. We calculated the RR and MD separately for dichotomous and continuous variables. A random effects model was used for pooling because of the clinical heterogeneity of CHM, which includes the complexity of ingredients and different therapeutic methods. We analyzed the pre- and posttreatment data separately to avoid biases from estimations of the values of change from baseline. We assessed heterogeneity using the χ^2 test, with a p value < 0.10 indicating a significant difference. Subgroup analyses were conducted to investigate the source of heterogeneity. Funnel plot asymmetry was used to investigate publication bias when there were at least 10 studies. The total effect was tested using the Z test, and $p < 0.05$ was used to identify significant effects.

3. Results

3.1. Characteristics of Included Studies. The initial search identified 2,109 studies published before October 2014 (Figure 1). Review of the titles and abstracts resulted in the inclusion of 243 studies. Of these, 211 studies were excluded after review of the full text because the studies were duplicates and animal experiments, had a small sample size, had a Jadad score < 3, or did not report the clinical stage. Notably, 90 Chinese studies were excluded because of a low Jadad score, which suggests that Chinese studies in this field are of poor quality. A total of 31 studies were finally included in the meta-analysis, of which 28 were retrieved from Chinese databases and 3 from English databases.

Of the total 2,805 patients who were enrolled in these studies, 1,391 received CTC therapy and 1,319 received chemotherapy alone. Ninety-five patients withdrew or dropped out (Table 1).

All trials were described as randomized and had two parallel groups. Concealment of allocation was not reported in any of the Chinese studies. The blinding process was described in the 3 English studies and in only 1 Chinese study. Eight studies reported withdrawals and dropouts. The risk of bias, as assessed using the tool from the Cochrane Collaboration, is shown in Figures 2 and 3.

All recruited patients in the 31 studies were diagnosed using pathological sections, and all studies had a Jadad score of 3, 4, or 5, indicating that the baseline characteristics were comparable among studies (Table 1).

3.2. Survival. One study reported 2-year survival with better survival in the CTC group than in the chemotherapy group, but the difference was not significant (RR = 1.15, 95% CI = 0.86–1.53) [26]. However, the median survival time differed significantly between these groups in another study (WMD = 1.90, 95% CI = 0.77–3.03) [25]. However, neither trial was of high quality.

3.3. Immediate Tumor Response. The immediate tumor response was investigated in 20 studies (1,282 patients). CTC therapy was associated with a significantly higher rate of CR or PR (RR = 1.15, 95% CI = 1.06–1.25, and $p = 0.0006$) (Figure 4) [22, 24–26, 28–35, 37, 40, 43–47, 51]. The exclusion of any one study did not influence the estimated treatment effect. The funnel plot indicated publication bias (Figure 5).

3.4. Decrease in Chemotherapy Toxicity. Chemotherapy-induced nausea and vomiting (CINV) is one of the most serious and unwanted side effects of chemotherapy [53–55].

Table 1: Characteristics of the included studies for the use of CHM as an adjunct for chemotherapy in breast cancer patients.

Study	Number of participants/dropouts	TNM stage	Duration (weeks)	Control group interventions	CHM interventions	Outcome(s)	Jadad scale score
Dang and Wang 2010 [22]	48/0	I–III	9	CTF	Aidi injection	Tumor response, cardiotoxicity, KPS, and chemotoxicity	3
Barton et al. 2013 [23]	210/44	I–IV	52	TE/TEC	Ginkgo Biloba	Cognitive dysfunction	5
Fang 2009 [24]	60/0	III/IV	3	CTF	Shenqi Wuweizi pill	Tumor response, KPS, and chemotoxicity	3
Fu and Kou 2007 [25]	88/0	IV	8	NP	Aidi injection	Tumor response, KPS, chemotoxicity, and median survival	3
Hong et al. 2014 [26]	91/7	II–IV	18	TEC	Xihuang pill	Tumor response, KPS, and overall survival	3
Huang et al. 2003 [27]	66/0	II–IV	4	CMF	Bazhen decoction	KPS and chemotoxicity	3
Huang et al. 2007 [28]	60/0	IV	6	CTF	Jianpi Xiaoji decoction	Tumor response, KPS, and immune system	3
Huang et al. 2008 [29]	60/0	III/IV	6	CTF	Shenqi Fuzheng injection	Tumor response, KPS, and chemotoxicity	3
Huang et al. 2013 [30]	60/0	IV	18–24	CEF	Huangqi injection	Tumor response, KPS, chemotoxicity, and median survival	3
Li et al. 2003 [31]	101/0	I–III	6~9	CMF	Rukang I prescription	Tumor response, KPS, and chemotoxicity	3
Li and Gong 2006 [32]	52/0	I–III	9	CEF	Aidi injection	Tumor response and chemotoxicity	3
Lu 2010 [33]	60/0	III/IV	6	CAF	Huangqi injection	Tumor response	3
Lv et al. 2014 [34]	54/0	IV	12–16	FAC	Yiqi Huoxue Huayu decoction	Tumor response, KPS, chemotoxicity, and immune system	3
Ni 2006 [35]	57/0	IV	13	Docetaxel + THP	Gaolisheng injection	Tumor response and chemotoxicity	3
Pérol et al. 2012 [36]	430/27	I–IV	8–12	FAC	Cocculine	Chemotherapy-induced emesis	5
Qi 2010 [37]	40/0	II–IV	6	TE	Yiqi Yangxue Shugan decoction	Tumor response, KPS, quality of life, and chemotoxicity	3
Qin 2013 [38]	78/0	I–IV	21	CAF	Fuzheng Qiqi Jiedu decoction	KPS	3
Semiglazov et al. 2006 [39]	352/21	I–III	16–24	CMF	Mistletoe extract	Quality of life	5
Shen 2007 [40]	100/0	IV	6	NVB + THP	Aiyishu injection	Tumor response and KPS	3
Sun et al. 2010 [41]	86/0	II–IV	3	CAF/AC	Zaofan pill	Chemotoxicity	3
Wang 2007 [42]	60/0	II-III	9	CEF	Taohong Siwu decoction	Quality of life and chemotoxicity	3
Wang 2010 [43]	40/0	II–IV	8	TE	Yiqijianpi Huayujiedu decoction	Tumor response, quality of life, and chemotoxicity	3
Wen et al. 2010 [44]	60/0	IV	3	TA	Fuzheng Xiaoyan prescription	Tumor response, quality of life, chemotoxicity, and immune system	3
Xiong 2012 [45]	48/0	IV	6	NVB + CAP	Fuzhengxiaoji decoction	Tumor response and KPS	3
Yang 2004 [46]	59/0	IV	6	NVB + THP	Aidi injection	Tumor response, KPS, and immune system	3
Yang et al. 2008 [47]	59/4	III B-IV	6–8	NP	Guben Yiliu II decoction	Tumor response and KPS	3
Yi et al. 2008 [48]	60/0	IV	12	DOX	Ginkgo Biloba	Cardiotoxicity	3
Zhang et al. 2010 [49]	80/0	I/II/III	18	CEF	Huangqi Taohong decoction	Immune system	3
Zhang et al. 2011 [50]	45/0	III-IV	6	CTF	Fuzheng Quyu Jiedu prescription	Immune system and quality of life	3
Zhang and Li 2013 [51]	96/0	II-III	3-4	CTF	Tiaogan Jianpi prescription	Tumor response	3
Zhong 2009 [52]	40/0	I–IV	6	TE	Shugan Tiaoli Chongren decoction	Quality of life, KPS, and chemotoxicity	3

KPS: Karnofsky performance score.

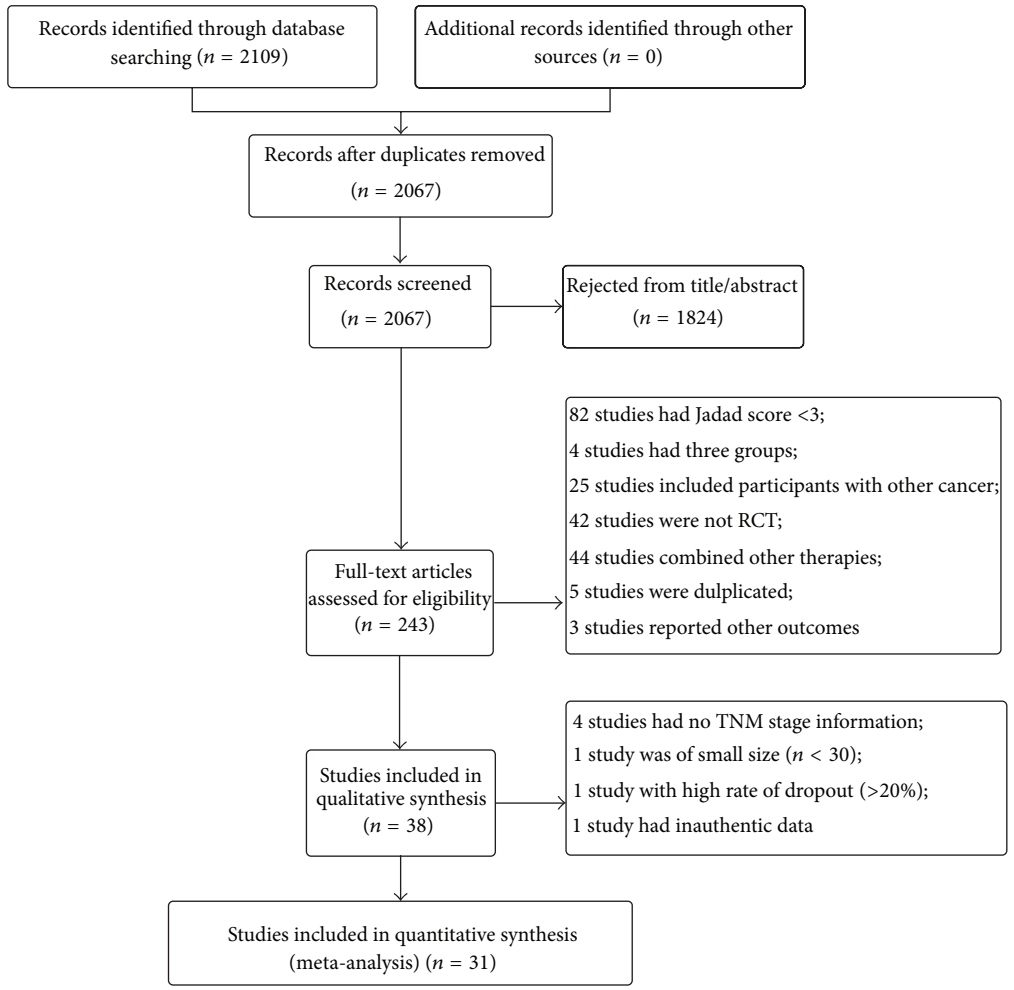

Figure 1: Study flow diagram of the selection process.

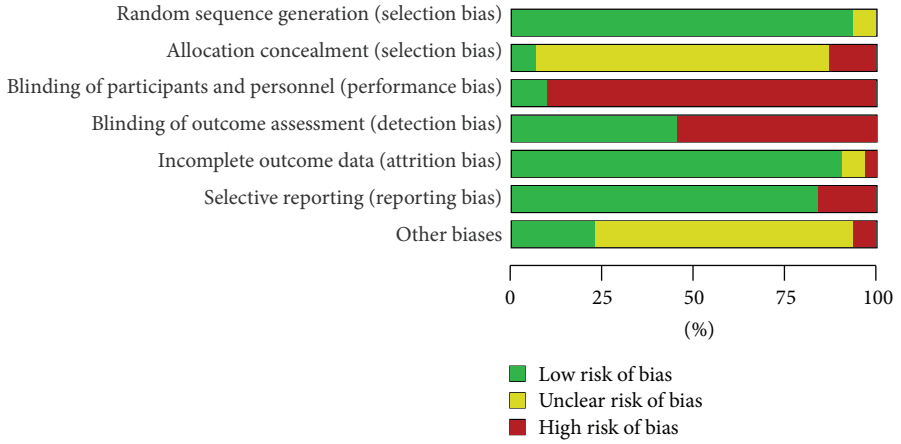

Figure 2: Risk of bias in included studies.

The frequency of grade II–IV CINV was significantly lower in the CTC group than in the chemotherapy group (RR = 0.53, 95% CI = 0.37–0.77, and $p = 0.0009$, 8 studies, 428 patients) (Figure 6) [22, 27, 30, 34, 42–44, 52]. The frequency of grade III-IV CINV was significantly lower in patients undergoing CTC therapy (RR = 0.23, 95% CI = 0.13–0.42, and $p < 0.00001$, 10 studies, 581 patients) (Figures 7 and 8) [22, 27, 30–32, 34, 42–44, 52]. However, one study, conducted in France, reported no differences in scores for nausea, vomiting, or global emesis on the Functional Living Index-Emesis tool

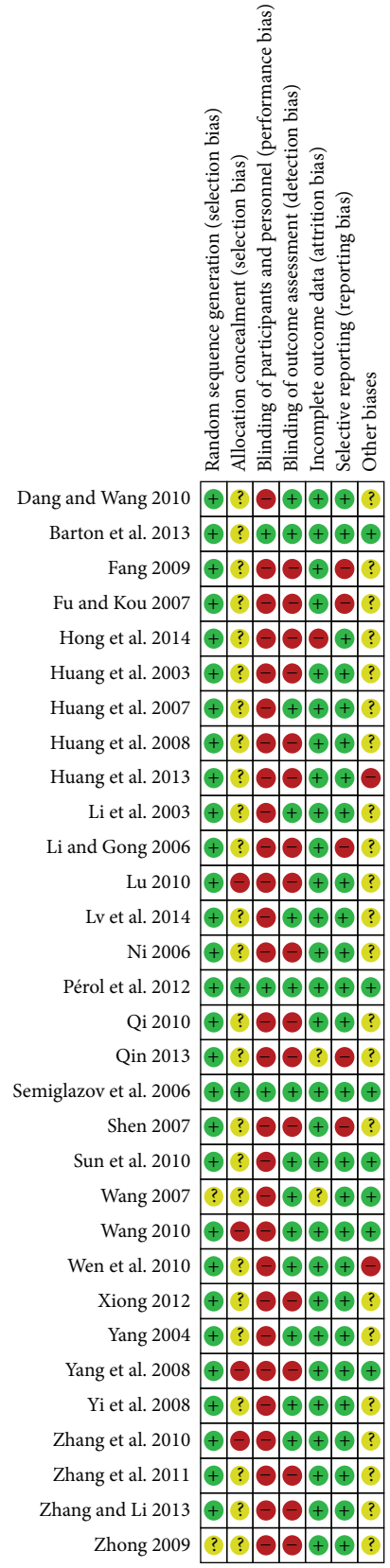

Figure 3: Summary of the risk of bias in included studies.

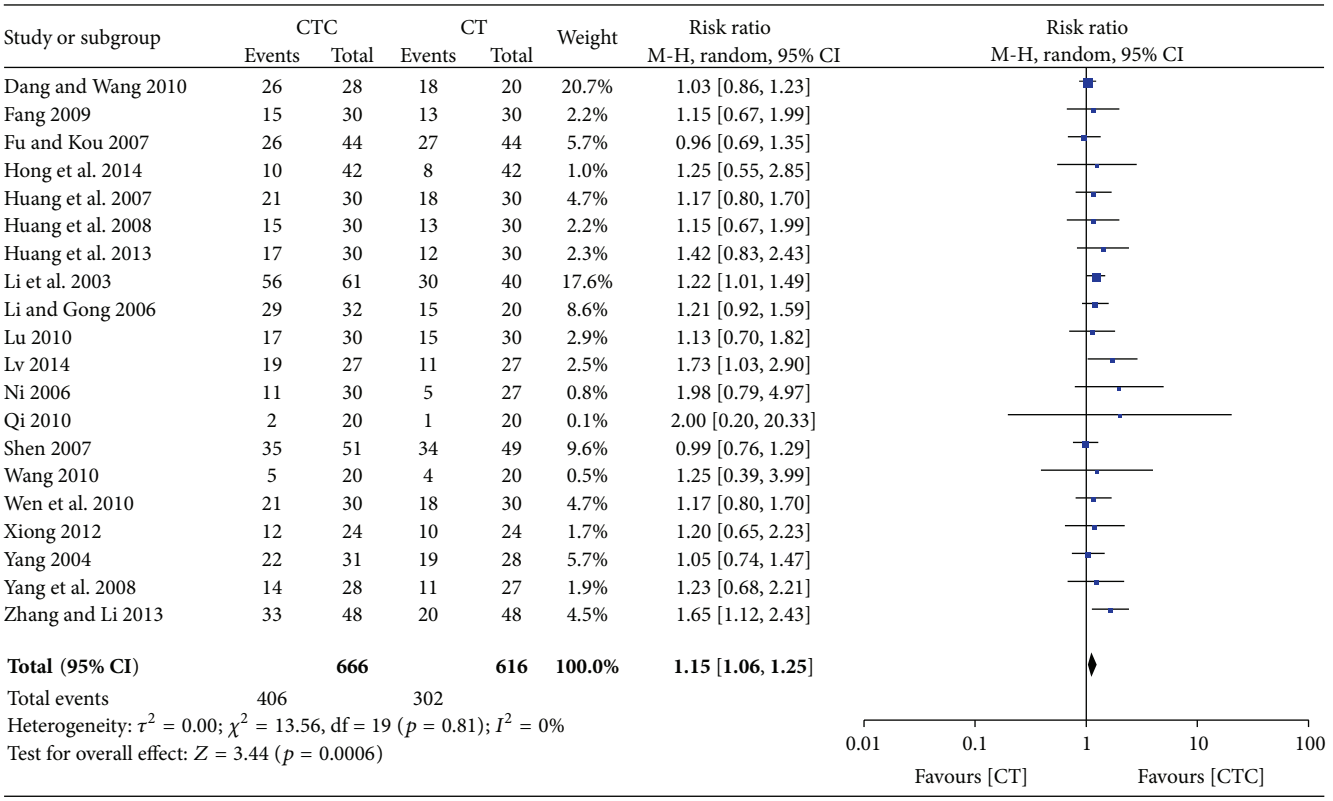

Figure 4: Immediate tumor response in breast cancer (CR + PR).

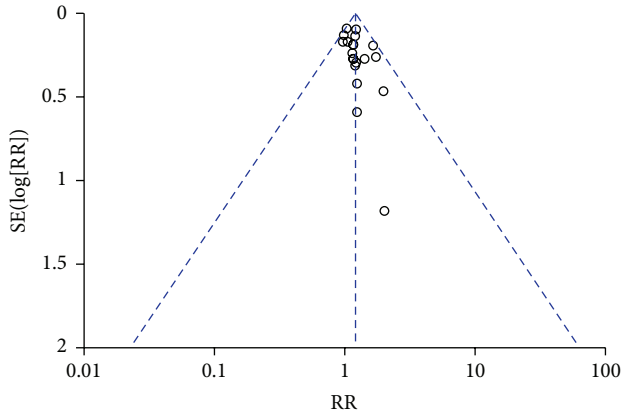

Figure 5: Funnel plot of immediate tumor response in breast cancer (CR + PR).

between the two arms (WMD = 0.02, 95% CI = −0.29–0.33) [36], and another study reported a significant difference [41]. These two studies were not pooled for analysis because of the different data types.

Grade III-IV-induced reductions in white blood cell (WBC) counts were significantly less frequent in the CTC group (RR = 0.68, 95% CI = 0.58–0.78, and $p < 0.00001$, 11 studies, 653 patients) (Figures 9 and 10) [24, 27, 29–32, 34, 42–44, 52]. Grade III-IV-induced reductions in WBC counts were also significantly less frequent in the CTC group (RR = 0.29, 95% CI = 0.17–0.47, and $p < 0.00001$, 9 studies, 530 patients) (Figure 11) [27, 30–32, 34, 42–44, 52].

Figure 12 shows that grade I–IV-induced reductions in platelets decreased in the CTC group (RR = 0.52, 95% CI = 0.33–0.80, and $p = 0.003$, 6 studies, 314 patients) [30, 34, 42–44, 52].

Grade I–IV-induced reductions in hemoglobin were significantly less frequent in participants undergoing combined treatment (RR = 0.63, 95% CI = 0.44–0.90, and $p = 0.001$, 3 studies, 154 patients) (Figure 13) [34, 42, 52].

3.5. Performance Status. Different types of KPS data were calculated in the studies. The first type was the improvement or stabilization of the KPS using a cutoff value of a 10-point change. The second type was the pre- and posttreatment KPS values. KPS improvement (≥10-point increase) was significantly better in the CTC group (RR = 1.81, 95% CI = 1.49–2.19, and $p < 0.00001$, 13 studies, 850 patients) (Figures 14 and 15) [24, 25, 27–30, 34, 35, 38, 40, 44–46]. The rate of improvement and stabilization (change in KPS of >0) was also significantly higher in participants receiving CTC (RR = 1.36, 95% CI = 1.26–1.47, and $p < 0.00001$, 13 studies, 850 patients) (Figures 16 and 17) [24, 25, 27–30, 34, 35, 38, 40, 44–46].

Pre- and posttreatment KPSs were reported in 6 studies. The difference in pretreatment KPS was not significant between the two arms (MD = −0.24, 95% CI = −1.32–0.84, and $p = 0.67$, 6 studies, 368 patients) (Figure 18) [22, 26, 31, 37, 47, 52]. The posttreatment KPS was significantly higher in the CTC group than in the chemotherapy group

Study or subgroup	CTC Events	CTC Total	CT Events	CT Total	Weight	Risk ratio M-H, random, 95% CI
Dang and Wang 2010	16	28	17	20	26.2%	0.67 [0.46, 0.97]
Huang et al. 2003	9	37	13	29	15.8%	0.54 [0.27, 1.09]
Huang et al. 2013	6	30	14	30	13.2%	0.43 [0.19, 0.96]
Lv et al. 2014	4	27	10	27	9.5%	0.40 [0.14, 1.12]
Wang 2007	12	30	12	30	17.8%	1.00 [0.54, 1.86]
Wang 2010	4	20	9	20	9.9%	0.44 [0.16, 1.21]
Wen et al. 2010	2	30	15	30	6.0%	0.13 [0.03, 0.53]
Zhong 2009	0	20	3	20	1.6%	0.14 [0.01, 2.60]
Total (95% CI)		**222**		**206**	**100.0%**	**0.53 [0.37, 0.77]**
Total events	53		93			

Heterogeneity: $\tau^2 = 0.10$; $\chi^2 = 11.58$, df = 7 ($p = 0.12$); $I^2 = 40\%$

Test for overall effect: $Z = 3.33$ ($p = 0.0009$)

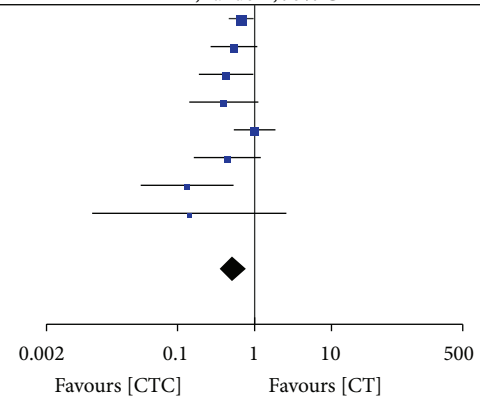

Figure 6: Nausea and vomiting during treatment for breast cancer (toxicity grades II–IV).

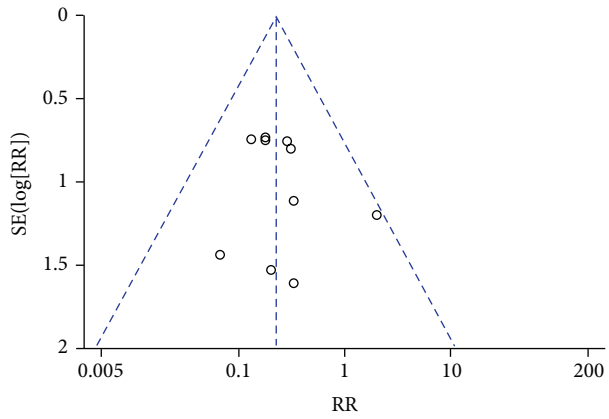

Figure 7: Funnel plot of nausea and vomiting during treatment for breast cancer (toxicity grades III-IV).

(MD = 6.31, 95% CI = 3.66–8.97, and $p < 0.00001$, 6 studies, 368 patients) (Figure 19).

3.6. Immunostimulation. Figures 20–24 show that, in the pooled studies, the differences in pretreatment T lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$) and NK cell levels between the two arms were not significant ($CD3^+$, MD = −1.09, 95% CI = −2.34–0.17, $p = 0.09$, and $I^2 = 0\%$; $CD4^+$, MD = −0.28, 95% CI = −1.08–0.52, $p = 0.50$, and $I^2 = 0\%$; $CD8^+$, MD = −0.12, 95% CI = −0.10–0.76, $p = 0.79$, and $I^2 = 0\%$; $CD4^+/CD8^+$, MD = −0.02, 95% CI = −0.11–0.08, $p = 0.71$, and $I^2 = 0\%$; NK cells, MD = −0.71, 95% CI = −2.36–0.94, $p = 0.40$, and $I^2 = 0\%$).

CTC therapy showed an advantage for $CD3^+$ and $CD4^+/CD8^+$ cells posttreatment (MD = 7.56, 95% CI = 6.28–8.85, $p < 0.00001$, and $I^2 = 5\%$, 6 studies, 358 patients, and MD = 0.26, 95% CI = 0.16–0.37, and $p < 0.00001$, 6 studies, 358 patients, resp.) (Figures 25 and 26) [28, 34, 44, 46, 49, 50]. However, the result indicates that the posttreatment NK cell level was not significantly different between the CTC and chemotherapy groups (MD = 2.30, 95% CI = −0.18–4.78, and $p = 0.07$, 2 studies, 134 patients) (Figure 27) [34, 49]. Heterogeneity existed among the included studies in terms of $CD4^+$ and $CD8^+$ cells posttreatment.

CTC therapy also resulted in significantly better posttreatment $CD4^+$ levels (MD = 7.30, 95% CI = 3.67–10.93, $p < 0.0001$, and $I^2 = 94\%$, 6 studies, 358 patients) (Figure 28). A subgroup analysis indicated that TNM stage may have affected the homogeneity. In the pooled studies, there were no significant differences in posttreatment $CD8^+$ levels between the two arms (MD = 1.41, 95% CI = −3.31–6.13, $p = 0.56$, and $I^2 = 96\%$, 6 studies, 356 patients) (Figure 29). The differences in sample size may have contributed to the heterogeneity of the studies.

3.7. QoL. Six studies reported on QoL, but we were not able to pool these results because each study used a different scale and data type. The study by Semiglazov et al. was of high quality and low risk, and the QoL was assessed using 3 FACT-G subscales (physical, emotional, and functional well-being) [39]. The intervention group exhibited improvements in the FACT-G total score and in the physical, emotional, and functional well-being scores, and the placebo group had poorer scores. Four studies that reported significantly improved QoL using combined therapy, as assessed using the Chinese version of the FACT-G, were of mixed quality [37, 42, 50, 52]. Two studies reported QoL as assessed using the Chinese version of the EORTC QLQ C-30. Zhong observed improvements in the physical and emotional subscales and the overall health QoL score with CTC therapy [52]. Wang reported significant within-group improvements in some subscales in both groups, but there were no details of comparisons between groups [43].

3.8. Other Outcomes. One high-quality study evaluated CTC therapy for the prevention of chemotherapy-related cognitive dysfunction and reported no significant difference between the two arms [23]. Two studies that reported chemotherapy-related cardiotoxicity had significant heterogeneity, and CTC

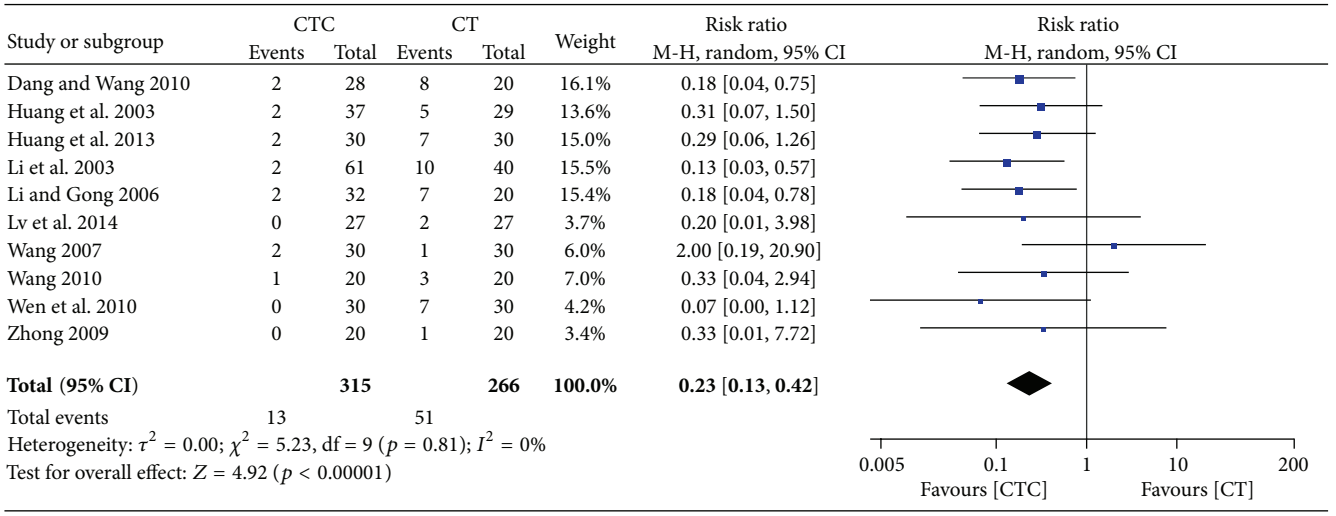

Figure 8: Nausea and vomiting during treatment for breast cancer (toxicity grades III-IV).

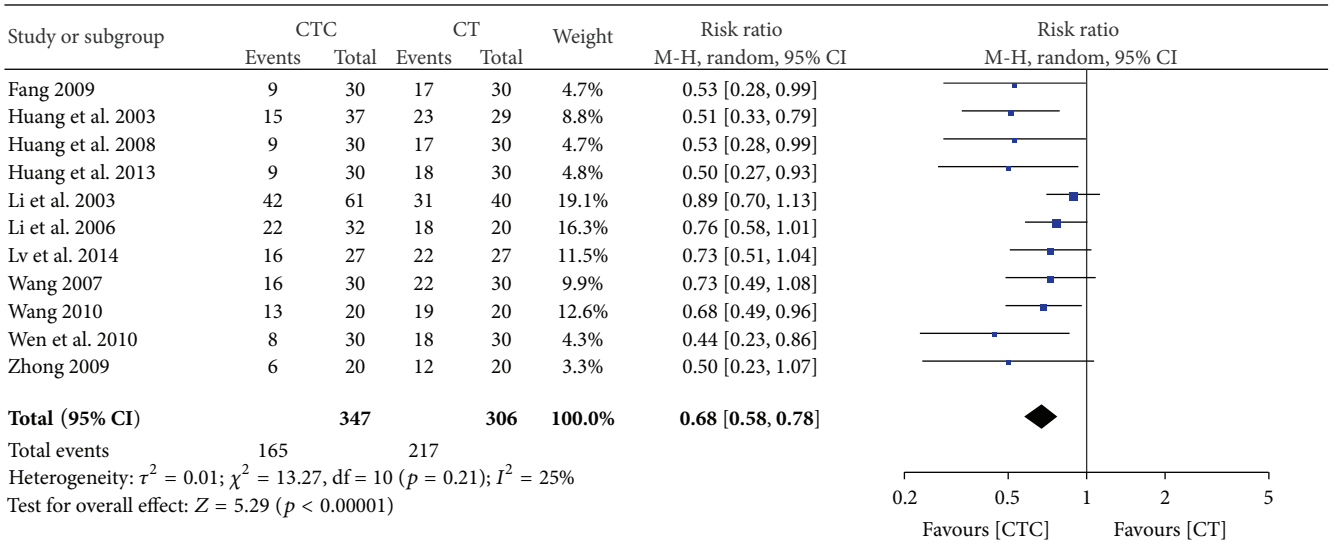

Figure 9: Reductions in WBCs during breast cancer treatment (toxicity grades I–IV).

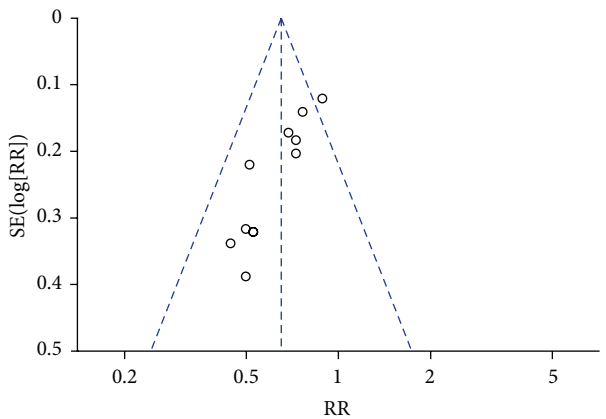

Figure 10: Funnel plot of the reduction in WBCs during breast cancer treatment (toxicity grades I–IV).

did not have a significant advantage in the prevention of creatinine kinase-MB isoenzyme (WMD = −21.11, 95% CI = −52.65–10.42, and $p = 0.19$) (Figure 30) [22, 48].

4. Discussion

Treatment of breast cancer using CHM has been described in Chinese medical texts for more than 2,000 years. Accepting TCM as science rather than myth remains a challenge in Western countries despite the recent increase in the use of CHM.

