Evidence-Based Complementary and Alternative Medicine

Natural Products as a Source for New Leads in Depression Treatment

Lead Guest Editor: Ke Ma
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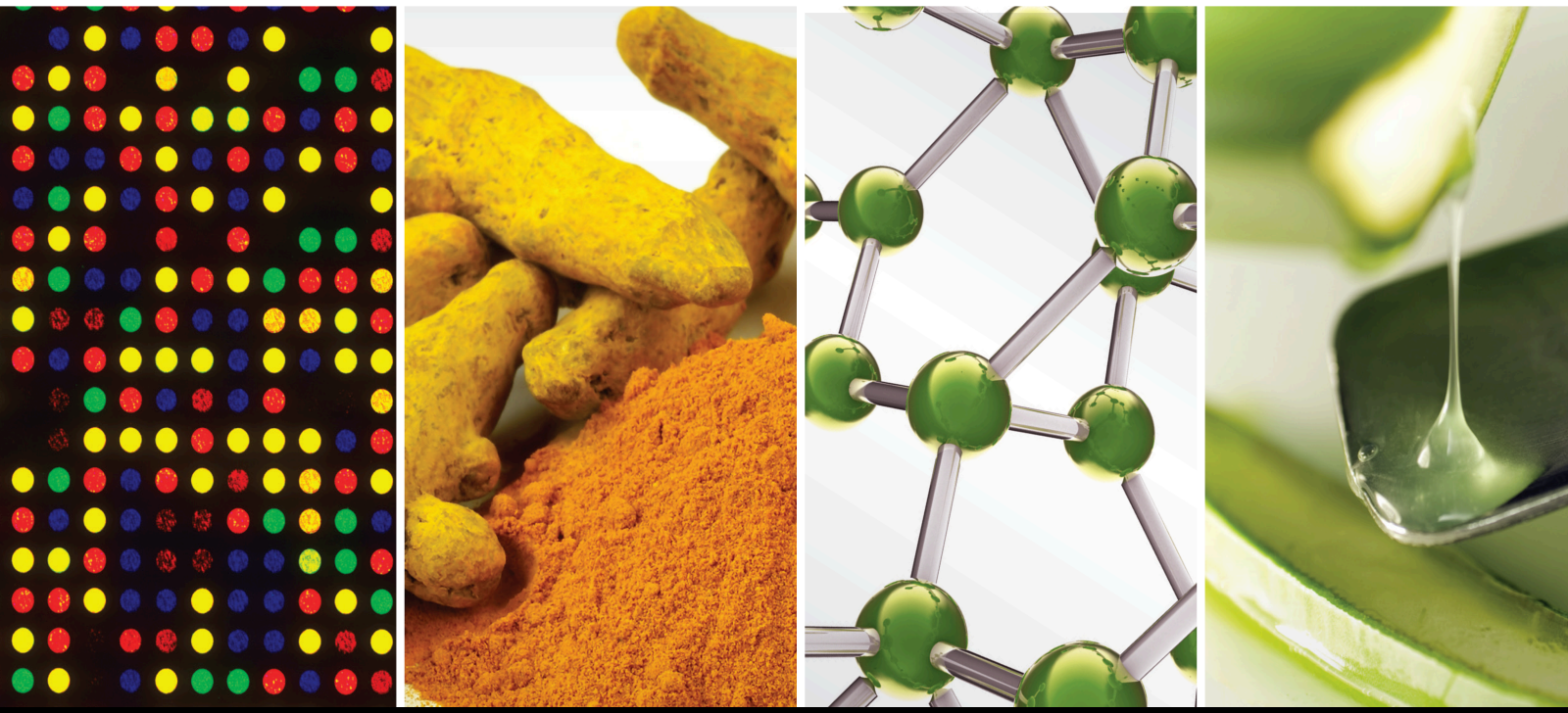

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Editorial

Natural Products as a Source for New Leads in Depression Treatment

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Depression is a mental health disorder, which varies from mild to severe changes in mood and affects physical, mental, and behavioral health. Recent evidence indicates that depressive disorders may represent an interactive matrix of reciprocally interactive pathophysiology influenced by various factors like gene or environment. The sustained stress to the individuals with genetic susceptibility leads to the deficits of HPA axis, imbalanced monoamine/cytokine, decreased neurogenesis, and the altered dynamics of connectivity in the reward and motivational circuitry. These mechanisms reduce neuroplasticity and impair the functional integrity of regulation neurocircuitry. Therefore, preventive and therapeutic strategies have been considered to avoid and treat this disease. In this context, the drugs regulating neurotransmitters, including selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, and tricyclic and monoamine oxidase inhibitors, are the most commonly prescribed antidepressants. However, these antidepressants only have relative success and can originate several side effects, particularly in chronic use. For these reasons, over the years, researchers have been searching for alternative therapeutic strategies, particularly involving the use of natural products.

In this Special Issue, we invited investigators to contribute the latest original research and review articles investigating in vitro, in vivo, nonclinical, and clinical/translational studies addressing the use of traditional Chinese medicine formula, Chinese herbs, and natural products, as either extracts or isolated compounds, in depression treatment. In this Special Issue, six original research articles and two review articles were published regarding the natural products as a source for new leads in depression treatment.

The research article by Y. Choi et al. demonstrated the potential of *Tetragonia tetragonioides* (TTK) in treating depressive symptoms and the alterations in the brain of the animal model of depression. TTK protected the mouse brain glial loss in the prefrontal cortex induced by a gliotoxin, L-AAA. It is suggested that TTK may be one of the potential candidates for treating depression. Further research studies are required to understand the effect of TTK on depressive disorders.

The research article by H. Zhu et al. reported Xiaoyaosan can improve depressive-like behavior in rats by suppressing the activation of the TLR4/NLRP3 inflammasome signaling pathway, thereby inhibiting immunoinflammatory activation and reducing the levels of inflammatory cytokines in the colon. The observed improvement in depressive symptoms may have resulted from the downregulation of the levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, and NLRP3 inflammasome-related NLRP3, ASC, and caspase-1 proteins, leading to the subsequent downregulation of the levels of the inflammatory cytokines IL-6, IL-1β, and TNF-α.

The research results from M. Qian et al. showed Chaihu Shugan San was superior to fluoxetine in regulating the appearance and HPA axis function of model rats. In addition, Chaihu Shugan San and fluoxetine have similar effects in improving blood theology, and both can alleviate the hypercoagulable state of blood via the platelet 5-hydroxytryptamine receptor 2A (5-HT2A) pathway in rats of depression. It was also observed that Chaihu Shugan San can

improve the blood state of depressed rats by restoring liver coagulation-anticoagulation balance and endothelium-related functions.

The research article by M. Jin et al. *So-Ochim-Tang-Gamibang* (SOCG) controlled the levels of HPA axis hormones in a rat model of chronic stress-induced depression. SOCG suppressed the stress-induced increase of the circulating plasma levels of the HPA axis hormones, decreased the ACTH and CRH levels in the pituitary and hypothalamus, respectively, and increased the hippocampal expression of GR, which is an important receptor for the GC hormone. In SOCG-treated depressed rats, an upregulation of the hippocampal expression of the HPA axis-related signaling molecules CREB and ERK was observed; this may lead to the increase of BDNF expression.

The research article by J. Li et al. showed that traditional Chinese medicine (TCM) as a complementary therapy combined with WM was accepted by more than half of the patients with postpartum depression (PPD) and it potentially could lead to a reduced medical expenditure in treating the disease. The study also discussed the associated sociodemographic and medical factors regarding TCM use of PPD patients. The results of our study may be helpful to clinical practitioners as well as health-policy decision-makers while considering the integration of TCM with western medicine in patients with PPD.

The research article by B. Yan et al. suggested acupuncture has a promising application prospect due to its unique advantages for the treatment of chronic pain with depression comorbidity, which can be used in patients suffering from some certain chronic pain with depression comorbidity with poorer response to the conventional medication or suffering from serious side effects. High-quality RCTs are needed to support the current clinical application of acupuncture for the treatment of chronic pain with depression comorbidity and to broaden the clinical application.

The review article by B. Shang et al. summarized the clinical symptoms, etiology, pathogenesis, and therapeutic medication of lily disease and modern Yin-deficient internal heat depression and discusses the relationship between them. Furthermore, the relationship between coronavirus disease 2019 (COVID-19) and lily disease was discussed from the etiology, pathogenesis, and treatment. It provides new ideas for the treatment of COVID-19 and the treatment of psychological problems after recovery.

Another review article by S. Munir et al. investigated the bioactive compounds from plants and marine sources that contain the indole moiety, which can serve as potent antidepressants. The prospects of naturally occurring as well as synthetic indole alkaloids for the amelioration of anxiety and depression-related disorders, structure-activity relationship, and their therapeutic prospects have been discussed.

Thus, with contributions from research groups from diverse countries, this Special Issue presented recent experimental findings and reviews on natural and semisynthetic products with relevant potential for the prevention and treatment of depression.

Conflicts of Interest

The Guest Editors declare that there are no conflicts of interest regarding the publication of this Special Issue.

Acknowledgments

The Guest Editors of this Special Issue acknowledge all contributors to the success of this interesting collection of studies concerning natural products as a source for new leads in depression treatment. Specifically, the Guest Editors would like to thank all authors of the articles of this Special Issue for their valuable scientific works, reviewers for their constructive criticism and time spent that made this Special Issue possible, and the editorial board of this journal for inviting them to edit this Special Issue. The authors acknowledge the support provided by the National Natural Science Foundation of China (81903948), Shandong Provincial Natural Science Foundation (ZR2019BH027 and ZR2019ZD23), and Shandong Province Universities' Development Plan for Youth Innovation Teams (2019-9-202, 201, and 2019KJK013).

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Research Article

Tetragonia tetragonioides Relieves Depressive-Like Behavior through the Restoration of Glial Loss in the Prefrontal Cortex

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Tetragonia tetragonioides, which is a halophyte and grows widely in Asian-Pacific regions, has been used for the treatment of digestive disorders in traditional oriental medicine. This study examined the potential antidepressant effect of *Tetragonia tetragonioides* in an astroglial degeneration model of depression, which was established based on the postmortem study of depressive patients' brain presenting diminished astrocytes in the prefrontal cortex. C57BL/6 male mice were exposed to glial ablation in the prefrontal cortex by the administration of the gliotoxin, L-alpha-aminoadipic acid (L-AAA) to induce depression. *Tetragonia tetragonioides* at doses of 100 mg/kg and 300 mg/kg, imipramine at a dose of 15 mg/kg, and distilled water were orally administrated to mice for 18 days. Behavioral tests including the open field test (OFT), sucrose preference test (SPT), forced swimming test (FST), and tail suspension test (TST) were carried out after 2 days of L-AAA injection. The expression levels of GFAP and NeuN in the prefrontal cortex were determined by immunohistochemistry. Mice subjected to glial ablation in the prefrontal cortex displayed decreased sucrose consumption in SPT and increased immobility time in FST and TST. Treatment with imipramine and *Tetragonia tetragonioides* remarkably ameliorated the behavioral despair induced by L-AAA. In addition, immunohistochemistry analysis showed that treatment with *Tetragonia tetragonioides* significantly restored the glial loss as indicated by the elevated GFAP expression level. These findings suggest that *Tetragonia tetragonioides* exerts an antidepressant effect through the restoration of glial loss under conditions of depression and can be a candidate for an antidepressant agent.

1. Introduction

A depressive disorder is identified as characteristic symptoms of despair, anhedonia, loss of appetite or sleep disturbance, decreased energy and concentration, and feeling of guilt and worthlessness [1–5]. According to the epidemiological survey of mental disorders in Korea in 2011, the lifetime prevalence of major depressive disorder was reported to be 6.7% [6]. As per the report on death and causes of death in Korea, the suicide rate was 24.3 per 100,000 persons in 2017 [7]. Needs for effective and immediate treatments for depression with fewer adverse events are gradually increasing, and various local herbs have been tested for this purpose [8–10].

In the last few years, pathology of glial cells has been studied with emphasis to understanding the mechanisms behind brain disorders [11]. Especially, decreased density of glial cells in the prefrontal and the cingulate area was consistently reported in depressed patients [12]. Abnormalities in glial cells, especially astrocytes, play an important role in mediating major depressive disorders. The previous review summarized that the expression of the glial fibrillary acidic protein (GFAP) and representative astrocyte related proteins was significantly decreased in subjects with major

depressive disorders [13, 14]. In animal studies, chronic and acute stress induced depressive-like symptoms in behavior and alterations in astrocytes in the brain [15].