This review has several limitations. First, we did not identify studies in languages other than Chinese and English. CAM use is reportedly high in East Asia, where CHM originated, with use rates of 29–83% in South Korea and 50% in one study in Japan [56, 57]. Therefore, additional studies

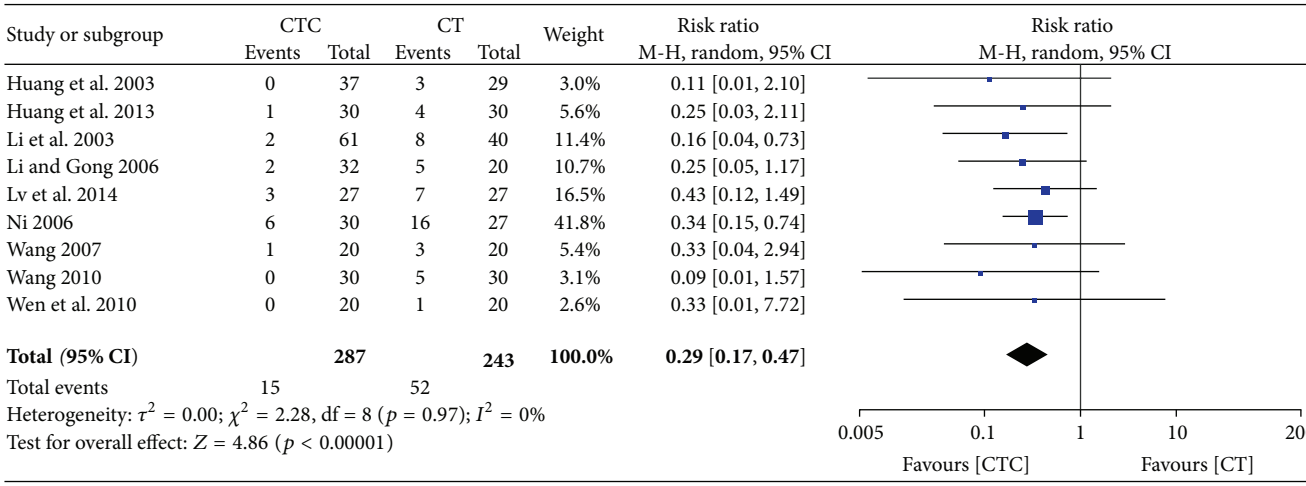

Figure 11: Reduction in WBCs during breast cancer treatment (toxicity grades III-IV).

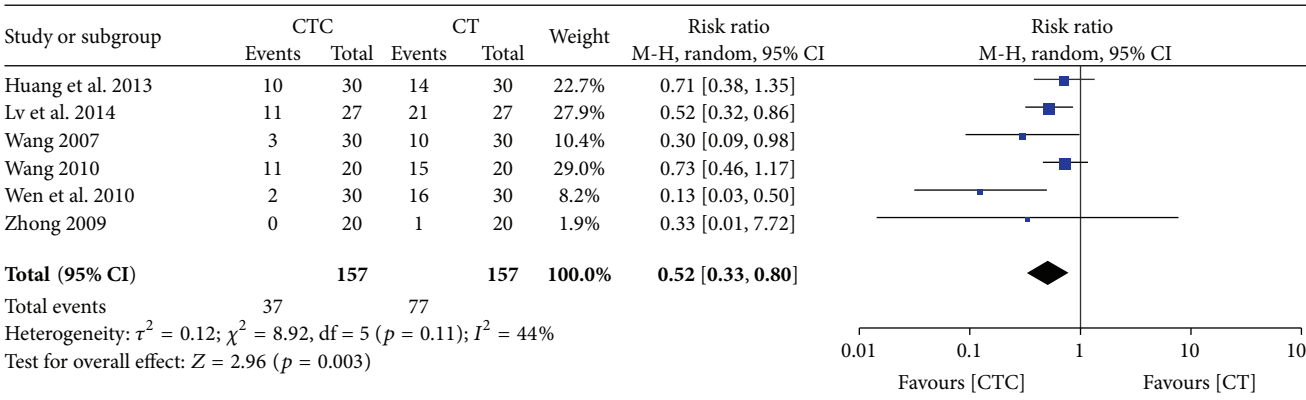

Figure 12: Reduction in platelets during breast cancer treatment (toxicity grades I–IV).

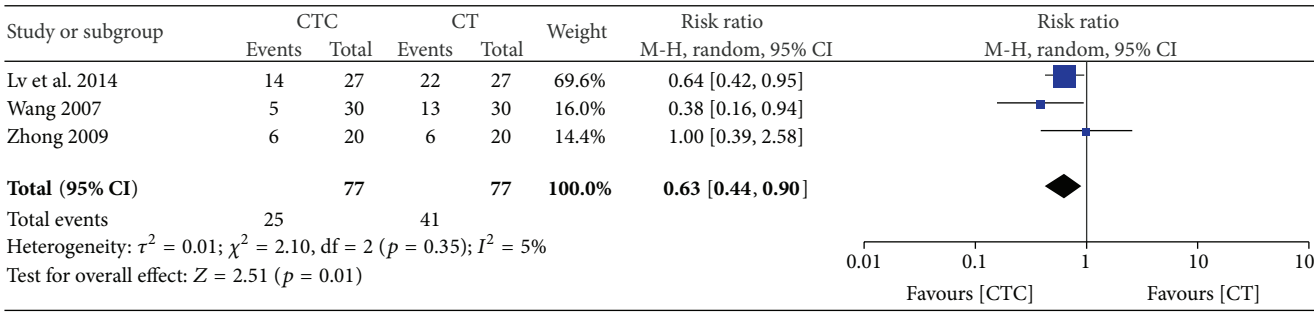

Figure 13: Reduction in hemoglobin during breast cancer treatment (toxicity grades I–IV).

should be identified from or conducted in these areas to further investigate the efficacy of CHM. Second, all of the included Chinese studies had relatively small sample sizes, ranging from 40 to 101 participants. None of these studies reported the details of sample size calculation. Third, the Chinese trials did not clearly report allocation concealment or blinding, and none of the Chinese studies were placebo controlled or double blinded, which could have resulted in bias and an overestimation of CTC efficacy [58]. Publication bias may also have existed. The asymmetry of the funnel plot may be the result of an insufficient number of trials and significant statistical heterogeneity (Figures 5, 7, 15, and 17). There were also different data types and assessment methods for outcomes, which may have resulted in statistical heterogeneity.

We also cannot ignore the low quality of the included trials; however, that may not be a sound reason to exclude a systematic review. A systematic review embraces the features of

Study or subgroup	CTC Events	CTC Total	CT Events	CT Total	Weight	Risk ratio M-H, random, 95% CI
Fang 2009	9	30	5	30	4.0%	1.80 [0.68, 4.74]
Fu and Kou 2007	15	44	13	44	10.0%	1.15 [0.62, 2.13]
Huang et al. 2003	12	37	3	29	2.8%	3.14 [0.97, 10.08]
Huang et al. 2007	15	30	8	30	7.8%	1.88 [0.94, 3.75]
Huang et al. 2008	9	30	5	30	4.0%	1.80 [0.68, 4.74]
Huang et al. 2013	14	30	7	30	6.6%	2.00 [0.94, 4.25]
Lv et al. 2014	15	27	8	27	8.3%	1.88 [0.96, 3.67]
Ni 2006	11	30	2	27	1.9%	4.95 [1.20, 20.36]
Qin 2013	27	39	11	39	12.8%	2.45 [1.43, 4.22]
Shen 2007	26	51	19	49	19.2%	1.31 [0.84, 2.05]
Wen et al. 2010	16	30	6	30	6.0%	2.67 [1.21, 5.88]
Xiong 2012	13	24	8	24	8.2%	1.63 [0.83, 3.19]
Yang 2004	17	31	8	28	8.4%	1.92 [0.98, 3.74]
Total (95% CI)		**433**		**417**	**100.0%**	**1.81 [1.49, 2.19]**
Total events	199		103			

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 9.36$, df = 12 ($p = 0.67$); $I^2 = 0\%$
Test for overall effect: $Z = 5.98$ ($p < 0.00001$)

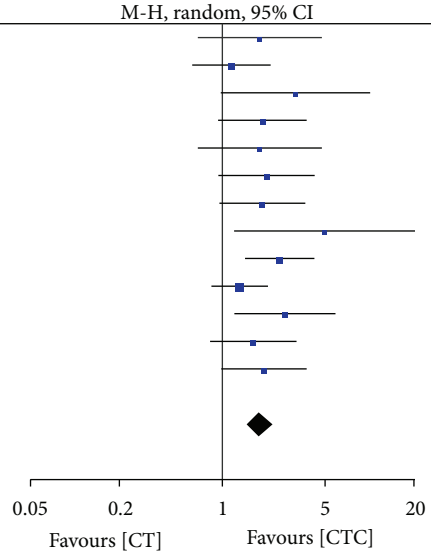

Figure 14: Improvement in KPS during breast cancer treatment.

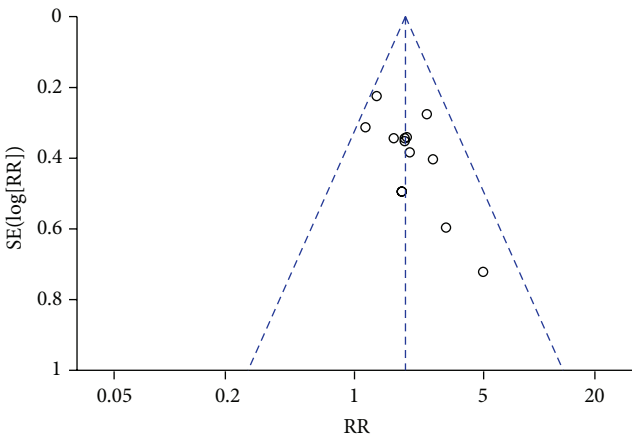

Figure 15: Funnel plot of improvement in KPS during breast cancer treatment.

systematization and comprehensiveness, which differentiate it from a normal review. In addition, the CHM used differed significantly among trials. Inevitably, the pharmacological actions of these treatments would not be the same. A random effects model was used for pooling because of the clinical heterogeneity. Because of this limitation, we cannot draw a convincing conclusion. Nevertheless, the problems with the current studies identified in this review are significant, and a great deal of work needs to be done to evaluate the efficacy of CAM using a modern and rigorous methodology.

Only 3 studies evaluated the Zheng TCM pattern, which is another key limitation of the included studies [37, 42, 52]. Zheng, also known as syndrome or pattern, is the core concept in TCM, and it describes the entire physiological and/or pathological pattern of the patient [59]. Zheng is usually evaluated through a comprehensive analysis of clinical signs and symptoms. TCM practitioners collect the signs by inspection, auscultation, olfaction, inquiry, pulse, and palpation. TCM practitioners in clinical practice prescribe CHM based on Zheng.

CHM therapy is more efficacious when based on the correct judgment of the Zheng classification according to the Chinese medical system. One clinical study found that the therapeutic effect of CHM for the treatment of irritable bowel syndrome was more sustainable when based on the TCM pattern than on standard treatment [60]. The key role of Zheng in TCM should not be ignored despite the controversial results reported by other clinical studies, which indicate that the efficacy of Zheng-based treatment is not advantageous over standard treatment [61–63]. Patients are not administered the same CHM for a long period of time in real practice, and the treatments reported in clinical trials did not follow a pattern that is commonly used in actual clinical practice because Zheng is dynamic during the treatment course. The biggest challenge in the exploration of Zheng-based CHM therapy using an RCT is the standardization of Zheng. Currently, the process of Zheng is highly subjective, and a nationwide and objective process is needed to improve its use. Randomized, multicenter trials should be conducted for this purpose. Analyses of Zheng at the molecular level may also enable acceptance of TCM on a scientific basis for the West.

Table 2 lists the herbal medicines that were commonly used for the treatment of breast cancer in the identified studies in this review. The pooled data in this review demonstrated that the adjunctive use of CHM with chemotherapy may improve immediate tumor response and performance status and reduce the occurrence of adverse events associated with chemotherapy. We were unable to verify whether CHM helped stimulate the immune system, as measured using $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ cells, because of the mixed quality and significance of the included studies. The evidence is too limited to make any confident conclusions.

Study or subgroup	CTC Events	CTC Total	CT Events	CT Total	Weight	Risk ratio M-H, random, 95% CI
Fang 2009	23	30	15	30	3.7%	1.53 [1.02, 2.31]
Fu and Kou 2007	39	44	31	44	12.8%	1.26 [1.01, 1.57]
Huang et al. 2003	31	37	18	29	6.0%	1.35 [0.98, 1.85]
Huang et al. 2007	25	30	18	30	5.5%	1.39 [1.00, 1.94]
Huang et al. 2008	23	30	15	30	3.7%	1.53 [1.02, 2.31]
Huang et al. 2013	26	30	18	30	5.8%	1.44 [1.04, 2.00]
Lv et al. 2014	25	27	18	27	7.4%	1.39 [1.04, 1.85]
Ni 2006	28	30	16	27	5.7%	1.57 [1.14, 2.18]
Qin 2013	38	39	24	39	9.5%	1.58 [1.23, 2.04]
Shen 2007	48	51	40	49	27.3%	1.15 [0.99, 1.34]
Wen et al. 2010	26	30	16	30	4.6%	1.63 [1.13, 2.34]
Xiong 2012	21	24	13	24	3.9%	1.62 [1.09, 2.40]
Yang 2004	25	31	15	28	4.1%	1.51 [1.02, 2.21]
Total (95% CI)		**433**		**417**	**100.0%**	**1.36 [1.26, 1.47]**
Total events	378		257			

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 11.05$, df = 12 ($p = 0.52$); $I^2 = 0\%$
Test for overall effect: $Z = 7.75$ ($p < 0.00001$)

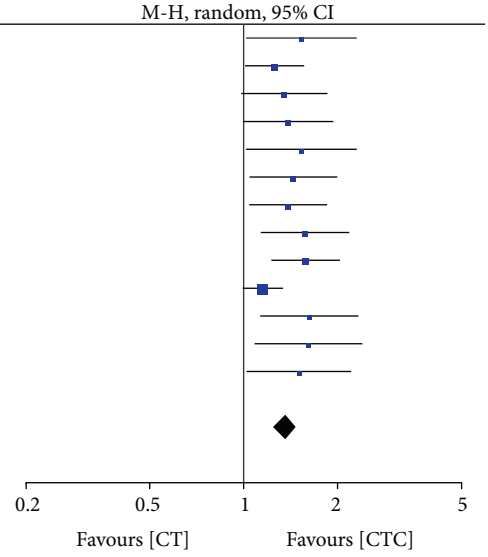

Figure 16: Improvement and stabilization of performance status during breast cancer treatment.

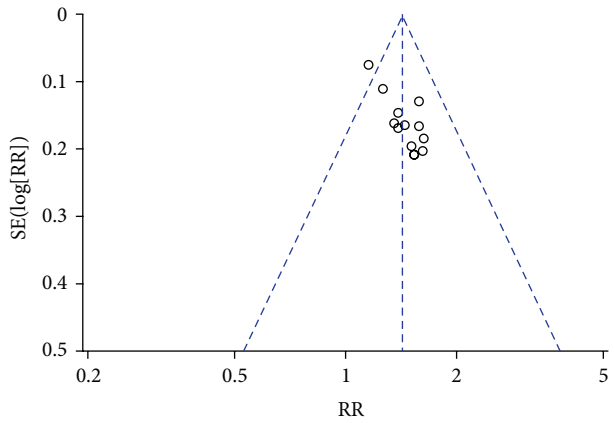

Figure 17: Funnel plot of improvement and stabilization of performance status during breast cancer treatment.

Table 2: Herbal medicines commonly used in the treatment of breast cancer.

Chinese herbal medicine	Frequency Count	Frequency %
Radix Astragalus	20	9.22
Rhizoma Atractylodis Macrocephalae	12	5.53
Poria	10	4.61
Angelica	8	3.69
Codonopsis pilosula	8	3.69
Radix Glycyrrhizae	7	3.23
Ligustrum lucidum	7	3.23
Oldenlandia diffusa	6	2.76
Pericarpium Citri Reticulatae	6	2.76
Panax	6	2.76
Pseudobulbus Cremastrae seu Pleiones	6	2.76

These results suggest that combined therapy has potential benefits for breast cancer patients. The finding of CHM efficacy as an adjunctive therapy for breast cancer is similar to the findings of other reviews for hepatocellular carcinoma, non-small-cell lung cancer, colorectal cancer, and nasopharyngeal carcinoma [58, 64–66]. A recent systematic review involving 8 RCTs showed that CHM combined with conventional therapy for breast cancer was efficacious in improving QoL and decreasing hot flashes, but this study did not identify as many clinical trials as it could have [67]. Breast cancer patients undergoing chemotherapy and/or endocrine therapy were included in that review, and the effect of CHM for breast cancer should have been examined separately in those two groups. In addition, the review focused on the effects on QoL and hot flashes but did not evaluate other cancer-related symptoms. Finally, the reviewers only presented a narrative synthesis without a meta-analysis, which made the conclusion unconvincing.

GRADE should be applied to judge the evidence and make recommendations regarding the application of CHM in the treatment of breast cancer. The present study suggests that recommendations for CHM combined with chemotherapy could be made for breast cancer, but TCM may be too complex to be immediately adopted by physicians in Western countries. The most fundamental and often-overlooked challenge is the lack of a 1:1 correlation between modern allopathic and Chinese holistic medical approaches [68]. We cannot make specific recommendations despite the rapid increase in the use of CHM and reported potential benefits because of the complexity of this system and the variable data.

Current evidence on the use of CHM as an adjunctive treatment with chemotherapy for breast cancer remains equivocal. Our findings highlight the poor quality of Chinese

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Dang and Wang 2010	72.87	4.69	28	71.89	5.03	20	14.9%	0.98 [−1.83, 3.79]
Hong et al. 2014	56.98	6.35	42	55.4	6.12	42	16.5%	1.58 [−1.09, 4.25]
Li et al. 2003	70.78	4.4	61	71.19	4.53	40	36.7%	−0.41 [−2.20, 1.38]
Qi 2010	79.49	4.5	20	81.27	3.67	20	18.1%	−1.78 [−4.32, 0.76]
Yang et al. 2008	74.64	7.44	28	74.81	8.02	20	5.9%	−0.17 [−4.64, 4.30]
Zhong 2009	77.5	6.38	20	79.5	6.86	27	8.1%	−2.00 [−5.81, 1.81]
Total (95% CI)			**199**			**169**	**100.0%**	**−0.24 [−1.32, 0.84]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 4.78$, df = 5 ($p = 0.44$); $I^2 = 0\%$
Test for overall effect: $Z = 0.43$ ($p = 0.67$)

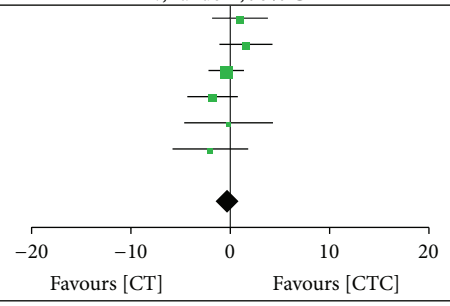

Figure 18: KPS before breast cancer treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Dang and Wang 2010	71.55	4.47	28	68.37	4.96	20	17.2%	3.18 [0.45, 5.91]
Hong et al. 2014	69.7	7.2	42	56.72	6.55	42	16.7%	12.98 [10.04, 15.92]
Li et al. 2003	77.35	4.17	61	70.95	4.95	40	19.0%	6.40 [4.54, 8.26]
Qi 2010	84.9	4.47	20	79.88	4.78	20	16.9%	5.02 [2.15, 7.89]
Yang et al. 2008	73.2	6.69	28	68.5	5.57	27	16.0%	4.70 [1.45, 7.95]
Zhong 2009	86	5.03	20	80.5	7.59	20	14.2%	5.50 [1.51, 9.49]
Total (95% CI)			**199**			**169**	**100.0%**	**6.31 [3.66, 8.97]**

Heterogeneity: $\tau^2 = 8.76$; $\chi^2 = 26.65$, df = 5 ($p < 0.0001$); $I^2 = 81\%$
Test for overall effect: $Z = 4.66$ ($p < 0.00001$)

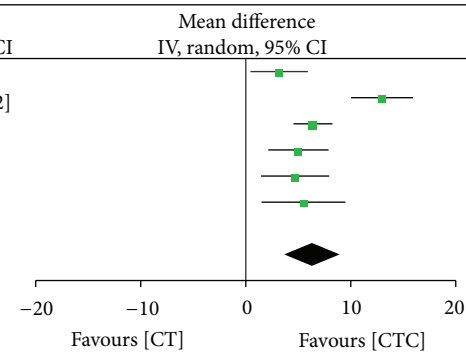

Figure 19: KPS after breast cancer treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Huang et al. 2007	51.9	8.7	30	52.7	9.1	30	7.8%	−0.80 [−5.31, 3.71]
Lv et al. 2014	55.9	7.3	27	56.8	6.3	27	11.9%	−0.90 [−4.54, 2.74]
Wen et al. 2010	51.9	8.7	30	52.7	9.1	30	7.8%	−0.80 [−5.31, 3.71]
Yang 2004	51.3	7.1	31	52.4	7	28	12.2%	−1.10 [−4.70, 2.50]
Zhang et al. 2010	70.85	7.56	40	70.48	8.49	40	12.7%	0.37 [−3.15, 3.89]
Zhang et al. 2011	55.33	3.41	23	56.94	2.8	22	47.6%	−1.61 [−3.43, 0.21]
Total (95% CI)			**181**			**177**	**100.0%**	**−1.09 [−2.34, 0.17]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.02$, df = 5 ($p = 0.96$); $I^2 = 0\%$
Test for overall effect: $Z = 1.69$ ($p = 0.09$)

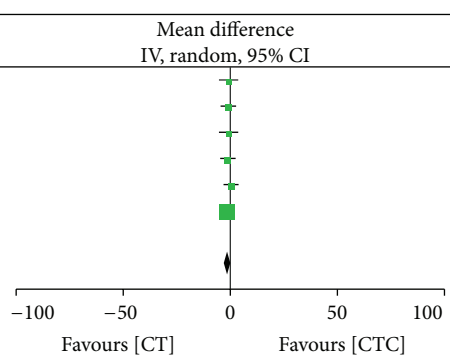

Figure 20: $CD3^+$ before treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Huang et al. 2007	47.7	8.5	30	46.8	9	30	3.3%	0.90 [−3.53, 5.33]
Lv et al. 2014	41.5	6.5	27	42.8	6.8	27	5.1%	−1.30 [−4.85, 2.25]
Wen et al. 2010	47.7	8.5	30	46.8	9	30	3.3%	0.90 [−3.53, 5.33]
Yang 2004	47.6	6.5	31	46.3	6.3	28	6.0%	1.30 [−1.97, 4.57]
Zhang et al. 2010	43.5	7.53	40	41.94	5.26	40	7.9%	1.56 [−1.29, 4.41]
Zhang et al. 2011	36.28	1.9	23	36.91	1.21	22	74.5%	−0.63 [−1.56, 0.30]
Total (95% CI)			**181**			**177**	**100.0%**	**−0.28 [−1.08, 0.52]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 3.91$, df = 5 ($p = 0.56$); $I^2 = 0\%$
Test for overall effect: $Z = 0.68$ ($p = 0.50$)

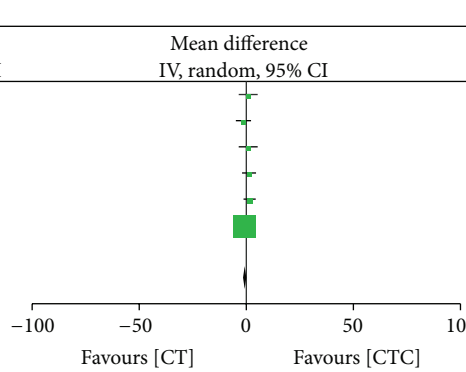

Figure 21: $CD4^+$ before treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Huang et al. 2007	29.6	7.6	30	29.5	2.7	30	9.3%	0.10 [−2.79, 2.99]
Lv et al. 2014	30.5	7.3	27	30.8	6.3	27	5.9%	−0.30 [−3.94, 3.34]
Wen et al. 2010	29.6	7.6	30	29.5	2.7	30	9.3%	0.10 [−2.79, 2.99]
Yang 2004	28.9	3.8	31	29.3	3.7	28	21.1%	−0.40 [−2.32, 1.52]
Zhang et al. 2010	28.84	5.83	40	30.66	7.29	40	9.3%	−1.82 [−4.71, 1.07]
Zhang et al. 2011	28.4	2.48	23	28.1	1.99	22	45.1%	0.30 [−1.01, 1.61]
Total (95% CI)			**181**			**177**	**100.0%**	**−0.12 [−1.00, 0.76]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.86$, df = 5 ($p = 0.87$); $I^2 = 0\%$
Test for overall effect: $Z = 0.26$ ($p = 0.79$)

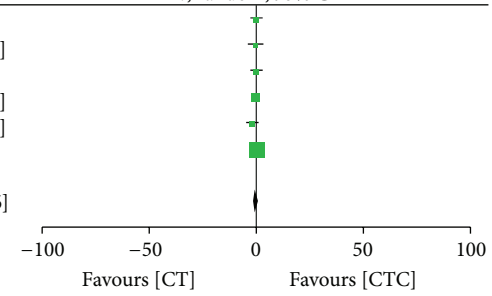

Figure 22: $CD8^+$ before treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Huang et al. 2007	1.8	0.4	30	1.8	0.5	30	17.7%	0.00 [−0.23, 0.23]
Lv et al. 2014	1.51	0.37	27	1.62	0.41	27	21.4%	−0.11 [−0.32, 0.10]
Wen et al. 2010	1.8	0.4	30	1.8	0.5	30	17.7%	0.00 [−0.23, 0.23]
Yang 2004	1.5	0.5	31	1.5	0.4	28	17.6%	0.00 [−0.23, 0.23]
Zhang et al. 2010	1.61	0.48	40	1.57	0.51	40	19.8%	0.04 [−0.18, 0.26]
Zhang et al. 2011	1.27	0.76	23	1.31	0.61	22	5.8%	−0.04 [−0.44, 0.36]
Total (95% CI)			**181**			**177**	**100.0%**	**−0.02 [−0.11, 0.08]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 1.11$, df = 5 ($p = 0.95$); $I^2 = 0\%$
Test for overall effect: $Z = 0.37$ ($p = 0.71$)

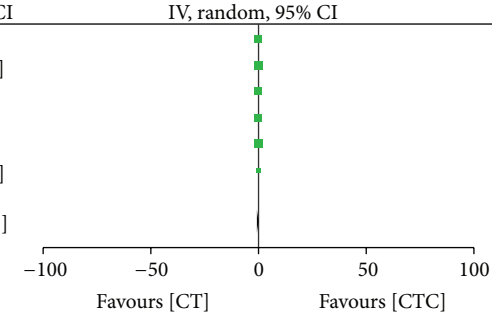

Figure 23: $CD4^+/CD8^+$ before treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Lv et al. 2014	13.5	4.77	27	13.8	5.22	27	38.2%	−0.30 [−2.97, 2.37]
Zhang et al. 2010	11.52	4.58	40	12.48	4.98	40	61.8%	−0.96 [−3.06, 1.14]
Total (95% CI)			**67**			**67**	**100.0%**	**−0.71 [−2.36, 0.94]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.15$, df = 1 ($p = 0.70$); $I^2 = 0\%$
Test for overall effect: $Z = 0.84$ ($p = 0.40$)

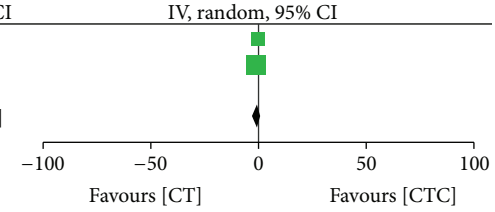

Figure 24: Natural killer cell level before treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Huang et al. 2007	52.1	4.9	30	43.8	9	30	11.8%	8.30 [4.63, 11.97]
Lv et al. 2014	60.9	6.7	27	52.9	7.3	27	11.4%	8.00 [4.26, 11.74]
Wen et al. 2010	52.1	4.9	30	43.8	9	30	11.8%	8.30 [4.63, 11.97]
Yang 2004	52.4	6.9	31	42.2	5.9	28	14.8%	10.20 [6.93, 13.47]
Zhang et al. 2010	79.36	8.42	40	72.03	7.94	40	12.3%	7.33 [3.74, 10.92]
Zhang et al. 2011	56.93	3.03	23	50.91	3.66	22	37.9%	6.02 [4.05, 7.99]
Total (95% CI)			**181**			**177**	**100.0%**	**7.56 [6.28, 8.85]**

Heterogeneity: $\tau^2 = 0.12$; $\chi^2 = 5.24$, df = 5 ($p = 0.39$); $I^2 = 5\%$
Test for overall effect: $Z = 11.55$ ($p < 0.00001$)

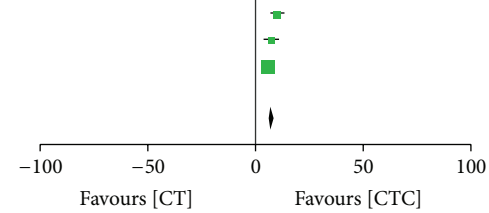

Figure 25: $CD3^+$ after breast cancer treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Huang et al. 2007	1.9	0.6	30	1.6	0.4	30	15.8%	0.30 [0.04, 0.56]
Lv et al. 2014	1.75	0.58	27	1.55	0.47	27	13.3%	0.20 [−0.08, 0.48]
Wen et al. 2010	1.9	0.6	30	1.6	0.4	30	15.8%	0.30 [0.04, 0.56]
Yang 2004	1.6	0.5	31	1.4	0.3	28	24.3%	0.20 [−0.01, 0.41]
Zhang et al. 2010	1.78	0.56	40	1.49	0.4	40	23.1%	0.29 [0.08, 0.50]
Zhang et al. 2011	1.53	0.15	23	1.18	0.87	22	7.7%	0.35 [−0.02, 0.72]
Total (95% CI)			**181**			**177**	**100.0%**	**0.26 [0.16, 0.37]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.98$, df = 5 ($p = 0.96$); $I^2 = 0\%$
Test for overall effect: $Z = 5.05$ ($p < 0.00001$)

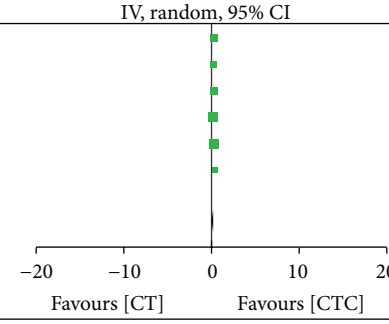

Figure 26: $CD4^+/CD8^+$ after breast cancer treatment.

Study or subgroup	CTC Mean	CTC SD	CTC Total	CT Mean	CT SD	CT Total	Weight	Mean difference IV, random, 95% CI
Lv et al. 2014	15.9	3.25	27	12.5	4.13	27	57.0%	3.40 [1.42, 5.38]
Zhang et al. 2010	13.46	5.46	40	12.62	6.92	40	43.0%	0.84 [−1.89, 3.57]
Total (95% CI)			**67**			**67**	**100.0%**	**2.30 [−0.18, 4.78]**

Heterogeneity: $\tau^2 = 1.79$; $\chi^2 = 2.21$, df = 1 ($p = 0.14$); $I^2 = 55\%$
Test for overall effect: $Z = 1.81$ ($p = 0.07$)

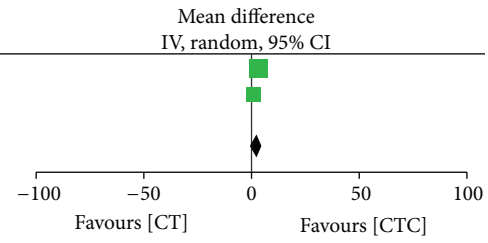

Figure 27: Natural killer cell level after breast cancer treatment.

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean difference IV, random, 95% CI
13.1.1 TNM stage IV								
Huang et al. 2007	45.3	2.8	30	35.6	4.8	30	17.2%	9.70 [7.71, 11.69]
Lv et al. 2014	46.9	7.1	27	39.4	4.1	27	16.0%	7.50 [4.41, 10.59]
Wen et al. 2010	45.3	2.8	30	35.5	4.8	30	17.2%	9.80 [7.81, 11.79]
Yang 2004	46.7	6.5	31	35.1	5.4	28	16.1%	11.60 [8.56, 14.64]
Subtotal (95% CI)			**118**			**115**	**66.4%**	**9.70 [8.41, 10.98]**
Heterogeneity: $\tau^2 = 0.23$; $\chi^2 = 3.46$, df = 3 ($p = 0.33$); $I^2 = 13\%$								
Test for overall effect: $Z = 14.80$ ($p < 0.00001$)								
13.1.2 TNM stage (I–IV)								
Zhang et al. 2010	46.14	7.51	40	42.88	6.95	40	15.9%	3.26 [0.09, 6.43]
Zhang et al. 2011	37.26	2.06	23	35.16	2.09	22	17.7%	2.10 [0.89, 3.31]
Subtotal (95% CI)			**63**			**62**	**33.6%**	**2.25 [1.12, 3.38]**
Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 0.45$, df = 1 ($p = 0.50$); $I^2 = 0\%$								
Test for overall effect: $Z = 3.89$ ($p = 0.0001$)								
Total (95% CI)			**181**			**177**	**100.0%**	**7.30 [3.67, 10.93]**
Heterogeneity: $\tau^2 = 18.93$; $\chi^2 = 83.67$, df = 5 ($p < 0.00001$); $I^2 = 94\%$								
Test for overall effect: $Z = 3.94$ ($p < 0.0001$)								
Test for subgroup differences: $\chi^2 = 72.70$, df = 1 ($P < 0.00001$); $I^2 = 98.6\%$								

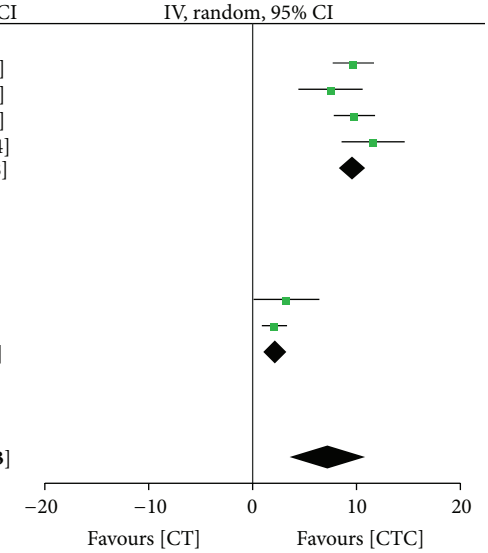

Figure 28: $CD4^+$ after breast cancer treatment.

studies, and additional well-designed RCTs addressing the role of CHM are warranted. The lack of molecular-based evidence for CHM and Zheng has resulted in a limited understanding and acceptance of CHM and TCM in Western countries. We believe that researchers should immediately explore a CHM-based cure, and CHM should be applied to routine care as soon as conclusive data are available [69]. For researchers devoted to the promotion of TCM or CHM, numerous barriers need to be addressed, including the standardizations of the Zheng classification and herbal agents, appropriate study designs, and the identification of the mechanisms of CHM at the molecular level.