Immense efforts have been made to generate the valid and insightful model of depression, and the chronic unpredictable stress model is one of the well-documented animal models for depression [16]. Based on the postmortem research studies reporting a loss of glia in prefrontal and the cingulate area from depressed patients [17], Banasr and Duman proved that glial ablation in the prefrontal cortex induced depressive-like behaviors [18]. The authors provoked the astrocytic degeneration by infusing L-alpha-aminoadipic acid (L-AAA), a gliotoxin specific for astrocytes [19], into prefrontal cortex. Infusion of L-AAA only led to the loss of glia and not neurons. Infusions of ibotenic acid, which is toxic for neurons, did not induce the depressive-like behaviors.

Tetragonia tetragonioides (Pall.) Kuntze (TTK), commonly called as New Zealand spinach, belongs to the family of Aizoaceae, and widely grows in Korea, Japan, southeast China, and New Zealand [20, 21]. In Korea, TTK grows abundantly on the Jeju Island [22]. In traditional oriental medicine, TTK has been documented to impart the protective effect in conditions of digestive troubles, and the antiulcerogenic activity was also reported [23]. Extract of TTK includes various antioxidative compounds [24], and TTK is reported to have antioxidant and anti-inflammatory effects [25]. 70% ethanol extract of TTK demonstrated potential to treat menopause-associated symptoms and metabolic disturbances [26]. Moreover, TTK extract ameliorated the depressive-like symptoms and regulated the serotonin level in ovarietomized rats [27]. The antidepressant effect of TTK in the depressive models has not been tested, and previous studies indicated the possibility that TTK may be effective on treating depressive disorders.

To address this topic, a depression model of astroglial degeneration was used to investigate the ameliorative effects of TTK on depressive symptoms. Furthermore, we examined the possible mechanism behind the antidepressant-like effects of TTK by examining the expression of the GFAP in the prefrontal region in mice brain.

2. Materials and Methods

2.1. Animals. Fifty male C57Bl/6 mice, aged seven weeks, were purchased from Orient Bio Inc., Korea. The mice were housed in acrylic cages (20 cm × 27 cm × 12 cm) under standard experimental conditions at constant temperature (22 ± 2°C), humidity (60 ± 10%), and light (12-hour light and dark cycle, with lights off at 6 pm). Animals were allowed free access to food and water and had a period of acclimation before the start of each experiment. The Kyung Hee University Medical Center Institutional Animal Care and Use Committee approved all the procedures (KHMC-IACUC 16-033). All efforts were made to minimize animal suffering during the progression of the experiments.

2.2. Drugs and Reagents. TTK was obtained from Jeju, Korea. The whole dried TTK (600 g) was boiled twice with 30% ethanol, and the extract was filtered through a filter paper. The filtrate was concentrated using a rotary evaporator and lyophilized to yield a powder (91 g). The powder was stored at 4°C until use. Mouse monoclonal antibodies against β-actin and GFAP were obtained from Santa Cruz Biotechnology (CA, USA). Neuronal-specific nuclear protein (NeuN) was purchased from Millipore Inc. (Bedford, MA, USA). Horseradish peroxidase-conjugated antimouse secondary antibody was purchased from Pierce Biotechnology (Rockford, IL, USA). Acetonitrile and methanol were purchased from Honeywell Burdick and Jackson (Morristown, NJ, USA) and were of HPLC grade. Analytical-grade formic acid (99% purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Deionized water (>18 mΩ) was obtained via a pure water purification system (Human Co., Korea).

2.3. LC-QTOF-MS Analysis. Isoferulic acid and 4-hydroxybenzoic acid were selected for reference standards based on previous studies. Approximately 50 mg of TTK powder was shaken with 1 mL of methanol by a vortex mixer for 30 seconds. The supernatants were filtered through a 0.2 μm polytetrafluoroethylene syringe filter (Thermo Scientific). Finally, the filtrate was transferred to a LC sample vial before use.

The liquid chromatography-mass spectrometry system consisted of a Thermo Scientific Vanquish UHPLC system (Thermo Fisher Scientific, Sunnyvale, CA, USA) with an Acclaim RSLC 120 C18 (2.1 mm × 100 mm, 1.7 μm; Agilent Technology, Ca, USA) and a TripleTOF 5600+ mass spectrometer system (TripleTOF MS; QTOF, Sciex, Foster City, CA, USA). The QTOF MS, equipped with a DuoSprayTM ion source, was used to complete the high-resolution experiment. The LC gradient used a mobile phase A containing 0.05% formic acid and 2.5 mM ammonium formate in water and a mobile phase B of acetonitrile. The flow rate was kept constant at 0.4 mL/min, and the injection volume was 1 μL. The gradient elution system began at 5% B for 0.8 min, 5–20% B from 0.8 to 2.5 min, 20–32% B from 2.5 to 5.5 min, 32–38% from 5.5 to 8, 38–45% B for 2 min, 45–60% B for 2 min, 60-95% B for 4 min, then increased to 100% B at 20.0 min, held at 100% B for 3 min, and then returned to the initial conditions for reequilibration.

Mass data acquisition was performed with a TripleTOF 5600+ in the negative ion mode using the following parameters: source temperature was set at 450°C with a curtain gas flow of 25 L/min (GS1 and GS2 both 50), the ion spray voltage was set at −4500 V, declustering potential was 30 V, and the collision energy was 10 V. High-purity nitrogen gas was used for the nebulizer/DuoSprayTM and curtain gases. The QTOF and information-dependent acquisition (IDA) scan were operated with a mass range of 50–1500 m/z. Precursor and product ion calibrations were performed in both high sensitivity and high-resolution modes using a calibrant delivery system prior to analysis. Data acquisition and processing were carried out using Analyst TF 1.7, PeakVeiw2.2, and MasterView (Sciex, Foster City, CA, USA).

2.4. Cannula Implantation. Mice were anesthetized using intraperitoneal injection of 100 mg/kg ketamine + 10 mg/kg xylazine, and guide cannula (RWD Life Science Co., Ltd., Shenzhen, China) were bilaterally implanted into the prefrontal cortex region of mice brain using a stereotaxic apparatus (Vernier Stereotaxic Instrument, Leica Biosystems, Nussloch, Germany) by the following coordinates: 1.7 mm anteroposterior, ±0.25 mm dorsolateral, and depth −2.5 mm from the bregma [28]. After seven days of recovery, we infused L-AAA (100 μg/μl; Sigma) bilaterally using injection cannula and a microdriven pump (Pump 11 Elite Nanomite, Harvard Apparatus, Holliston, MA, USA). We administered infusion once daily for 2 days at a rate of 0.1 μl/min for 6 minutes.

2.5. Drug Administration. Mice were randomly assigned into five groups: control group with distilled water (DW), negative control group with L-AAA infusion and DW, positive control group with L-AAA infusion and 15 mg/kg imipramine, and two experimental groups with L-AAA infusion and 100 mg/kg or 300 mg/kg TTK. After the adaptation period, oral administration was continued until the animals were sacrificed. Experimental procedure that followed the time schedule is shown in Figure 1.

2.6. Behavioral Test. The open field test (OFT) was carried out to assess the locomotor activity [29]. Briefly, mice were placed in the center of a white, acrylic, plastic square box (50 cm × 50 cm) and allowed to freely explore the apparatus for 10 min. The total distance travelled, recorded by video camera, was evaluated using a computer-aided control system (SMART 3.0, Panlab Harvard Apparatus).

The sucrose preference test (SPT), tail suspension test (TST), and forced swimming test (FST) were conducted to measure the depressive behavior of the mice. In SPT, mice were habituated for 48 hours to 1% sucrose, and following a 4-hour of deprivation period, preference for sucrose or water was determined for 1 hour. The bottles of sucrose and water were identical [30]. For TST, mice were suspended 50 cm above the floor by adhesive tape placed 1 cm from the tip of the tail for 6 min. Immobility time was defined after the 2 min mark, as the duration of time the animal was hung passively and completely motionless during the remaining 4-minute period [31]. In FST, mice were placed in an inescapable open cylindrical container (diameter 20 cm, height 35 cm), with 15 cm of water maintained at 25°C for a total of 6 min. Immobility time was defined as the time the mouse ceased struggling and floated motionless during the last 4 min of the 6-minute test, following an initial 2 min of activity [32].

2.7. Immunohistochemistry. Mice were quickly anesthetized with diethyl ether and then perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde solution. The brains were fixed in 4% paraformaldehyde for 24 h, followed by PBS containing 20% sucrose for 24 h. Typically, 10 μm thick coronal sections of each brain were embedded in optimal cutting temperature (OCT) compound and cut with a cryostat. After washing in PBS, the sections were incubated for 1 h at room temperature with 1% normal horse serum in PBS and incubated overnight at 4°C with the primary antibody against the GFAP and NeuN at 1 : 500 dilutions in PBS, containing 2.5% normal horse serum. After washing in PBS, sections were incubated for 1 h at room temperature with the biotinylated secondary antibody (1 : 50) in PBS containing 2% normal horse serum and subsequently incubated with ABC reagents (Vector Laboratories, CA, USA) in PBS. After washing again in PBS, the sections were incubated in 3,3'-diaminobenzidine tetrahydrochloride (DAB; Dako, CA) and mounted in permount mounting medium (Fisher Scientific International, USA). GFAP and NeuN-positive cells were detected using the Olympus BX51 microscope (Olympus, Tokyo, Japan)

2.8. Enzyme-Linked Immunosorbent Assay (ELISA). Prefrontal cortex of some mice were dissected and stored at −80°C for ELISA. Tissue lysates were quantified according to the mouse ELISA kit (ThermoFisher, Waltham, Massachusetts, U.S, CAS: BMS607-3) manufacturer's user guide. Protein level of tumor necrosis factor-alpha (TNF-α) was measured at an absorbance of 450 nm (pg/ml) through a microplate reader.

2.9. Statistical Analysis. Statistical differences of the mean were examined using one-way analysis of variance (ANOVA), followed by Dunnett's test for intergroup comparisons. For all the analyses, a P value less than 0.05 was considered statistically significant. The analysis was conducted using SPSS 22.0 (IBM Inc., Armonk, NY, USA).

3. Results

3.1. Chromatogram of TTK Extract and Reference Standards. Extracted ion chromatogram of 4-hydroxybenzoic acid and isoferulic acid from reference standards and TTK powder is shown in Figure 2. Peak of 4-hydroxybenzoic acid is seen at 2.71 min and that of isoferulic acid is seen at 4.10 min. In TTK power, 4-hydroxybenzoic acid (RT: 2.71) and isoferulic acid (RT: 4.17) was confirmed to exist from the MS/MS spectrum, and the peak at 3.93 is expected to ferulic acid.

3.2. Effect of TTK on the Behavior Tests in L-AAA Injected Mice. The locomotor activity of mice was measured in an open field test (Figure 3(a)). There was no significant difference observed among the groups with respect to the total distance moved (F (4,42) = 1.627, P = 0.185). Thus, it was apparent that L-AAA infusion, administration of imipramine, and TTK did not affect the locomotor activity of mice.

The depressive-like behaviors of mice were measured by the sucrose preference test, tail suspension test, and forced swimming test. The result of the sucrose preference test is presented in Figure 3(b). The percentage of sucrose preference was significantly reduced in the L-AAA + DW group, compared with the control group (P = 0.003). TTK at doses

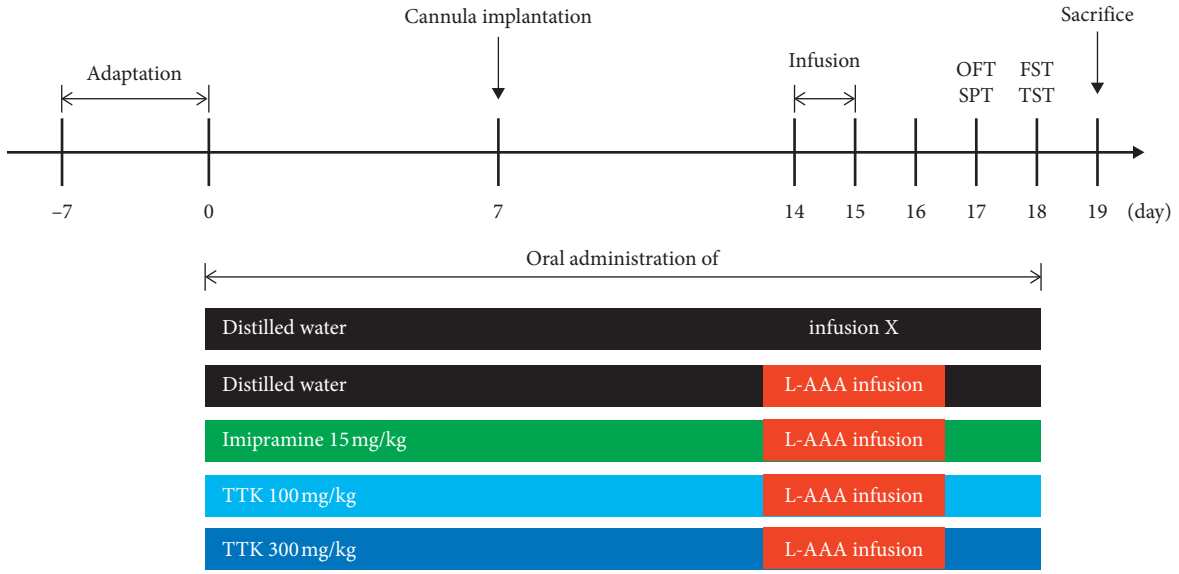

Figure 1: Experimental schedule. 50 mice were randomly divided into five groups ($n = 10$ for each group): control (sham surgery + distilled water), negative control (L-AAA injection + distilled water), positive control (L-AAA injection + imipramine 15 mg/kg), low-dose (L-AAA injection + TTK 100 mg/kg), and high dose (L-AAA injection + TTK 300 mg/kg). After 2 weeks of administration of drugs or distilled water, cannula implantation and infusion of L-AAA were performed. Subsequently, behavior tests including the open field test (OFT), tail suspension test (TST), forced swimming test (FST), and sucrose preference test (SPT) were carried out.