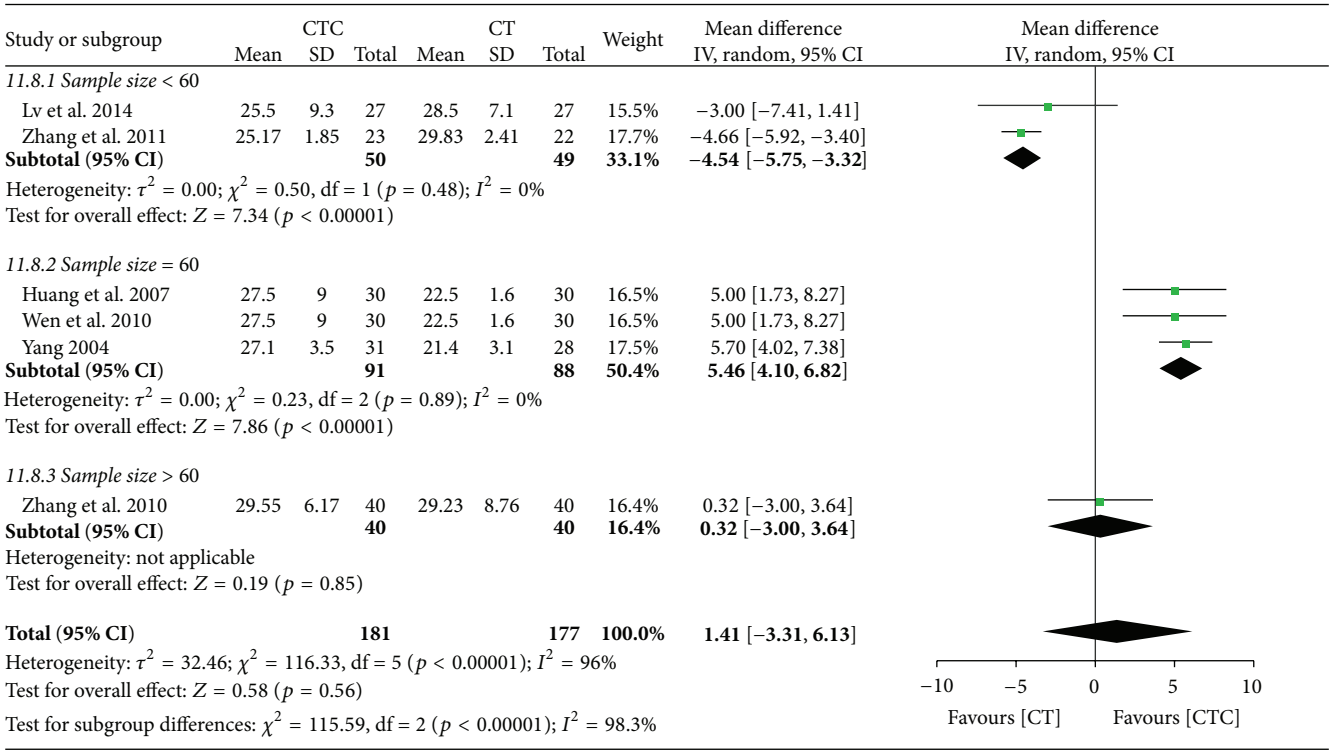

Figure 29: $CD8^+/CD8^+$ after breast cancer treatment.

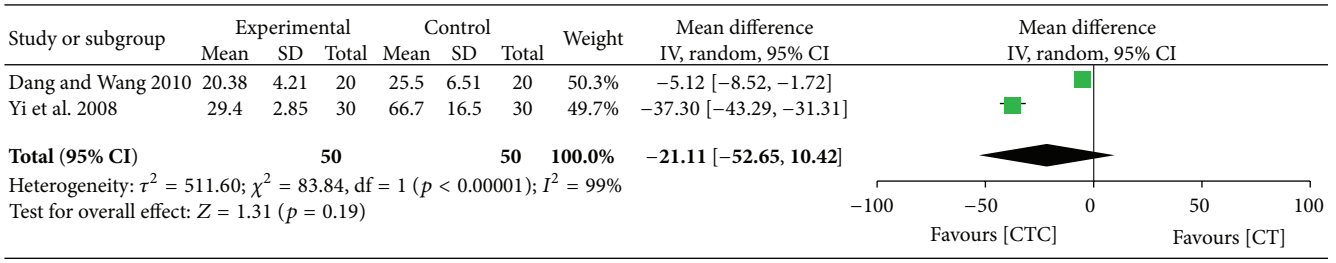

Figure 30: CK-MB (U/L) after breast cancer treatment.

Disclosure

The authors were not employed or contracted by the funder. The funder did not play a role in study design, data collection, or analysis.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Xu Sun, Xing Zhang, Jia-Yun Nian, Jiao Guo, and Yi Yin contributed equally to this work. Xu Sun, Xiao-Min Wang, and Yi Yin drafted the protocol (unpublished). Xing Zhang, Jia-Yun Nian, and Jiao Guo developed and ran the search strategy. Xing Zhang, Jia-Yun Nian, and Jiao Guo obtained copies of studies. Xu Sun, Xing Zhang, and Jia-Yun Nian selected which studies to include. Gan-Lin Zhang, Ming-Wei Yu, and Yi Zhang extracted data from studies. Gan-Lin Zhang, Pei-Yu Cheng, and Lin Yang entered data into RevMan. Xu Sun, Guo-Wang Yang, and Jin-Ping Li conducted the analysis. Xu Sun, Xiao-Min Wang, and Yi Yin interpreted the analysis. Xu Sun, Gan-Lin Zhang, and Ming-Wei Yu drafted the final review.

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Research Article

Effect of Yi Gong San Decoction on Iron Homeostasis in a Mouse Model of Acute Inflammation

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We investigated the effect of Yi Gong San (YGS) decoction on iron homeostasis and the possible underlying mechanisms in a mouse model of acute inflammation in this study. Our findings suggest that YGS regulates iron homeostasis by downregulating the level of HAMP mRNA, which may depend on regulation of the IL-6/STAT3 or BMP/HJV/SMAD pathway during acute inflammation.

1. Introduction

Iron plays a pivotal role in cell survival and proliferation. It is thus an important source of nutrition in the competition between microbial pathogens and their hosts [1]. In humans, host defense responses to infectious agents modulate local and systemic iron availability, which disrupts infections such as malaria and tuberculosis [2]. Increased production of inflammatory cytokines can directly induce changes in iron homeostasis, which are characterized by reduction of both iron absorption and macrophage iron release. Hepatic bactericidal protein (hepcidin) provides a first line of defense at mucosal barriers, although it is less potent than many other antimicrobial peptides. Hepcidin impairs iron absorption and macrophage iron release and acts as a major hormonal regulator of iron homeostasis [1, 3]. The bone morphogenic protein (BMP)/hemojuvelin (HJV)/SMAD pathway is the major regulator of hepcidin expression that responds to iron status. Additionally, inflammation stimulates hepcidin via the interleukin- (IL-) 6/STAT3 pathway with support by activation of the BMP/HJV/SMAD pathway [4]. The expression of hepcidin in isolated primary hepatocytes increases in response to infection/inflammation stimulated by IL-6, IL-1, and LPS [5]. LPS is a component of the outer membrane of Gram-negative bacteria and elicits a potent inflammatory response when administered intravenously or intraperitoneally [6]. LPS induces hepcidin and causes hypoferraemia within hours of administration in both humans [7] and mice [3, 8]. Therefore, we investigated iron homeostasis in a mouse model of LPS-induced acute inflammation.

YGS originated from *Pediatric Medicine Card Straight Strategics*, a classic book of traditional Chinese medicine (TCM) that was written approximately 900 years ago. It has the functions of tonifying splenic Qi and gasification stagnation. YGS is based on Si Jun Zi decoction (SJZD) combined with Citri Unshius Pericarpium. SJZD can decrease serum levels of IL-6, CRP, and TNF. Early application of SJZD during enteral nutritional therapy can enhance the immune function of patients with gastrointestinal tumours [9]. In addition, YGS has been traditionally used in Korea to treat a variety of inflammatory diseases; pretreatment with YGS inhibited TNF-α and IL-6 production by LPS-stimulated mouse peritoneal macrophages [10]. Thus, YGS may maintain iron homeostasis by regulating the production

of inflammatory cytokines. This study aimed to determine the effect of YGS on iron homeostasis and elucidate the underlying mechanisms to facilitate its clinical application.

2. Materials and Methods

2.1. Animals. Six-week-old C57BL/6 female mice were purchased from SLRC Laboratory Animals (Shanghai, China). The mice were housed in an environmentally controlled animal care facility and were used for experiments after 4 days of acclimation. Experiments were carried out according to the China Council on Animal Care guidelines after approval by the Institutional Animal Care Committee of Shanghai University of Traditional Chinese Medicine, Shanghai, China.

2.2. Preparation of YGS. YGS is composed of five different herbs: Ginseng Radix et Rhizoma (Ren-Shen), Glycyrrhizae Radix et Rhizoma (Gan-Cao), Citri Reticulatae Pericarpium (Chen-Pi), Atractylodis Macrocephalae Rhizoma (Bai-Zhu), and Poria (Fu-Ling). All of the five herbs were provided by our hospital pharmacy. After being soaked together for 1 h, the five herbs in equal dose were water decocted for 20 min, concentrated by distillation, dried through 60°C vacuum decompression, and homogenised into 100-mesh powders. 1 g dry powder was equal to 4.1 g herb (performed at the College of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, China). The dosage given to mice was 10.57 g/kg which was equally effective to clinical routine dosage (each herb 15 g, total 75 g), calculated according to the surface area formula. The powder was dissolved in double-distilled water to a final concentration of 1.057 g/mL.

2.3. Multicomponent Analysis of YGS Powder

2.3.1. Sample Preparation. 0.3 g of accurately weighed fine powder was placed in a 50 mL centrifuge tube and ultrasonically extracted with 25 mL of 50% methanol (v/v) for 20 min. After centrifugation at 14000 rpm for 10 min, the supernatant was obtained and used as the test solution.

2.3.2. UPLC/QTOF MS Conditions. Chromatographic separation was performed on Waters ACQUITY I-Class UPLC (Waters, Milford, MA, USA) equipped with a binary solvent manager, a sample manager, and a column manager. A Waters HSS T3 column (2.1 × 100 mm, 1.7 μm) together with a Waters on-line filtrate 35°C was used. The mobile phase consisted of acetonitrile (B) and water containing 0.1% formic acid (v/v) (A) following a gradient elution program: 0–2 min: 15%–25% (B); 2–18 min: 25%–47% (B); 18–18.5 min: 47%–75% (B); 18.5–20 min: 75%–90% (B); 20–22 min: 90% (B); 22–22.1 min: 90%–15% (B); 22.1–26 min : 15% (B). The flow rate was set at 0.4 mL/min. 2 μL of the test solution was injected for UPLC analysis.

High-accuracy mass spectrometric data were recorded on a Waters Xevo G2-S QTOF mass spectrometer (Waters, Manchester, UK). Tune parameters were set for MS^E experiments: capillary voltage, 2.5 kV (negative mode) and 2.0 KV (positive mode); sampling cone, 60 V; source offset voltage, 60 V; source temperature, 120°C; desolvation temperature, 450°C (negative mode) and 350°C (positive mode); cone gas flow, 30 L/h (negative mode) and 20 L/h (positive mode); desolvation gas, 900 L/h (negative mode) and 800 L/h (positive mode). The mass analyzer scanned over a mass range of 100–1500 Da within 0.1 s under a low collision energy at 6 V. High collision energy ramp of 20–90 V for negative mode and 40–90 V for positive mode was employed. Data calibration was performed using an external reference (LockSpray™) constant infused at 1 ng/μL of leucine enkephalin (LE; Sigma-Aldrich, St. Louis, MO, USA) at a flow rate of 5 μL/min, and with reference to the ion *m/z* 554.2615. Data acquisition was controlled by MassLynx V4.1 software (Waters Corporation, Milford, USA). Automatic metabolites characterization was performed using UNIFI 1.8 (Waters, Milford, USA) by the search of the TCM library.

2.4. Detection of Total Iron Concentration in YGS Powder. Total iron concentration in YGS Powder was determined by using an Agilent Technologies 7700 Series ICP-MS system equipped with ASX-500 Series ICP-MS Autosampler. The procedure was performed as previously described [11].

2.5. Animal Model. To induce acute inflammation, mice were injected with LPS (*Escherichia coli* serotype O127:B8, 1.5 mg/kg intraperitoneally; Sigma-Aldrich, Inc., USA) and sacrificed at 3, 6, 9, and 12 h thereafter. A CTL group was also established. All animals were given an equivalent volume of double-distilled water via intragastric administration for 7 successive days before being injected with LPS. To evaluate the effect of YGS on iron homeostasis *in vivo* and the underlying mechanisms, YGS (10.57 g/kg) was given to mice via intragastric administration for 7 successive days. Mice were injected with LPS on the following day and sacrificed at 3, 6, 9, and 12 h. A YGS control group was also established.

2.6. Specimen Collection. Mice were immediately killed with isoflurane, and blood was collected into serum separator tubes through cardiac puncture. The abdomen was then opened and liver samples were taken for tissue iron determination and ribonucleic acid (RNA) and protein isolation. Samples were stored at −80°C for later analysis.

2.7. Determination of Liver Iron. Liver tissue was processed as follows. About 100 to 200 mg of liver tissue was accurately weighed in a 1.5 mL centrifuge tube. Following the addition of 1 mL nitric acid solution, each centrifuge tube was vortex mixed after blending into the microwave digestion instrument resolution organisation and made up to 1.5 mL using nitric acid solution. Samples were then centrifuged at room temperature at 12,000 rpm for 3 min and then transferred to 96-well plates. After incubation for 10 min at room temperature, an ultraviolet spectrophotometer was used to determine the OD at 535 nm of the samples. Iron content was calculated using the following formula: iron concentration (μg/g wet weight) = $[(A_t - A_b) * (n + 0.75W) * \text{Fes}\,(1 + V_e)]/[(A_s - A_b) * W * V_e \times 1.1]$ (A_T = sample absorbance; A_b = absorbance blank hole; A_s = standard absorbance; Fes = standard iron content

Table 1: PCR primers, product sizes, and annealing temperatures.

Gene	PCR primer sequence	Temperature (°C)
β-actin	5′-AGCTGAGAGGAAATCGTGCG-3′ 5′-GTGCCACCAGACAGCACTGTG-3′	59.8
HAMP	5′-AGCACCACCTATCTCCATCAAC-3′ 5′-TGTCTCTCTTCCTTCTCTTCTGC-3′	57.0
IL-6	5′-GGAGAGGAGACTTCACAGAGGA-3′ 5′-ATTTCCACGATTTCCCAGAGA-3′	57.0
BMP6	5′-CAGGAGCATCAGCACAGAGA-3′ 5′-GTCACCACCCACAGATTGC-3′	59.8
HJV	5′-TGCTAACCTTGGGAGTCACG-3′ 5′-TCCTCTGCTACCCTGATGGA-3′	59.8

(μg); W = wet weight; n = join tissue samples of acid volume (mL); V_e = acid extraction volume (mL)).

2.8. Determination of Serum Iron Levels. About 200 μL of whole blood from the posterior orbital venous plexus was placed in serum separation tubes and centrifuged at 3000 rpm for 25 min at 4°C to separate the serum; approximately 40 μL of serum was produced from each sample. In accordance with the manufacturer's instructions, 50 μL of liquid iron buffer was added to the wells of a 96-well plate, as were 10 μL aliquots of the standard and the test samples. A trace ultraviolet spectrophotometer A1 value wavelength (560 nm) was used. We added 1 μL of iron chromogenic agent in each hole, 37°C, 10 min, using trace ultraviolet spectrophotometer A2 value wavelength (560 nm). Serum iron (μg/dL) was calculated using the following formula: 500 × (A2a − A1a)/(A2b − A1b) (A2a, A2 specimens; A1a, A1 specimens; A2b, A2 standard samples; A1b, A1 standard samples). Serum and liver iron levels were measured by colourimetric assay (MI 48188, Pointe Scientific Inc., USA) using a NanoDrop2000 Spectrophotometer (Thermo Scientific Inc., USA).

2.9. IL-6 Assay. Serum IL-6 levels were determined using an ELISA kit according to the manufacturer's instructions (mouse IL-6 ELISA kit, M6000B; R&D Systems, Inc., USA). A standard dilution series of 3.12, 6.25, 12.5, 25, 50, and 100 pg/L was created. To each well of a 96-well plate was added 100 μL of standard diluent RDIW together with 100 μL of standard or sample. Following incubation at room temperature for 2 h, liquid was aspirated and wells were washed four times. Next, 200 μL of IL-6 polymer was added, followed by incubation at room temperature for 2 h. Liquid was aspirated and wells were washed four times. Next, 200 μL of substrate solution was added to each well, followed by incubation in the dark at a room temperature for 20 min. Next, 50 μL of liquid was added to each well, and the plates were incubated at room temperature for 30 min. IL-6 levels were then determined using an ultraviolet spectrophotometer to read OD values (450 nm wavelength, calibration wavelengths of 540 and 570 nm). The standard curve was used to calculate IL-6 levels.

2.10. Determination of mRNA Levels. Total RNA was isolated using RNAiso Plus (D9108A, TaKaRa Bio Inc., Japan) and reverse-transcribed by PrimeScript reverse transcriptase using a real-time polymerase chain reaction (RT-PCR) system (TaKaRa Bio Inc., Japan). β-actin, HAMP, IL-6, BMP6, and HJV mRNA levels were measured using CFX96 RT-PCR Detection System (Bio-Rad, Inc., USA) with SYBR Premix Ex Taq Kit (DRR420A, Bio-Rad, Inc., USA). Expression levels were normalised to that of the housekeeping gene β-actin. The primers used are shown in Table 1.

2.11. Sodium Dodecyl Sulfate- (SDS-) Polyacrylamide Gel Electrophoresis and Western Blot Analysis. Livers were removed, rinsed in ice-cold phosphate-buffered saline, and used to prepare total protein extracts with RIPA Lysis Buffer (Beyotime Institute of Biotechnology, China) plus 1 mM phenylmethanesulfonyl fluoride and 0.1 to 2.0 mM sodium orthovanadate (Beyotime Institute of Biotechnology, China). Total protein extracts were separated in a 10% SDS-polyacrylamide gel and blotted onto nitrocellulose membranes (Bio-Rad, Inc., USA). The membranes were immunoblotted with antibodies to the following: phospho-STAT3, STAT3, β-actin, phospho-SMAD1/5/8, SMAD5 (Cell Signaling Technology, Inc., USA), and HJV (R&D Systems, Inc., USA). Anti-rabbit IgG, anti-mouse IgG, and anti-goat IgG (Cell Signaling Technology, Inc., USA) were used as secondary antibodies. Antigen-antibody complexes were visualised using an Immune-Star™ Western C™ Kit (Bio-Rad, Inc., USA) and analyzed using Image Lab™ software (Bio-Rad, Inc., USA).

2.12. Statistical Analysis. All statistical analyses were performed using SPSS 22.0 software (IBM Inc., USA). Multiple comparisons were performed by one-way analysis of variance (ANOVA) followed by the Bonferroni correction. If data could not be compared using an equal variance t-test, a Kruskal-Wallis test and one-way ANOVA were used, followed by Student-Newman-Keuls or the Dunn *post hoc* test. Correlation coefficients were determined using Spearman's rank-order correlation method.

3. Results

3.1. Multicomponent of YGS Powder. By optimizing the gradient elution program, satisfactory separation of major peaks was achieved in both negative and position ion modes, as

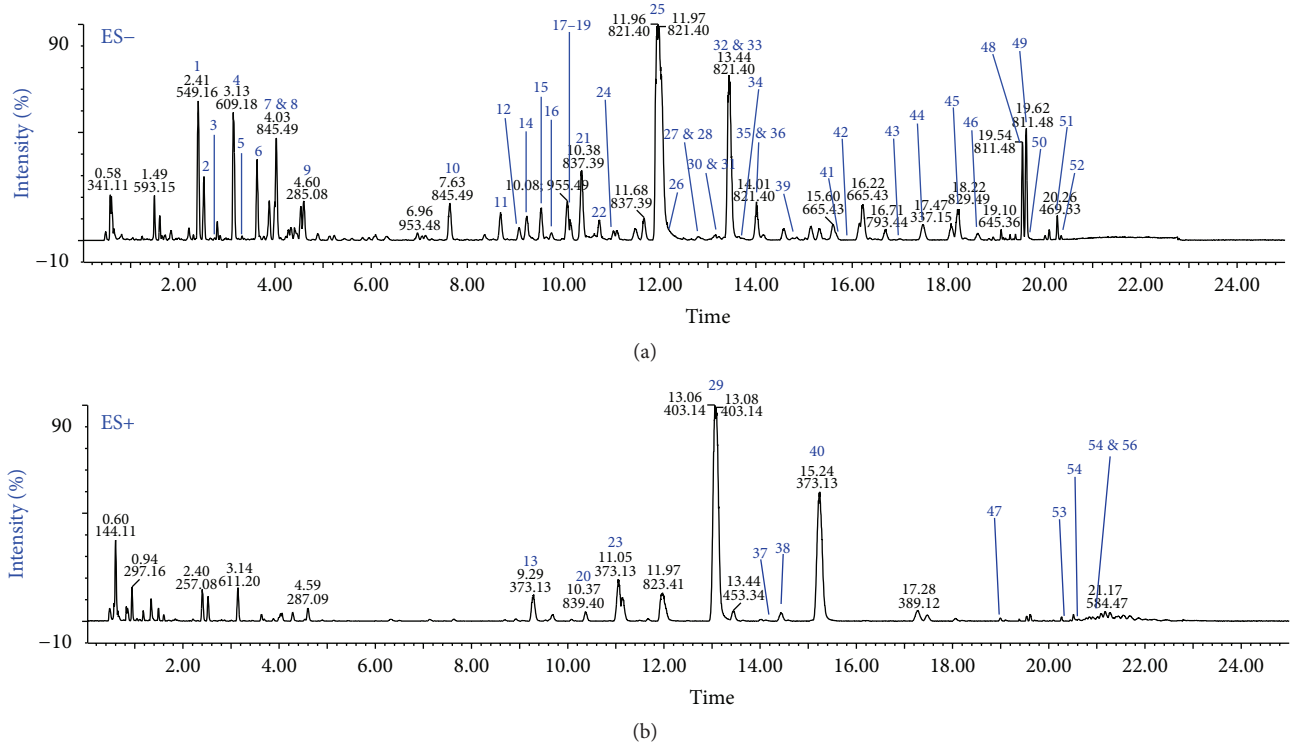

Figure 1: Base peak chromatograms of YGSP in negative mode (a) and positive mode (b).

shown in Figure 1. The obtained MS^E data were further imported into UNIFI software for automatic components characterization. By comparison with the TCM library, 56 peaks were identified or tentatively characterized by element composition and fragment matching analyses (Table 2). Among them, 24 saponins should be from Glycyrrhizae Radix et Rhizoma, 19 saponins from Ginseng Radix et Rhizoma, 5 compounds from Citri Reticulatae Pericarpium, 5 from Poria, and 3 from Atractylodis Macrocephalae Rhizoma.

In contrast, compounds **7**, **8**, **15**, **25**, **32**, and **35**, from Glycyrrhizae Radix et Rhizoma or Ginseng Radix et Rhizoma, gave strong ion response in the negative ion mode, whilst the protonated precursors of compounds **23**, **29**, and **40**, from Citri Reticulatae Pericarpium, were strong in positive ion mode. These compounds with strong responses as well as abundant secondary product ions lead to credible identification results. However, the compounds characteristic of Poria and Atractylodis Macrocephalae Rhizoma generated rather weak signals of precursor ions, without MS/MS fragments.

3.2. The Iron Element Level in YGS Decoction Could Not Directly Affect the Results. The iron concentration in YGS Powder was 2.56 mg per 100 g lyophilized powder weight. The dosage of YGS decoction given to the mice was 10.57 g/kg. The weight of the mice was from 18 g to 20 g. So, the iron taken by the mice from YGS decoction was not over 0.0015 mg. Mice were fed a standard iron rodent laboratory diet (232 mg iron/kg). Thus, precious little iron in YGS decoction could not directly affect the results.

3.3. YGS Increased Serum Iron by Decreasing Liver Iron Retention. In light of the influence of YGS on imbalanced iron homeostasis mediated by LPS, serum and liver iron concentrations were determined following preventive intervention with YGS. YGS alone had no significant effect on serum or liver iron levels. However, when compared with the LPS-treated control, YGS pretreatment significantly reduced the ability of LPS to decrease the serum iron level (Figure 2(a)) and iron retention in the liver (Figure 2(b)) at both 3 and 6 h after LPS injection ($P < 0.05$). Therefore, 3 and 6 h were used in subsequent analyses.

3.4. YGS Blockade of LPS-Mediated Hepcidin Induction via the IL-6/STAT3 Signaling Pathway. LPS injection leads to the production of the inflammatory cytokine IL-6 [12], which has been identified as a major inducer of hepcidin through the IL-6/STAT pathway [3]. Inhibiting LPS-induced secretion of IL-6 decreases hepcidin levels. Hence, in this study, a mouse model was used to evaluate the effect of YGS on LPS-induced IL-6 release. Compared with the LPS-treated control, YGS pretreatment significantly reduced the ability of LPS to increase serum IL-6 levels (Figure 3(a)) at 3 h, as well as IL-6 mRNA expression (Figure 3(b)) at both 3 and 6 h ($P < 0.05$). Hence, YGS blocked IL-6 increase after LPS injection *in vivo*. LPS-mediated HAMP mRNA expression (Figure 3(c)) was

Table 2: Information of 56 compounds identified from YGNP by UPLC/QTOF MS.

Number	Retention time (min)	Identification	TCM
1	2.42	Licurazide or its isomer	GC
2	2.52	Isoliquiritin or its isomer	GC
3	2.75	Ferulic acid	GC
4	3.14	Hesperidin or neohesperidin	CP
5	3.32	20-*O*-Glucopyranosyl ginsenoside Rf	RS
6	3.63	Licurazide or its isomer	GC
7	4.01	Ginsenoside Re	RS
8	4.03	Ginsenoside Rg1	RS
9	4.60	Licochalcone B	GC
10	7.64	Ginsenoside Rf	RS
11	8.74	Uralsaponin U/N or licorice saponin G2	GC
12	9.08	Ginsenoside 20(*S*)-Rg2	RS
13	9.28	Sinensetin or its isomer	CP
14	9.31	Ginsenoside Ra1 or Ra2	RS
15	9.53	Ginsenoside Rb1	RS
16	9.81	Ginsenoside F1	RS
17	10.13	Ginsenoside Ro	RS
18	10.14	Ginsenoside Ra2	RS
19	10.15	Ginsenoside Rb2, Rb3, or Rc	RS
20	10.37	Glabrolide or isoglabrolide	GC
21	10.42	Uralsaponin U/N or licorice saponin G2	GC
22	10.74	Ginsenoside Rb2, Rb3, or Rc	RS
23	11.05	Sinensetin or its isomer	CP
24	11.08	Uralsaponin U/N or licorice saponin G2	GC
25	11.95	Glycyrrhizic acid or its isomer	GC
26	12.08	Ginsenoside Rd	RS
27	12.80	Glycyrrhizic acid or its isomer	GC
28	12.82	Licorice saponin K2 or its isomer	GC
29	13.07	Nobiletin	CP
30	13.17	Ginsenoside Ro	RS
31	13.22	Licoflavone A or its isomer	GC
32	13.44	Glycyrrhizic acid or its isomer	GC
33	13.48	Licorice saponin K2 or its isomer	GC
34	13.64	Licobenzofuran	GC
35	14.01	Glycyrrhizic acid or its isomer	GC
36	14.04	Licorice saponin K2 or its isomer	GC
37	14.11	Atractylenolide I or its isomer	BZ
38	14.61	Uralsaponin C/P or licorice saponin J2	GC
39	14.75	Ginsenoside Rg3 or its isomer	RS
40	15.23	Sinensetin or its isomer	CP
41	15.68	Uralsaponin V/W or licorice saponin C2	GC
42	15.89	Poricoic B or its isomer	FL
43	16.99	Licoflavone A or its isomer	GC
44	17.47	Licoflavone A or its isomer	GC
45	18.20	Ginsenoside 20(S)-Rg3	RS
46	18.61	Ginsenoside 20(R)-Rg3	RS
47	18.99	3β-Hydroxyatractylon or atractylenolide II	BZ
48	19.54	Ginsenoside Rg4 or Rg6	RS
49	19.62	Ginsenoside Rg4 or Rg6	RS
50	19.71	Stractylenolide I or its isomer	BZ

Table 2: Continued.

Number	Retention time (min)	Identification	TCM
51	20.27	Glycyrrhetinic acid or its isomer	GC
52	20.36	Glycyrrhetinic acid or its isomer	GC
53	20.44	Poricoic B or its isomer	FL
54	20.63	Dehydropachymic acid	FL
55	21.03	Pachymic acid	FL
56	21.09	Trametenolic acid or its isomer	FL

Note: GC (Gan-Cao): Glycyrrhizae Radix et Rhizoma; RS (Ren-Shen): Ginseng Radix et Rhizoma; CP (Chen-Pi): Citri Reticulatae Pericarpium; FL (Fu-Ling): Poria; BZ (Bai-Zhi): Atractylodis Macrocephalae Rhizoma.

also significantly decreased by YGS at both 3 and 6 h ($P < 0.05$), although YGS alone did not significantly reduce HAMP mRNA levels. YGS inhibited STAT3 phosphorylation only at 3 h ($P < 0.05$) (Figures 3(d) and 3(e)).

3.5. BMP/HJV/SMAD Signaling Pathway May Contribute to Maintenance of Iron Homeostasis by YGS. There is a crosslink between the iron and cytokine-dependent pathways of hepcidin upregulation. The integrity of the BMP/HJV/SMAD pathway is required to activate hepcidin [13]. Hence, we determined whether YGS downregulates hepcidin through BMP/HJV/SMAD pathway. Compared with the CTL group, LPS resulted in a significant increase in the liver HJV mRNA level (Figure 4(b)) and P-SMAD1/5/8 (Figures 4(c) and 4(d)) and HJV (Figures 4(c) and 4(e)) protein levels at both 3 and 6 h, and BMP6 mRNA expression (Figure 4(a)) only at 6 h ($P < 0.05$). Moreover, YGS pretreatment significantly inhibited the increase in the BMP mRNA level (Figure 4(a)) and P-SMAD1/5/8 (Figures 4(c) and 4(d)) and HJV (Figures 4(c) and 4(e)) protein levels only at 6 h ($P < 0.05$).

4. Discussion

Metabolic iron homeostasis is regulated by several factors but is most closely related to hepcidin, the central mediator of iron homeostasis. As it binds to its target iron-export protein, ferroportin (Fpn), hepcidin stimulates internalisation and degradation of Fpn and reduces the quantity of Fpn on the small intestinal mucosa and in macrophages [14]. This process serves to control dietary iron absorption, iron release from storage sites, and iron bioavailability in the body, therefore regulating the balance among iron absorption, iron utilisation, and iron storage [15].

In inflammatory conditions, hepcidin is augmented by increased levels of the inflammatory factor IL-6. Upon binding to its membrane-bound receptor glycoprotein (gp)80 on hepatocytes, IL-6 further interacts with the gp130 membrane glycoprotein to induce STAT3 (signal transducer and activator of transcription) phosphorylation [12]. Phosphorylated STAT3 enters the nucleus and upregulates transcription of the gene encoding hepcidin. This increases the circulating hepcidin level, followed by enhanced Fpn internalisation and degradation [1]. As a result, dietary iron absorption and iron release from storage sites are restricted.

The spleen is a larger concept in TCM than in Western medicine. According to TCM theory, the spleen has the central function of transporting and transforming nutrient substances, and this function plays a major role in erythropoiesis. Hence, it is plausible that iron absorption, transport, and transformation are controlled by the spleen. Furthermore, we hypothesised that an imbalance of iron homeostasis might be improved by promoting movement of splenic Qi [16]. YGS, a representative TCM prescription for promoting the movement of splenic Qi, was investigated because it has been reported to regulate the expression of inflammatory factors.

We first investigated the effect of YGS on LPS-induced imbalanced iron homeostasis in mice. Injection of 1.5 mg/kg of LPS into mice resulted in serum iron deficiency and liver iron retention within 3, 6, 9, or 12 h. Both the minimum serum iron level and maximum liver iron level occurred at 6 h. Serum and liver iron levels differed significantly between groups with and without YGS pretreatment before LPS injection, particularly at 3 and 6 h. Thus, YGS pretreatment blocked the ability of LPS to decrease serum iron levels and increase liver iron levels. This finding suggests that YGS can adjust the abnormal iron distribution under inflammatory conditions.

We next investigated the mechanisms underlying the maintenance of iron homeostasis by YGS. LPS injection leads to the production of the inflammatory cytokine IL-6, which is a major hepcidin inducer [12]. Inhibition of LPS-induced secretion of IL-6 decreases hepcidin levels. IL-6 directly regulates hepcidin through induction and subsequent promoter binding of STAT3, which is necessary for IL-6-mediated hepcidin induction [17]. Hence, we first investigated the STAT3 pathway. We observed the downregulation of IL-6 mRNA in mice pretreated with YGS prior to LPS administration at both 3 and 6 h. However, serum IL-6 was downregulated only at 3 h. The kinetics of mRNA and protein levels differed, likely because serum IL-6 protein has a longer half-life than IL-6 mRNA [18]. In addition, the use of incremental doses or prolonged delivery of YGS may result in more persistent downregulation of serum IL-6 levels; this warrants further research.

As expected, HAMP mRNA levels at 3 h were downregulated due to the decreased IL-6 mRNA and protein levels. p-STAT3 levels also decreased at 3 h, which indicates that YGS may maintain iron homeostasis by regulating IL-6/STAT3/hepcidin pathway. However, HAMP mRNA levels at 6 h were also downregulated by YGS pretreatment, although serum IL-6 and p-STAT3 levels were unaffected.

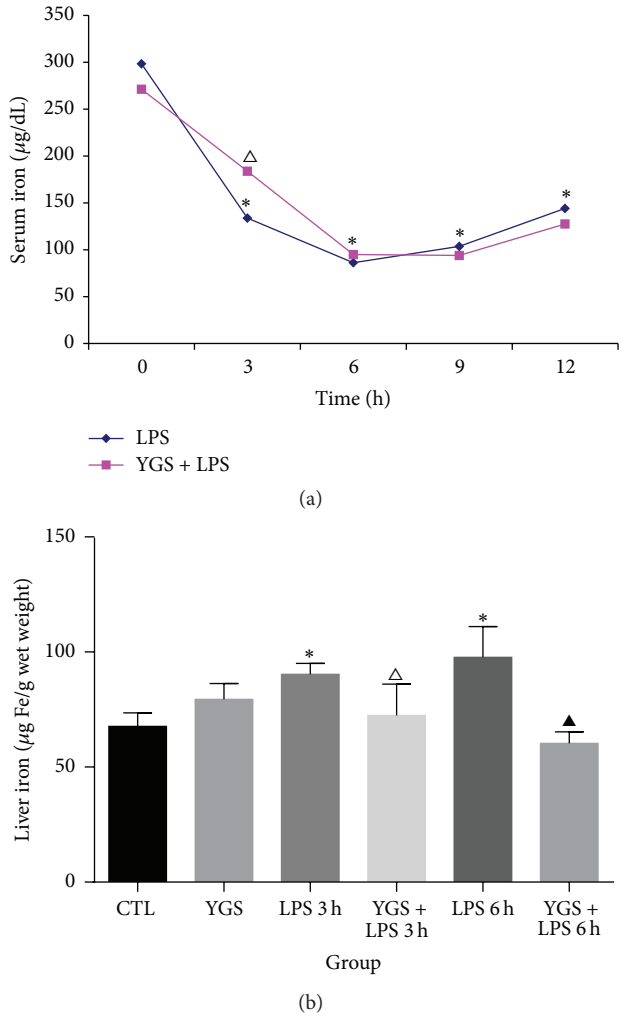

Figure 2: Effects of YGS on LPS-induced imbalanced iron homeostasis. (a) Colourimetric analysis of serum iron levels at 3, 6, 9, and 12 h after LPS administration. (b) Colourimetric analysis of liver iron levels at 3 and 6 h after LPS administration. This experiment was repeated twice, and the results are shown as mean plus or minus SD; $n = 6$. $^{*}P < 0.05$ versus the control group; $^{\triangle}P < 0.05$ versus the LPS 3 h group; $^{\blacktriangle}P < 0.05$ versus the LPS 6 h group.

Therefore, YGS may have inhibited inflammation caused by hepcidin production via another signaling pathway.

The BMP signaling pathway is involved in regulating hepcidin expression in the liver. BMPs are potent inducers of hepcidin production. The interactions of BMPs with BMP receptors result in the phosphorylation of a subset of SMAD proteins (SMAD1/5/8) and subsequent formation of a heteromeric complex with SMAD4, which translocates to the nucleus and induces the transcription of target genes [19]. HJV, a member of the repulsive guidance molecule (RGM) family, acts as a BMP coreceptor and triggers the binding of BMP ligands to BMP receptors to enhance hepcidin expression. Regulation of BMP/HJV/SMAD signaling occurs also after the phosphorylation of SMAD1/5/8, which ensures fine-tuning at the cytosolic level. STAT3-inducible hepcidin expression is also influenced by BMP-dependent SMAD activation. The BMP/HJV/SMAD signaling pathway modulates IL-6-inducible STAT3 pathway [12]. Thus, it may cooperate and enhance IL-6-dependent stimulation and may represent a connecting component between iron status and inflammation.

As mentioned earlier, we further explored BMP/HJV/SMAD pathway. BMP6 mRNA and P-SMAD1/5/8 and HJV protein levels were downregulated at 6 h by YGS pretreatment, which was correlated with the HAMP mRNA levels. Thus, we speculated that regulation of the BMP/HJV/SMAD pathway by YGS may contribute to the downregulation of hepcidin, resulting in the restoration of iron homeostasis. Our findings show that YGS regulates iron homeostasis by downregulating HAMP mRNA, which may depend on the regulation of the IL-6/STAT3 or BMP/HJV/SMAD pathway during acute inflammation.

Anaemia of chronic disease (ACD) is the most common anaemia secondary to various chronic infections, chronic inflammation, and malignancies. Increased production of inflammatory cytokines can directly induce changes in iron homeostasis, which are characterized by the reduction of both iron absorption and macrophage iron release, resulting in ACD [20, 21]. In contrast to iron deficiency anaemia, hypoferraemia in ACD is not due to iron deficiency; instead, the impaired iron absorption and utilisation regulatory mechanisms result in imbalanced iron homeostasis [22]. The changes of iron metabolism in the mice caused by LPS injection through hepcidin-induced inflammation pathway, which is one of the important ACD pathogenesis, imitate ACD pathological state although the mice can not be anaemic [6, 23]. So, if YGS decoction could improve this mimic ACD pathological state, it is possible to be used to prevent or treat ACD. Further studies on the YGS decoction are maybe important in science and application.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Qin Zheng and Yu Guan contributed equally to this work. Meihong Luo designed and performed the research, analyzed data, and wrote this paper; Qin Zheng and Yu Guan performed research, analyzed data, and wrote the paper; Lemin Xia, Zhicheng Wang, Yiling Jiang, Xiaofeng Zhang, Jianying Wang, Guohua Wang, Yiqiong Pu, and Jing Xia performed the research.

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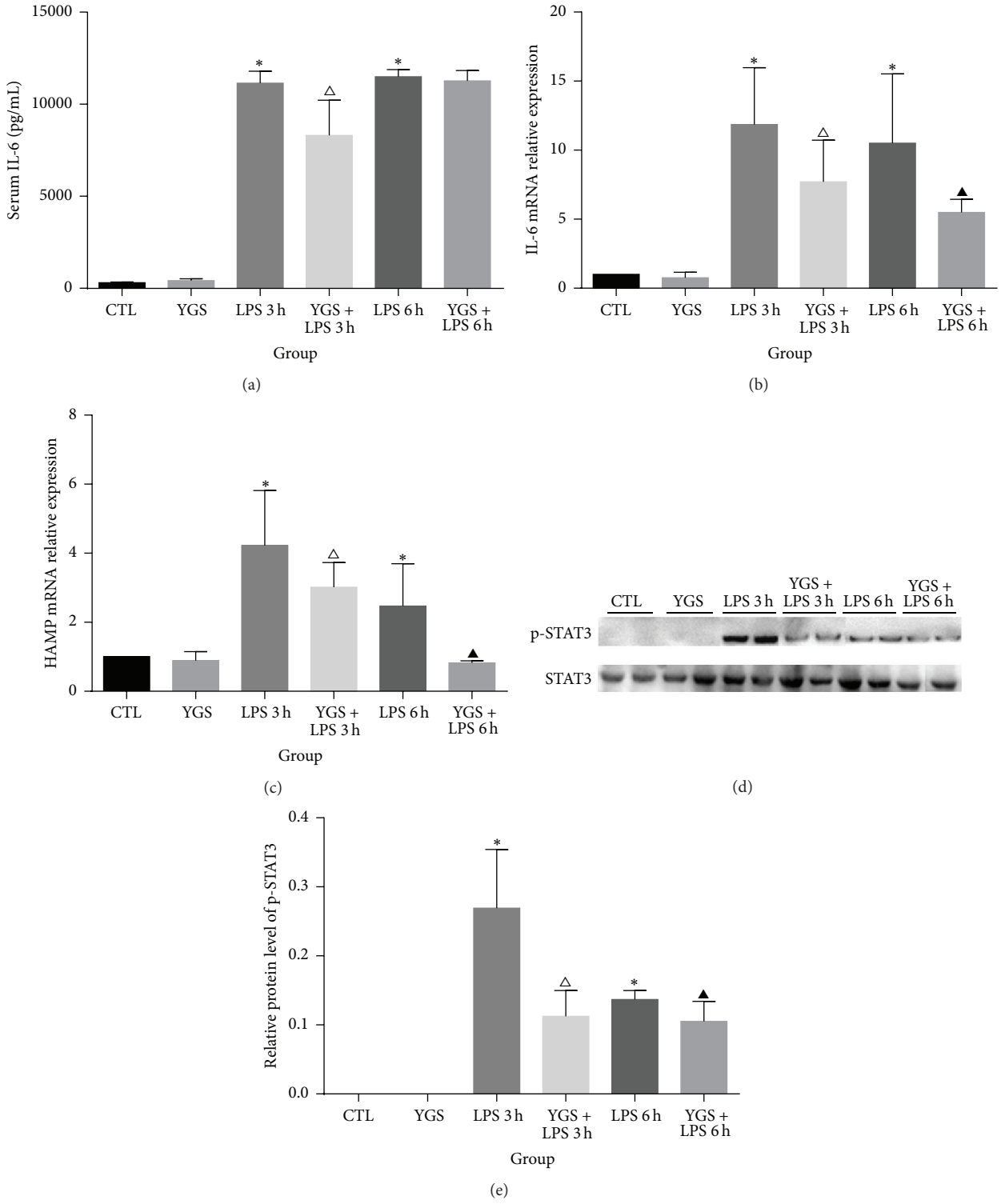

Figure 3: Levels of serum and liver IL-6 protein and liver HAMP mRNA and p-STAT3 protein. (a) ELISA of serum IL-6 levels. (b) Real-time PCR analysis of liver IL-6 and HAMP mRNA levels. (c) Western blotting analysis of p-STAT3 and STAT3 protein levels. (d, e) Western blotting analysis of p-STAT3/STAT3 protein levels expressed as densitometry values. This experiment was repeated twice, and the results are shown as mean plus or minus SD; $n = 6$. $^{*}P < 0.05$ versus the control group; $^{\triangle}P < 0.05$ versus the LPS 3 h group; $^{\blacktriangle}P < 0.05$ versus the LPS 6 h group.

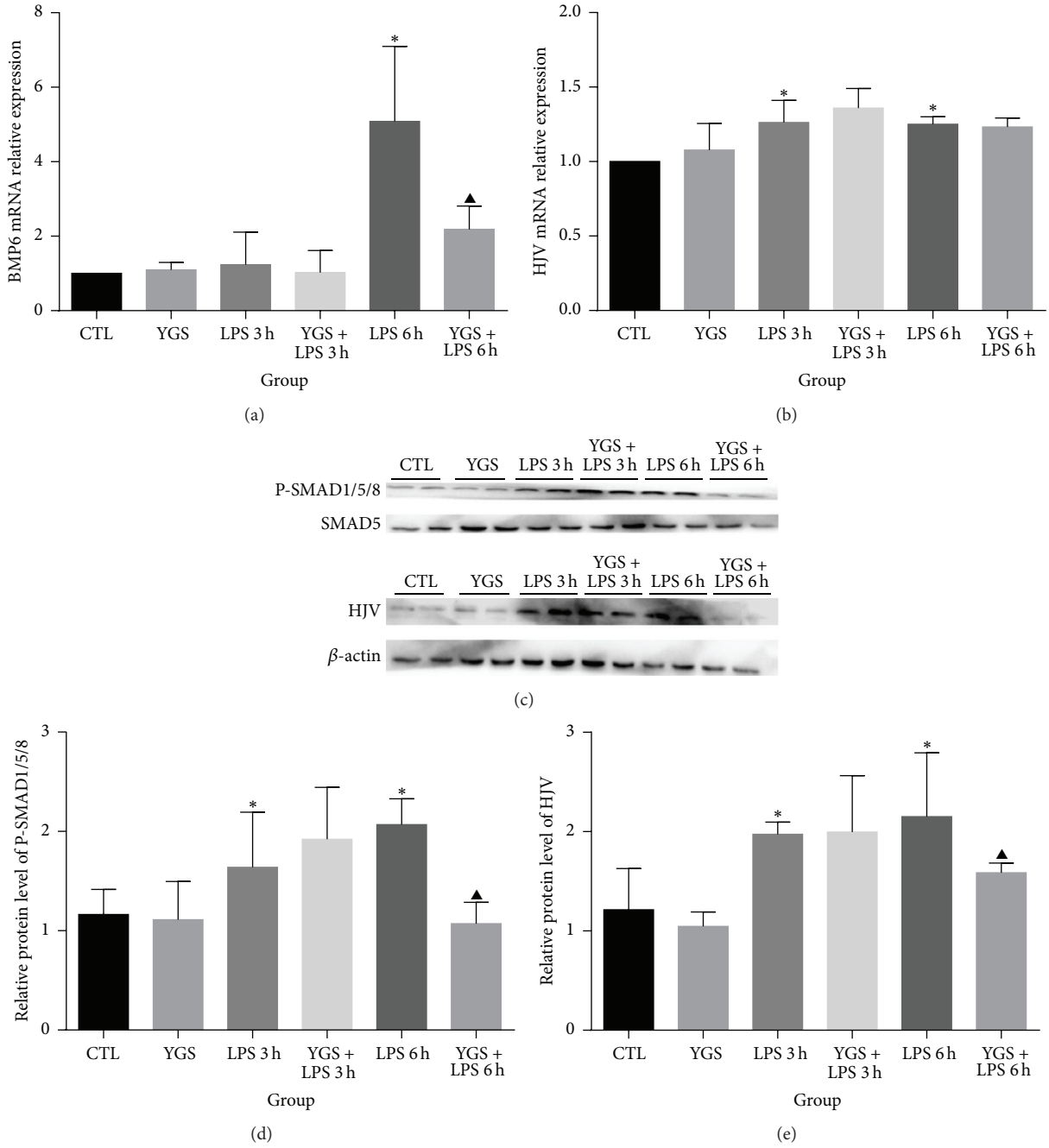

Figure 4: Hepatic levels of BMP6 and HJV mRNA and p-SMAD1/5/8 and HJV protein. Real-time PCR analysis of liver BMP6 (a) and HJV (b) mRNA levels at 3 and 6 h after LPS administration. Western blotting analysis of p-SMAD1/5/8 (c, d) and HJV (c, e) protein levels expressed as densitometry values. This experiment was repeated twice, and the results are shown as means plus or minus SD; $n = 6$. $^{*}P < 0.05$ versus the control group; and $^{\blacktriangle}P < 0.05$ versus the LPS 6 h group.

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Research Article

Protective Effects of Sheng-Mai-San on Right Ventricular Dysfunction during Chronic Intermittent Hypoxia in Mice

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Right ventricular (RV) dysfunction and failure contribute to the increasing morbidity and mortality of cardiovascular diseases; however, current treatment strategies are grossly inadequate. Sheng-Mai-San (SMS) has been used to treat heart diseases for hundreds of years in China, and its protective effects on RV have not been observed. The present study was to investigate the protective effects of SMS aqueous extract on RV dysfunction in chronic intermittent hypoxia (CIH) mice model. The results showed that CIH mice model presented RV dysfunction and maladaptive compensation after 28-day-CIH and SMS treatment significantly reversed these changes. Diastolic function of RV was restored and systolic dysfunction was attenuated, including elevation of RV stroke volume and fractional shortening, as well as pulmonary circulation. Structurally, SMS treatment inhibited RV dilation, cardiomyocytes vacuolization, ultrastructure abnormalities, and the expression of cleaved caspase-3. Of importance, SMS showed remarkable antioxidant activity by decreasing the levels of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), increasing the levels of superoxide dismutase (SOD) and heme oxygenase-1 (HO-1), as well as inhibiting the overexpression of 3-NT in RV. Our results indicate that SMS preserve RV structure and function in CIH-exposed mice by involving regulation in both ROS and Reactive Nitrogen Species (RNS) production.

1. Introduction

Impaired right ventricular (RV) function is associated with the increasingly increased morbidity and mortality of cardiovascular diseases and plays an important role in rising global health problems [1–4]. According to the results of clinical studies, patients with RV failure on the basis of other heart problems, such as left heart failure [5], acute myocardial infarction [6], or pulmonary hypertension and embolus [7–9], tend to suffer from inferior prognosis. So far, there are inadequate treatment strategies for RV dysfunction and decompensation [10].

Sheng-Mai-San (SMS) is a well-known Chinese herbal formula, which has over eight hundred years of application history in China. It is composed of Panax ginseng, Ophiopogon japonicus, and Schisandra chinensis and has been proved to be beneficial to Deficiency of both Qi and Yin Syndrome (DQYS), which is closely related to heart and lung diseases [11, 12]. An increasing number of studies have reported that it is beneficial to improve life quality and prolong life-span of the patients with pulmonary heart diseases through long-term administration of SMS [13–15]. However, the protective mechanisms of SMS on chronic dysfunctional RV have not been clarified.

It has been reported that people exposed to high altitudes for long term are susceptible to RV dysfunction [16, 17], and such pathological process can be simulated by experimental animals induced by chronic intermittent hypoxia (CIH) [18, 19]. Our group member has also demonstrated that CIH mice can simulate the main clinical features of DQYS [20] and has the characteristics of pulmonary heart diseases (unpublished). Several risk factors are involved in impairing RV function [21–23], and oxidative stress, the primary injury inflicted by CIH [24], is regarded as the crucial factor giving

rise to RV decompensation and maladaptive compensation [25, 26]. Considering the beneficial effects of SMS on cardiac diseases, together with the reports of its antioxidant activity [11, 27], the present study was designed to test the hypothesis that SMS exerts protective effects on RV impairment through suppression of oxidative stress.

2. Materials and Methods

2.1. Preparation of SMS Aqueous Extract and Its Quality Control. The herbal materials of SMS were purchased from Nanjing Traditional Chinese Medicine Out-Patient Department in Nanjing, Jiangsu, and were identified by Professor Chun-gen Wang of Nanjing University of Chinese Medicine. A voucher specimen was deposited in Department of Complex Prescription of TCM, China Pharmaceutical University. The extraction procedure of SMS was carried out as previously described in our published literature [28]. Briefly, the three ingredients, including Radix ginseng (60 g), Radix ophiopogonis (180 g), and Fructus schisandrae (90 g), were mixed together and immersed in 10-fold, 8-fold, and 6-fold volumes of water (1 : 10, 1 : 8, and 1 : 6 w/v) to decoct for 1 h at 100°C, respectively. After filtrating with 8 layers of gauze, the three extractions were combined and concentrated to approximately 100 mL and stored at −20°C. SMS sample was then diluted by double distilled water to the required concentrations of oral administration before use. A high-performance liquid chromatography (HPLC) fingerprint analysis method has been established in our laboratory [28] and a HPLC-DAD-MS/MS analysis was used to identify the main constituents of SMS extracts referring to the published method of quality control in the present study.

2.2. Animal and Experimental Protocol. All animal welfare and experimental procedures complied with Chinese Institutional regulations. The experimental protocols were approved by the Animal Ethics Committee of the School, Chinese Materia Medica, China Pharmaceutical University. Eight-week-old male ICR mice (25-26 g) were purchased from Experimental Animal Center of Yangzhou University (Yangzhou, Jiangsu, China). All animals were housed in a temperature (23 ± 1°C), humidity (30%–40%), and light controlled (12 h light/dark cycle) room with food and water *ad libitum*.

Mice were randomly divided into four groups (10–12 mice per group) and received distilled water (control and model groups) or SMS (1.1 g/kg for SMS1 group and 5.5 g/kg for SMS2 group). In addition to the control group, all groups were exposed to chronic intermittent hypoxia (CIH) (nadir 7% to peak 8% oxygen, 20 min per day) in a chamber. All animals were sacrificed after echocardiography examination at the time of 28 days. Tissue samples were rapidly excised and stored at −70°C refrigerator.

2.3. Echocardiography. Mice were anesthetized by 4% chloral hydrate (0.1 mL/kg, i.p.) before echocardiography by Vevo2100 imaging system (VisualSonics Inc., Toronto, ON, Canada) with a 30 MHZ probe. Stable images were obtained in M, B, and Doppler Mode. RV inner dimension (RVID), RV stroke volume (RVSV), RV fractional shortening (RVFS), tricuspid valve early and late diastolic filling velocities (TV E/A ratio), pulmonary arterial velocity time integral (PA-VTI), pulmonary arterial preejection time (PA-PET), and pulmonary arterial ejection time (PA-ET) were measured.

2.4. Histology. Heart sections from formalin-fixed and paraffin-embedded tissues were prepared at 5 μm thickness using a routine procedure. Tissue sections were stained with hematoxylin/eosin for general histology. A morphological analysis was used for semiquantitatively determining the extent of RV injury. Briefly, 5 visions (upper left, lower left, upper right, lower right, and middle) were observed under low magnification per section. Images were acquired by DFC 450C light microscope (Leica Microsystems Ltd., Wetzlar, Germany). The pathologist was unaware of the group assignment of individual mice.

2.5. Electron Microscopy. Right ventricle sample was fixed in paraformaldehyde (4%) solution (with 2.5% glutaraldehyde) as the previously described method [29, 30]. After 24 h, the tissue was sliced to prepare ultrathin samples to assess ultrastructure. Images were acquired by JEM-1001 transmission electron microscope (JEOL Ltd., Tokyo, Japan).

2.6. Enzyme-Linked Immunosorbent Assay (ELISA). RV samples for ELISA analysis were prepared following the manufacturer' instructions. Expressions of malondialdehyde (MDA), superoxide dismutase (SOD), heme oxygenase-1 (HO-1), and 4-hydroxynonenal (4-HNE) were detected by ELISA (Nanjing Jian Cheng Biotech Co. Ltd., Nanjing, China).

2.7. Immunohistochemistry. 8 μm sections were prepared from frozen hearts and mounted on coated slides. The sections were first incubated in blocking buffer (1% BSA in PBS containing 0.3% Triton-X-100) for 1 h and incubated with the primary antibody against cleaved caspase-3 (1 : 300, rabbit anticleaved caspase-3; Abcam, Cambridge, UK) and 3-nitrotyrosine (3-NT, 1 : 300, mouse anti-NT; Abcam, Cambridge, UK) for 24 h. The sections were then washed by PBS and incubated in DAB substrate. The tissue was finally counterstained with hematoxylin and xylene before being washed with ethanol. Images were collected by DFC 450C light microscope (Leica Microsystems Ltd., Wetzlar, Germany).

2.8. Statistical Analysis. All data were presented as mean ± standard error of mean (SEM). Differences among groups were measured by one-way analysis of variance (ANOVA) followed by Dunnett's test (Prism 5, GraphPad, CA, USA). A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Effects of SMS on General Parameters. As the results show, the death rate of model group is higher than that of SMS treatment groups (1.1 or 5.5 g/kg), and administration with SMS significantly enhanced the survival rate. Compared with the mice in control group, right ventricle weight (RVW) in model group markedly increased while pretreatment with

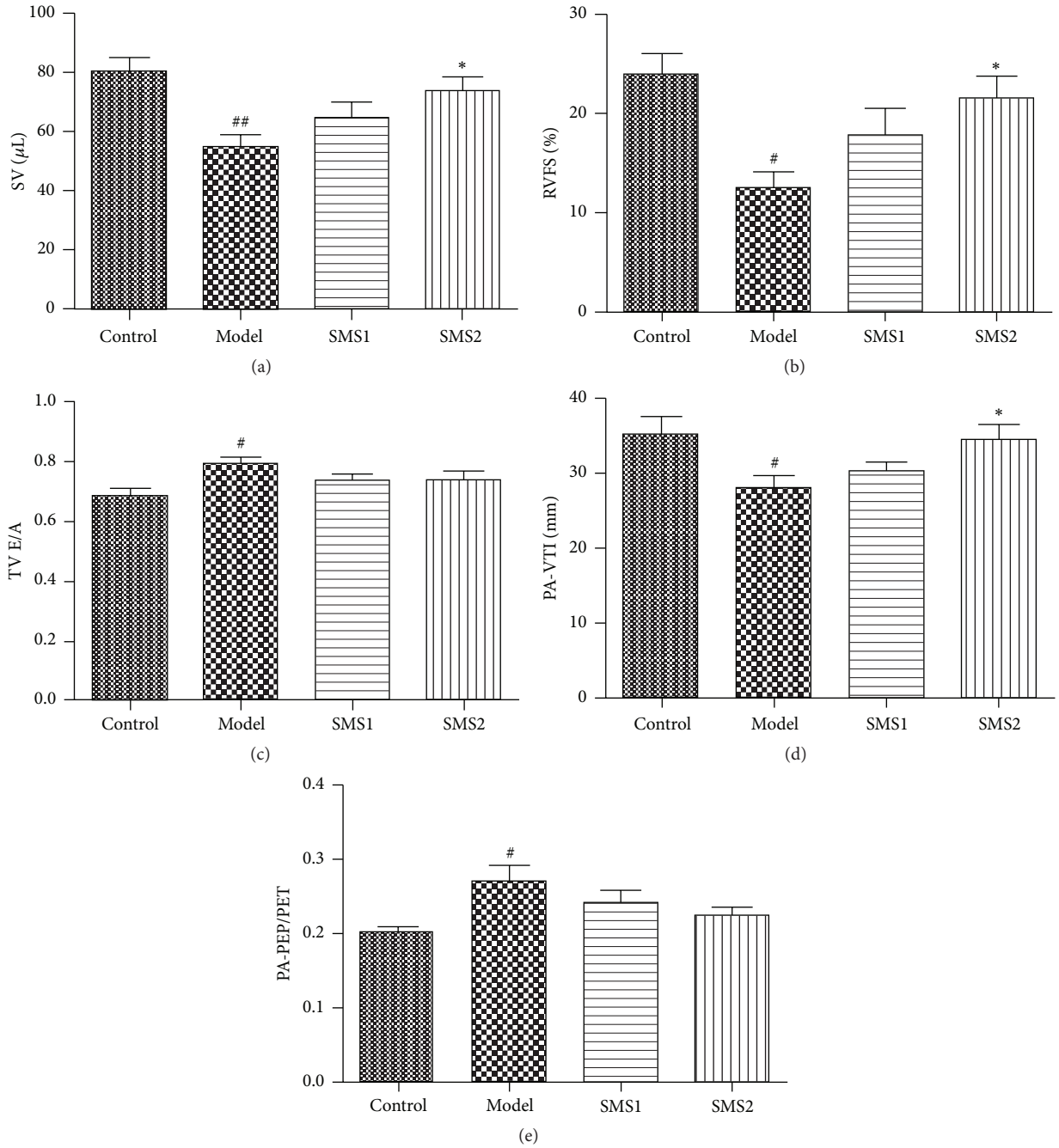

Figure 1: Echocardiographic assessment of RV function ($n = 6$). Functional parameters were SV (a), RVFS (b), TV E/A ratio (c), PA-VTI (d), and PA-PEP/PET (e). SMS1 (1.1 g/kg) and SMS2 (5.5 g/kg) prevented the occurrence of RV and pulmonary vascular dysfunction. SV, stroke volume; FS, fractional shortening; TV E/A, tricuspid valve early and late diastolic filling velocities; PA-VTI, pulmonary arterial velocity time integral; PA-PEP/ET, pulmonary arterial preejection time and ejection time. Values are presented as mean ± SEM; $^{\#}P < 0.05$ versus control, $^{\#\#}P < 0.01$ versus control, and $^{*}P < 0.05$ versus model.

SMS (1.1 or 5.5 g/kg) reversed this abnormal performance significantly (Table 1).

3.2. Effects of SMS on RV Function. RV function was evaluated by echocardiography. As the results show, right ventricular stroke volume (RVSV) and right ventricular fractional shortening (RVFS) of 28-day-CIH mice severely declined. In contrast, 4-week pretreatment with SMS (SMS2, 5.5 g/kg) prevented right ventricular from systolic dysfunction and the mice maintained normal levels of RVSV and RVFS (Figure 1, $P < 0.05$). In addition, CIH caused a significant rise in the tricuspid valve early and late diastolic filling velocities (TV E/A) ratio, which was prevented by administration of SMS, but without significance compared with the mice in CIH group

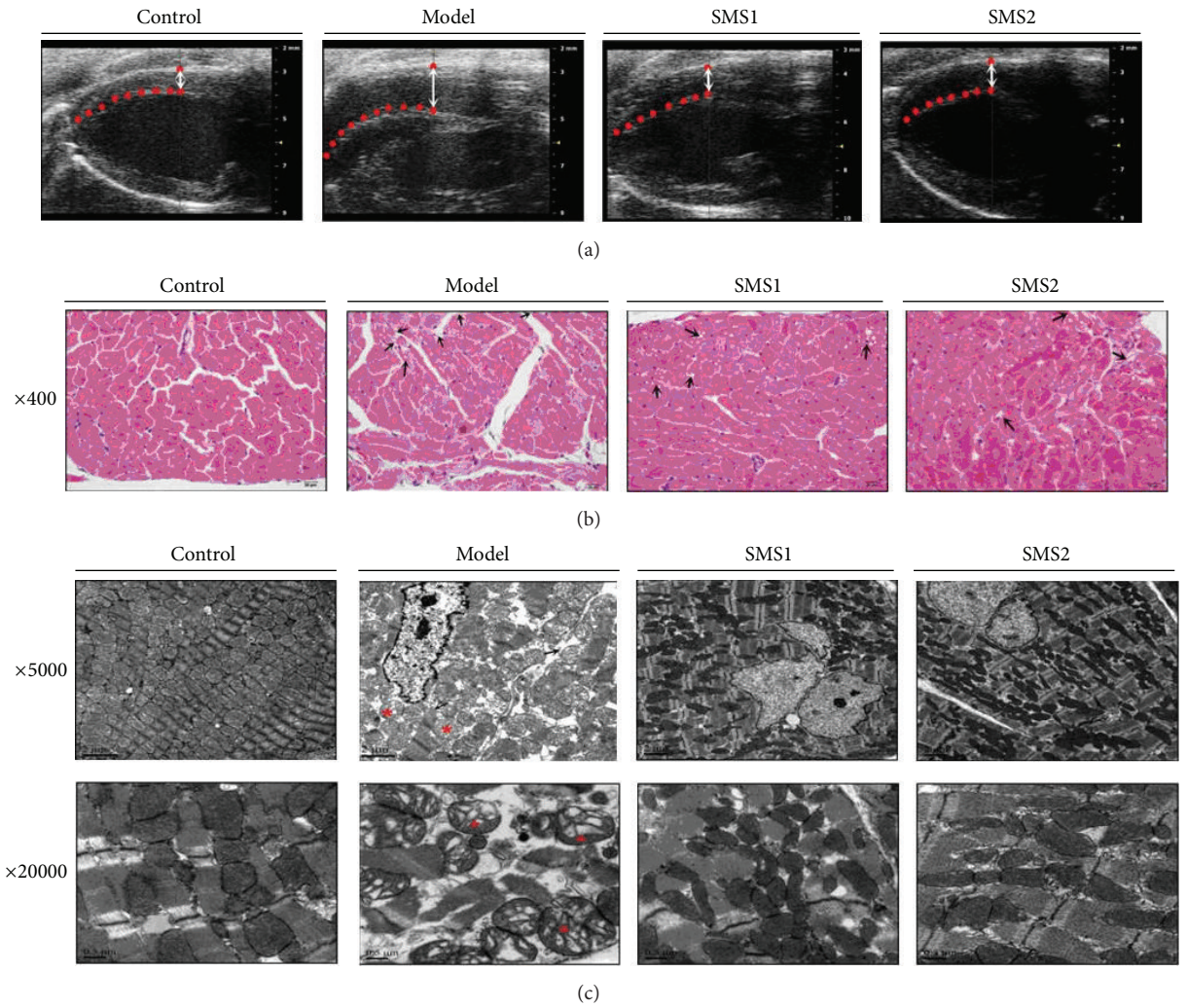

Figure 2: Beneficial effects of SMS on CIH-induced changes of RV structure in mice. RVID was determined by ultrasound ((a), $n = 7$). Dotted lines represented the interventricular septum. Histological examination of RV sections with H&E staining ((b), $n = 3$). Serve vacuolization was evidenced in 28-day-CIH mice (arrows shown). Compared with CIH mice, SMS2 (5.5 g/kg) treatment prevented this injury. Magnification, ×400; scale bar, 20 μm. Myocardial ultrastructure examination in all groups by transmission electron microscopy ((c), $n = 3$). Images were acquired under magnification ×5000; scale bar, 2 μm, and magnification, ×20000; scale bar, 0.5 μm. SMS successfully improved disruption or dissolution of myofilaments, disordered sarcomere and swollen mitochondria (stars shown). Values are presented as mean ± SEM.

Table 1: General parameters of mice ($n = 8$–10).

Group	Survivors/total mice	RVW, mg	RVW/HW, %
Control	10/10	45.0 ± 3.0	33.2 ± 2.4
Model	8/12	54.1 ± 2.1$^{\#}$	36.7 ± 2.4
SMS1	10/11	47.5 ± 2.3	33.1 ± 1.7
SMS2	10/10	46.4 ± 1.8	34.5 ± 1.7

RVW, right ventricular weight; RVW/HW, the ratio of right ventricular weight and heart weight; SMS1, SMS-treated group (1.1 g/kg); SMS2, SMS-treated group (5.5 g/kg). Values are presented as mean ± SEM. $^{\#}P < 0.05$ versus control.