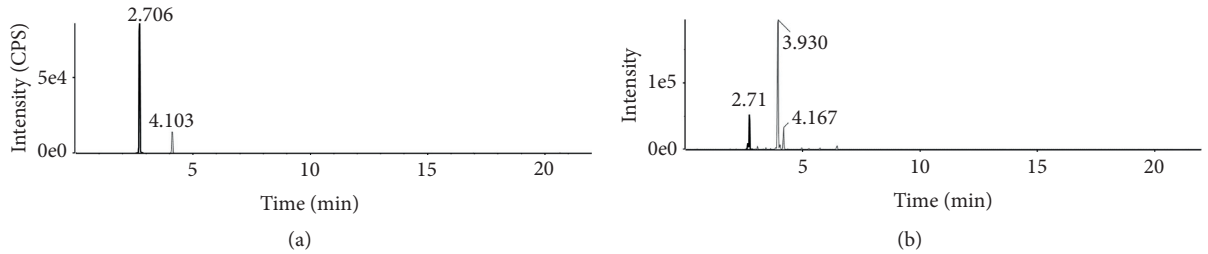

Figure 2: Extracted ion chromatogram (XICs) of 4-hydroxybenzoic acid and isoferulic acid from (a) reference standards and (b) TTK 30% ethanol extract. The peak at 2.71 is expected to 4-hydroxybenzoic acid, and the peak at 4.1 is expected to isoferulic acid. In Figure 2(b), the peak at 3.93 is expected to ferulic acid.

of 100 mg/kg ($P = 0.003$) and 300 mg/kg ($P = 0.002$) and imipramine at a dose of 15 mg/kg ($P = 0.016$) showed a significant increase in sucrose preference compared with the L-AAA + DW group.

Figures 3(c) and 3(d) show the effect of L-AAA infusion on the immobility times of mice in FST and TST, respectively. The L-AAA + DW group displayed significantly increased immobility time compared to the control group in FST ($P = 0.037$) and TST ($P = 0.004$). TTK at doses of 100 mg/kg ($P = 0.008$) and 300 mg/kg ($P = 0.006$) and imipramine at a dose of 15 mg/kg ($P = 0.016$) showed a significant decrease in immobility times compared with the L-AAA + DW group in FST (Figure 3(c)). TTK at doses of 100 mg/kg ($P = 0.018$) and 300 mg/kg ($P = 0.003$) significantly decreased the duration of immobility time, whereas imipramine at a dose of 15 mg/kg ($P = 0.177$) did not show a significant decrease compared with the L-AAA + DW group in TST (Figure 3(d)).

3.3. Effect of TTK on GFAP and NeuN Expression in L-AAA Injected Mice. The outcomes of immunoreactivity of GFAP in the prefrontal region are demonstrated in Figure 4. Slides from control mice displayed moderate GFAP immunoreactivity in the prefrontal region (Figure 4(a)), whereas slides from the L-AAA + DW group revealed lowered immunoreactivity patterns (Figure 4(b)). Notably, slides from mice treated with TTK and imipramine also displayed a moderate

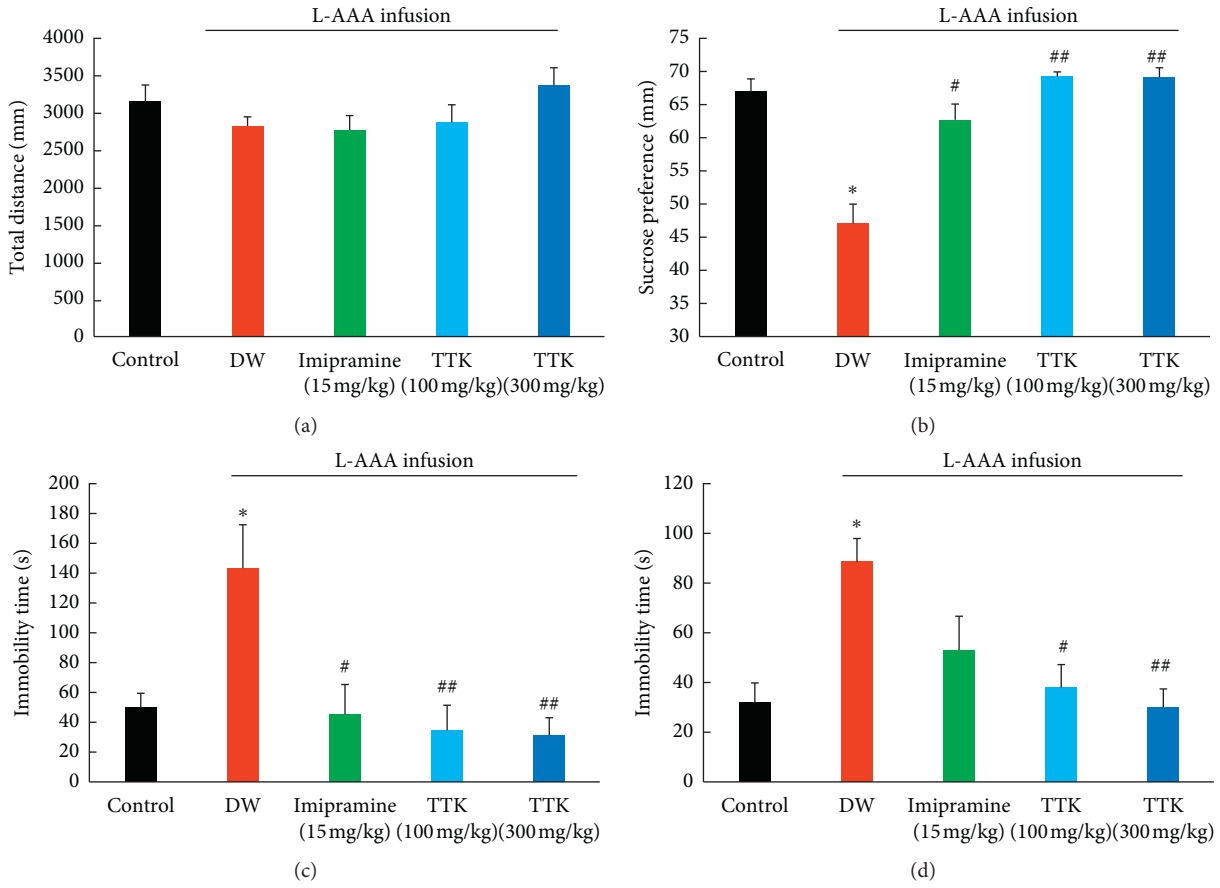

FIGURE 3: Effect of TTK in the behavior test. (a) Total distance in the open field test (OFT), (b) sucrose preference in the sucrose preference test (SPT), (c) immobility time in the forced swimming test (FST), and (d) immobility time in the tail suspension test (TST). $^{*}P<0.05$ as compared with the control group; $^{\#}P<0.05$ as compared with L–AAA+DW group; $^{\#\#}P<0.01$ as compared with L–AAA+DW group.

level of GFAP immunoreactivity in the prefrontal region (Figures 4(c)–4(e)), similar to the slides prepared from the control mice.

The outcomes of immunoreactivity of NeuN in the prefrontal region are shown in Figure 5. Slides from all mice displayed moderate NeuN immunoreactivity in the prefrontal region. There was no significant difference observed in the NeuN expression among the groups.

3.4. Effect of TTK on TNF-α in L-AAA Injected Mice. The protein level of TNF-α determined by ELISA is shown in Figure 6. The protein level of TNF-α in the prefrontal cortex significantly increased in the L-AAA + DW group compared to the control group. In contrast, the protein level of TNF-α in the prefrontal cortex of mice treated with both low dose and high dose of TTK and imipramine were significantly lower than that of mice treated with DW.

4. Discussion

The major finding of our study is that TTK can alleviate the depressive symptoms induced by the astroglial degeneration model of depression in mice. Mice treated with TTK showed an increased activity and sucrose preference in behavior tests, compared with those treated with DW. TTK appeared to protect the decrease of GFAP expression induced by L-AAA injection in prefrontal cortex. Therefore, our results indicate that TTK may offer a potential alternative treatment for treating depressive disorders.

Increased immobility times in the TST and FST of L-AAA-infused mice reflect a state of despair observed in depression. Their decreased sucrose preference also reflects a loss of interest. In the present study, TTK imparted protection in sucrose preference in SPT and decreased immobile times in FST and TST without affecting mouse locomotor activity. Pathology of astrocytes contributes significantly to major depressive disorders [33, 34]. In the prefrontal cortex, astrocytes regulate glutamate levels, and blockade of astrocytic glutamate uptake is related to symptoms of anhedonia [35]. Also, low adenosine triphosphate abundance released by astrocyte in the brain modulates the symptoms of despair presented by increased immobility time [36]. Infusion of L-AAA in the prefrontal cortex of mice also reported to decrease the brain levels of induced glutamate and glutamine [37] and not only induce astrocytic degeneration.

These results from this study show that TTK attenuated the depressive-like symptoms produced by the astroglial degeneration model of depression. After the glial ablation model of depression was developed [18], the antidepressant-

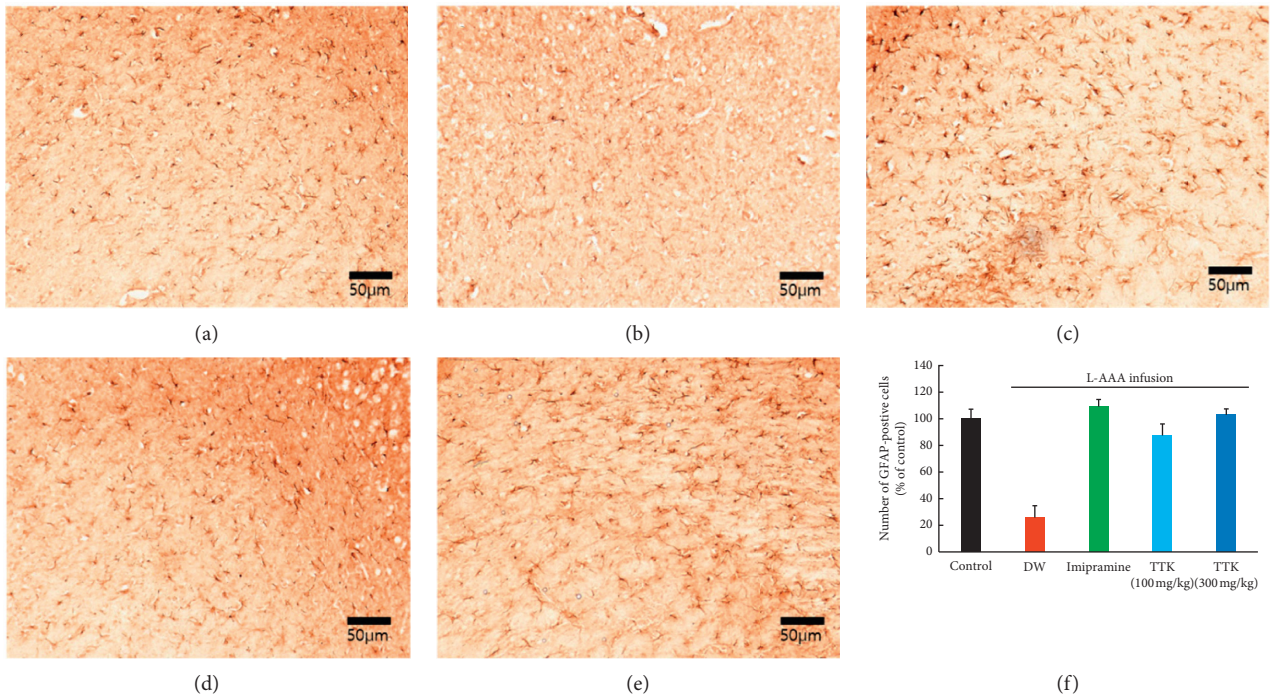

Figure 4: Effect of TTK on expression of GFAP-positive cells in the prefrontal cortex of mice treated with L-AAA as determined by immunohistochemistry. Representative coronal sections of the mice brain in (a) sham surgery + distilled water, (b) L-AAA + distilled water, (c) L-AAA + imipramine (15 mg/kg), (d) L-AAA + TTK (100 mg/kg), and (e) L-AAA + TTK 300 mg/kg.