(Figure 1, $P < 0.05$). Furthermore, the levels of pulmonary arterial velocity time integral (PA-VTI) of CIH mice significantly were reduced while the levels of pulmonary arterial preejection time and ejection time (PA-PEP/PET) were increased, which indicated the decline of pulmonary circulation function. Compared with the model group, SMS pretreatment preserved the normal levels of these two parameters indicating its effects on modulating pulmonary circulation function (Figure 1).

3.3. Effects of SMS on RV Structure. CIH induced a significant increase of RV inner dimension (RVID) compared with the control group ($P < 0.05$), and treatment with SMS (SMS2, 5.5 g/kg) prevented the increase of RVID markedly (Figure 2(a)). In addition, the alterations of RV were observed by light microscopic examination of RV sections stained with hematoxylin and eosin, immunohistochemistry, and transmission electron microscopy. Compared with the RVs of control mice, the RVs of CIH-treated mice showed a number

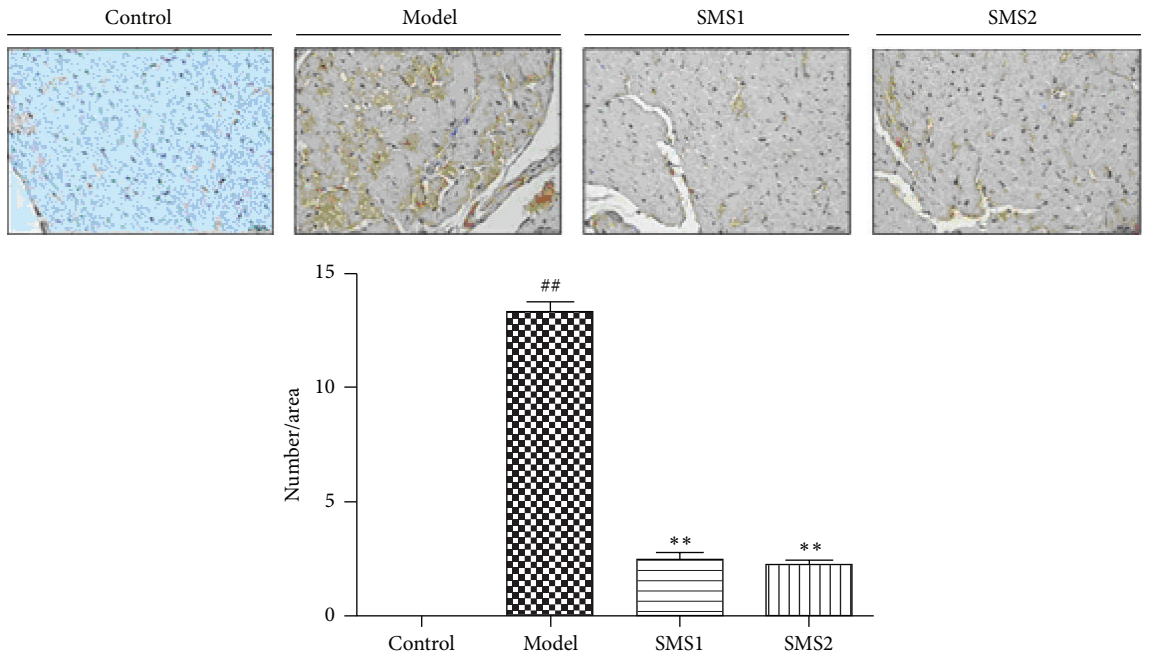

Figure 3: SMS reduced cleaved (active) caspase-3 expression in CIH-induced mice. Positive areas were brownish (arrows shown); magnification, ×400. Values are presented as mean ± SEM ($n = 4$); $^{\#\#}P < 0.01$ versus control and $^{**}P < 0.01$ versus model.

of vacuoles in the cardiomyocytes (arrows shown) and a mild degree of inflammation (Figure 2(b)). Neither fibrosis nor necrosis occurred in all groups. The histopathological damage induced by CIH was minimal in mice that received SMS treatment. The ultrastructures of RV were shown at two magnifications (×5000; ×20000) and dissolution of myofilaments, disruption sarcomeres, and disarranged swollen mitochondria appeared with abnormal cristae (stars shown). SMS treatment protected against CIH-induced mitochondria damage.

Then, we determined the levels of cleaved caspase-3 expression by immunohistochemistry (Figure 3). The results showed increased expression levels of cleaved (active) caspase-3 in CIH-treated mice and indicated that there existed apoptosis-like pathology in RV cardiomyocytes. SMS treatment presented lower levels of cleaved caspase-3 expression compared with the CIH group (SMS1 and SMS2 groups in Figure 3).

3.4. Effects of SMS on Oxidative Stress. Cardiac oxidative stress was determined by enzyme-linked immunosorbent assay, as well as immunohistochemistry. As the results showed, the levels of MDA and 4-HNE in RV homogenates increased significantly, while SMS treatment completely prevented these alterations. Compared with the mice of CIH group, there were less decreases of SOD and HO-1 levels in SMS treatment groups (SMS1, SMS2), indicating the antioxidant properties of SMS (Figure 4). Additionally, we evaluated the levels of 3-NT in different groups through quantifying the positive stains in 60 × 60 μm squares in five random areas of each group (Figure 5). The results showed that the positive points substantially increased in CIH-treated mice, while the positive points of SMS treatment (SMS1, SMS2) groups decreased significantly (Figure 5). The levels of 3-NT in four groups were consistent with the results of the oxidative stress factors MDA and 4-HNE.

4. Discussion

Deficiency of both Qi and Yin Syndrome (DQYS) is one of the common syndromes in cardiovascular diseases, and Sheng-mai San (SMS) is the representative prescription for the treatment of this syndrome. Experimental studies have proved that SMS can prevent heart ischemia, apoptosis, and so forth [31], which are in accord with its clinical application. In the present study, a chronic intermittent hypoxia (CIH) mice model [20] simulating the clinical features of DQYS was used, and the function and morphology of right ventricle (RV) in CIH-treated mice were furtherly observed, and the protective effects of SMS on impaired RV were also evaluated.

RV dysfunction and maladaptive remodeling are risk factors contributing to RV or left ventricular (LV) failure [1, 2] and are related to the increasing morbidity and mortality of cardiovascular diseases. In the present study, we found that RV remodeled in mice after 28-day-CIH (nadir 7% to peak 8% oxygen, 20 min per day) and the mortality of CIH-treated mice significantly increased, which were consistent with the reported references [32, 33]. By contrast, SMS administration attenuated systolic dysfunction by the elevation of RV stroke volume, fractional shortening, and pulmonary circulation. In addition, diastolic function of RV was also restored moderately as detected by echocardiography. The findings suggested that SMS exerted a protective effect on impaired RV induced by CIH.

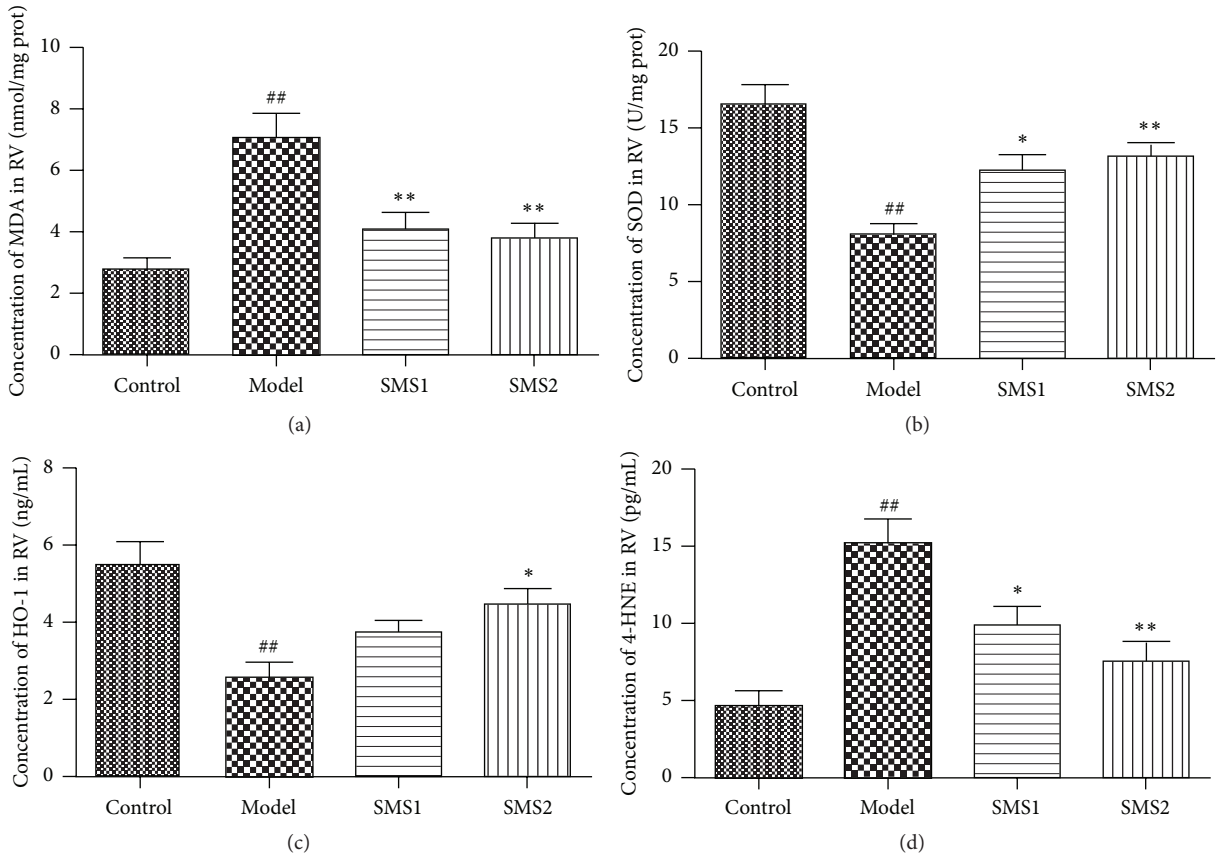

Figure 4: Influence of SMS on CIH-induced changes on malondialdehyde (MDA, (a)), superoxide dismutase (SOD, (b)), heme oxygenase-1 (HO-1, (c)), and 4-hydroxynonenal (4-HNE, (d)) in RV. Values are presented as mean ± SEM (n = 7-8) $^{\#\#}P < 0.01$ versus control; $^{*}P < 0.05$ versus model; $^{**}P < 0.01$ versus model.

Oxidative stress is regarded as an important pathophysiologic process of RV dysfunction and maladaptive compensation, although the exact mechanism of CIH giving rise to RV dysfunction is largely unknown [21, 25, 26]. In general, oxidative stress occurs from an imbalance between the formation of ROS and the antioxidant defense systems, and RNS also has a harmful effect on the cardiovascular system during this process. Substantial and persistent ROS and RNS will initiate signal pathways including apoptosis, inflammation, or fibrosis [23, 24]. There are correlative complex signal pathways between ROS and RNS of different origins in the heart. In the present study, we examined the activity of antioxidant enzymes except the content of lipid peroxidation products MDA and 4-HNE. As the results show, SMS remarkably reduced oxidative stress in RV by downregulation of the content of MDA and 4-HNE and upregulation of the activity of SOD and HO-1. Notably, overexpression of 3-NT in RV was also prevented by SMS administration. ROS and RNS in cardiomyocytes are derived from mitochondria [34, 35], NADPH oxidase [21, 36], xanthine oxidase [24], and uncoupled nitric oxide synthase [37, 38]. The results suggested that SMS is able to reduce oxidative stress by involving regulation of both ROS and RNS production.

The structure of the ventricular chamber is integral for optimal function and movement of blood flow in RV [22]. RV dilation reflected remodeling of the maladaptive decompensation processes within the context of lower cardiac cell regeneration [21, 39, 40]. CIH can make RV regress from compensation to decompensation [24]. In the short term, RV hypertrophy occurs to maintain the normal level of RV function [41]. However, RV hypertrophy will not be sustainable and becomes maladaptive decompensation over the long term. During long-term CIH exposure, remodeling and hypertrophy lead to adverse outcomes, including metabolic disorder, endothelial dysfunction, and excessive autonomous nervous system activity [24, 42, 43]. In the present study, histopathology examination of heart tissue demonstrated that CIH induced severe vacuolization, while necrosis or fibrosis has not been detected. Further transmission electron microscopy analysis showed swollen mitochondria, the cristae of mitochondria blurred, broken, or dissolved, and dissolution of myocardial myofilaments, suggesting that apoptosis might occur in RV. Next, we evaluated caspase-3 protein expression using western blotting analysis, which plays a central role in apoptosis [44]. The results showed that cleaved caspase-3 protein expression level of CIH-treated group significantly increased compared with that of the control group, further suggesting the feature of cardiomyocytes apoptosis. Compared with the model group, SMS prevented the occurrence of vacuolar cardiomyocytes

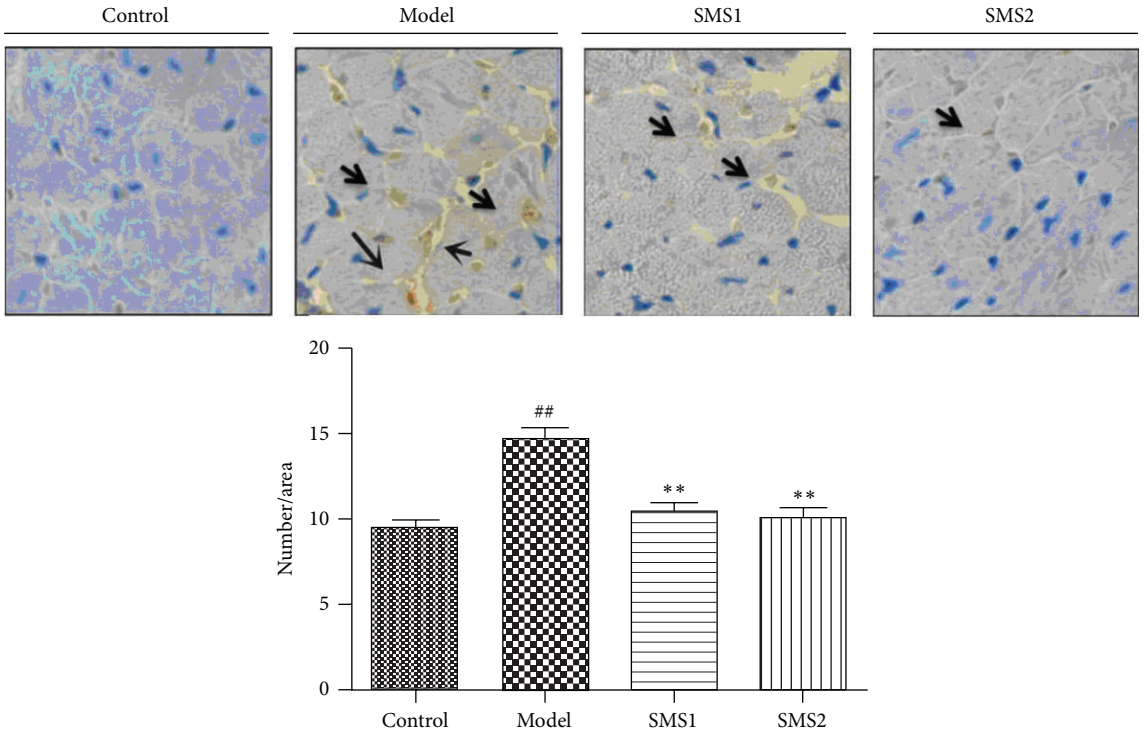

FIGURE 5: SMS reduced cardiac 3-nitrotyrosine (3-NT) expression in CIH-induced mice. Representative images are in 60 × 60 μm squares (magnification, ×400). Positive stains of 3-NT are shown by arrows and quantitative analysis was used. Values are presented as mean ± SEM ($n = 4$), and five areas of each sample were observed, respectively. $^{\#\#}P < 0.01$ versus control; $^{**}P < 0.01$ versus model.

and swollen mitochondria as well as downregulating caspase-3 protein expression, and the protective effects of SMS on cardiomyocytes apoptosis were confirmed.

PA-VTI can reflect RV ejection and systolic pressure when there is no obstruction in RV outflow tract [45, 46]. The ratio of PEP/ET is dependent on pulmonary arterial pressure, as well as the stiffness of RV [46]. Compared with the model group, SMS treatment (SMS2, 5.5 g/kg) restored pulmonary arterial function by regulating PEP/ET moderately and preventing the reduction of PA-VTI, which suggested that SMS mildly prevented the development of RV stiffness.

In conclusion, the present study showed that 28-day-CIH results in RV dysfunction and structural abnormalities while SMS administration can prevent these alterations from happening, possibly due to its antioxidant activity. The beneficial effects of SMS successfully preserved RV systolic ejection and prevented maladaptive compensation of RV, providing promising therapeutic strategies for the management of RV hypertrophy and failure in patients.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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Review Article

The Role of *Nigella sativa* and Its Active Constituents in Learning and Memory

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The loss of the ability for learning and memory is a prominent feature of dementia, which affects millions of individuals all over the world, due to either neurodegenerative diseases or brain injury. Although a lot of information is known about the pathology involved, treatment remains elusive at best. The Black Seed of *Nigella sativa* has been historically and religiously used for thousands of years for preventing and treating many different kinds of diseases. This review article looks at *Nigella sativa* and its potential role in facilitating learning and memory. The possible use of this seed's extract or compounds isolated from it, such as thymoquinone, for treating damaged brain neural tissue is discussed. The evidence presented in this paper appears to be supporting the hypothesis that this plant and/or its bioactive constituents can enhance learning and memory in health and disease in animals and humans.

1. *Nigella sativa* History and Its Importance

Being an established historical and religion-based remedy for wide ranging health problems, *Nigella sativa* (NS), which belongs to the family Ranunculaceae, is one of the herbal medicines that has been extensively investigated and gaining worldwide recognition [1]. NS is a native dicotyledonous plant to southern Europe, North Africa, and Asia Minor, and being widely cultivated in Pakistan and India, thus, becoming a household traditional medicinal plant in the region [2]. Over the years, immigration helped the plant cultivation to spread extensively throughout Eastern Europe and North America. It is also known as the Black Seed because when the seeds are exposed to air, they turned black [1]. Among the Muslim community, this plant is referred to as *Habbatus Sauda*, *Alhabahat Alsawda*, and *Alkamoun Alaswad* in reference to the colour of its seeds [3]. In some other parts of the world, it is also known as *Shuniz*, *Khodhira*, Black Cumin, or Black Caraway [4].

NS has a long history of folklore usage in different civilizations and has been recognized as a "miracle cure" for its ability to treat various diseases and assist the body in its own natural healing process [1]. In ancient texts and historical documents, NS has been mentioned as a notable healer for a range of ailments. Archeological evidence about the earliest cultivation of NS is scanty but there are studies, which reported that NS seeds have been found in several sites from ancient Egypt, including in the tomb of Tutankhamen. It is also known as a beauty secret since ancient times as Queen Nefertiti, who was praised for her exquisite complexion, was a devoted user of NS oil [5]. The earliest written reference is in the book of Isaiah of the Bible, in which it is referred to as "*Ketzah*" in Hebrew, a spice for bread and cakes [6].

For the Muslim community, the traditional practice of its usage is primarily due to the authentic prophetic statement that NS is a cure for all, except death; that was quoted by a renowned Muslim scholar, Al-Bukhari [7]. Thus, the glorified status of NS among the Muslim community is as

Habbat Albarakah, with the term "*Albarakah*" signifying its "blessed" status [3]. Besides that, various Muslim scholars also gave ample credit to the healing properties of the NS and, hence, its importance in the "Prophetic Medicine" tradition. The Persian physician and philosopher Ibn Sina, commonly known in the West as Avicenna, had mentioned NS in his famous medical treatise "Canon of Medicine," which is considered as a hallmark in the history of human medicine and was used as the main medical text until the 17th century in Europe. In his writings, he stated that NS has preventative and restorative features as it stimulates the body's energy and helps in recovery from fatigue or dispiritedness. Ibn Sina also recommended NS as a remedy for fever, common colds, headache, toothache, skin diseases, wounds, fungus, parasites, and worms as well as against bites and stings by poisonous animals [5].

NS has been reported to have many therapeutic properties such as immunopotentiation, bronchodilatation, and being antitumor, antihistaminic, antidiabetic, antihypertensive, anti-inflammatory, antimicrobial, hepatoprotective, and gastroprotective, which are attributed to its quinone constituents in the seeds [8–10]. Identification of the therapeutic features of NS came from researches in various fields starting in the early 1970s [11]. Nonetheless, there are comparatively only a few studies that scientifically support its positive role in treating central nervous system (CNS) related ailments. However, considering its significant antioxidant, anti-inflammatory, and immunomodulatory properties, consuming NS could be one of the promising health strategies to help prevent the oxidative damage to cells, particularly in the brain regions related to memory functions [12]. Thus, this review article looks at NS and its potential role in facilitating learning and memory. The possible use of this seed's extract or compounds isolated from it, such as thymoquinone (TQ), for treating neurodegenerative disease is discussed. The evidence presented in this paper appears to be supporting the hypothesis that this plant and/or its bioactive constituents can enhance learning and memory in health and disease in animals and humans.

2. Bioactive Constituents of *Nigella sativa*

Literature revealed that from ancient times it has been known that the medicinally significant component of the NS plant is the *Nigella sativa* oil (NS oil) (Figure 1). The efficacy of the NS oil is mostly attributed to its quinone constituents in the NS fixed and essential oil, which is especially endowed with thymoquinone (TQ), a significant bioactive constituent making up 30–48% of the total compounds [13]. Other functional components of the NS oil include *p*-cymene, carvacrol, thymohydroquinone (THQ), dihydrothymoquinone (DHTQ), α-thujene, thymol, *t*-anethole, β-pinene, α-pinene, and γ-terpinene.

Among these, TQ has received the most attention and is mostly attributed to the learning and memory enhancing effects of NS. It has been shown to ameliorate diabetes-induced cognitive decline by preventing oxidative stress [14]. TQ has also been reported to restore oxidative balance, mitochondrial dysfunction, and cholinesterase activity caused by Aβ administration to PC 12 cells [15]. It exhibited a neuroprotective effect in hippocampal slices and cultured rat primary neurons treated with Aβ [16, 17]. It is further shown to inhibit apoptosis induced by Aβ in primary cultured cerebellar granule neurons [18]. In addition, TQ and THQ are usually present in the form of glycosidically bound aglycones, which easily cross the blood-brain barrier, hence, possibly related to its neuroprotective effects [19]. TQ has also been shown to inhibit nonenzymatic peroxidation in ox brain phospholipid liposomes with a 10 times higher potency than NS oil [20]. Taken together, TQ appears to be the major neuroprotective constituent present in NS oil.

The other bioactive compounds, that is, thymol and carvacrol, also attenuated Aβ- and scopolamine-induced cognitive impairments in rats [21]. Both of the aforesaid bioactive compounds along with γ-terpinene and *p*-cymene are shown to inhibit the acetylcholinesterase activity while γ-terpinene alone is found to be a good inhibitor of lipid peroxidation [19, 22]. Notably, a nutraceutical containing thymol and *p*-cymene has been patented for cognitive enhancement properties [23]. Therefore, it appears that the cholinergic modulation properties of NS may be mediated by constituents other than TQ.

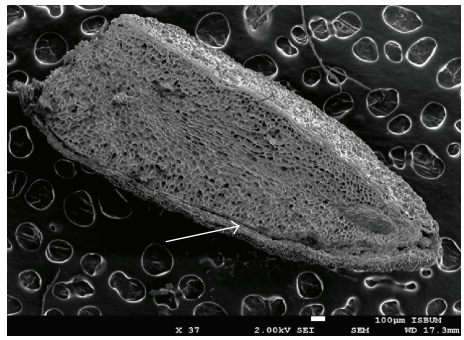
Flavonoids are present in NS seeds and have been widely studied [24–26]. Emerging evidence suggests that flavonoids are able to induce improvements in memory, learning, and cognition. Flavonoids have been shown to modulate critical neuronal signaling pathways involved in processes of memory and, therefore, are likely to affect synaptic plasticity and long-term potentiation (LTP) mechanisms, which is widely considered as a mechanism for memory [27]. Briefly, flavonoid-induced improvements in behaviour have been associated with specific changes in protein expression in the hippocampus. Hippocampal elevation of NR2B-containing N-methyl-D-aspartate (NMDA) receptor at synaptic sites is correlated with the levels of the adhesion molecule of polysialylated form of the neural adhesion molecule (PSA-NCAM) in the dentate gyrus of the hippocampus, with both proteins linked to efficient and persistent LTP and spatial learning [28].

3. Effects of *Nigella sativa* on Learning and Memory

Learning and memory are the most important executive functions performed by the human brain, the loss of which is a prominent feature in dementia. Dementia can be caused by aging, physical and/or chemical injuries, or neurodegenerative diseases, which in most cases would affect the quality of learning and memory of the concerned individuals. The latter include health problems such as Alzheimer's disease (AD) or Parkinson's disease (PD), which are characterized by the accumulation of protein aggregates on the surface or inside the neurons. Disturbances, which cause oxidative stress and elevated cortisol levels, can lead to neurodegeneration that may subsequently induce a fall in cognitive ability. Any chemical, natural, or synthetic substances that enhances executive functions of the brain is of immense clinical significance.

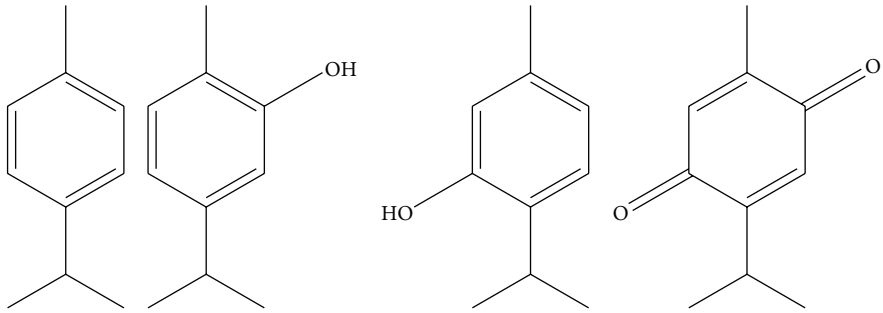
Nigella sativa seeds

(a)



NS seed cut surface as seen by scanning electron microscopy

(b)



(c)

Figure 1: (a) Each *Nigella sativa* (NS) seed shows characteristic corrugations of its surface. (b) NS essential oil resides in vesicles just beneath the black seed coat (as shown by white arrow). (c) NS oil is mainly composed of monoterpenes (having 10 carbon atoms) having phenolic groups that provide the basis for its antioxidant activity.

In comparison to studies involving other plant materials, established reports on the effects of NS seeds and/or its constituents on the CNS and on behavioural actions are few, most of which focused on the spatial memory [29]. Spatial memory involves memory for spatial information by which the brain functions in recognizing, codifying, storing, and recovering information about objects or routes. It has working memory and reference memory components and normally associated with exploratory behaviour and curiosity, which represent the need to acquire information when facing new environments [30].

It is well known that cholinergic neurons are degenerated in AD and, notably, acetylcholine (ACh) as a neurotransmitter plays a role in facilitating learning and memory, and, therefore, its decreased release will result in memory impairment. Hence, elevation of ACh via the inhibition of its degradation by acetylcholinesterase (AChE) is a currently used strategy for its management. Pharmacological studies demonstrated that NS is involved in AChE inhibition activity, the principal enzyme involved in the hydrolysis of Ach, thus, retaining its effects in the encoding of new memories.

3.1. Studies on Animals. The involvement of the central cholinergic enhancement (via AChE inhibition) is reflected from the alleviating effect by NS hydroalcoholic extract against scopolamine-induced amnesia [31]. The mnemonic effect, cholinergic modulation, and oxidative stress mitigation were attributed to the oil present in NS [29]. A study has also reported that extract of NS could prevent scopolamine-induced deficit memory in rats, as the animals showed better performance in passive avoidance test and decreased AChE activity in the hippocampus and cortex tissue of the brain [32]. Following scopolamine administration, NS treated group decreased the AChE activity and oxidative stress of the brain cortex tissues in rats, as evidenced by significant decrease in total sulfhydryl (SH) and increase in malondialdehyde (MDA) and thiol concentrations [33]. Worth noting is the fact that NS oil tended to mimic the effects of donepezil, an AChE inhibitor, which is known to have positive effects by decreasing MDA and brain tumor necrosis factor-alpha (TNF-α) content as well as increasing glutathione brain contents. Oral pretreatment of NS oil could significantly reverse the amnesic effect of scopolamine-induced deficit of spatial and nonspatial working memory impairment in the T-maze alternation task and object recognition test, respectively [34].

Induced neurotoxicity by Aβ-peptide, a protein type which is commonly accumulated in AD, could be protected by NS oil and its aqueous fraction via antioxidant effect in rat primary cerebellar neurons [35]. Its oil further showed beneficial effect on memory in animal model of chronic

hypoperfusion without altering the hippocampal plasticity and preserving the ultrastructural constituents [36–38].

Some inferences are also drawn from works done on diabetes, which is characterized by hyperglycemia, and reported to be associated with cognitive decline. A study conducted by Khan and colleagues has shown that TQ, the active principle of NS, has neuroprotective properties on cognitive impairment and related dementias [39]. Rats pretreated with 3 mg/kg body weight of TQ for 15 days after streptozotocin- (STZ-) induced cognitive impairment have been found to significantly decrease latency and path length in the Morris Water Maze (MWM) behaviour test and restored antioxidant enzymes viz. glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase. NS extract has also been shown to ameliorate spatial memory disturbances linked with diabetes in rodents as shown through the use of passive avoidance and Y-maze tests, indicated by improved initial latency, step-through latency, and alternation behaviour [40]. Importantly, in diabetic rats, the aqueous extract of NS is shown to have adaptogenic effect via normalizing the hypothalamus-pituitary-adrenal (HPA) gland axis and oxidative stress [41, 42]. These actions probably underlie the aforementioned protective effect of NS in diabetic rats.

Our research team has also reported the possible beneficial effects of NS oil administration on the spatial memory performance (SMP) of male adult rats using the radial arm maze (RAM) apparatus, one of the standard apparatuses used in behavioural-based research to assess spatial memory [43]. From the finding, it is reasonable to suggest that treatment with NS oil could enhance the learning ability and memory of the rats, especially the working memory.

Eysenck and Calvo suggested that anxiety could also partly impair memory performances, depending on certain circumstances [44]. For instance, anxious individuals have less attentional capacity for task performance and, thus, do not perform as well as nonanxious individuals on tasks that make substantial demands on working memory [45]. NS has been also demonstrated to produce antianxiety effect in different tests which used behavioural models for exploration-induced anxiety. One study confirmed this hypothesis; NS daily treatment for four weeks exhibited increase in the open field activity and produced antianxiety behaviour when tested in elevated plus maze. Treatment with NS also increased levels of serotonin/5-hydroxytryptamine (5-HT) and decreased the levels of hydroxyindoleacetic acid (5HIAA) in the brain, both inducing the coordination of behaviour including reducing anxiety via the production of serotonin [46].

Epilepsy, a neuro-related disease characterized by seizures, can also lead to poor cognitive functions. In the pentylenetetrazole- (PTZ-) induced epileptic model, the NS hydroalcoholic extract was reported to be beneficial by preventing the learning and memory decline [47]. In addition, glycation, the nonenzymatic reaction between sugar and protein, is the phenomenon that is long known to underlie several aging linked physiological alterations. It is suggested that NS may affect the glycation process, although the phenomenon remains elusive, and hence, is worth investigating.

Thyroxine plays an important role in growth, development, and function of the brain. In neonatal animals, hypothyroidism linked with learning and memory impairments could be reversed by hydroalcoholic extract of NS, which is attributed to its antioxidant effects. Comparable with vitamin C, NS treatment reduced the time latency, increased the time spent in target quadrant in MWM test, and significantly increased the time latency for entering the dark compartment in passive avoidance test [48, 49]. This data reflects the neuronal growth promoting effect of NS and should be evaluated in CNS retardation studies.

3.2. Studies on Humans. Literature reveals that NS possesses mnemonic/nootropic properties. In elderly humans, its commercially available capsule (500 mg for 9 weeks) was also shown to enhance the executive functions in various memory related tests such as logical memory, digit span, letter cancellation, Rey-Osterrieth complex figure, trail making, and stroop tests [50]. The effects of NS on mood, anxiety, and cognition have also been investigated in human subjects [51]. Volunteers were assessed for cognition with modified California verbal learning test-II (CVLT-II), mood with Bond-Lader scale, and anxiety with State-Trait Anxiety Inventory (STAI). Four weeks daily consumption of one NS capsule of 500 mg as a nutritional supplement stabilized mood, decreased anxiety, and improved memory.

4. Conclusions

The neuroprotection plus cholinergic modulation by NS provides a good example of the emerging multitarget approach towards treating complex ailments such as AD. Though the literature has revealed several reports addressing the effects of NS and its bioactive constituents on learning and memory, its mechanism of action still remains elusive. Long-term potentiation (LTP), amyloid precursor protein cleaving enzymes, glutamatergic system, GABAergic neurotransmission, mitochondrial membrane, and enzymes are other important modulators of learning and memory, which need to be investigated in the context of the aforementioned mnemonic/nootropic effects of NS.

Taken together, these mentioned reports in this review are strongly suggestive of the neuroprotective potential of NS and/or its bioactive constituents in animals and humans. It appears that enough data has been accumulated to support NS as a potential candidate for a drug discovery programme against neurodegeneration related diseases and brain injury affecting learning and memory.

Conflict of Interests

All authors have no commercial or financial interests in the products described in this research paper.