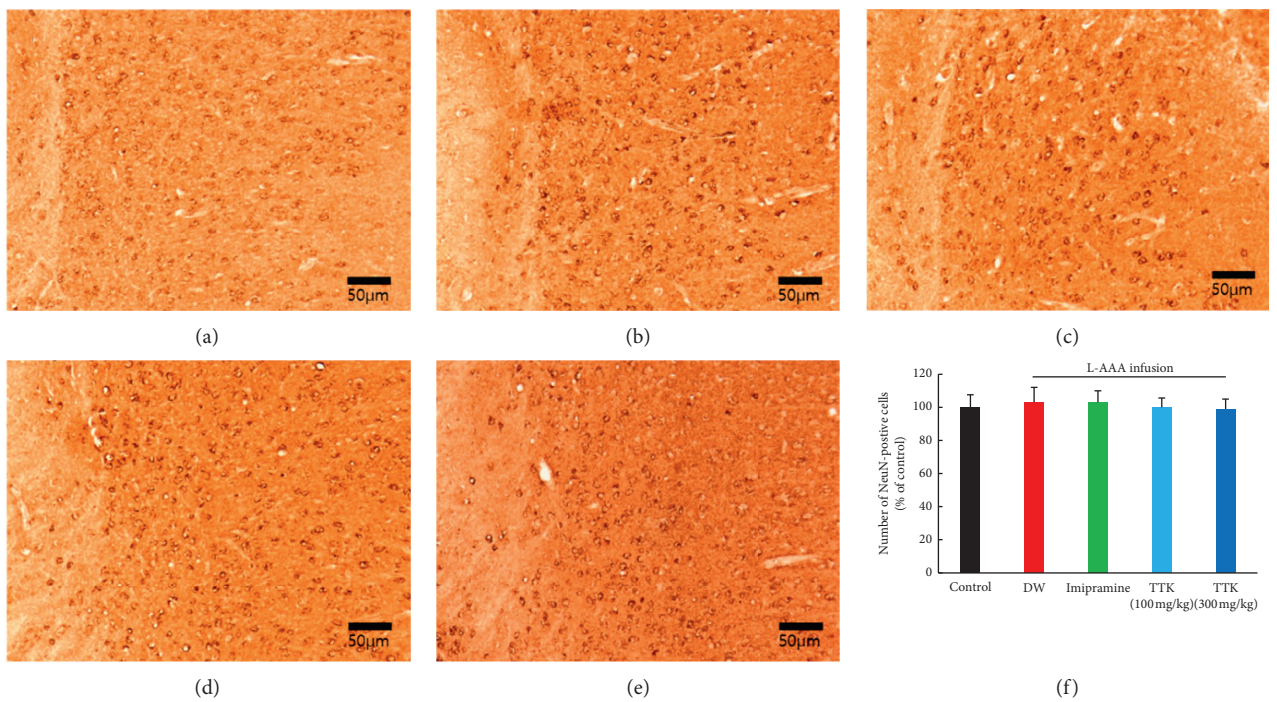

Figure 5: Effect of TTK on expression of NeuN-positive cells in the prefrontal cortex of mice treated with L-AAA as determined by immunohistochemistry. Representative coronal sections of the mice brain in (a) sham surgery + distilled water, (b) L-AAA + distilled water, (c) L-AAA + imipramine (15 mg/kg), (d) L-AAA + TTK (100 mg/kg), and (e) L-AAA + TTK 300 mg/kg.

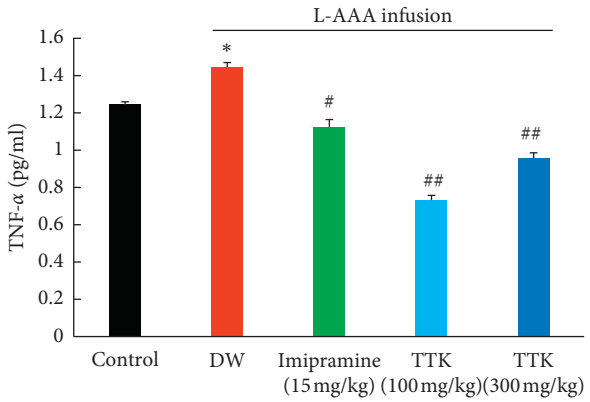

FIGURE 6: Effect of TTK on the level of TNF-α in the prefrontal cortex of mice treated with L-AAA. $^{*}P<0.05$ as compared with the control group; $^{\#}P<0.05$ as compared with L−AAA+DW group; $^{\#\#}P<0.01$ as compared with L−AAA+DW group.

like effect of mGluR5 antagonist MTEP [38], prefrontal cortex deep brain stimulation [39], Y5 receptor antagonist Lu AA33810 [40], Harmine [41], ZL006 [42], and ginsenoside Rf [43] were tested. In a manner similar to a previous study, infusion of L-AAA induced depressive-like behavior, and administration of imipramine [38] reversed the depressive behavior and led to a decrease in the number of GFAP-positive cells. In this study, we demonstrated that L-AAA infusion induced a decreased expression of GFAP in the prefrontal region, which was ameliorated with the administration of TTK. TTK imparted a protective effect against the reduction of GFAP expression induced by L-AAA infusion. Numerous evidences demonstrate that a decrease in the number of glial cells in the prefrontal region reflects the pathology of depression, and it is encouraging to note that treatment with TTK recovered the glial ablation immediately.

The antidepressant effect of TTK is presumably related to inhibition of neuroinflammation. In this study, TTK reduced the level of TNF-α, which was similar to the previous report on the effect of TTK in estrogen-deficient rats [26]. TNF-α is one of the key proinflammatory cytokines, which is relevant to pathogenesis of depression [44, 45] through activating hypothalamopituitary-adrenocortical (HPA) axis. Administration of TNF-α was reported to induce depressive-like behavior in mice, which was diminished by treatment with typical antidepressants such as fluoxetine and imipramine [46]. The imipramine and fluoxetine also reduced the production of TNF-α both in human patients and in the animal model of depression [47–49]. Also, in the astroglial degeneration model of depression, L-AAA-injected mice showed elevated TNF-α [50]. The reversal of TNF-α by TTK suggests that TTK may elicit the antidepressant-like effect by participating in anti-inflammatory activity.

In the previous study, 1% and 2% TTK 70% ethanol extract decreased the immobility time in ovariectomized rats [27]. In this study, oral administration of 100 mg/kg and 300 mg/kg 30% ethanol extracts showed the antidepressant-like effect in the L-AAA induced depression model. Typically, 300 mg/kg TTK seems to be superior in relieving the depressive behavior compared to 100 mg/kg TTK in the behavior test, indicating a dose-dependent response. Ferulic acid is one of the active components of the extract from TTK [51]. Recently, the antidepressant-like effects of ferulic acid have been reported in various models of depression [41, 52–55]. As far as underlying mechanisms are considered, activity in response to neuroinflammation in the prefrontal cortex [53] and elevation of the neurotrophic factor in the prefrontal cortex and hippocampus [41] have been reported. In this study, TTK reversed depression-like behavior and protected the glial ablation. Ferulic acid may be the major component of TTK responsible for exerting an antidepressive effect, and quantitative comparison between the effect of single compound and extract of TTK needs further investigations.

There are several limitations to our study. First, the antidepressant-like effect of the new natural product was only tested in the astroglial degeneration model of depression. As the transitory glial ablation effect of L-AAA lasts for three days [18, 56], the glial ablation model of depression was used to test the acute effect of the drugs. In this study, after infusion of L-AAA, drug administration was performed twice before the conduction of behavior tests. We designed this study to test the preventive and acute effect of TTK. Further research studies are required to explore the long-term effect of TTK in another depression model such as the chronic unpredictable stress model. Second, the mechanism behind the antidepressive effect of TTK was not examined enough in this study. Considering the previous studies about the antioxidant and anti-inflammatory effects of TTK, the effect of TTK on another antidepressant mechanism in prefrontal cortex related to depression is also expected. Third, as TTK is the natural product, which contains various compounds, the active compound involved in exhibiting the antidepressive effect should be proved in the further research studies.

5. Conclusions

The present study demonstrates the potential of TTK in treating depressive symptoms and the alterations in the brain of the animal model of depression. TTK protected the mouse brain glial loss in the prefrontal cortex induced by a gliotoxin, L-AAA. It is suggested that TTK may be one of the potential candidates for treating depression. Further research studies are required to understand the effect of TTK on depressive disorders.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Yujin Choi and Yunna Kim equally contributed to this work.Yujin Choi: Conceptualization, Investigation, Data curation, Formal analysis, Writing original draft; Yunna Kim: Conceptualization, Investigation, Data curation, Formal analysis, Writing review & editing; Hwa-young Lee: Conceptualization, Investigation, Writing review & editing; Seung-Hun Cho: Conceptualization, Supervision, Writing review & editing.

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Research Article

Xiaoyaosan Exerts Therapeutic Effects on the Colon of Chronic Restraint Stress Model Rats via the Regulation of Immunoinflammatory Activation Induced by the TLR4/NLRP3 Inflammasome Signaling Pathway

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Depression is the neurological manifestation most commonly associated with gastrointestinal diseases. The release of inflammatory cytokines mediated by TLR4/NLRP3 inflammasome signaling-induced immunoinflammatory activation may represent a common pathogenic process underlying the development of gastrointestinal diseases and depression. Clinical studies have indicated that Xiaoyaosan (XYS) can relieve depressive behavior by improving gastrointestinal symptoms. We previously demonstrated that XYS can reduce colonic inflammation in a rat model of chronic unpredictable mild stress; however, the precise anti-inflammatory mechanisms involved remain unclear. Here, we investigated whether XYS can ameliorate depressive behavior through regulating the TLR4/NLRP3 inflammasome signaling pathway, thereby inhibiting immunoinflammatory activation and reducing colonic proinflammatory cytokine levels. Fifty-two healthy male Sprague–Dawley rats were randomly divided into four groups (control, model, XYS, and fluoxetine). The latter three groups were subjected to 21 days of chronic restraint stress to generate a model of stress-induced depression. XYS and fluoxetine were administered intragastrically. Behavioral changes in the rats were assessed after 21 days. Serum and colon samples were collected, and the relative levels of the inflammation indicators IL-6, IL-1β, and TNF-α were determined by ELISA. Pathological changes in colon tissue were assessed by hematoxylin and eosin staining. The levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 were detected by immunohistochemistry, while the gene and protein expression levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, NLRP3, ASC, and caspase-1 were detected by quantitative polymerase chain reaction (qPCR) and Western blotting. The results indicated that XYS could improve the depressive-like behavior and the weight loss of rats with stress-induced depression. Furthermore, depressed rats treated with XYS exhibited decreased expression levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, NLRP3, ASC, and caspase-1 in colonic tissue; reduced colon and serum concentrations of the inflammatory factors IL-6, IL-1β, and TNF-α; and lowered levels of colonic inflammation.

1. Introduction

Depression is the leading cause of disability worldwide and a major contributor to the overall global disease burden. According to a recent World Health Organization (WHO) report, between 2005 and 2015, 322 million people suffered from depression worldwide and the number of affected patients increased by 18.4% [1]. The main clinical feature of these patients is significant and persistent depression, which may be associated with a high incidence of comorbid somatic symptoms such as gastrointestinal motility disorder and insomnia. Depression can impair the function of certain parts of the gastrointestinal tract, thereby increasing the risk of gastrointestinal complications. Furthermore, depression is the neurological manifestation that is most commonly associated with gastrointestinal diseases, such as irritable bowel syndrome and inflammatory bowel disease [2, 3]. Indeed, several studies have shown that the prevalence of mental disorders in patients with gastrointestinal symptoms is approximately 60–85% [4–6].