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Research Article

The Difference between the Two Representative Kampo Formulas for Treating Dysmenorrhea: An Observational Study

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In Kampo medicine, two different formulas are effective for treating dysmenorrhea—tokishakuyakusan and keishibukuryogan; however, the criteria by which specialists select the appropriate formula for each patient are not clear. We compared patients treated with tokishakuyakusan and those with keishibukuryogan and proposed a predictive model. The study included 168 primary and secondary dysmenorrhea patients who visited the Kampo Clinic at Keio University Hospital. We collected clinical data from 128 dysmenorrhea patients, compared the two patient groups and selected significantly different factors as potential predictors, and used logistic regression to establish a model. An external validation was performed using 40 dysmenorrhea patients. Lightheadedness, BMI < 18.5, and a weak abdomen were significantly more frequent in the tokishakuyakusan group; tendency to sweat, heat intolerance, leg numbness, a cold sensation in the lower back, a strong abdomen, and paraumbilical tenderness and resistance were more frequent in the keishibukuryogan group. The final model fitted the data well. Internally estimated accuracy was 81.2%, and a leave-one-out cross-validation estimate of accuracy was 80.5%. External validation accuracy was 85.0%. We proposed a model for predicting the use of two Kampo formulas for dysmenorrhea, which should be validated in prospective trials.

1. Introduction

Dysmenorrhea is the most common gynecological disorder in women, regardless of age and nationality [1]. Patients with dysmenorrhea have strong lower abdominal or lower back pain that begins during or just before the menstrual period. Dysmenorrhea is thought to be caused by an excess or imbalance of prostanoids, and possibly other eicosanoids, released from the endometrium during menstruation. As a result, the uterine basal tone increases, with frequent and dysrhythmic contraction. Pain is induced by uterine hypercontractility, reduced uterine blood flow, and increased peripheral nerve hypersensitivity [2].

The standard treatment for dysmenorrhea is nonsteroidal anti-inflammatory drugs (NSAIDs) or oral contraceptives (OCs) [3, 4]. Up to 30% of patients, however, do not respond sufficiently to NSAIDs, and 10% to 20% respond to neither NSAIDs nor OCs [1]. Furthermore, NSAIDs are contraindicated in patients with a peptic ulcer or gastritis. OCs are contraindicated in those with any thrombotic predisposing factor, breast cancer, migraine with aura, or pregnancy. For these reasons, various alternative treatments have been

examined, such as acupressure, vitamin B1, vitamin E, use of a hot pack, transcutaneous electrical nerve stimulation, and behavioral interventions [5].

Kampo, Japanese traditional medicine, is a leading alternative medicine [5, 6] and is popular in Japan, particularly for treating women's health issues. Two Kampo formulas are commonly used for treating dysmenorrhea [7, 8]—tokishakuyakusan and keishibukuryogan—and both have been shown to be effective in randomized placebo-controlled trials [9, 10]. In the Japanese national health insurance system, both formulas are indicated for dysmenorrhea and other gynecological conditions, including irregular menstruation, menopause, and infertility.

Kampo formulas are prescribed according to traditional pattern-based diagnosis [11], which is used in addition to Western diagnosis [12]. In Kampo medicine, pattern diagnosis refers to the unique clinical classification of the patient, which takes into account symptoms, general constitution, and other factors. The patient is differentially diagnosed with chronic health conditions, including dysmenorrhea, on the basis of disharmony in any of the following areas: the eight categories (excess-deficiency, heat-cold, interior-exterior, and yin-yang) and body constituents (qi, blood, and fluid) [13]. Tokishakuyakusan is traditionally prescribed for patients diagnosed with "deficiency," "cold," "interior," "yin," "blood deficiency," and "fluid disturbance" [10], while keishibukuryogan is used for patients diagnosed with "excess," "tangled heat and cold," "interior," "yang," and "blood stasis."

However, pattern diagnosis in traditional medicine is a subtle art; it takes years to master the skills required to choose the appropriate formulas, and to our knowledge, it has not yet been reported whether the prescription of Kampo formulas by specialists can be predicted without knowledge of traditional pattern diagnosis. Moreover, it is not known how subjective symptoms and objective findings differ between patients who are prescribed the different Kampo formulas.

In this study, we compared the subjective symptoms and objective findings in patients prescribed tokishakuyakusan with those in patients prescribed keishibukuryogan and used this information to derive a model that can predict the selection of either of the two formulas by specialists in Kampo medicine.

2. Methods

2.1. Patient Enrollment. This observational study included primary and secondary dysmenorrhea patients who were first-time visitors to the Kampo Clinic at Keio University Hospital, between May 2008 and December 2015. All patients were treated with either of the two formulas—tokishakuyakusan or keishibukuryogan. Patients who were treated with both formulas were excluded. Patients over 50 years of age were also excluded. The Institutional Review Board at Keio University School of Medicine approved this study.

2.1.1. Comparison and Model-Development Analysis. In this analysis, we included patients who made their first visit between May 2008 and March 2013. Patients who were prescribed tokishakuyakusan were included in the "TSS" group, and those who were prescribed keishibukuryogan were in the "KBG" group. We used a browser-based questionnaire during this part of the study; the questionnaire is explained in detail in Section 2.2.

2.1.2. External Validation Analysis. The predictive model was validated using a different data set (the external validation group), obtained from patients who made their first visit to Kampo Clinic at Keio University Hospital between April 2013 and December 2015. We did not use the browser-based questionnaire system during this part of the study. The systems used in the medical interview were reviewed using a paper-based questionnaire, and this database was entirely separate from that used in the comparison and model-development analysis; however, the items in the questionnaire were identical.

2.2. Data Collection. In 2008, Keio University first introduced a browser-based questionnaire to collect information about patients' subjective symptoms, as well as their age, sex, body mass index (BMI), lifestyle, Western diagnosis (based on the international classification of diseases (ICD-10)), traditional medicine pattern-based diagnosis (based on ICD-11 beta version) [11], and Kampo formulas prescribed by Kampo specialists. Kampo specialists from representative Universities and Kampo institutions in Japan (Keio University, Chiba University, Toyama University, Jichi Medical University, Tokyo Women's Medical University, Tohoku University, Kameda Medical Center, and Aso Iizuka Hospital) prepared the questionnaire after repeated discussions. Using this questionnaire, which comprises 128 binary questions, we collected information about our patients' subjective symptoms, as described in our previous report [14].

BMI was assessed in 2 ways: as a sequential variable (crude BMI) and as binary variables: "slim" (yes/no) and "obese" (yes/no). Patients with a BMI < 18.5 were considered slim, and those with a BMI ≥ 25 were considered obese, as defined by the Japan Society for the Study of Obesity.

Data regarding each objective factor, including abdominal and tongue findings, were also collected as binary variables. Specifically, abdominal findings included nine items; one of these—abdominal strength—contained three mutually exclusive categories: weak, intermediate, and strong. Here, however, we used binary variables to code the abdominal strength: "weak abdomen" (yes/no) and "strong abdomen" (yes/no). Abdominal strength is determined by abdominal examination, whereby the doctor presses the palm of his/her hand onto the patients' abdomen to assess both the degree of resistance offered by the muscles and the thickness of the abdominal muscle wall and fat [15]. Other abdominal findings were also expressed in binary form, namely, epigastric discomfort, palpable abdominal aortic pulsation, hypochondrial resistance and discomfort, splashing sound in the epigastric region, paraumbilical tenderness and resistance, rectus muscle tension, weakness of the lower abdomen, and abdominal distension. Tongue findings included teeth marks on the edges of the patient's tongue and dilatation of the sublingual veins.

2.3. Comparison of Tokishakuyakusan with Keishibukuryogan. We compared each subjective and objective item between the TSS and KBG groups. We used Fisher's exact test for comparison of binary variables and Wilcoxon's rank sum test and two-sample t-tests for continuous variables items, such as age and crude BMI. Missing data were ignored in the tests.

2.4. A Predictive Model for Prescription of the Two Kampo Formulas by Specialists

2.4.1. Selection of Potential Predictor Variables. We used variables with a p value < 0.05 in the analyses detailed in Section 2.3 as potential variables that could be used to predict which Kampo medicine would be prescribed. BMI had a p value < 0.05, but this information was missing for several patients; we therefore replaced the missing BMI data with the overall mean BMI during the model-development analysis.

2.4.2. Model-Fitting Procedure. We applied logistic regression to the 128 data points from the TSS and KBG groups [16]; the KBG group was designated as 1, and the TSS group as 0. Using logistic regression analysis, we calculated the probability of the patient belonging to the KBG group; $p > 0.5$ indicated that the patient was predicted to belong to the KBG group, and $p < 0.5$ that the patient was predicted to belong to the TSS group. We then performed a univariate analysis on the potential predictive variables, followed by a multivariate analysis. The model that contained all the potential predictive variables was considered the full model. To measure the effect size of each predictive variable, we computed the odds ratio (OR).

However, to avoid overfitting the predictive model, the predictive variables needed to be selected more strictly, which we achieved using the Akaike information criterion (AIC) [17]. We started with the full model and challenged all possible models; the model with the lowest AIC was considered the final model.

The variance inflation factor (VIF) was used to monitor multicollinearity. We also evaluated interactions between predictor variables in the final model by including interaction terms along with main-effect terms. None of the interactions were found to be significant, and they are not discussed further in this paper.

2.5. Internal and External Validations of the Final Model. Calibration of the model was assessed using the area under the receiver operating characteristic curve (AUC) and the Hosmer-Lemeshow test [18]. An AUC > 0.80 and a p value > 0.05 in the Hosmer-Lemeshow test were considered acceptable values. The final model was internally validated by leave-one-out cross-validation. We also externally validated the final model by applying it to the external validation group's data set.

2.6. Statistical Analyses. All statistical analyses were conducted using R software version 3.1.1 (The R Foundation for Statistical Computing; July 10, 2014; see also: http://www.r-project.org/). We used "glm" [19] from the package "stats," as well as the packages "DAAG" [20], "pROC" [21], and "ResourceSelection" [22]. Data are shown as mean ± standard deviation. We used a significance level of 5% for all tests but made no adjustment for multiple testing.

Table 1: Frequently used Kampo formulas in 222 patients with dysmenorrhea.

Formulas	Number
Keishibukuryogan	73
Tokishakuyakusan	67
Kamishoyosan	20
Anchusan	19
Goreisan	18
Saikokeishikankyoto	13
Tokakujokito	10
Yokukansan	8
Saikokaryukotsuboreito	8
Tokikenchuto	7
Jumihaidokuto	6
Shosaikoto	6
Tokishigyakukagoshuyushokyoto	6
Bukuryoingohangekobokuto	6
Byakkokaninjinto	6
Daisaikoto	5
Hochuekkito	5
Shakuyakukanzoto	5
Hangekobokuto	4
Rikkunshito	4
Others (45 kinds of formulas)	60
Total	356

Five patients from the keishibukuryogan group and 7 from the tokishakuyakusan group were excluded from the comparison and model-development analysis (see Figure 1). A total of 127 patients were prescribed 2 or more formulas, and 356 formulas were prescribed in total.

3. Results

3.1. Participant Information. We assessed the eligibility of 290 dysmenorrhea patients—222 patients for the comparison and model-development analysis and 68 patients for the external validation analysis.

Among the 222 candidate patients for the comparison and model-development analysis, 127 had been prescribed two or more formulas (a total of 356 formulas were prescribed; Table 1). Tokishakuyakusan and keishibukuryogan were the most frequently used formulas, and 135 patients (61%) were prescribed either or both of these. None of the patients withdrew from the study. We excluded two patients who were aged over 50 years and six patients who were prescribed both tokishakuyakusan and keishibukuryogan or related formulas. Finally, we used data from 128 patients in the comparison and model-development analysis, comprising 60 who were prescribed only tokishakuyakusan (TSS group) and 68 who were prescribed only keishibukuryogan (KBG group; Figure 1: the comparison and model-development set).

Of the 68 candidate patients for the external validation analysis, 28 were excluded because they were not prescribed

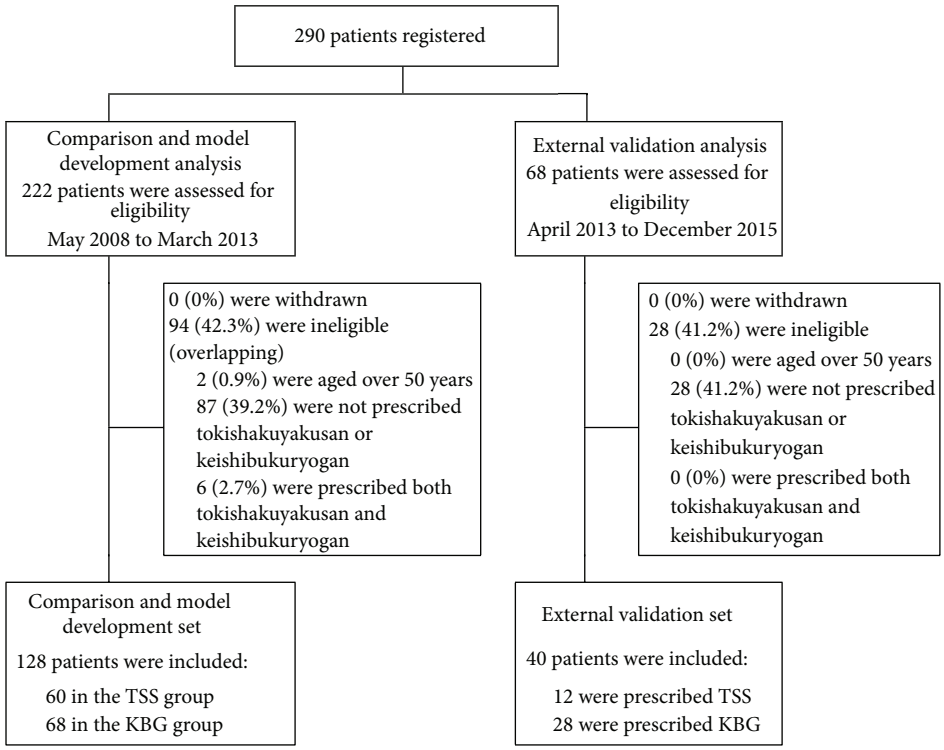

Figure 1: Patients' flow chart. Patients who were prescribed tokishakuyakusan only were included in the "TSS" group, and those who were prescribed keishibukuryogan only were included in the "KBG" group.

either tokishakuyakusan or keishibukuryogan. The data from the remaining 40 patients were used for external validation of the final model (Figure 1: the external validation set).

Table 2 summarizes the characteristics of the patients included in the two analyses. The frequency of OC use and diagnosed diseases were significantly higher in the external validation set than in the comparison and model-development set.

3.2. Comparison between the Characteristics of the TSS and KBG Groups. We compared the characteristics of patients in the TSS group with those of patients in the KBG group (Table 3). The BMI was significantly lower in the TSS group; correspondingly, the binary variable "slim" was significantly more frequently present. Endometriosis or adenomyosis, which leads to secondary dysmenorrhea, was found in 13.3% of TSS patients and in 22.1% of KBG patients; however, this was not significantly different. The remainder of the patients in each group was diagnosed with primary dysmenorrhea.

Five subjective symptoms and three objective findings significantly differed between the TSS and KBG groups (Table 4; Appendix Table, at Supplementary Material available online at http://dx.doi.org/10.1155/2016/3159617). Lightheadedness was more frequent in patients in the TSS group; tendency to sweat, heat intolerance, leg numbness, and a cold sensation in the lower back were more frequent in patients in the KBG group. A weak abdomen was more frequent in the TSS group, whereas a strong abdomen, as well as paraumbilical tenderness and resistance, was more frequent in the KBG group. There was no significant difference between the two groups in terms of the other 123 subjective symptoms, seven abdominal findings, or two tongue findings.

3.3. A Predictive Model for Prescription of the 2 Kampo Formulas by Specialists. We performed univariate analyses of the five subjective symptoms and three abdominal findings that had shown a significant difference between the two groups, as well as of the variable "slim" (Table 5: univariate). We included a categorical variable "slim," rather than the continuous variable crude BMI, as linearity cannot be achieved on the logit scale when using crude BMI. We calculated AIC and AUC for each univariate model; all models had AIC > 150 and AUC < 0.8 (Figure 2: univariate models).

We developed the full model using these nine potential predictive variables. The AIC for the full model was 127.9, and the AUC was 0.88 (95% CI: 0.82–0.94, Figure 2: full model). The Hosmer-Lemeshow test indicated that the full model fitted the comparison and model-development set ($p = 0.1982$).

After challenging all possible models, four subjective symptoms and three abdominal findings were included in the final model. The AIC for this model was 125.1, which was lower than that of the full model (Table 5: multivariate). None of the VIF values exceeded 2.0; thus, there was no collinearity in the model.

3.4. Internal and External Validations of the Final Model. The AUC was computed as 0.88 (95% CI: 0.81–0.93, Figure 2: final model). The Hosmer-Lemeshow test indicated that the final model fit the comparison and model-development set

Table 2: Baseline characteristics of the patients included in the study.

	Comparison and model- development set May 2008 to March 2013	External validation set April 2013 to December 2015	p value
Number of patients	128	40	N/A
Age at consultation			
Mean ± SD	32.8 ± 8.3	35.0 ± 7.0	0.108*
Median	33	37	0.129†
Range	12–50	22–47	
N/A	0 (0.0)	0 (0.0)	
Age at menarche, years			
Mean ± SD	12.3 ± 1.5	12.7 ± 2.1	0.245*
Median	12	12	0.355†
Range	9–17	10–21	
N/A	2 (1.6)	1 (2.5)	
Menstrual cycle, days			
Mean ± SD	29.1 ± 4.8	28.2 ± 2.6	0.162*
Median	28	28	0.076†
Range	16–60	24–40	
N/A	22 (17.2)	9 (22.5)	
Bleeding period, days			
Mean ± SD	5.9 ± 1.6	5.6 ± 1.5	0.366*
Median	6	5	0.196†
Range	3–14	3–10	
N/A	13 (10.2)	3 (7.5)	
BMI, kg/m^2			
Mean ± SD	20.8 ± 3.1	20.8 ± 2.8	0.905*
Median	20.4	20.6	0.572†
Range	15.9–39.8	16.2–31.6	
<18.5 (slim)	27 (21.1)	5 (12.5)	0.259‡
≥25 (obese)	11 (8.6)	2 (5.0)	0.735‡
N/A	3 (2.3)	0 (0.0)	
Use of OCs			
No	117 (91.4)	31 (77.5)	0.025‡
Yes	11 (8.6)	9 (22.5)	
Delivery			
No	116 (90.6)	35 (87.5)	0.556‡
Yes	12 (9.4)	5 (12.5)	
Abortion			
No	114 (89.1)	35 (87.5)	0.778‡
Yes	14 (10.9)	5 (12.5)	
Diagnosed organic disease			
No	105 (82.0)	25 (62.5)	0.016‡
Endometriosis	14 (10.9)	11 (27.5)	0.019‡
Adenomyosis	10 (7.8)	10 (25.0)	0.009‡
Infertility (primary and secondary)			
No	123 (96.1)	45 (87.5)	0.146‡
Yes	5 (3.9)	5 (12.5)	

N/A, not available; BMI, body mass index; OCs, oral contraceptives.
Findings are expressed as mean ± SD, median, range, or number with percentage in parentheses.
p values were calculated using *t-test, †Wilcoxon's rank sum test, and ‡Fisher's exact test.

Table 3: Baseline characteristics of the patients included in the comparison and model-development analysis.

	The comparison and model development set		p value	Other formulas
	TSS group	KBG group		
Number of patients	60	68	N/A	86
Age at consultation				
Mean ± SD	33.3 ± 7.9	32.5 ± 8.7	0.595*	33.0 ± 8.1
Median	33	33	0.742†	33
Range	17–50	12–50		13–49
N/A	0 (0.0)	0 (0.0)		0 (0.0)
Age at menarche, years				
Mean ± SD	12.2 ± 1.5	12.3 ± 1.6	0.753*	12.7 ± 1.6
Median	12	12	0.932†	12
Range	9–16	9–17		9–17
N/A	0 (0.0)	2 (2.9)		2 (2.3)
Menstrual cycle, days				
Mean ± SD	29.0 ± 5.7	29.3 ± 3.6	0.772*	28.9 ± 4.5
Median	28	28	0.771†	28
Range	16–60	25–45		17–60
N/A	6 (10.0)	16 (23.5)		10 (11.6)
Bleeding period, days				
Mean ± SD	5.8 ± 1.3	5.9 ± 1.9	0.841*	5.5 ± 1.6
Median	6	6	0.747†	5
Range	3–9	3–14		3–14
N/A	4 (6.7)	9 (13.2)		7 (8.1)
BMI, kg/m^2				
Mean ± SD	19.8 ± 2.3	21.6 ± 3.5	0.001*	20.6 ± 2.9
Median	19.4	21.0	0.000†	20.0
Range	15.9–26.7	17.0–39.8		15.6–30.1
<18.5 (slim)	21 (35.0)	6 (8.8)	0.000‡	26 (30.2)
≥25 (obese)	2 (3.3)	9 (13.2)	0.060‡	7 (8.1)
N/A	2 (3.3)	1 (1.5)		4 (4.7)
Use of OCs				
No	54 (90.0)	63 (92.6)	0.754‡	77 (89.5)
Yes	6 (10.0)	5 (7.4)		9 (10.5)
Delivery				
No	54 (90.0)	62 (91.2)	1‡	75 (87.2)
Yes	6 (10.0)	6 (8.8)		11 (12.8)
Abortion				
No	53 (88.3)	61 (89.7)	1‡	74 (86.0)
Yes	7 (11.7)	7 (10.3)		12 (14.0)
Diagnosed organic disease				
No	52 (86.7)	53 (77.9)	0.251‡	81 (94.2)
Endometriosis	6 (10.0)	8 (11.8)	0.785‡	4 (4.7)
Adenomyosis	2 (3.3)	8 (11.8)	0.103‡	2 (2.3)
Infertility (primary and secondary)				
No	59 (98.3)	64 (94.1)	0.370‡	81 (94.2)
Yes	1 (1.7)	4 (5.9)		5 (5.8)

TSS, tokishakuyakusan; KBG, keishibukuryogan; N/A, not available; BMI, body mass index; OCs, oral contraceptives.
Findings are expressed as mean ± SD, median, range, or number with percentage in parentheses.
p values were calculated using *t-test, †Wilcoxon's rank sum test, and ‡Fisher's exact test.

Table 4: Comparison of subjective symptoms and objective findings between the TSS and KBG groups.

	The comparison and model development set		p value
	TSS group (n = 60)	KBG group (n = 68)	
Subjective symptoms			
Tendency to sweat			
No	52 (86.7)	40 (58.8)	0.001
Yes	8 (13.3)	28 (41.2)	
Heat intolerance			
No	54 (90.0)	50 (73.5)	0.023
Yes	6 (10.0)	18 (26.5)	
Leg numbness			
No	59 (98.3)	59 (86.8)	0.019
Yes	1 (1.7)	9 (13.2)	
Cold sensation in lower back			
No	57 (95.0)	56 (82.4)	0.030
Yes	3 (5.0)	12 (17.6)	
Lightheadedness			
No	30 (50.0)	46 (67.6)	0.049
Yes	30 (50.0)	22 (32.4)	
Objective findings			
Weak abdomen			
No	28 (46.7)	60 (88.2)	0.000
Yes	32 (53.3)	8 (11.8)	
Strong abdomen			
No	58 (96.7)	50 (73.5)	0.003
Yes	2 (3.3)	18 (26.5)	
Paraumbilical tenderness and resistance			
No	31 (51.7)	10 (14.7)	0.000
Yes	29 (48.3)	58 (85.3)	

TSS, tokishakuyakusan; KBG, keishibukuryogan.
Only factors with p value < 0.05 were included. Findings are expressed as number with percentage in parentheses. p values were calculated using Fisher's exact test.

Table 5: Effects of potential predictor variables and predictor variables in the final model.

	Univariate		Multivariate (final model)		
	Estimates	OR (95% CI)	Estimates	OR (95% CI)	p value
(Intercept)			−1.356	0.258 (0.075, 0.752)	0.019
Subjective symptoms					
Tendency to sweat	1.515	4.550 (1.945, 11.684)	0.930	2.533 (0.844, 8.210)	0.106
Heat intolerance	1.176	3.240 (1.248, 9.531)			
Leg numbness	2.197	9.000 (1.617, 168.588)	2.448	11.561 (1.844, 228.900)	0.029
Cold sensation in lower back	1.404	4.071 (1.216, 18.571)	1.559	4.752 (0.870, 35.614)	0.095
Lightheadedness	−0.738	0.478 (0.231, 0.974)	−1.019	0.361 (0.128, 0.971)	0.047
Objective findings					
Slim (BMI < 18.5)	−1.716	0.180 (0.061, 0.460)			
Weak abdomen	−2.148	0.117 (0.045, 0.274)	−1.498	0.224 (0.068, 0.666)	0.009
Strong abdomen	2.346	10.440 (2.827, 67.728)	2.077	7.984 (1.732, 60.429)	0.017
Paraumbilical tenderness and resistance	1.825	6.200 (2.753, 14.968)	2.183	8.870 (2.921, 31.644)	0.000

OR, odds ratio; CI, confidence interval; BMI, body mass index.

Table 6: Internal and external validation of the final model.

	Accuracy (%)
Internal validation	
Internal estimate	81.2
Cross-validation estimate	80.5
External validation	85.0

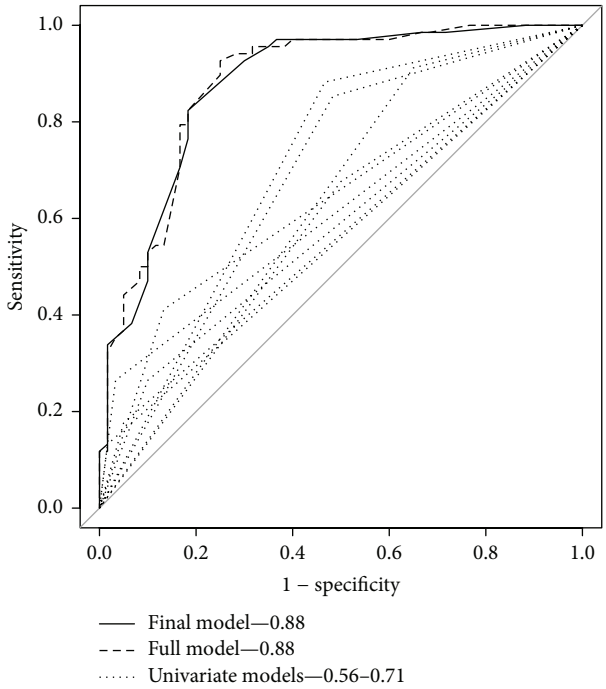

Figure 2: Model calibration using a receiver operating characteristic curve. The full model (broken line) included 9 predictive variables, and the final model (black line) included 7 predictive variables. Each univariate model was drawn using dotted lines. The final model had an area under the curve of 0.88 (95% CI: 0.81–0.93), and the full model had an area under the curve of 0.88 (95% CI: 0.82–0.94). The univariate models had areas under the curve of 0.56–0.71 (95% CI: not shown).

($p = 0.2519$) better than did the full model. The internal estimate of accuracy of the final model was 81.2%, and the leave-one-out cross-validation estimate of accuracy was 80.5% (Table 6: internal validation). When we applied this final model to the set of 40 external validation analysis patients, we found a proper prediction rate of 85.0% (Table 6: external validation).

4. Discussion

Here, we have reported the differences in both subjective symptoms and objective findings between patients who had been prescribed tokishakuyakusan and those who had been prescribed keishibukuryogan. We extracted five subjective symptoms and four objective findings that were significantly different between these two groups. These items are compatible with the traditional medicine pattern diagnosis for each Kampo formula. Tokishakuyakusan is used for patients diagnosed with a "deficiency," "cold," "interior," "yin," "blood deficiency," and "fluid disturbance" pattern. From among these selected factors, a lower BMI and weak abdomen indicate a "deficiency" and a "yin" pattern. Lightheadedness indicates a "blood deficiency" or a "fluid disturbance" pattern. Conversely, keishibukuryogan is used for patients diagnosed with an "excess," "tangled heat and cold," "interior," "yang," and "blood stasis" pattern. Higher BMI and a strong abdomen indicate an "excess" and a "yang" pattern. A tendency to sweat, heat intolerance, and a cold sensation in the lower back indicate a "tangled heat and cold" pattern. Leg numbness, as well as paraumbilical tenderness and resistance, indicates a "blood stasis" pattern. Both formulas are used for an "interior" pattern; however, we found no item with $p < 0.05$ that indicated an "interior" pattern.

Based on this differentiation, we have developed a predictive model, our final model, which fitted the data well. The final model quantified the tacit knowledge of Kampo specialists in selecting an appropriate Kampo formula for dysmenorrhea. During model selection, a subjective symptom—heat intolerance—and an objective finding—BMI—were eliminated from the final model, whereas all three abdominal findings were included in the final model. These results suggest that abdominal findings are important for specialists in selecting a Kampo treatment from among these two candidate formulas.

The selection of the appropriate formula is important in clinical situations. Each formula has specific characteristics and has been studied based on clinical experience. For example, tokishakuyakusan has been studied for its effect on infertility in rats and mice [23–26]. Keishibukuryogan has been studied for its effect on uterine myoma, not only in rats and mice, but also in humans [27–29].

Furthermore, the efficacy of each of these formulas is different from that of their individual crude constituents; thus, the combination of components is important [30]. For instance, tokishakuyakusan consists of six crude components: Japanese Angelica root, peony root, hoelen, Atractylodes rhizome, Alisma rhizome, and Cnidium rhizome. In contrast, keishibukuryogan consists of five crude components: cinnamon bark, peony root, hoelen, peach kernel, and moutan bark. Peony root is one of the crude drugs that tokishakuyakusan and keishibukuryogan have in common. A decoction of peony root has been used to treat many painful or inflammatory conditions, such as cholangitis, bronchiolitis, rhinorrhea, and muscle cramps. It has been reported to have an anticontraction effect, by suppressing the increase of intracellular calcium ion concentration, and anti-inflammatory effects, by inhibiting the production of prostaglandin E2, leukotriene B4, and nitric oxide [31]. However, some studies found that the isolated crude drug did not act as an anticontraction agent on uterine smooth muscle [32, 33].

The present study has some limitations. Our study involved many Kampo specialists, who may vary in their definitions of each finding. Such variations should be

standardized with the advent of modern devices that can objectively examine a patient's tongue [34], abdominal wall [35], or pulse [36]. These objective findings will be incorporated into our model in the future to improve data reliability.

Second, clinical efficacy was not considered as part of this model development. More than 80% of our patients improved to at least some degree after Kampo treatment (data not shown), but retrospective validation of efficacy using medical charts was difficult and incomplete. Whether any formula is truly appropriate should be defined only by its carefully assessed efficacy. Moreover, we considered only the two representative Kampo formulas and did not consider other minor formulas. Although we performed a small external validation or our model, we excluded 41.2% of patients who were treated with minor formulas. If we apply our model in a clinical situation, approximately 40% of patients, who were treated with minor formulas, would have been prescribed either of the two major formulas. In the future, the effectiveness and safety of this model in a clinical situation should be evaluated using a prospective study design.

5. Conclusions

We compared the subjective symptoms and objective findings between patients who were prescribed either of the two major Kampo formulas used to treat dysmenorrhea (tokishakuyakusan and keishibukuryogan) and used this to develop a model that could predict the selection of either of these formulas for a patient by Kampo specialists. The effectiveness and safety of this model should be validated in prospective trials.

Disclosure

A part of this work was presented at the 33rd Annual Conference on Obstetrics and Gynecological Kampo Medicine Research.

Conflict of Interests

The authors declare that there is no conflict of interests regarding this work.

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Review Article

Liuwei Dihuang Pills Enhance the Effect of Western Medicine in Treating Diabetic Nephropathy: A Meta-Analysis of Randomized Controlled Trials

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Objectives. To assess the effectiveness and adverse effects of adding Liuwei Dihuang Pills (LDP) to Western medicine for treating diabetic nephropathy. *Methods.* Studies were retrieved from seven electronic databases, including PubMed, Embase, The Cochrane Library, CBM, CNKI, Chinese Scientific Journal Database (VIP), and Wanfang Data until November 2015. Study selection, data extraction, quality assessment, and data analyses were conducted according to Cochrane standards. Meta-analysis was performed on the overall therapeutic efficacy of hyperglycemia and renal functions, and the study also analyzed adverse events. *Results.* A total of 1,275 patients from 18 studies were included. The methodological quality of these included trials was generally low. We found that adding LDP can lower patients' FBG (MD: −0.36 [−0.46, −0.25], $P < 0.00001$), PBG (MD: −1.10 [−1.35, −0.85], $P < 0.00001$), and HbA1c (MD: −0.14 [−0.49, 0.21], $P = 0.43$). There were also improvements in lowering patients' BUN (MD: −0.67 [−0.89, −0.45], $P < 0.00001$), SCr (MD: −0.96 [−1.53, −0.39], $P < 0.00001$), 24 h UTP (SMD: −1.26 [−2.38, −0.15], $P < 0.00001$), UAER (MD: −26.18 [−27.51, −24.85], $P < 0.00001$), and UmAlb (SMD: −1.72 [−2.67, −0.77], $P < 0.00001$). *Conclusion.* There is encouraging evidence that adding LDP to Western medicine might improve treatment outcomes of diabetic nephropathy, including hyperglycemia and renal functions. However, the evidence remains weak. More rigorous high-quality trials are warranted to substantiate or refute the results.

1. Introduction

Diabetic nephropathy (DN) is a widely recognized microvascular complication of diabetes and almost the leading cause of end-stage kidney failure worldwide responsible for morbidity and mortality [1]. If the damage to the kidney and proteinuria is irreversible, it will evolve into End-Stage Renal Disease. However, exact pathogenesis of DN is still unclear and it is difficult for us to cure DN. At present diet management, control of blood pressure and blood sugar, and blood fat treatment are the foundation treatment for DN. Furthermore, an adequate control of high blood pressure and treatment of microalbuminuria are the major therapeutic targets [2]. To achieve adequate blood pressure control, a combination therapy with different classes of antihypertensive agents is often necessary, especially including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) [3]. ACEIs and ARBs have been demonstrated to protect renal function of DN but are not enough to delay or retard the progression of DN; therefore, exploring feasible drugs is the hotspot of medical research at present.

Currently, with increasing application of complementary and alternative medicine (CAM) worldwide, traditional Chinese medicine (TCM) has become more popular and has drawn more attention [4–7]. TCM has lots of advantages over the conventional medical approaches in the prevention of diabetic complications because of less toxicity and/or side effects [8–10]. TCM is becoming increasingly popular and widely used among patients with DN [11].

Liuwei Dihuang Pills (LDP), a traditional Chinese herbal formula containing six commonly used herbs (*Rehmannia*

glutinosa, *Cornus officinalis* Sieb., Common Yam Rhizome, *Alisma orientalis*, Tree Peony Bark, and *Poria cocos*), are widely used to DN-related symptoms in clinical practice for centuries in China. Three of the six ingredients in the formula are nutrients, while the other three facilitate drainage, enrich yin, nourish the kidney (Shen), and thereby address the root cause of diabetes according to the Chinese medicine theory [12]. Currently, LDP combined with Western medicine has been widely used as an alternative and effective method to treat or prevent diabetic nephropathy in China. Up to now, lots of studies have been published about the effects of LDP combined with conventional drugs for diabetic nephropathy. It is necessary for us to compare the effect of such combinations to the use of Western medicine alone. This report aims to evaluate the beneficial and adverse effects of LDP combined with conventional drugs for the treatment of DN in randomized trials.

2. Materials and Methods

2.1. Database and Search Strategies. Initial searches were performed by 2 authors independently. We selected all the clinical trials about LDP used for treating DN in the Chinese National Knowledge Infrastructure (CNKI), Chinese Scientific Journal Database (VIP), Wanfang Data, Chinese Biomedical Literature Database (CBM), PubMed, Embase, and the Cochrane Central Register of Controlled Trials in The Cochrane Library. All of those searches ended on November 30, 2015. Search terms used were diabetes mellitus, DN, and chronic kidney impairment, combined with LDP. Additionally, we checked bibliographies of retrieved articles and prior reviews on the subject for additional references. We contacted the authors of included trials for missing information when necessary.

2.2. Inclusion and Exclusion Criteria. All the randomized controlled trials (RCTs) based on LDP combined with Western drugs or Western conventional therapeutics compared with corresponding Western drugs or Western conventional therapeutics in patients with DN were included. No restrictions on language, population characteristics, or publication type were enforced. The primary outcome measure that the current study examined was the overall clinical efficacy of LDP for hyperglycemia and impairments of renal functions. A secondary outcome that was assessed was frequency of adverse events. Articles that duplicated the same groups of participants in another publication were excluded.

2.3. Data Extraction and Quality Assessment. Two authors conducted the literature searching (Q. H. Wang and Y. X. Yi), two conducted study selection (Q. H. Wang and S. H. Wang), and two conducted data extraction (Y. X. Yi and Z. L. Qiu) independently. The extracted data included authors, title of the study, year of publication, patients' characteristics and the studies' designs, sample sizes, details of intervention, details of control interventions, outcomes, adverse effects for each study, and intervention durations. Discrepancies were resolved by discussion and consensus was reached through a third party (L. Lin). We assessed the methodological

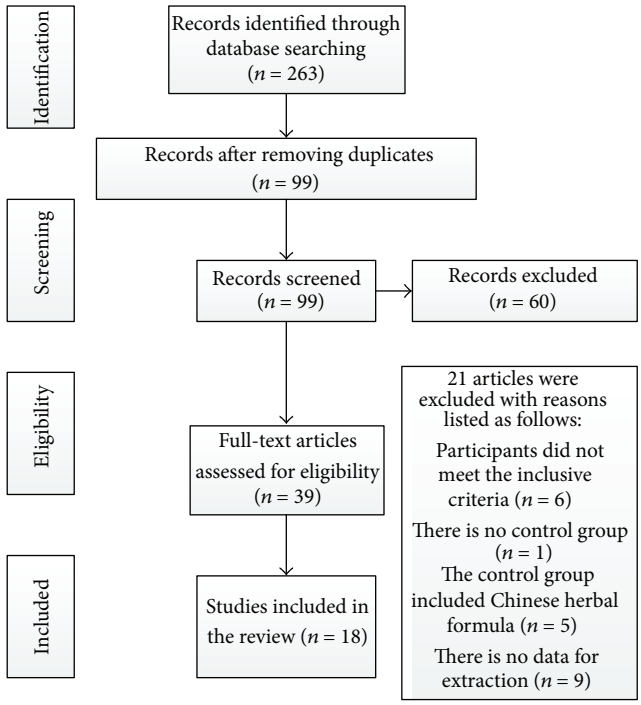

Figure 1: Study selection process.

quality of trials using the criteria of the Cochrane Handbook for Systematic Review of Interventions, version 5.2.3 (Q. H. Wang and S. H. Wang) [13]. The items included the following 6 aspects: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), and selective reporting (reporting bias).

2.4. Data Synthesis. All data analyses were conducted using the Review Manager 5.2 software provided by the Cochrane Collaboration. The outcomes were analyzed as continuous variables using fixed- or random-effect models, and the results for overall clinical efficacy were reported as weighted mean difference (WMD), 95% confidence intervals (95% CIs), and pooled odds ratio (OR). Meta-analysis was performed if the intervention, control, and outcome were the same or similar. It was considered to be indicators of a substantial level of heterogeneity when $I^2 > 50\%$ or $P < 0.1$. In the absence of significant heterogeneity, data were pooled using a fixed-effect model ($I^2 > 50\%$), and, otherwise, data were pooled using a random-effect model ($50\% < I^2 > 50\%$[14]. The relative strength of treatment efficacy was illustrated by forest plots.

3. Results

3.1. Description of Included Trials. 99 trials were screened out from electronic and manual searches in the seven databases. The screening process is summarized in a flow diagram (Figure 1). Only 18 RCTs [15–32] were included. All of the 18 trials were conducted in China and published in Chinese

Table 1: Characteristics and methodological quality of included studies.

Reference (year)	Study design	Participants T/C	Intervention (herbs included)	Control	Outcome measure	Treatment duration (days)
Cao (2013) [18]	RCT	36/36	LDP treatment (6 g, bid) plus acarbose (100 mg, tid)	Acarbose (100 mg, tid)	24 h UTP; FBG; PBG; UmAlb	60
Lou (2014) [32]	RCT	60/60	LDP treatment (8 pills, tid) plus telmisartan (80 mg, qd) plus routine treatment	Telmisartan (80 mg, qd) plus routine treatment	24 h UTP	360
Li and Zhao (2011) [26]	RCT	30/30	LDP treatment (8 pills, tid) plus routine treatment	Routine treatment	UAER	90
He et al. (2009) [20]	RCT	17/18	LDP treatment (8 pills, tid) plus Benazepril Hydrochloride (10 mg, qd)	Benazepril Hydrochloride (10 mg, qd)	24 h UTP	90
Song et al. (2004) [28]	RCT	41/31	LDP treatment (9 g, tid) plus routine treatment	Routine treatment	FBG, PBG, UAER	90
Chen and Ling (2004) [15]	RCT	38/30	LDP treatment (6 g, tid) plus captopril (12.5 mg, tid) plus routine treatment	Captopril (12.5 mg, tid) plus routine treatment	UAER	90
Liu (2012) [21]	RCT	30/30	LDP treatment (9 g, bid) plus routine treatment	Routine treatment	BUN, Scr	60
Kong and Zeng (2014) [17]	RCT	25/25	LDP treatment (8 pills, tid) plus routine treatment	Routine treatment	FBG, PBG, HbA1c	360
Chen et al. (2009) [23]	RCT	34/34	LDP treatment (10 g, bid) plus routine treatment	Routine treatment	UAER, FBG, BUN, Scr	60
Wang (2015) [24]	RCT	50/50	LDP treatment (9 g, bid) plus Losartan Potassium (50 mg, qd)	Losartan Potassium tablets (50 mg, qd)	Scr, BUN, UmAlb, UAER	56
Wang et al. (2013) [16]	RCT	33/30	LDP treatment (6 g, bid) plus valsartan dispersible (80 mg, qd) plus routine treatment	Valsartan dispersible (80 mg, qd) plus routine treatment	UAER, UmAlb	28
Yin et al. (2010) [29]	RCT	17/17	LDP treatment (6 g, bid) plus routine treatment	Routine treatment	FBG, UmAlb	56
Li (2013) [22]	RCT	37/38	LDP treatment (8 pills, tid) plus enalapril (5 mg, bid)	Enalapril (5 mg, bid)	FBG, UmAlb, HbA1c	42
Zhong et al. (2010) [30]	RCT	21/21	LDP treatment (6 g, bid) plus routine treatment	Routine treatment	Scr	21
Zhao (2010) [25]	RCT	30/30	LDP treatment (8 pills, tid) plus routine treatment	Routine treatment	24 h UTP	90
Ma (2015) [31]	RCT	74/74	LDP treatment (8 pills, tid) plus Losartan Potassium (50 mg, qd)	Losartan Potassium tablets (50 mg, qd)	UAER, Scr, BUN	180
Deng et al. (2006) [19]	RCT	22/22	LDP treatment (10 g, tid) plus Perindopril (2 mg, qd) plus routine treatment	Perindopril (2 mg, qd) plus routine treatment	UmAlb	56
Lv and Wang (2006) [27]	RCT	45/41	LDP treatment (8 pills, tid) plus routine treatment	Routine treatment	24 h UTP, UAER, Scr	112

between 2004 and 2015. The characteristics of the 18 included trials were summarized in Table 1.

The 18 RCTs involved 1257 patients with diabetic nephropathy. 14 trials [15–28] specified two diagnostic criteria of diabetic nephropathy, one trial [15] used 1985 WHO criteria for the diagnosis of diabetes mellitus, 2 trials [16, 17] used Chinese Guidelines for the Prevention of Diabetes Mellitus-2007, 11 trials [18–28] used 1999 WHO criteria for the diagnosis of diabetes mellitus and 1987 Mogensen Criteria for the diagnosis of diabetic nephropathy, and the other four trials [29–32] only demonstrated patients with diabetic nephropathy without detailed information.

The interventions included LDP combined with antihypertensive or routine treatment drugs. The controls included three types of groups, including antihypertensive drugs, routine treatment, and antihypertensive drugs combined

Table 2: Quality assessment of included randomized controlled trials.

Included trials	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Cao (2013) [18]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
Lou (2014) [32]	Table of random numbers	Unclear	Unclear	Unclear	No	No	Unclear
Li and Zhao (2011) [26]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
He et al. (2009) [20]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
Song et al. (2004) [28]	Table of random numbers	Unclear	Unclear	Unclear	No	No	Unclear
Chen and Ling (2004) [15]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
Liu (2012) [21]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
Kong and Zeng (2014) [17]	Order of hospital registration	Unclear	Unclear	Unclear	No	No	Unclear
Chen et al. (2009) [23]	Order of hospital registration	Unclear	Single blinding	Unclear	No	No	Unclear
Wang (2015) [24]	Drawing lots	Unclear	Unclear	Unclear	No	No	Unclear
Wang et al. (2013) [16]	Coin tossing	Unclear	Unclear	Unclear	No	No	Unclear
Yin et al. (2010) [29]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
Li (2013) [22]	Table of random numbers	Unclear	Unclear	Unclear	No	No	Unclear
Zhong et al. (2010) [30]	Table of random numbers	Unclear	Unclear	Unclear	No	No	Unclear
Zhao (2010) [25]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
Ma (2015) [31]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
Deng et al. (2006) [19]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear
Lv and Wang (2006) [27]	Unclear	Unclear	Unclear	Unclear	No	No	Unclear

with routine treatment. The range of participants' mean ages in the various RCTs was from 39.9 to 66.4 years. The total treatment duration was in the range of 21 to 360 days. The outcome measures included Scr, BUN, UmAlb, UAER, 24 h urine protein, FBG, PBG, and HbA1c. Adverse effect was described in detail. The main finding of the trials showed that the use of LDP plus Western drugs had beneficial effects for the prevention and therapy of diabetic nephropathy.

3.2. Methodological Quality of Induced Trials. The quality assessments are summarized in Table 2. The randomized allocation of participants was mentioned in all RCTs. However, only 6 trials [16, 22, 24, 28, 30, 32] stated the methods for sequence generation by using random number tables or coin tossing or drawing lots. Two trials [17, 23] described inappropriate methods of randomization by order of hospital registration. The others did not mention the sequence generation processes adequately. Thus we failed to judge whether it was conducted properly or not because of insufficient information. No trial stated the double-blind principle and allocation concealment. Only one trial [23] mentioned single blinding but gave no details of either participants' or investigators' or assessors' blinding. Only one trial [16] reported dropouts or withdrawals. Selective reporting was generally unclear in the RCTs due to lacking data for the current research team. All the trials did not mention follow-up. The pretrial estimation of sample size was not mentioned in all trials. We tried to contact the authors for further information but regrettably we have got no information.

3.3. Effect of Interventions

3.3.1. Improvement of Hyperglycemia. We analyzed the RCTs that studied the benefits of LDP for FPG, PBG, and HbA1c.

(1) Improvement of FPG. Six studies [17, 18, 22, 23, 28, 29] including 371 participants used the levels of FPG as an outcome measure. These trials showed homogeneity in the consistency of the trial results (chi-square = 7.00; $P = 0.22$; $I^2 = 29\%$). Therefore, a fixed-effect model should have been used for statistical analysis. A meta-analysis showed a significant difference for the LDP combined with conventional therapies group (MD: −0.36 [−0.46, −0.25]; $P < 0.00001$), which demonstrated that LDP combined with conventional therapies group was superior to the conventional therapies taken by the control groups (Table 3).

Table 3: Analysis of the score of FBG.

Trials		MD (95% CI)	*P* value
LDP treatment plus Western drugs versus Western drugs			
LDP treatment plus acarbose versus acarbose	1	−1.05 [−1.78, −0.32]	0.005
LDP treatment plus enalapril versus enalapril	1	−0.39 [−0.53, −0.25]	<0.00001
LDP treatment plus routine treatment versus routine treatment	1	−0.66 [−1.27, −0.05]	0.03
LDP treatment plus routine treatment versus routine treatment	1	−0.15 [−1.48, 1.18]	0.83
LDP treatment plus routine treatment versus routine treatment	1	−0.20 [−0.41, 0.01]	0.06
LDP treatment plus routine treatment versus routine treatment	1	−0.31 [−0.76, 0.14]	0.17
Meta-analysis	6	−0.36 [−0.46, −0.25]	<0.00001

Table 4: Analysis of the score of PBG.

Trials		MD (95% CI)	*P* value
LDP treatment plus Western drugs versus Western drugs			
LDP treatment plus acarbose versus acarbose	1	−1.49 [−2.93, −0.05]	0.04
LDP treatment plus routine treatment versus routine treatment	1	−1.49 [−2.93, −0.05]	0.68
LDP treatment plus routine treatment versus routine treatment	1	−1.10 [−1.35, −0.85]	<0.00001
Meta-analysis	3	−1.10 [−1.35, −0.85]	<0.00001

Table 5: Analysis of the score of HbA1c.

Trials		MD (95% CI)	*P* value
LDP plus antihypertensive drugs versus antihypertensive drugs			
LDP treatment plus enalapril versus enalapril	1	−0.13 [−0.50, 0.24]	0.49
LDP treatment plus routine treatment versus routine treatment	1	−0.20 [−1.15, 0.75]	0.68
Meta-analysis	2	−0.14 [−0.49, 0.21]	0.43

(2) Improvement of PBG. Three studies [17, 18, 28] including 194 participants used the levels of PBG to measure the outcome. The trials showed homogeneity in the consistency of the trial results (chi-square = 0.79; $P = 0.67$; $I^2 = 0\%$). Therefore, a fixed-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP combined with conventional therapies compared with conventional therapies in decreasing the level of PBG (MD: −1.10 [−1.35, −0.85]; $P < 0.00001$) (Table 4).

(3) Improvement of HbA1c. Two studies [17, 22] including 112 participants used the levels of HbA1c to measure the outcome. The trials showed homogeneity in the consistency of the trial results (chi-square = 0.07; $P = 0.79$; $I^2 = 0\%$). Therefore, a fixed-effect model should have been used for statistical analysis. A meta-analysis showed that HbA1c measurements also showed greater efficacy for LDP combined with conventional therapies group (−0.14 [−0.49, 0.21]; $P = 0.43$) (Table 5).

3.3.2. Improvement of Renal Functions. We analyzed the RCTs that measured blood urea nitrogen, serum creatinine, UAER, 24 h urine protein quantitation, and UmAlb.

(1) Improvement of BUN. Four studies [21, 23, 24, 31] including 376 participants used the levels of BUN to measure the outcome. The trials showed heterogeneity in the consistency of the trial results (chi-square = 10.94; $P = 0.01$; $I^2 = 73\%$). Therefore, a random-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP combined with conventional therapies compared with conventional therapies in decreasing the level of BUN (MD: −0.67 [−0.89, −0.45]; $P < 0.00001$) (Table 6).

(2) Improvement of Scr. Six studies [21, 23, 24, 27, 30, 31] including 458 participants used the levels of Scr to measure the outcome. Four studies including 210 participants [21, 23, 27, 30] compared the combination of LDP treatment plus routine treatment drugs with routine treatment drugs. The trials showed homogeneity in the consistency of the trial results (chi-square = 3.40; $P = 0.33$; $I^2 = 12\%$). Therefore, a fixed-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP plus routine treatment drugs compared with routine treatment drugs in decreasing the level of Scr (MD: −0.60 [−0.89, −0.30]; $P < 0.0001$) (Table 7). Two studies [24, 31] including 248 participants compared the combination of LDP treatment plus Losartan Potassium with Losartan Potassium. The trials showed heterogeneity in the consistency of the trial results (chi-square = 22.72; $P < 0.00001$; $I^2 = 96\%$). Therefore, a random-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP combined with Losartan Potassium compared with Losartan Potassium in decreasing the level of Scr (MD: −1.14 [−2.45, 0.18]; $P = 0.09$) (Table 7).

Table 6: Analysis of the score of BUN.

Trials		MD (95% CI)	*P* value
LDP plus routine treatment drugs versus routine treatment drugs			
LDP treatment plus routine treatment versus routine treatment	1	−1.54 [−3.07, −0.01]	0.05
LDP treatment plus routine treatment versus routine treatment	1	−0.57 [−1.98, 0.84]	0.43
LDP treatment plus Losartan Potassium plus Losartan Potassium	1	−0.88 [−1.15, −0.61]	<0.00001
LDP treatment plus Losartan Potassium plus Losartan Potassium	1	−0.08 [−0.51, 0.35]	0.71
Meta-analysis	4	−0.63 [−1.24, −0.02]	0.04

Table 7: Analysis of the score of serum creatinine.

Trials		MD (95% CI)	*P* value
LDP plus conventional drugs versus conventional drugs			
LDP treatment plus routine treatment versus routine treatment	1	−0.85 [−1.35, −0.36]	0.0008
LDP treatment plus routine treatment versus routine treatment	1	−0.21 [−0.72, 0.30]	0.41
LDP treatment plus routine treatment versus routine treatment	1	−0.65 [−1.29, −0.01]	0.05
LDP treatment plus routine treatment versus routine treatment	1	−0.71 [−1.34, −0.08]	0.03
Meta-analysis	4	−0.59 [−0.87, −0.32]	<0.0001
LDP treatment plus Losartan Potassium versus Losartan Potassium	1	−1.81 [−2.19, −1.42]	<0.00001
LDP treatment plus Losartan Potassium versus Losartan Potassium	1	−0.46 [−0.86, −0.07]	0.02
Meta-analysis	2	−1.14 [−2.45, 0.18]	0.09

Table 8: Analysis of the score of 24 h UTP.

Trials		SMD (95% CI)	*P* value
LDP plus Western drugs versus Western drugs			
LDP treatment plus acarbose versus acarbose	1	−0.48 [−0.95, −0.01]	0.04
LDP plus Benazepril Hydrochloride versus Benazepril Hydrochloride	1	−0.80 [−1.49, −0.11]	0.02
LDP plus routine treatment versus routine treatment	1	−0.89 [−1.55, −0.24]	0.007
LDP plus routine treatment versus routine treatment	1	−0.67 [−1.19, −0.15]	0.01
Meta-analysis	4	−0.67 [−0.95, −0.38]	<0.00001
LDP plus telmisartan plus routine treatment versus telmisartan plus routine treatment	1	−3.48 [−4.05, −2.90]	<0.00001
Meta-analysis	1	−3.48 [−4.05, −2.90]	<0.00001

(3) Improvement of 24 h UTP. Five studies [18, 20, 25, 27, 32] including 327 participants used the levels of 24 h UTP to measure the outcome. Four studies [18, 20, 25, 27] including 207 participants compared the combination of LDP treatment plus conventional therapies with conventional therapies. The trials showed homogeneity in the consistency of the trial results (chi-square = 1.20; $P = 0.75$; $I^2 = 0\%$). Therefore, a fixed-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP plus conventional therapies compared with conventional therapies in decreasing the level of 24 h UTP (SMD: −0.67 [−0.95, −0.38]; $P < 0.00001$) (Table 8). One study [32] including 120 participants compared the combination of LDP plus telmisartan plus routine treatment with telmisartan plus routine treatment. The homogeneity in the consistency of the trial results is not applicable ($Z = 11.90$; $P < 0.00001$). A meta-analysis showed a significant beneficial effect of LDP treatment plus telmisartan plus routine treatment compared with telmisartan plus routine treatment in decreasing the levels of 24 h UTP (SMD: −3.48 [−4.05, −2.90]; $P < 0.00001$) (Table 8).

(4) Improvement of UmAlb. Five studies [16, 19, 22, 24, 29] including 321 participants used the levels of UmAlb to measure the outcome. Three studies including 202 participants [16, 24, 29] compared the combination of LDP treatment plus conventional therapies with conventional therapies. The trials showed homogeneity in the consistency of the trial results (chi-square = 0.66; $P = 0.72$; $I^2 = 0\%$). Therefore, a fixed-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP plus conventional therapies compared with conventional therapies in decreasing the level of UmAlb (SMD: −1.37 [−1.68, −1.06]; $P < 0.00001$) (Table 9). One study [19] including 44 participants compared the combination of LDP treatment plus Perindopril plus routine treatment with Perindopril plus routine treatment. The homogeneity in the consistency of the trial results is not applicable ($Z = 7.76$; $P < 0.00001$). A meta-analysis showed a significant beneficial effect of LDP treatment plus Perindopril plus routine treatment compared with Perindopril plus routine treatment in decreasing the levels of UmAlb (SMD: −4.70 [−5.89, −3.52]; $P < 0.00001$). Another study [22] including 75

Table 9: Analysis of the score of UmAlb.

Trials		SMD (95% CI)	*P* value
LDP plus conventional drugs versus conventional drugs			
LDP plus captopril plus routine treatment versus captopril plus routine treatment	1	−1.52 [−2.07, −0.98]	<0.00001
LDP plus Losartan Potassium versus Losartan Potassium	1	−1.25 [−1.68, −0.82]	<0.00001
LDP plus routine treatment versus routine treatment	1	−1.47 [−2.24, −0.70]	0.0002
Meta-analysis	3	−1.37 [−1.68, −1.06]	<0.00001
LDP plus Perindopril plus routine treatment versus Perindopril plus routine treatment	1	−4.70 [−5.89, −3.52]	<0.00001
Meta-analysis	1	−4.70 [−5.89, −3.52]	<0.00001
LDP plus enalapril versus enalapril	1	−0.27 [−0.73, 0.18]	0.24
Meta-analysis	1	−0.27 [−0.73, 0.18]	0.24

Table 10: Analysis of the score of UAER.

Trials		MD (95% CI)	*P* value
LDP plus Western drugs versus Western drugs			
LDP plus routine treatment versus routine treatment	1	−51.30 [−64.13, −38.47]	<0.00001
LDP plus routine treatment versus routine treatment	1	−34.40 [−44.40, −24.40]	<0.00001
LDP plus routine treatment versus routine treatment	1	−24.88 [−37.94, −11.82]	0.0002
LDP plus routine treatment versus routine treatment	1	−44.40 [−46.59, −42.21]	<0.00001
Meta-analysis	4	−43.65 [−45.73, −41.58]	<0.00001
LDP plus Losartan Potassium plus Losartan Potassium	1	−27.76 [−30.70, −24.82]	<0.00001
LDP plus Losartan Potassium plus Losartan Potassium	1	−24.81 [−31.67, −17.95]	<0.00001
Meta-analysis	2	−27.30 [−30.01, −24.60]	<0.00001
LDP plus captopril plus routine treatment versus captopril plus routine treatment	1	−42.84 [−56.40, −29.28]	<0.00001
LDP plus valsartan dispersible plus routine treatment versus valsartan dispersible plus routine treatment	1	−3.51 [−5.81, −1.21]	0.003
Meta-analysis	2	−4.61 [−6.88, −2.34]	<0.0001

participants compared the combination of LDP plus enalapril with enalapril. The homogeneity in the consistency of the trial results is not applicable ($Z = 1.17$; $P = 0.24$). A meta-analysis showed a significant beneficial effect of LDP plus enalapril compared with enalapril in decreasing the levels of UmAlb (SMD: −0.27 [−0.73, 0.18]; $P = 0.24$) (Table 9).

(5) Improvement of UAER. Eight studies [15, 16, 23, 24, 26–28, 31] including 625 participants used the levels of UAER to measure the outcome. Four studies [23, 26–28] including 246 participants compared the combination of LDP treatment plus routine treatment drugs with routine treatment drugs. The trials showed homogeneity in the consistency of the trial results (chi-square = 13.04; $P = 0.005$; $I^2 = 77\%$). Therefore, a random-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP combined with routine treatment compared with routine treatment in decreasing the level of UAER (MD: −43.65 [−45.73, −41.58]; $P < 0.00001$) (Table 10). Two studies [24, 31] including 248 participants compared the combination of LDP treatment plus Losartan Potassium with Losartan Potassium. The trials showed homogeneity in the consistency of the trial results (chi-square = 0.60; $P = 0.44$; $I^2 = 0\%$). Therefore, a random-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP combined with Losartan Potassium compared with Losartan Potassium in decreasing the level of UAER (MD: −27.30 [−30.01, −24.60]; $P < 0.00001$) (Table 10). Two studies [15, 16] including 131 participants compared the combination of LDP treatment plus antihypertensive drugs plus routine treatment with antihypertensive drugs plus routine treatment. The trials showed heterogeneity in the consistency of the trial results (chi-square = 31.42; $P < 0.00001$; $I^2 = 97\%$). Therefore, a random-effect model should have been used for statistical analysis. A meta-analysis showed a significant beneficial effect of LDP treatment plus antihypertensive drugs plus routine treatment compared with routine treatment with antihypertensive drugs in decreasing the level of UAER (MD: −4.61 [−6.88, −2.34]; $P < 0.0001$) (Table 10).

3.4. Publication Bias. The number of trials was too small to conduct any sufficient additional analysis of publication bias.

3.5. Adverse Effects. In total, five of the eighteen trials mentioned the presence or absence of adverse effects [18, 22, 24, 31, 32]. Two trials reported the incidence of adverse events, whereas 3 trials reported that no adverse events occurred in the treatment groups compared with the control groups. One trial [22] reported slight cough ($n = 1$) in the treatment

group. Another trial [18] indicated that the treatment group presented with hypoglycemia ($n = 1$) and gastrointestinal tract reaction ($n = 1$) and that the control group presented with hypoglycemia ($n = 2$) and gastrointestinal tract reaction ($n = 2$). In general, adverse events were rare and minor.

4. Discussion

There is no specific treatment of diabetic nephropathy. Currently, comprehensive treatments including diet management, control of blood pressure, control of blood sugar, and lipid adjusting treatment have been commonly used. Some corresponding measures taken to intervene actively against some risk factors may reduce proteinuria and delay the occurrence of albuminuria. It is considered that active treatment can prevent or delay the progress of diabetic nephropathy at any stage, especially in the early period. ACEI and ARB drugs are widely used in the treatment of DN, but they are not enough to delay or retard the progression of DN. TCM has lots of advantages over the conventional medical approaches in the prevention and treatment of DN [9, 10].

Based on the meta-analyses of the outcome, LDP may have positive effects for lowering FBG, PBG, and HbA1c and improving renal function. LDP as an adjunctive treatment to conventional drugs significantly improved blood sugar and renal function in patients with DN. However, due to the low-quality methodology and potential publication bias, we cannot draw a definite conclusion of the beneficial effectiveness of LDP combined with Western drugs in preventing and treating diabetic nephropathy. Our positive findings should be interpreted conservatively.

Firstly, in terms of the current evaluative standards, all of the included studies were of low methodological quality. Bias may exist in many areas, such as an unclear method of random sequence, no mention of allocation concealment, and being double-blind. It is also possible that bias was produced by the link of distribution, implementation and measurement, statistics, and reporting.

Secondly, five of the eighteen trials mentioned the presence or absence of adverse effects. The three trials only mentioned that there were no adverse effects in their study. The other two trials reported the presence of adverse effects. One trial [22] reported slight cough ($n = 1$) in the treatment group. Another trial [18] indicated that the treatment group presented with hypoglycemia ($n = 1$) and gastrointestinal tract reaction ($n = 1$) and that the control group presented with hypoglycemia ($n = 2$) and gastrointestinal tract reaction ($n = 2$). The safety of LDP needs to be rigorously monitored in future clinical trials. Therefore, conclusions about the safety of LDP cannot be made from this study due to the limited and inadequate evidence in included trials. Large-scale clinical trials with long-term follow-up appear warranted.

Thirdly, none of the eighteen included trials mentioned health related quality of life, economic index, or compliance with treatments. A recent investigation of 20 public hospitals and integrative medical hospitals in Beijing demonstrated that $4 million can be saved in medical expenses if prescriptions of Chinese herbal medicine were increased by 1% [33]. In view of this finding, we should pay attention to the health economics indices of LDP treatment.

5. Conclusions

The results suggest that Liuwei Dihuang Pills added to other routine treatment have a therapeutic potential for people with diabetic nephropathy. However, due to the poor quality of included studies, the reported effectiveness and safety of Liuwei Dihuang Pills for diabetic nephropathy cannot be taken as confirmative conclusion. More rigorous RCTs will be needed to present a high level of evidence for the effectiveness of Liuwei Dihuang Pills in treating diabetic nephropathy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Lan Lin, Qiuhong Wang, Yongxin Yi, and Shihan Wang contributed equally to this paper.

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Review Article

Huangqi Jianzhong Tang for Treatment of Chronic Gastritis: A Systematic Review of Randomized Clinical Trials

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To assess the clinical effects and safety of Huangqi Jianzhong Tang (HQJZ) for the treatment of chronic gastritis (CG), three English databases and four Chinese databases were searched through the inception to January 2015. In randomized controlled trials (RCTs) comparing HQJZ with placebo, no intervention and western medicine were included. A total of 9 RCTs involving 979 participants were identified. The methodological quality of the included trials was generally poor. Meta-analyses demonstrated that HQJZ plus conventional medicine was more effective in improving overall gastroscopy outcome than western medicine alone for treatment of chronic superficial gastritis with the pooling result of overall improvement [OR 3.78 (1.29, 11.06), $P = 0.02$]. In addition, the combination of HQJZ with antibiotics has higher overall effect rate than antibiotics alone for the treatment of CG [OR 2.60 (1.49, 4.54), $P = 0.0007$]. There were no serious adverse events reported in both the intervention and controlled groups. HQJZ has the potential of improvement of the patients' gastroscopy outcomes, *Helicobacter pylori* clearance rate, traditional Chinese Medicine syndromes, and overall effect rate alone or in combination use with conventional western medicine for chronic atrophic gastritis. However, due to poor methodological quality, the beneficial effect and safeties of HQJZ for CG could not be confirmed.

1. Introduction

Chronic gastritis (CG) is defined as chronic inflammatory cells infiltration in gastric mucosa [1]. They are classified into chronic nonatrophic gastritis (CSG) and chronic atrophic gastritis (CAG) based on the endoscopic appearances and histopathologic patterns of the gastric mucosa. *Helicobacter pylori* (Hp) infection in the stomach lining is the most common and likely causes, leading to some gastric glandular cells which can be lost and eventually replaced by intestinal and fibrous tissues or even worse associated with gastric cancer during their long process of the disease [1, 2].

CG is a kind of the most common digestive system diseases in clinical practice, with estimated 50% of the world population having the Hp infection [3, 4]. And there is a lack of effective drug for CG with about 20% recurrence rate [5]. Huangqi Jianzhong Tang (HQJZ), a traditional Chinese Medicine (TCM), is commonly used for treatment of CG in China. Here a systematic review and meta-analysis of randomized controlled trials were conducted to evaluate its therapeutic effects on the treatments of CG patients.

2. Materials and Methods

2.1. Searching Strategy. Two authors (Yue Wei and Li-Xin Ma) identified the citations by searching three English electronic databases (PubMed, Embase, and Cochrane Library) and four Chinese electronic databases (China National Knowledge Infrastructure (CNKI), Chinese Biomedicine (SinoMed), Chinese Scientific Journals Database (VIP), and Wanfang database) from their inception through January 2015. Conference proceedings and dissertations were also searched from CNKI and Wanfang databases for unpublished trials. Searching strategies were made through the way of

text word, key words, and MeSH terms. The following terms (Chinese equivalent) were used individually or in combination with each other including "atrophic gastritis", "chronic atrophic gastritis", "chronic gastritis", "chronic", "atrophic", "gastritis", "precancerous lesions of gastric cancer", "intestinal metaplasia", "dysplasia", "Chronic superficial gastritis", "superficial gastritis", "chronic non atrophic gastritis", "non atrophic gastritis", "huangqi jianzhong Formula", "huangqi jianzhong decoction", "huangqi jianzhong tang", "huangqi jianzhong capsules", "huangqi jianzhong pills", "huangqi jianzhong tablets", and "random". There is no restriction for publication language and time. We retrieved the titles and abstract using the reference management software NoteExpress V 3.0.