It has been suggested that depression may represent a chronic low-level inflammatory response involving neuroimmune-endocrine factors [7]. An increasing number of studies have found that specific depressive symptoms, particularly somatic symptoms, are associated with changes in inflammation-related immune system components [8]. Human immunity is divided into natural (innate) and adaptive immunity. The former occurs via pattern recognition receptors (PRRs), which recognize highly conserved pathogen-associated molecular patterns (PAMPs) and produce corresponding immune responses. Three types of PRRs have been identified, namely, Toll-like receptors (TLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs) [9]; of these, TLR4 and the inflammasome-associated NOD-like receptor pyrin domain containing 3 (NLRP3) are most closely linked to depression.

Studies have indicated that TLR4 activation and the inflammatory factors it mediates are closely related to several depressive symptoms and may be directly linked to their occurrence and development [10]. Long-term chronic stress can activate NLRP3, producing a corresponding inflammatory response that contributes to the pathogenesis of depression [11, 12]. TLRs are important mediators of innate immune responses in the intestinal mucosa and play an important role in maintaining microecological homeostasis in the intestinal tract [13]. Recent studies have shown that NLRP3 is involved in maintaining the stability of the intestinal environment and is also closely related to the occurrence and development of inflammatory bowel disease [14].

In humans, the immune system is the first line of defense against both exogenous and endogenous threats, responding rapidly to attack through the regulation of the levels of both proinflammatory and anti-inflammatory factors. The accumulation of inflammatory cytokines can lead to depression, as well as other psychiatric and gastrointestinal diseases, and reducing the levels of these factors can show good therapeutic effects. In summary, the release of inflammatory cytokines mediated by TLR4/NLRP3 inflammasome signaling pathway-induced immunoinflammation may represent a common pathogenic mechanism underlying the development of gastrointestinal diseases and depression.

Xiaoyaosan (XYS), a classic agent used in traditional Chinese medicine (TCM), was first described by the "Taiping Huimin Heji Jufang" of the Song Dynasty (960–1127 AD). XYS is a decoction composed of the following eight commonly used Chinese herbs: Radix Angelicae Sinensis, Radix Paeoniae Alba, Poria, Radix Bupleuri, Radix Glycyrrhizae, Rhizoma Atractylodis Macrocephalae, Herba Menthae, and Rhizoma Zingiberis Recens. XYS has been used in clinical practice for more than 1,000 years in China and is commonly used to treat diseases such as depression, functional dyspepsia, hepatitis, cirrhosis, gastroduodenal ulcer, chronic gastritis, premenstrual tension, and perimenopausal syndrome [15]. Pharmacological studies have shown that XYS can protect the liver, enhance gastrointestinal peristalsis, regulate the central nervous system (CNS) and endocrine function, and help resist stress [16]. A recent analysis of the use of antidepressants indicated that more than 55% of the Chinese medicines used for treating depression targeted the spleen-stomach meridian [17]. For instance, the Radix Acanthopanacis Senticosi capsule can reportedly relieve colitis and improve depressive symptoms [18]. As depression has been reported to induce injury in the colon through oxidative stress and inflammation, we chose depression-induced colitis as a target in this study [18]. We have previously demonstrated that XYS treatment can reduce colonic inflammation in a rat model of chronic unpredictable mild stress (CUMS) [19]; however, the underlying anti-inflammatory mechanism remains unclear. In this study, we hypothesized that XYS could improve depressive behavior by regulating the TLR4/NLRP3 inflammasome signaling pathway, thereby inhibiting immunoinflammatory activation and, consequently, reducing the levels of inflammatory cytokines in the colon.

2. Materials and Methods

2.1. Animals and Grouping. Healthy, specific pathogen-free male Sprague–Dawley rats (license no. SCXK (YUE) 2013-0001) weighing 200 ± 20 g were purchased from the Experimental Animal Center of Guangzhou University of Traditional Chinese Medicine. The rats were maintained in plastic cages at a room temperature of $22 \pm 2^{\circ}$C and a relative humidity of $35 \pm 5\%$. The rats were allowed to drink deionized water and eat conventional food ad libitum. After 7 days of adaptive feeding, the rats were randomly divided into 4 groups: a control group, a chronic restraint stress model group, an XYS treatment group, and a fluoxetine treatment group, with 13 rats allocated to each group. The animal experiments were approved by the Ethics Committee of the Guangzhou University of Traditional Chinese Medicine (ethical protocol no. 20180829001) and conformed with the guidelines of the National Institutes of Health for the Care and Use of Animals.

2.2. Drug Preparation and Intervention. The XYS prescription consisted of eight herbal medicines, as shown in Table 1. XYS Chinese herbal pieces were provided by Beijing Tongrentang (Bozhou) Pieces Co., Ltd. (Bozhou, China). The

Table 1: Composition of Xiaoyaosan.

English name	Chinese name	Latin name	Scientific name	Part used
Chinese thorowax root	Chai Hu	Radix Bupleuri	*Bupleurum chinense* DC	Root
Chinese angelica root	Dang Gui	Radix Angelicae Sinensis	*Angelica sinensis* (Oliv.) Diels	Root
White peony root	Bai Shao	Radix Paeoniae Alba	*Paeonia lactiflora* Pall.	Root
Large head atractylodes rhizome	Bai Zhu	Rhizoma Atractylodes Macrocephalae	*Atractylodes macrocephala* Koidz.	Rhizoma
Poria cocos	Fu Ling	Poria	*Poria cocos* (Schw.) Wolf	Sclerotia
Fresh ginger rhizoma	Sheng Jiang	Zingiberis Recens	*Zingiber officinale* Rosc.	Rhizoma
Peppermint	Bo He	Herba Menthae	*Mentha haplocalyx* Briq.	Stems and leaves
Liquorice root	Gan Cao	Radix Glycyrrhizae	*Glycyrrhiza uralensis* Fisch.	Root

XYS dry powder was prepared by Jiuzhitang Co., Ltd. (Changsha, China) in accordance with the process described in the 2015 edition of the "Chinese Pharmacopoeia" [20]. The Xiaoyaosan dry powder was prepared by mixing 100 g of *Bupleurum chinense*, 100 g of *Paeonia lactiflora*, 100 g of *Angelica sinensis*, 100 g of fried *Atractylodes macrocephala*, 100 g of *Poria cocos*, 80 g of *Glycyrrhiza uralensis*, 20 g of mint, and 100 g of ginger. We previously detected eight compounds in XYS by high-performance liquid chromatography–mass spectrometry (HPLC–MS/MS), and the results suggested that the eight compounds might be XYS quality control references [21]. The fluoxetine hydrochloride tablets used in this experiment were purchased from Patheon (France) and packaged by Lilly Suzhou Pharmaceutical Co., Ltd. (20 mg/tablet, Suzhou, China).

Treatments were administered orally to the rats in each group 30–60 min before modeling. The drug dosage was determined according to the mean adult bodyweight (at 60 kg/day). The volume of the gavage solution was 1 mL/100 g of bodyweight. Fluoxetine was administered intragastrically at a dose of 2 mg/(kg·day^{-1}). The XYS dosage was 2.224 g/(kg day^{-1}). The control group and the model group received an equal volume of deionized water.

2.3. Quality Control of XYS. XYS quality control was carried out according to the 2015 edition of the Chinese Pharmacopoeia [20]. As the pharmacopoeia standard states that 1 g of XYS should contain at least 4 mg of paeoniflorin, HPLC was used to detect the content of paeoniflorin in XYS as follows: 1 g of XYS was put into 25 mL of 70% methanol, sonicated for 30 min, and filtered through a 0.22 μm filter. The chromatographic column used was an Agilent ZORBAX Eclipse XDB C-18 (250 × 4.6 mm, 5 μm), and the column temperature was set at 350°C. The chromatographic conditions were A: 0–5 min 0.1%–70% methanol, 5–40 min 70%–10%, 40–45 min 10%–0%, and 40–50 min 0%; B: 0–5 min 0.1%–30% methanol, 5–40 min 30%–90%, 40–45 min 90%–100%, and 40–50 min 100%. The detection wavelength was 230 nm, the injection volume was 10 μL, and the flow rate was 1 mL/min. The paeoniflorin content was 5.3299 mg/g, and the retention time was 10.078 min, which conformed to the quality standard stipulated in the Pharmacopoeia (Figure 1).

2.4. Chronic Immobilization Stress (CIS) Modeling Procedure. Chronic restraint stress modeling was performed as previously described [22]. Rats in the model, XYS, and fluoxetine groups were bound to a special restraint frame. Two soft bands were attached to the chest and abdomen of each rat. The rats were then placed in a feeding box for 3 h/day. The restraint time was random, and the procedure was performed for 21 consecutive days. Rats in the normal group were fed in their respective feeding boxes for 21 consecutive days.

2.5. Bodyweight, Sugar Preference Test, Open-Field Test, and Forced Swim Test. The bodyweight of the rats in each group was determined at 08:00 every day using an electronic scale, and the mean weight of the rats in each group on days 0, 7, 14, and 21 was calculated and compared.

The analysis of sugar consumption was carried out on days 0 and 21 of modeling. Before the experiment, the rats in each group were fed in a single cage. Two bottles containing a 1% sucrose solution were placed in each cage, and the rats were allowed to drink freely for 24 h. Next, one bottle containing a 1% sucrose solution and one containing pure water were placed in each cage, and the rats were allowed to drink freely for 24 h. Then, the rats were deprived of food and water for 24 h. During the experiment, one bottle containing a 1% sucrose solution and one containing pure water were placed in each cage, and the rats were allowed to drink freely for 3 h. The weight of the sucrose solution and the pure water before and after the experiment was then recorded. The rate of sugar water consumption was calculated as sugar water consumption/total liquid consumption×100%. This experiment was carried out to evaluate the degree of loss of pleasure.

The open-field test (OFT) was performed on days 0 and 21 of the experiment. An open-field box was placed in the middle of the test room, and a camera was placed above the center grid and connected to a computer. Measurements were taken under quiet conditions. Before the experiment, rats were placed in the dark environment of the test room for 10 min to acclimatize. The operator then grasped the tail root of a rat and placed the rat in the middle compartment of the open-field box. Recording and timing were then synchronized. The time of residence in the central grid, the total number of passes, and the number of entries into the central

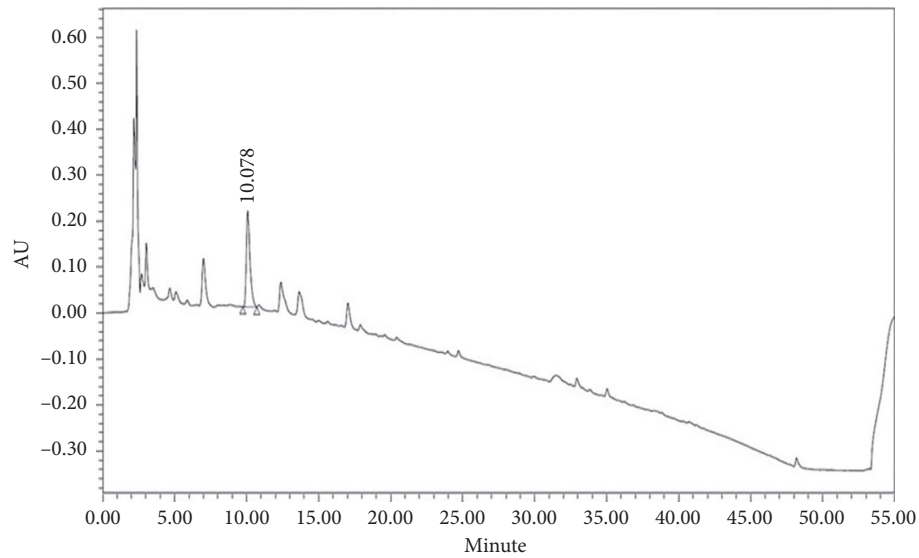


Figure 1: Quality control of Xiaoyaosan (quantitative paeoniflorin analysis).

zone were recorded within a 5 min period. After 5 min, the rats were removed, and the bottom of the box was wiped thoroughly with a towel dipped in clean water containing a low concentration of alcohol. The next rat was then observed.

The device for the forced swim test (FST) consisted of a transparent acrylamide resin barrel 40 cm high and 30 cm in diameter. The rats were placed in the barrel with water (23–25°C) to a depth of 32 cm to ensure that their hind limbs could not touch the bottom of the barrel. On the first day of the experiment, all the rats were subjected to a 15 min swim, dried, and then allowed to continue feeding. After 24 h, the forced swimming test was conducted for 5 min, and the immobility time (characterized as floating on the water surface, immobility, or making small movements) was recorded and analyzed. After each test, the barrel was filled with clean water to avoid cross fecal and olfactory interference.

2.6. Sample Collection. All the animals were euthanized on day 21 (after behavioral testing). Then, 1 cm of colon tissue (10 cm from the anus) was removed and immediately rinsed with normal saline and fixed in 10% formaldehyde. The colon tissue was then embedded in paraffin wax, sectioned to a thickness of 4 μm, and the sections were placed on poly-lysine-coated glass slides. Two other 1 cm colonic tissue samples were placed in an EP tube and stored at −80°C for later analysis. Blood samples (2-3 mL) were collected from the abdominal aorta, left at room temperature for 10–20 min, and then centrifuged at 3,500× g for 20 min at 4°C. The serum collected after centrifugation was then filtered, sterilized, inactivated, and stored at −20°C for subsequent analysis.

2.7. ELISA Analysis. The colon and serum levels of IL-1β (JER-01), TNF-α (JER-06), and IL-6 (JER-04) were determined using commercial ELISA kits (Joyee Biotechnics Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Absorbance was detected at 450 nm using a microplate reader (BioRad, USA). Finally, a standard curve was generated to determine the concentration of each factor.

2.8. Hematoxylin and Eosin Staining. Colonic tissue samples were fixed in a 10% formalin solution, decalcified, dehydrated, made transparent, and finally embedded in paraffin. Then, 5 μm-thick tissue sample sections were prepared using a microtome. The sections were dewaxed with xylene, passed through an ethanol series to water, and finally stained with hematoxylin and eosin (H&E). The results were assessed under a microscope.

2.9. Immunohistochemical Analysis. Immunohistochemistry was used to determine the localization of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 in colonic tissue. Colonic tissue samples were fixed in 4% paraformaldehyde for 30–60 min, washed with phosphate-buffered saline (PBS) twice for 2 min, and dehydrated at 5°C. The tissues were then embedded in paraffin, sliced into 4 μm-thick sections, and the sections were placed onto glass slides. Endogenous enzymes were inactivated using 3% hydrogen peroxide (H_2O_2) after routine dewaxing to water. The sections were then incubated with 0.01 mol/L citrate for antigen retrieval and blocked in a 3% bovine serum albumin (BSA) solution for 30 min. Subsequently, the sections were incubated with primary antibodies targeting TLR4 (Zen, 1 : 100), MyD88 (Zen, 1 : 100), NF-κB-p65 (Zen, 1 : 100), TAK1 (Zen, 1 : 100), IRAK1 (PTG, 1 : 250), and TRAF6 (Zen, 1 : 100) overnight at 4°C in a humid box. The sections were then slightly dried and incubated with an HRP-conjugated secondary antibody against the corresponding species and genera at room temperature for 50 min. The slides were then washed four times with PBS, 5 min each wash, followed by DAB and nuclear staining. Finally, the slides were washed in distilled water, dehydrated, made transparent, and sealed with resin. The sections were observed under a microscope. Cells with

brown granules were classified as positively stained. Immunohistochemical staining integrated optical density (IOD) was analyzed using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA). At least three random sections from each group were analyzed, and 200 fields of view were imaged whilst ensuring that the background lighting was consistent. Image-Pro Plus 6.0 software was then used to select the same brown-yellow color as a unified criterion for judging the positivity on all photographs. The positive IOD of each image was then determined.

2.10. RNA Isolation and RT-qPCR Analysis. First, 20–50 mg of tissue was placed into a mortar and quickly ground with a small amount of liquid nitrogen. Once the tissue had softened, more liquid nitrogen was added, and the procedure was repeated three times. Then, 1 mL of TRIzol was added, and the sample was transferred to a centrifuge tube and left at room temperature for 5 min. The sample was then centrifuged at 12,000 ×g for 10 min at 4°C. The supernatant was removed, 200 μL of chloroform was added, and the tube was capped, followed by vigorous shaking for 15 s. The sample was left at room temperature for 5 min and then centrifuged at 12,000 ×g for 15 min at 4°C. The colorless upper water phase was then removed (400 μL) and added to 500 μL of isopropanol. The tube was capped, and the contents were mixed by inversion 10 times; the tube was then left at room temperature for 10 min. Samples were then centrifuged at 12,000 ×g for 10 min at 4°C. The supernatant was discarded, and 1 mL of 75% ethanol was added. The sample was then washed and centrifuged at 7,500 ×g for 5 min at 4°C. The supernatant was removed and the sample air-dried. Finally, the RNA was dissolved in 30 μL of RNase-free water. Then, 4 μL of RNA was diluted 25-fold and used to determine the RNA concentration and purity.

qPCR was performed using the Brilliant III Ultra-Fast SYBR Green QPCR Master Mix Kit (Agilent Technologies) in a Stratagene Mx3000P Real-Time PCR System (Agilent Technologies). The reaction mixture included 10 μL of 2x SYBR Green QPCR Master Mix, 3.7 μL of RNase-free water, 0.3 μL of control fluorescent dye, 2 μL of each forward and reverse primer, and 2 μL of cDNA. The primer sequences (Generay Biotechnology Company, Shanghai, China) are listed in Table 2. The reaction conditions were as follows: an initial denaturation of 95°C for 3 min, followed by 40 cycles of denaturing at 95°C for 5 s and annealing at 60°C for 1 min. After cycling, a dissolution curve was measured under the following conditions: 95°C for 15 s, 55 °C for 1 min, and 95°C for 30 s. During heating from 55°C to 95°C, fluorescence was measured at each 0.5°C step. The amplification efficiency of the primers was measured using a serially diluted cDNA template. qPCR was performed for samples from each group. Reaction specificity was evaluated by analyzing the dissolution curve, and quantitative analysis of the fluorescence of the PCR products was carried out using the $2^{-\Delta\Delta CT}$ method.

2.11. Western Blot Analysis. The levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, NLRP3, ASC, and caspase-1 were quantified by Western blotting. First, proteins were extracted by cutting and grinding tissue samples in RIPA lysis buffer. The lysates were then centrifuged, and the supernatants were collected. Protein concentrations were determined using a BCA protein quantitation kit (GeneCopoeia). Proteins were then separated by SDS–PAGE and subsequently transferred onto methanol-pretreated PVDF membranes (Millipore Corporation, USA). The membranes were then blocked with 5% skimmed milk powder at room temperature for 1 h and incubated with the following antibodies: anti-TLR4 rabbit pAb (Zen, 1 : 1,000); anti-TRAF6 rabbit pAb (Zen, 1 : 1,000); anti-TAK1 rabbit pAb (Zen, 1 : 1,000); anti-NF-κB-p65 mAb (Zen, 1 : 1,000); anti-MYD88 rabbit pAb (Zen, 1 : 800); anti-IRAK1 (PTG, 1 : 1,000); anti-ASC (Bioss, 1 : 1,000); anti-NLRP3 (Bioss, 1 : 1,500); and anti-caspase-1 (Bioss, 1 : 800). The membranes were subsequently incubated with the appropriate secondary antibodies for 1 h (HRP-conjugated goat anti-rabbit IgG (Ubio, 1 : 5,000) and HRP-conjugated goat anti-mouse IgG (Ubio, 1 : 5,000)). GAPDH and β-tubulin (Ubio) were used as reference proteins.

2.12. Statistical Analysis. Data are expressed as means ± standard deviation (SD). SPSS 25.0 (SPSS Inc., Chicago, IL, USA) was used to test the data for normality and homogeneity of variance. Repeated measurement data were first analyzed by repeated-measures analysis of variance (ANOVA). The remaining data were analyzed using one-way ANOVA. The least significant difference (LSD) method was used for comparison. The artwork was created using GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. The Effect of XYS on the Bodyweight of Rats with CIS. On days 0 and 7, no difference in bodyweight was observed across the four groups. However, from day 14, the bodyweight of the XYS and fluoxetine groups showed a significant increase when compared with the model group; this difference was also evident on day 21 (day 14: $p < 0.01$, $F = 31.471$; day 21: $p < 0.01$, $F = 70.129$). The results showed that, from day 14, chronic stress led to significant weight loss in the three groups. However, compared with the model group, XYS and fluoxetine treatments alleviated the weight loss (Figure 2).

3.2. The Effect of XYS on the Depressive-Like Behavior of Rats with CIS. To evaluate depressive-like behaviors, we carried out several behavioral tests, including a sucrose preference test (SPT), an OFT, and a FST. On day 0, the sugar preference rate was not significantly different across the four groups ($p = 0.396$, $F = 1.027$). However, on day 21, the consumption rate in the model group was significantly lower than that of the XYS, fluoxetine, and control groups ($p < 0.01$, $F = 51.385$), suggesting that the animals in the model group were experiencing a marked loss of pleasure. This indicated that the model had been successfully established (Figures 3(a) and 3(b)). The results of the FST showed that the immobility time of the rats in

Table 2: Primer sequences used for RT-qPCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Irak1	TCCTAACAGAGGTGGAACAG	GATCCTCTAAGGAGCCATTG
Myd88	GTCGCATGGTGGTGGTTGTT	GGATCAGTCGCTTCTGTTGG
NF-κB-p65	AGCTATAACTCGCCTGGTGA	ATGTCCGCAATGGAGGAGAA
Tak1	GTATGGAGCCTGCTTGAATC	GGCATGAGCAGCAGTGTAAT
Tlr4	CAGAGCCGTTGGTGTATCTT	AGGCGATACAATTCGACCTG
Traf6	GAAGCACAGCAGTGTAATGG	CAGGTCTGCCTGTGTAGAAT
Gapdh	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA
Nlrp3	CGAGGTCTCTTCTCAAGTCT	CCAACCACAGTCTCTGAATG
Asc	GCACAGCCAGAACAGAACAT	AACTGCCTGGTACTGTCCTT
Casp1	CCTGTGCGATCATGTCACTA	AGCTGATGGACCTGACTGAA

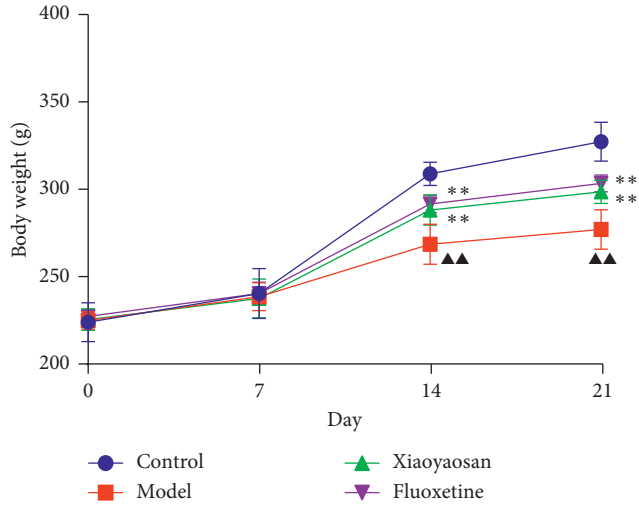


Figure 2: The bodyweight for each group. Data are expressed as means ± SD, $n = 8$. $p < 0.01$, compared with the normal group; $^{**}p < 0.01$, compared with the model group.