2.2. Inclusion/Exclusion Criteria

Types of Studies. Randomized controlled trials were included, as well as crossover randomized trials, but only the outcomes from the first period of treatment were extracted and analyzed. Quasi-randomized trials were excluded. Two authors screened the titles and abstracts by eliminating the duplications, animal test, and other mechanical studies. Then the full articles were retrieved and the relevant studies were included. The disagreements were settled by consulting the third author.

Types of Participants. The participants diagnosed with CG (containing CAG and CSG) by gastroscopy and pathology were included. There are no limitations for the age, sex, and comorbidities.

Types of Interventions. The patients in the experiment group were orally administered HQJZ, which were in any preparations such as pills, capsules, decoctions, and tablets. Treatment course was more than 2 weeks. Modified HQJZ changes based on TCM syndrome differentiations and treatment variations were acceptable. The controlled group could be placebo, with no intervention and western medicine. The trials of intervention of HQJZ ± western medicine ± supportive treatment were included.

Types of Outcome Measures. The primary outcome was the improvement of atrophy and intestinal metaplasia based on the gastroscopy and pathology, and the incidence of gastric cancer. The secondary outcome was the score of TCM syndromes, the clinical symptom improvement rate (stomachache, gastrectasia, dyspepsia, shapeless stools, etc.), quality of life (QOL), Hp clearance rate, and overall effect rate.

2.3. Assessment of Risk of Bias. Two authors (Yue Wei and Li-Xin Ma) independently assessed the quality of included trials using the Cochrane risk of bias table [6]. The following items were assessed: random sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other bias. In addition, estimation of sample size and consistency of the baseline characteristic were also considered for the assessment of the bias. Disagreements were resolved by discussion with a third author (Jin-Xiang Yang). The risk of bias was categorized as low, unclear, or high.

2.4. Data Analysis. Two reviewers independently conducted the screening of studies, and data extraction (Yue Wei and Li-Xin Ma). Epidata 3.1 was used for data extraction. Meta-analyses were performed using RevMan 5.2 software. We pooled data using odds ratio (OR) with 95% confidence interval (CI) for dichotomous outcomes or mean difference (MD) with 95% CI for continuous outcomes. If different measurement scales were used, standardized mean differences (SMD) were analyzed. For crossover trials, only the outcomes from the first period were included. Where data were not reported, the data was requested from the corresponding author. A fixed effects model was used unless there was evidence of heterogeneity. Heterogeneity was assessed by the chi-squared test and/or the I-squared statistic. The $\alpha \leq 0.1$ and/or $I^2 \geq 45\%$ was indicative of substantial heterogeneity. When heterogeneity was present, subgroup analysis and sensitivity analysis were conducted to evaluate the robustness of the results. Funnel plots were performed to detect publication bias.

3. Results

3.1. Description of Studies. The searching flow chart is presented in Figure 1. There were 9 randomized clinical trials (RCTs) ($N = 979$) in this systematic review. All RCTs were conducted in China and all studies published in full in Chinese. There was no multicentre trial. Two studies [7, 8] were conducted to evaluate the effects of HQJZ for the treatment of CAG. Three studies [9–11] assessed the effects of HQJZ for the treatment of CSG. And four trials [12–16] explored the effects of HQJZ for the treatment of CG. The sample size was from 60 [8] to 238 [13]. Participants are from 19 to 83 years old. The disease courses were from 1.5 months to 27 years, except for 1 trial [11] that did not mention the clinical course. Five trials reported the TCM syndrome differentiation and treatment variation, of which 4 trials [8, 9, 12, 13] reported the participants' syndrome of deficiency cold in spleen and stomach, and in another 1 trial [7], the participants have the syndrome of weakness in spleen and stomach. Almost all trials included reported that HQJZ was applied in the intervention group; only 1 trial [8] used its modified formulas. The courses of treatment were from 2 weeks [12] to 3 months [7].

The comparisons included the following: HQJZ versus western drugs (6 trials) [7, 8, 10–12, 14] and HQJZ + western drugs versus western drugs (3 trials) [9, 13, 15].

As for primary outcome reporting, seven trials reported the results of gastroscopy and pathology [7–12, 15] and two of them also reported the cure rate of Hp infection [11, 15]. While two trials did not report the pathology results [13, 14], one of the trials reported clinical symptoms and signs only [14]. In addition, 3 trials [7–9] reported the improvement effect in TCM syndromes. Table 1 lists the characteristics of the studies including interventions used in the control and treatment groups, outcomes, and methodological qualities.

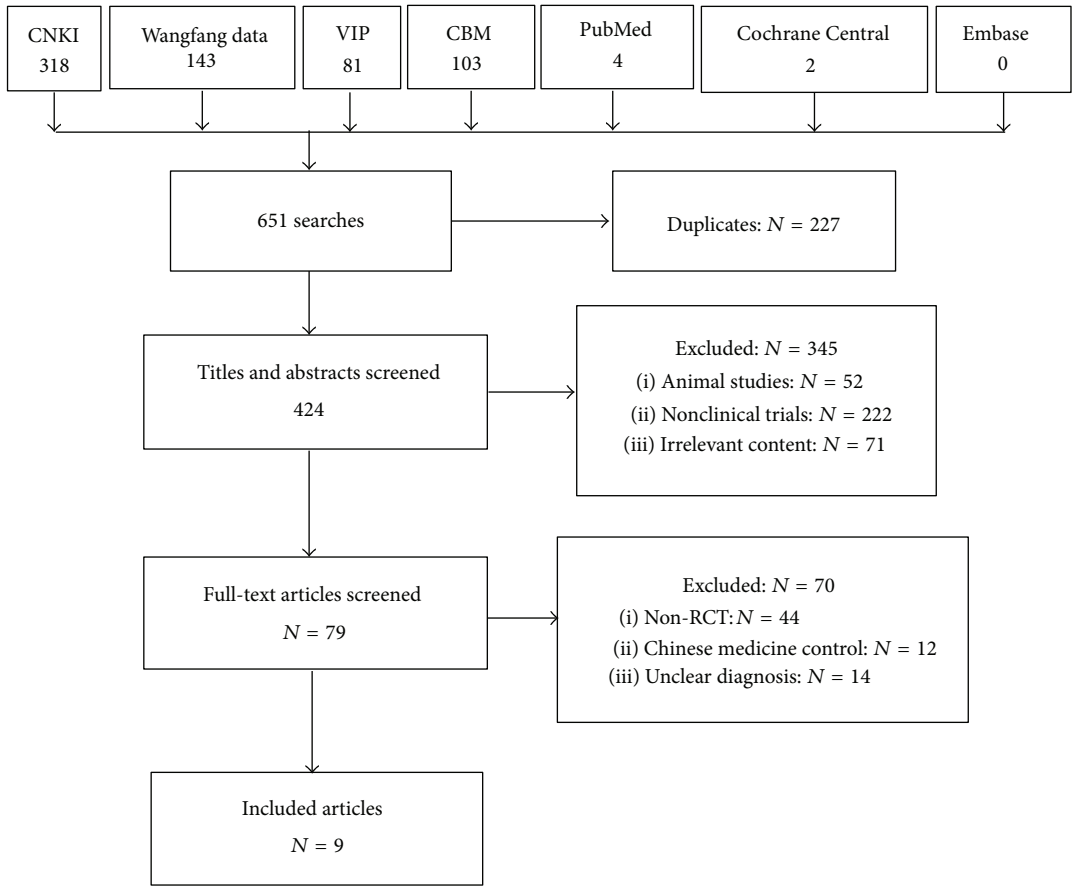

Figure 1: Flow chart of literature search.

3.2. Risk of Bias Assessment. Only three of the 9 trials (33.3%) described how subjects were randomly assigned into the intervention group and the controlled group. They all used a random number table [8, 9, 12]. The remaining six trials (66.7%) simply mentioned "randomization" but did not report the specific method.

None of the trials mentioned the allocation concealment and blindness. In addition, no trial reported their estimation of sample size, flow chart of the trial, and the utilization of intention-to-treat analysis. There was neither any information about trial registration nor incomplete outcome reporting. The risk bias assessment of the methodological quality lists is shown in Table 2.

3.3. Clinical Effect

3.3.1. Improvement of Atrophy and Intestinal Metaplasia under the Gastroscopy Pathology

HQJZ + Western Medicine versus Western Medicine. One trial reported the effect rate of overall improvement and pathology changes under the gastroscopy for patients with CSG [9]. Study showed that there were statistically significant differences for gastroscopy improvement rate [OR 3.78 (1.29, 11.06), $P = 0.02$] and pathology improvement rate [OR 2.83 (1.00, 7.98), $P = 0.05$] between the comparisons of HQJZ ± western medicine groups. See Table 2.

3.3.2. Incidence of Gastric Cancer, Clinical Symptom Improvement, and QOL. Our review did not find any assessment on the effects of incidence of gastric cancer, clinical symptom improvement rate, or QOL of HQJZ for patients with CG, CSG, or CAG among the included trials.

3.3.3. Improvement of TCM Syndromes

HQJZ versus Western Medicine. For the improvement of TCM syndrome effect of treatment on the patients with CAG [7, 8], meta-analysis showed that there was a statistically significant difference for the comparison between HQJZ and domperidone + vatacoenayme ([OR 6.67 (1.41, 31.59), $P = 0.02$]) [7] or HQJZ and domperidone [MD −5.85, (−7.71, −3.99), $P < 0.00001$] [8]. See Table 2.

HQJZ + Western Medicine versus Western Medicine. Only one trial reported the effects of TCM syndromes for the combination use of western medicine ± HQJZ for patients with CSG [9]. Study results showed statistically significant difference between the two groups [OR 9.75 (1.16, 82.11), $P = 0.04$]. See Table 2.

Table 1: An overview of the included studies.

Study ID	Age (years)	Classification of chronic gastritis	Type of syndrome	Course of disease (years)	Male (%)	Sample size N (n/n)	Intervention	Control medicine	Time of treatment (weeks)	Outcome measures
Chen and Lai 2013 [7]	21–62	Chronic atrophic gastritis	Weakness of spleen and stomach	1–14	58	117 (59/58)	HQJZ (and stagnation, added Costas, *Amomum villosum*, and blood stasis, added *Salvia miltiorrhiza*, *Panax notoginseng*, and yin-deficiency, added *Polygonatum*, dwarf lilyturf, and indigestion, added Jiaosanxian, and damp-heat, added *Coptis chinensis*, and cold-dampness, added *Atractylodes*)	Domperidone 10 mg tid, vatacoenayme 1 g tid	12	Overall effect (clinical symptoms, signs, manifestations of gastroscopy, and pathology), TCM syndrome effect (TCM symptoms and signs)
Fu et al. 2013 [8]	30–70	Chronic atrophic gastritis	Deficiency cold of spleen and stomach	1–10	53	60 (30/30)	HQJZ	Vatacoenayme 1 g tid	8	Overall effect (clinical symptoms, signs, manifestations of gastroscopy, and pathology), TCM syndrome effect (TCM symptoms and signs)
Zhang 2013 [9]	19–65	Chronic nonatrophic gastritis	Deficiency cold of spleen and stomach	1–11	56	80 (40/40)	HQJZ + western medicine (and loose stools, added parched hyacinth bean 15 g, *Coix* seed 15 g, and fullness, added citron 10 g, *Magnolia* 10 g, and stomachache, added Rhizoma *Corydalis* 10 g, and weakness, added red ginseng 10 g, and loss of appetite, added Jiaosanxian 15 g, *Amomum villosum* 6 g, and vomiting, added *Pinellia* 10 g, and acid regurgitation, heartburn, added Cuttlebone 18 g, fritillary bulb 15 g)	Omeprazole 20 mg bid for 4 weeks, or +domperidone 10 mg tid for 4 weeks, or +amoxicillin 0.5 g bid, metronidazole 0.4 g bid for 1 week	4	Overall effect (clinical symptoms, signs, manifestations of gastroscopy, and pathology), TCM syndrome effect (TCM symptoms and signs), Hp clearance, gastroscope, and pathology
Shi 2010 [10]	19–65	Chronic nonatrophic gastritis		1–27	55	120 (60/60)	HQJZ (added *Bupleurum* 10 g, Radix Aucklandiae 10 g)	Omeprazole 40 mg qd, domperidone 10 mg tid	4	Overall effect (clinical symptoms, signs, manifestations of gastroscopy, and pathology)
Li and Xu 2009 [11]	18–63	Chronic nonatrophic gastritis			47	72 (38/30)	HQJZ (added *Evodia rutaecarpa* 10 g)	Omeprazole 20 mg qd	4	Overall effect (clinical symptoms, signs, manifestations of gastroscopy, and pathology, Hp)

Table 1: Continued.

Study ID	Age (years)	Classification of chronic gastritis	Type of syndrome	Course of disease (years)	Male (%)	Sample size N (n/n)	Intervention	Control medicine	Time of treatment (weeks)	Outcome measures
L. Liu and Y. Liu 2014 [12]	30–76	Chronic gastritis	Deficiency cold of spleen and stomach	0.25–11	56	131 (67/64)	HQJZ + control medicine (added *lanceolata* 20 g, *Atractylodes* 15 g, *Poria cocos* 30 g, Tangerine Peel 8 g, *Pinellia* 10 g, bitter orange 15 g, corium stomachium galli 15 g, *Salvia* 15 g, *Panax notoginseng* powder 4 g, and Cuttlebone 8 g, and stomach fullness, added Radix Aucklandiae 10 g, and white and greasy fur, added *Pogostemon cablin* 10 g, Perrin 15 g, and loose stools, added yam 15 g, parched hyacinth bean 15 g, and eating little, added *Amomum villosum* (putted later) 8 g, and stomach cold pain, added Rhizoma *Corydalis* 15 g, *Evodia rutaecarpa* 8 g, and acid regurgitation, added Concha Arcae 15 g, Cuttlebone 15 g)	Clarithromycin 0.5 g bid, amoxicillin 0.5 g bid, and bismuth pectin 0.15 g qid	2	Overall effect (clinical symptoms, signs, manifestations of gastroscopy, and pathology)
Ni et al. 2013 [13]	21–66	Chronic gastritis	Deficiency cold of spleen and stomach	0.5–12	62	238 (120/118)	HQJZ + control medicine (and vomiting seriously, added dried ginger, *Pinellia*, Tangerine Peel, *Poria cocos,* and acid regurgitation, added *Coptis chinensis*, *Evodia rutaecarpa*, Cuttlebone, Concha Arcae, and stomach cold pain, seriously cold inside, vomiting, and cold limbs, added Lizhong Wan, and feeling cold, soreness, tiredness of waist and knee, added Fuzi Lizhong Wan, or added medicated leaven, *Atractylodes*, Tangerine Peel, agrimony, bitter orange, bergamot according to the symptoms)	Colloidal bismuth pectin 2 capsules tid	4	Overall effect (clinical symptoms, signs, and manifestations of gastroscopy)

Table 1: Continued.

Study ID	Age (years)	Classification of chronic gastritis	Type of syndrome	Course of disease (years)	Male (%)	Sample size N (n/n)	Intervention	Control medicine	Time of treatment (weeks)	Outcome measures
Li 2013 [14]	19–76	Chronic gastritis		0.125–10	61	76 (38/38)	HQJZ (added Xiangsha Liujunzi Tang, and stomachache like needling, added Fructus Toosendan, *Spatholobus suberectus* Dunn, and gastric acid and vomiting, added *Evodia rutaecarpa*, Cuttlebone, Concha Arcae, and loose stools and not warm hands and feet, added dried ginger, Eaglewood, combined spicebush, aconite, and loss of appetite, nausea, dry and bitter mouth, and yellowish fur, added *Gardenia*, bamboo shavings, and constipation, added *Fructus Cannabis*, rhubarb, and white and damp fur, hiccup, and slow pulse, added Calyx Kaki, clove, and eructation, added bristle *Inula*, Eaglewood)	Omeprazole 20 mg bid	Intervention 2; control medicine 4	Overall effect (clinical symptoms)
Tang and Hong 2003 [15]	20–68	Chronic gastritis		0.5–>5	53	85 (43/42)	HQJZ + furazolidone 0.1 g tid (added *lanceolata* 15 g, medicated leaven 10 g, and dandelion 30 g)	Amoxicillin (if allergy, metronidazole 0.1 g) 0.5 g, furazolidone 0.1 g, and sucralfate 1.0 g, tid	3	Overall effect (clinical symptoms, manifestations of gastroscopy, and pathology, Hp)

Table 2: Meta-analysis and pooled results of the main outcome in included studies.

Study ID	Adverse reaction	Risk of bias	Follow-up	Random method	Overall effect OR (95% CI)	TCM syndrome effect	Hp OR (95% CI)	Gastroscope OR (95% CI)	Pathology OR (95% CI)	Overall effect P value
Chen and Lai 2013 [7]	Not mentioned	Low	Not mentioned	Not mentioned	3.08 (1.10, 8.62)	OR 6.67 (1.41, 31.59)				0.03
Fu et al. 2013 [8]	Not found	Low	Not mentioned	Random number table	5.21 (1.28, 21.24)	MD −5.85 (−7.71, −3.99)				0.02
Zhang 2013 [9]	Not found	Low	Not mentioned	Random number table	3.58 (0.89, 14.39)	OR 9.75 (1.16, 82.11)	6.02 (1.43, 25.40)	3.78 (1.29, 11.06)	2.83 (1.00, 7.98)	0.07
Shi 2010 [10]	Not mentioned	Low	Not mentioned	Not mentioned	3.86 (1.41, 10.57)					0.009
Li and Xu 2009 [11]	Not mentioned	Low	Not mentioned	Not mentioned	1.64 (0.51, 5.33)					0.41
L. Liu and Y. Liu 2014 [12]	Not mentioned	Low	Not mentioned	Random number table	2.62 (0.99, 6.94)					0.05
Ni et al. 2013 [13]	Not found for intervention; rash and anaphylactoid purpura of legs for 1 case in control group; increase of eosinophil for 1 case	Low	Not mentioned	Not mentioned	2.80 (1.35, 5.82)					0.006
Li 2013 [14]	In intervention group, epigastric pain, fullness, belching, and poor appetite were all less than control group	Low	Not mentioned	Not mentioned	3.62 (0.90, 14.63)					0.07
Tang and Hong 2003 [15]	Most in control group, poor appetite, upper abdominal discomfort, nausea and vomiting, and so forth; especially used metronidazole, must add metoclopramide and anisodamine, and so forth, impacted quality of lives	Low	Not mentioned	Not mentioned	1.58 (0.25, 9.95)					0.63

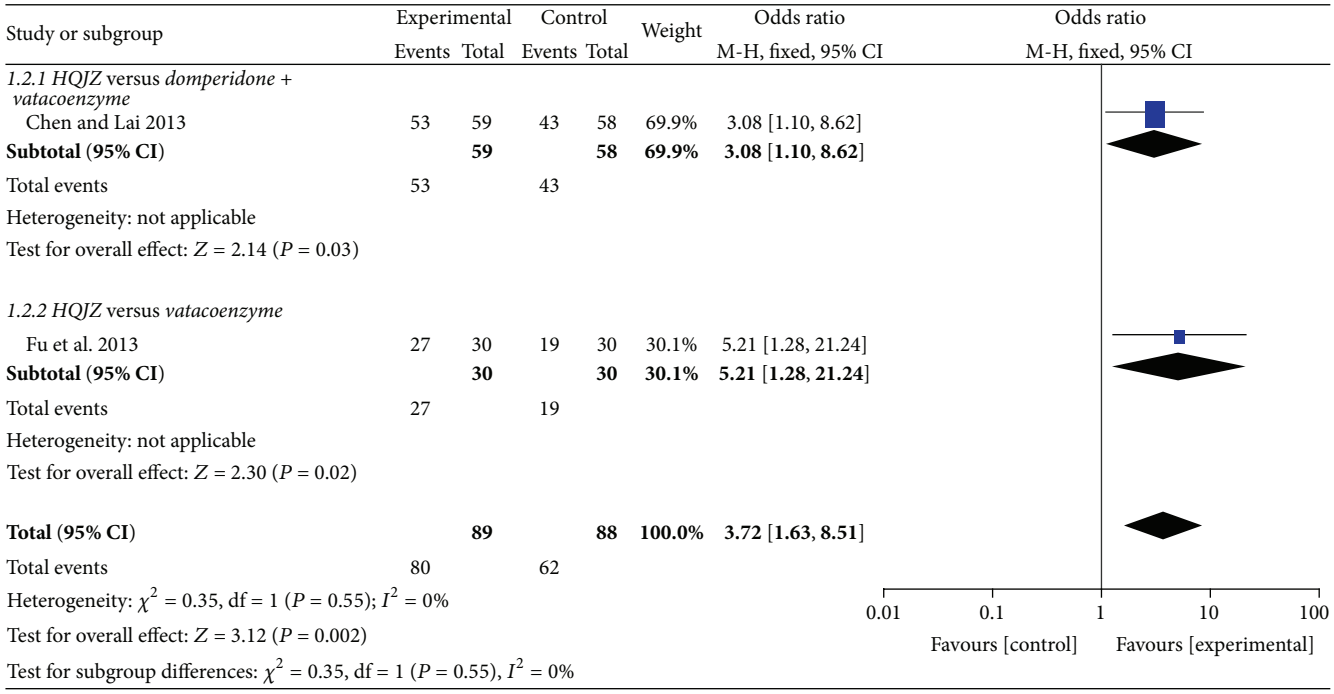

Figure 2: Forest plot of improvement of overall effect rate for patients with CAG.

3.3.4. Hp Clearance Rate

HQJZ + Western Medicine versus Western Medicine. We included one trial on the effects of Hp clearance rate for patients with CSG [9]. There was statistically significant difference between the comparisons of HQJZ ± western medicine (omeprazole and domperidone) groups [OR 6.02 (1.43, 25.40), $P = 0.01$]. See Table 2.

3.3.5. Overall Effect Rate

HQJZ versus Western Medicine. For the treatment of the patients with CAG [7, 8], two studies comparing the overall effects between HQJZ and domperidone or vatacoenayme were included in the pooling results. There was a statistically significant overall effect rate comparing HQJZ and western medicine [OR 3.72, 95% CI (1.63, 8.51), $P = 0.002$]. See Figure 2.

For the treatment of the patients with CSG [10, 11], two studies comparing the overall effects between HQJZ and omeprazole or domperidone were included in the pooling results. There was a statistically significant overall effect rate comparing HQJZ and western medicine [OR 2.73 (1.29, 5.81), $P = 0.009$]. See Figure 3.

We included one study comparing the overall effect rate between HQJZ and omeprazole for the treatment of the patients with CG (not classified as atrophic and nonatrophic) [14]. There was no statistically significant difference between HQJZ and omeprazole group [OR 3.62 (0.90, 14.63), $P = 0.07 > 0.05$].

HQJZ + Western Medicine versus Western Medicine. For the treatment of the patients with CSG [9], we included one trial comparing the overall effect rate of the combination of HQJZ plus omeprazole + domperidone with the omeprazole + domperidone. There was no statistically significant difference between the two groups [OR 3.58 (0.89, 14.39), $P = 0.07 > 0.05$].

There were three trials comparing the overall effect rate of combined intervention of western medicine ± HQJZ for patients with CG [12, 13, 15]. A statistically significant difference between the comparing groups was found [OR 2.60 (1.49, 4.54), $P = 0.0007$]. See Figure 4.

HQJZ plus Colloidal Bismuth Pectin versus Colloidal Bismuth Pectin. Results showed that there was statistically significant difference between the intervention group of HQJZ plus colloidal bismuth pectin and the controlled group of colloidal bismuth pectin [OR 2.80 (1.35, 5.82), $P = 0.006$] [13].

HQJZ plus Clarithromycin + Amoxicillin + Bismuth Pectin versus Clarithromycin + Amoxicillin + Bismuth Pectin. There was no statistically significant difference between the two groups of clarithromycin, amoxicillin, and bismuth pectin ± HQJZ [OR 2.62 (0.99, 6.94), $P = 0.05$] [12].

HQJZ + Furazolidone versus Furazolidone + Amoxicillin (Metronidazole) + Sucralfate. There was no statistically significant difference between the two groups of furazolidone, amoxicillin (or metronidazole if allergy), and sucralfate, ±HQJZ [OR 1.58 (0.25, 9.95), $P = 0.63 > 0.05$] [15].

3.3.6. Adverse Reaction. Five of the 9 trials mentioned adverse effects [8, 9, 13–15]. Two of them reported that there was not any adverse effect observed in HQJZ application [8, 9].

Study or subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds ratio M-H, fixed, 95% CI
4.1.1 HQJZ versus *omeprazole and domperidone*						
Shi 2010	54	60	42	60	49.2%	3.86 [1.41, 10.57]
Subtotal (95% CI)		**60**		**60**	**49.2%**	**3.86 [1.41, 10.57]**
Total events	54		42			
Heterogeneity: not applicable						
Test for overall effect: $Z = 2.62$ ($P = 0.009$)						
4.1.2 HQJZ versus *omeprazole*						
Li and Xu 2009	32	38	26	34	50.8%	1.64 [0.51, 5.33]
Subtotal (95% CI)		**38**		**34**	**50.8%**	**1.64 [0.51, 5.33]**
Total events	32		26			
Heterogeneity: not applicable						
Test for overall effect: $Z = 0.82$ ($P = 0.41$)						
Total (95% CI)		**98**		**94**	**100.0%**	**2.73 [1.29, 5.81]**
Total events	86		68			
Heterogeneity: $\chi^2 = 1.17$, df = 1 ($P = 0.28$); $I^2 = 14\%$						
Test for overall effect: $Z = 2.61$ ($P = 0.009$)						
Test for subgroup differences: $\chi^2 = 1.17$, df = 1 ($P = 0.28$), $I^2 = 14.3\%$						

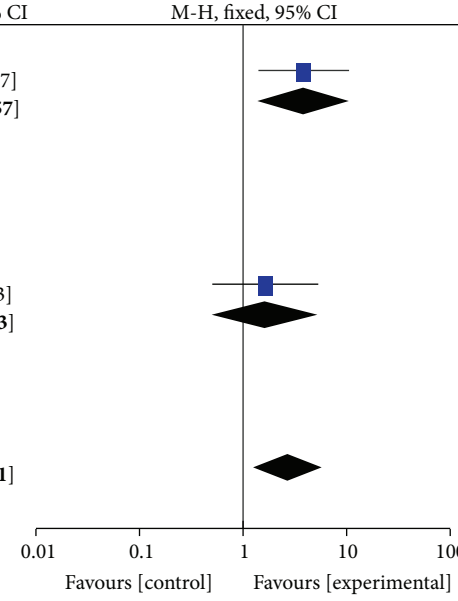

Figure 3: Forest plot of improvement of overall effect rate for patients with CSG.

Study or subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds ratio M-H, fixed, 95% CI
1.1.1 HQJZ+ amoxicillin + clarithromycin + bismuth pectin versus *amoxicillin + clarithromycin + bismuth pectin*						
L. Liu and Y. Liu, 2014	60	67	49	64	32.4%	2.62 [0.99, 6.94]
Subtotal (95% CI)		**67**		**64**	**32.4%**	**2.62 [0.99, 6.94]**
Total events	60		49			
Heterogeneity: not applicable						
Test for overall effect: $Z = 1.94$ ($P = 0.05$)						
1.1.2 HQJZ + colloidal bismuth pectin versus *colloidal bismuth pectin*						
Ni et al. 2013	108	120	90	118	56.2%	2.80 [1.35, 5.82]
Subtotal (95% CI)		**120**		**118**	**56.2%**	**2.80 [1.35, 5.82]**
Total events	108		90			
Heterogeneity: not applicable						
Test for overall effect: $Z = 2.76$ ($P = 0.006$)						
1.1.3 HQJZ+ furazolidone versus *furazolidone + amoxicillin (metronidazole) + sucralfate*						
Tang and Hong 2003	41	43	39	42	11.4%	1.58 [0.25, 9.95]
Subtotal (95% CI)		**43**		**42**	**11.4%**	**1.58 [0.25, 9.95]**
Total events	41		39			
Heterogeneity: not applicable						
Test for overall effect: $Z = 0.48$ ($P = 0.63$)						
Total (95% CI)		**230**		**224**	**100.0%**	**2.60 [1.49, 4.54]**
Total events	209		178			
Heterogeneity: $\chi^2 = 0.32$, df = 2 ($P = 0.85$); $I^2 = 0\%$						
Test for overall effect: $Z = 3.37$ ($P = 0.0007$)						
Test for subgroup differences: $\chi^2 = 0.32$, df = 2 ($P = 0.85$), $I^2 = 0\%$						

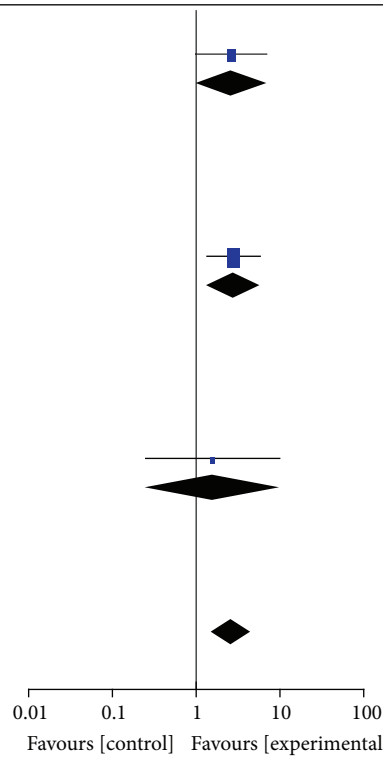

Figure 4: Forest plot of improvement of overall effect rate for patients with CG.

One trial [13] reported the adverse effects including rash and anaphylactoid purpura found in legs for 1 case and increase of eosinophils cell count in the blood test for 1 case in the controlled group and with no adverse effect found in intervention group. Another one trial [14] mentioned that the clinical symptoms of epigastric pain, fullness, belching, and poor appetite were observed both in intervention and in controlled groups, relatively minor in the intervention group than those in the controlled group. The other trial [15] reported that most of patients had poor appetite, upper abdominal discomfort, nausea and vomiting, and so forth in the control group especially for those patients who were given metronidazole, metoclopramide, and anisodamine (see Table 2).

4. Discussion

4.1. About HQJZ. The prescription of HQJZ is made of seven Chinese herbal drugs including *astragalus*, *cassia twig*, *white peony root*, *baked licorice*, *ginger*, *jujube*, and *maltose*. Reports on the effects of HQJZ for the treatment of patients with CG came from a TCM classic named *Synopsis of Golden Chamber*, written by Zhang Zhongjing, and dated back to more than 1800 years before (Eastern Han Dynasty of China) [16]. It has been used in the clinical scenario of patients with the abdominal upset or pain, with or without belching, abdominal bloating, nausea, vomiting, and loose stools or a feeling of fullness, of burning in the upper abdomen, or of cold and weakness in the limb. Up to now there are some evidences reporting its mechanism in the treatment of CG. Evidence from an animal test in rat models with spleen-asthenia showed that the HQJZ might regulate serum gastrin levels and significantly inhibit pepsinogen secretion of the chief cells and the acid secretion of the oxyntic mucosa [17]. Another experiment showed that the HQJZ could elevate the levels of substance P in gastric antrum and facilitates gastric emptying [18]. The results of the third experimental test demonstrated that HQJZ might set in motion mechanisms involving the improvement of energy metabolism in colonic mucosal injury induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) [19]. Evidences from the clinical trials also found that HQJZ may reduce fatigue by increasing the oxygen uptake and the systemic utility of oxygen among twelve senior male high school basketball players [20]. In one word, HQJZ may be a multitargeting management for the treatment of patients with CG.

4.2. Main Findings. 9 RCTs and 979 participants were included in this review. Firstly our meta-analysis of the overall effect rate found that HQJZ ± western medicine were more effective than western medicine for the treatment of CG. Secondly, HQJZ was more effective in improving the symptoms and signs than western medicine for patients with CAG; and these effects were also found when comparing the groups of HQJZ ± western medicine for the treatment of patients with CSG. Thirdly, studies showed that HQJZ plus western medicine had more effects on increasing Hp clearance rate and improving gastroscopic manifestation than western medicine for treatment of CSG.

4.3. Limitations of This Review. The following are some limitations existing in the included RCTs:

(1) The included studies had limitations in methodological qualities. Only 3 of the trials reported on how the participants are randomly assigned to the intervention groups. Six out of 9 trials (66.7%) simply mentioned "randomization," with none of the trials mentioning the use of allocation concealment, and blinding. 5 of the 9 trials mentioned adverse reaction. None of the trials mentioned follow-up.

(2) Although Hp infection is the most frequent cause of CG, there are many other causes of gastritis [21, 22]. In this review only 2 of the 9 trials made a clear statement in including patients diagnosed with CAG. Four studies did not classify CG into subtype of CSG and CAG based on pathology test. Instead, 9 RCTs reported TCM syndrome diagnosis, such as *deficiency and cold of spleen and stomach*, resulting in lower external validity and impaired clinical application of the results under these circumstances.

(3) CG has relatively minor manifestation in the process of the diseases. And no universally accepted classification system provides an entirely satisfactory description of all of the gastritis and gastropathies [23]. So there is a need to report explicitly the endoscopic appearances and histopathologic patterns of the gastric mucosa tests in RCTs. However only 1 of the trials reported gastroscopy and pathology separately. 2 of them did not contain pathologic outcome reporting. In addition, most of the trials even used the overall effect rate as the main outcome; this will lead to failure to quantitatively assess the effectiveness of HQJZ on the treatment of patients with CG.

5. Conclusions

HQJZ may have potential effects on the treatment of patients with CG. However, due to limitation of the methodological quality, we could not draw confirmed conclusion on its beneficial effect as well as its risks. Future clinical trials on evaluating the effects of HQJZ should be designed more rigorously in methodological quality.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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