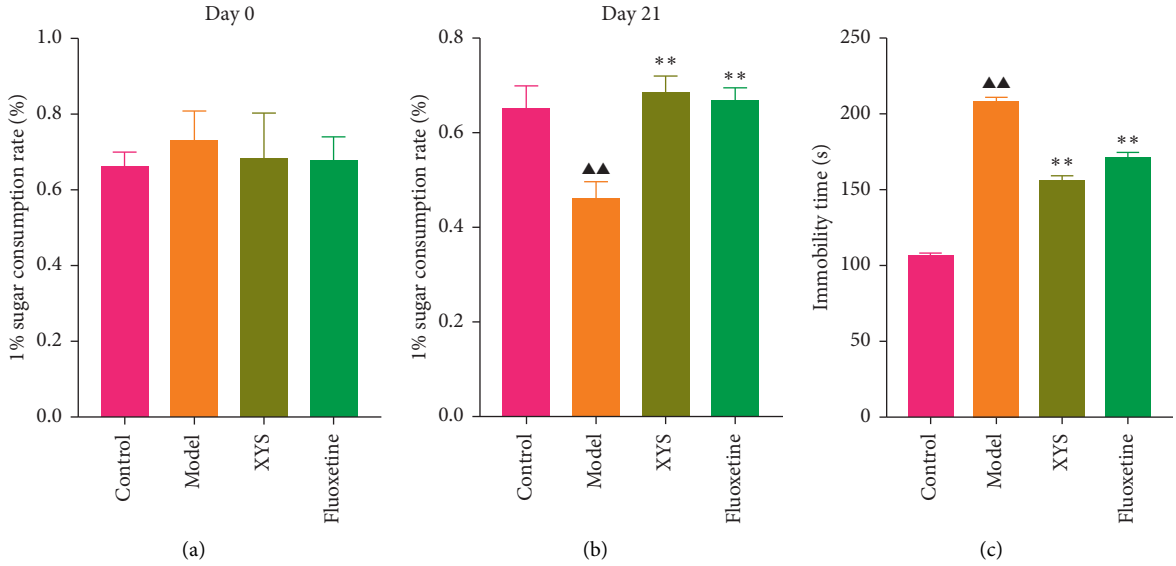


Figure 3: The sugar consumption rate (a)-(b) and forced swimming test (c). Data are expressed as means ± SD, $n = 8$. $^{▲▲}p < 0.01$, compared with the control group; $^{**}p < 0.01$, compared with the model group. XYS, Xiaoyaosan.

the depression model group was significantly increased ($p < 0.01$, $F = 35.389$), whereas that of the rats in the XYS and fluoxetine groups was decreased (all $p < 0.01$, $F = 35.389$) (Figure 3(c)). In the OFT, the total distance traveled in 5 min by the rats in the model group ($p < 0.01$, $F = 5.693$), as well as the number of times they entered the central area ($p < 0.01$, $F = 4.931$), was significantly reduced compared with that in the control group. However, compared with the model group, the total distance traveled in 5 min and the number of times the rats entered the central area increased after treatment with XYS ($p < 0.05$, $F = 4.931$) and fluoxetine ($p < 0.01$, $F = 4.931$). Fluoxetine treatment also increased the residence time in the central area ($p = 0.01$, $F = 7.455$) (Figure 4). Representative movement trails of the rats in each group during the OFT on day 21 are shown in Figure 4.

3.3. The Effect of XYS on Colon Pathology. As shown in Figure 5, the colon mucosa of the control group was intact, the epithelial cells were orderly arranged, no infiltration of inflammatory cells was seen, and the glands of the lamina propria were clear, well-arranged, and rich in goblet cells. In the model group, the colon mucosa was obviously absent, the glands in the lamina propria were damaged or absent, there were fewer goblet cells, and a large number of inflammatory cells were infiltrated. However, compared with the model group, treatment with XYS or fluoxetine could prevent epithelial hyperplasia around the colon mucosa, reduce the loss of goblet cells, decrease the infiltration of inflammatory cells, and restore basic goblet cell morphology.

3.4. The Effect of XYS on the Localization of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 in the Colon. Immunohistochemical analysis (Figure 6) showed that the colon cells of the control group were well arranged, and relatively few small brown granules, representing TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 staining, were evident in the cytoplasm. However, in the model group, the colon cell arrangement was irregular, the number of small brown granules representing the above proteins was significantly increased, and the staining was more intense. After 3 weeks of intragastric administration of XYS and fluoxetine, the expression of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 was significantly reduced in both treatment groups. Further quantitative analysis using IOD showed that the levels of TLR4 ($p < 0.01$, $F = 4.565$), MyD88 ($p < 0.01$, $F = 9.785$), NF-κB-p65 ($p < 0.05$, $F = 6.486$), TAK1 ($p < 0.01$, $F = 29.217$), IRAK1 ($p < 0.01$, $F = 4.835$), and TRAF6 ($p < 0.01$, $F = 16.399$) were significantly increased in the model group; however, XYS or fluoxetine treatment reduced the levels of TLR4 (XYS: $p < 0.05$, fluoxetine: $p < 0.01$, $F = 4.565$), MYD88 (all $p < 0.01$, $F = 9.785$), NF-κB-p65 (XYS: $p < 0.05$, fluoxetine: $p < 0.01$, $F = 6.486$), TAK1 (all $p < 0.01$, $F = 29.217$), and TRAF6 (all $p < 0.01$, $F = 16.399$); the level of IRAK1 was also decreased, although not significantly (Figure 6).

3.5. The Effects of XYS on IL-1β, IL-6, and TNF-α Levels in Serum and Colon. The levels of IL-1β, IL-6, and TNF-α were determined by ELISA. Compared with the control group, the serum levels of IL-1β, IL-6, and TNF-α in the model group displayed a significant increase ($p < 0.01$; $F = 5.007$, $F = 4.108$, and $F = 9.509$, respectively). After 3 weeks of XYS treatment, the levels of IL-1β, IL-6, and TNF-α had decreased significantly ($p < 0.01$; $F = 5.007$, $F = 4.108$, and $F = 9.509$, respectively) (Figures 7(a)–7(c)). The colon levels of IL-6, IL-1β, and TNF-α in the depression model group was significantly increased ($p < 0.01$; $F = 19.265$, $F = 38.3$, and $F = 57.968$, respectively) compared with the control group. However, XYS and fluoxetine treatment significantly reduced the secretion of IL-6, IL-1β, and TNF-α ($p < 0.01$; $F = 19.265$, $F = 38.3$, and $F = 57.968$, respectively) (Figures 7(d)–7(f)). The ELISA results showed that XYS could significantly reduce the levels of inflammatory factors in depressed rats.

3.6. The Effects of XYS on the mRNA and Protein Expression of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, NLRP3, Caspase-1, and ASC in the Rat Colon. The relative expression levels of genes coding for TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, NLRP3, caspase-1, and ASC were detected by qPCR. In this study, the mRNA levels of *Tlr4* ($p < 0.01$, $F = 25.762$), *Myd88* ($p < 0.01$, $F = 13.352$), NF-κB-p65 ($p < 0.01$, $F = 42.469$), *Tak1* ($p < 0.01$, $F = 35.626$), *Irak1* ($p < 0.01$, $F = 29.723$), *Traf6* ($p < 0.01$, $F = 4.840$), *Nlrp3* ($p < 0.01$, $F = 36.286$), caspase-1 ($p < 0.01$, $F = 22.024$), and *Asc* ($p < 0.01$, $F = 84.990$) were increased in the model group compared with the control group. However, compared with the model group, XYS treatment resulted in decreased levels of *Tlr4* ($p < 0.01$, $F = 25.762$), *Myd88* ($p < 0.01$, $F = 13.352$), NF-κB-p65 ($p < 0.01$, $F = 42.469$), *Tak1* ($p < 0.01$, $F = 35.626$), *Irak1* ($p < 0.01$, $F = 29.723$), *Traf6* ($p < 0.01$, $F = 4.840$), *Nlrp3* ($p < 0.01$, $F = 36.286$), caspase-1 ($p < 0.05$, $F = 22.024$), and *Asc* ($p < 0.01$, $F = 84.990$) mRNA in depressed rats (Figure 8(a)).

The protein expression levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 were analyzed by Western blotting. Compared with the control group, the expression levels of TLR4 ($F = 13.954$), MyD88 ($F = 8.393$), NF-κB-p65 ($F = 3.617$), TAK1 ($F = 18.689$), and TRAF6 ($F = 7.184$) were significantly increased (all $p < 0.01$) in the model group, as was that of IRAK1 ($p < 0.05$, $F = 10.157$). However, when compared with the model group, the levels of TLR4, MYD88, NF-κB-p65, TAK1, and TRAF6 were significantly decreased in the XYS treatment group ($p < 0.01$), as was that of NF-κB-p65 ($p < 0.05$). The protein levels of TLR4, TAK1, IRAK1 (all $p < 0.01$), MYD88, NF-κB-p65, and TRAF6 (all $p < 0.05$) were significantly decreased in the fluoxetine treatment group compared with the model group.

The expression levels of NLRP3, ASC, and caspase-1 were also assessed by Western blotting. Compared with the control group, the expression levels of NLRP3, caspase-1 (both $p < 0.01$), and ASC ($p < 0.05$) were significantly increased in the model group. However, compared with the model group, the protein expression levels of the three proteins were significantly decreased (NLRP3 and ASC: $p < 0.01$; caspase-1: $p < 0.05$) in the XYS treatment group.

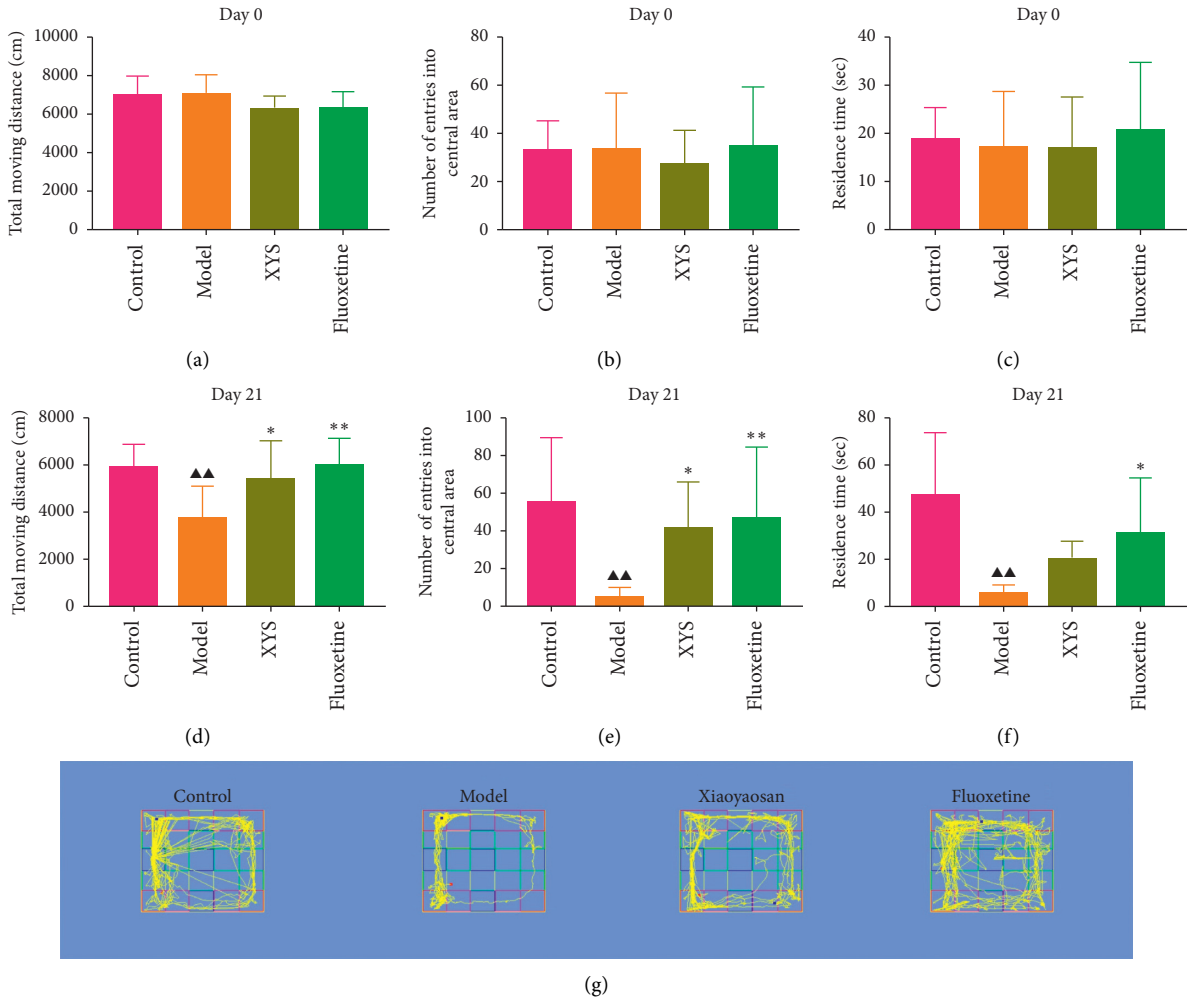


FIGURE 4: Changes in the depressive-like behaviors of rats with chronic stress-induced depression. The total distance traveled by rats in each group at day 0 (a); the total distance traveled by rats in each group on day 21 (d); the number of entries into the central area by rats in each group on day 0 (b); the number of entries into the central area by rats in each group on day 21 (e); the residence time of rats in each group on day 0 (c); and the residence time of rats in each group on day 21 (f). Data are expressed as means ± SD ($n = 8$), $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the model group; $^{\blacktriangle}p < 0.05$ and $^{\blacktriangle\blacktriangle}p < 0.01$ vs. the control group. XYS, Xiaoyaosan. (g) Representative movement trails of rats in each group on day 21 as assessed by video tracking software.

Similarly, the levels of NLRP3, ASC, and caspase-1 were all significantly decreased (all $p < 0.01$) in the fluoxetine group compared with those in the model group (NLRP3: $F = 15.779$; ASC: $F = 11.012$; caspase-1: $F = 8.463$). Combined, the Western blotting results showed that XYS could significantly reduce the levels of proteins in the TLR4 signaling pathway as well as those of NLRP3 inflammasome-related proteins (Figures 8(b) and 8(c)).

4. Discussion

To the best of our knowledge, this is the first study to report on the mechanism underlying the ameliorating effects of XYS on depression from the perspective of gastrointestinal inflammation based on the TLR4/NLRP3 inflammasome signaling pathway. This study offers new insights into the antidepressive mechanism of action of XYS in vivo and provides a basis for the clinical use of XYS in the treatment of depression.

In this study, we used colonic tissue to study depression and investigated key factors involved in the TLR4/NLRP3 inflammasome signaling pathway by H&E staining, immunohistochemistry, qPCR, and Western blotting. Our results showed that the expression levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, inflammasome-related NLRP3, ASC, and caspase-1 were all increased in rats of the depression model group. XYS and fluoxetine treatment led to the downregulation of these factors and subsequently also the levels of inflammatory cytokines such as IL-6, IL-1β, and TNF-α. The results also showed that chronic restraint stress can lead to the TLR4 signaling pathway/NLRP3 inflammasome-induced activation of

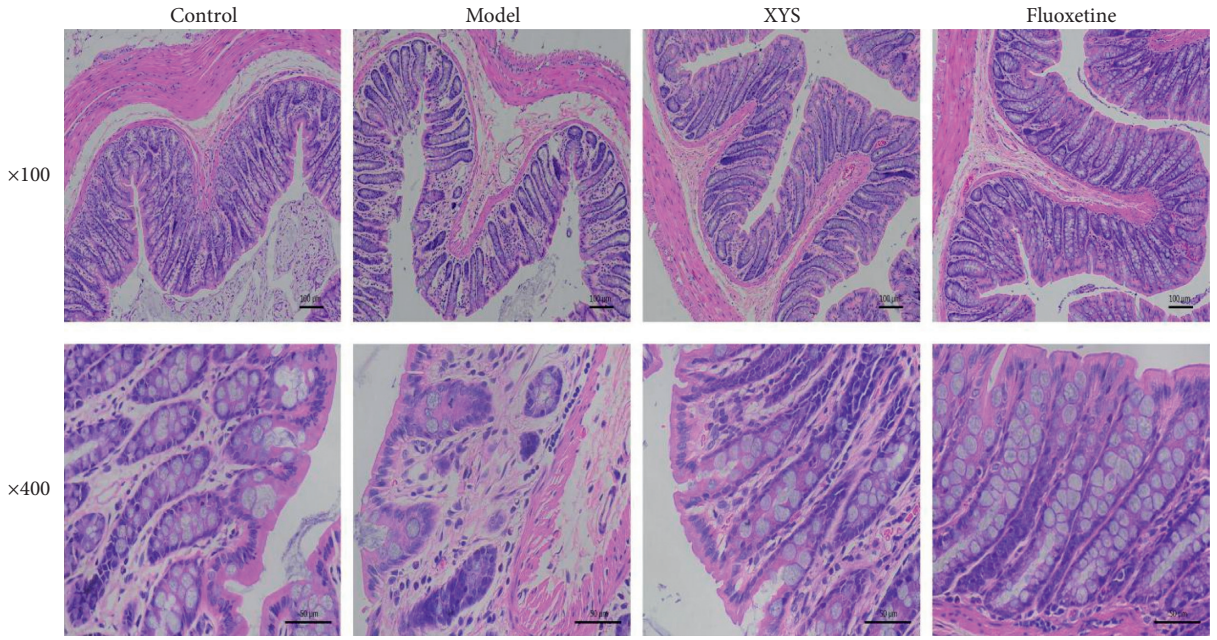
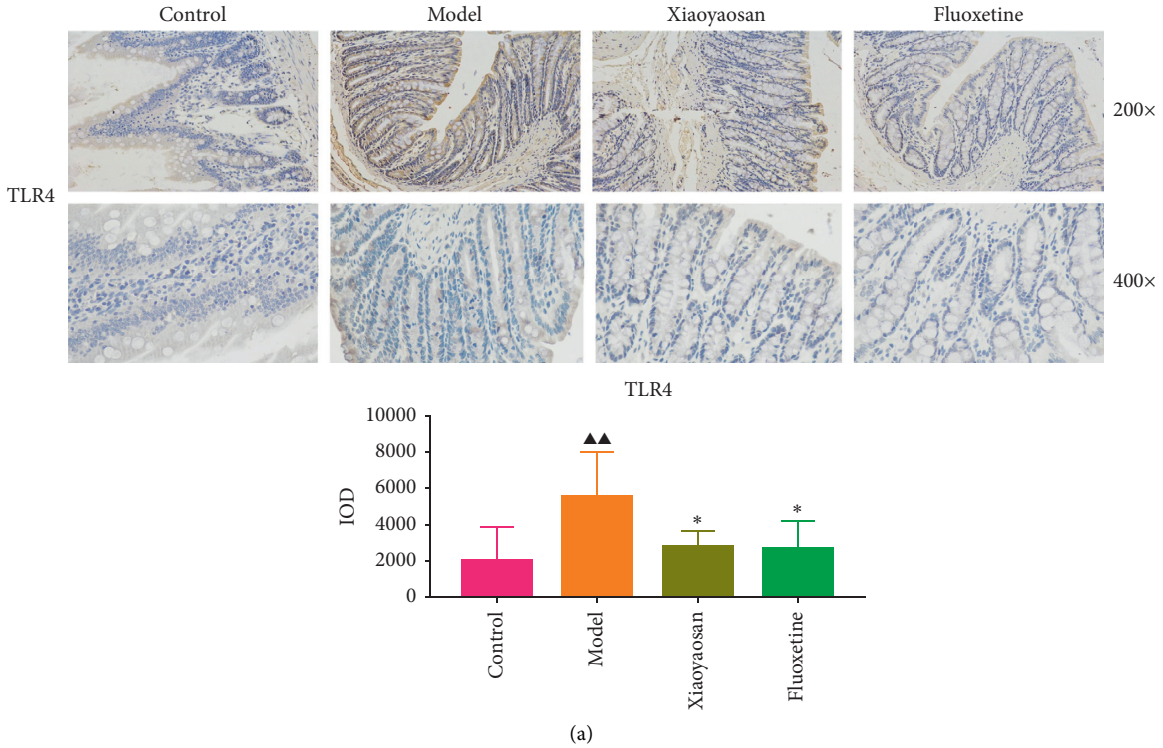
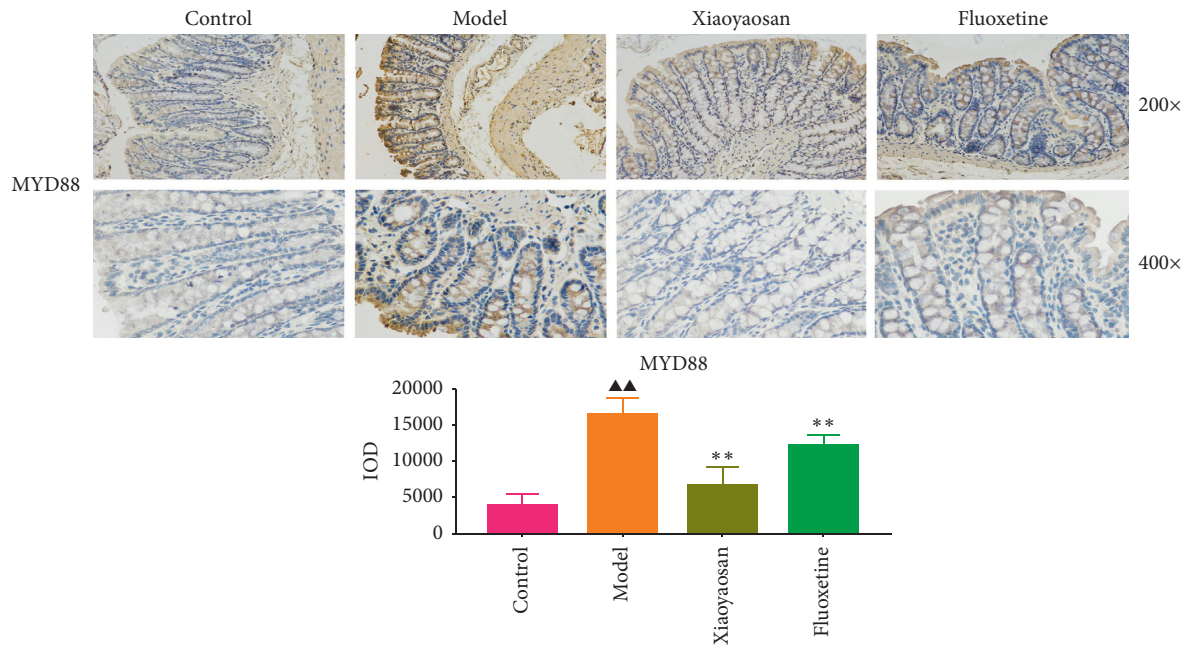


Figure 5: The effect of Xiaoyaosan (XYS) on colon pathology. Hematoxylin and eosin staining of the colon (×100 magnification, ×400 magnification).

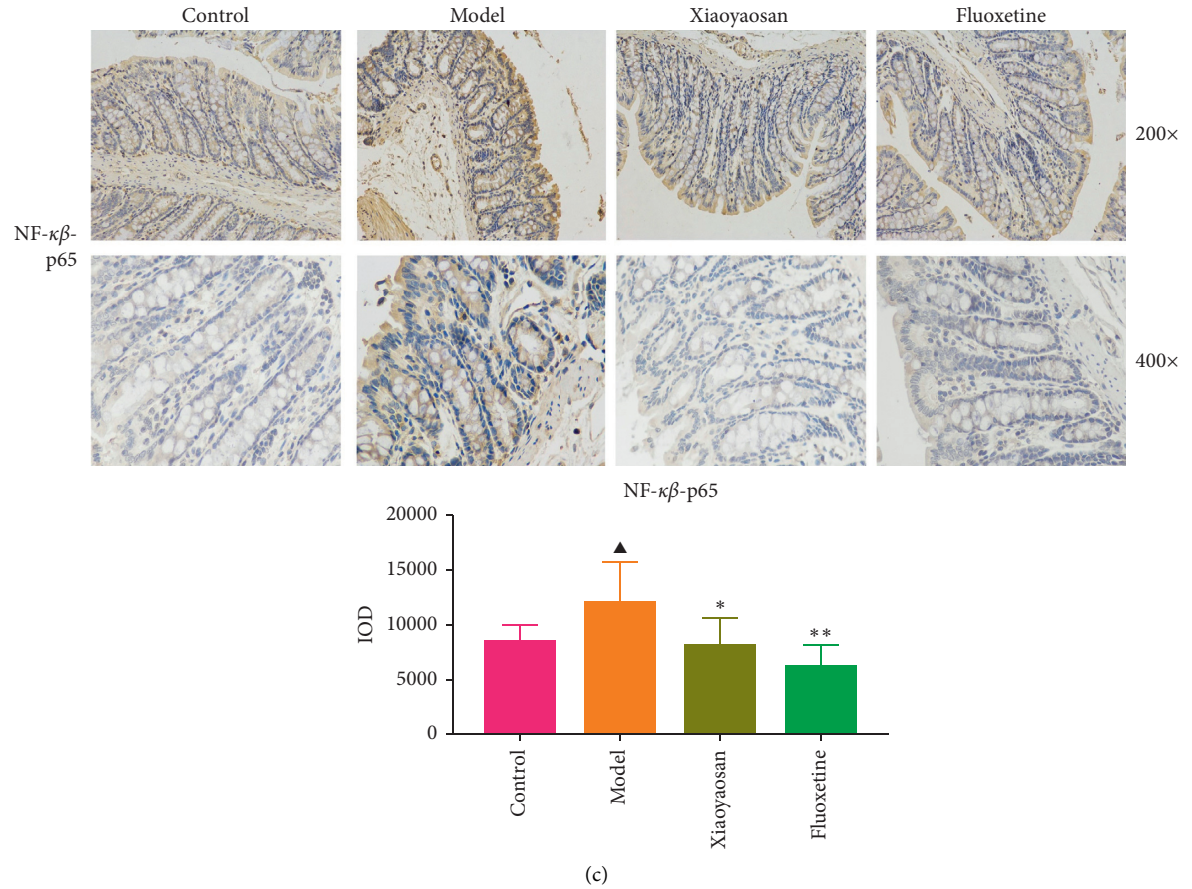


(a)

Figure 6: Continued.

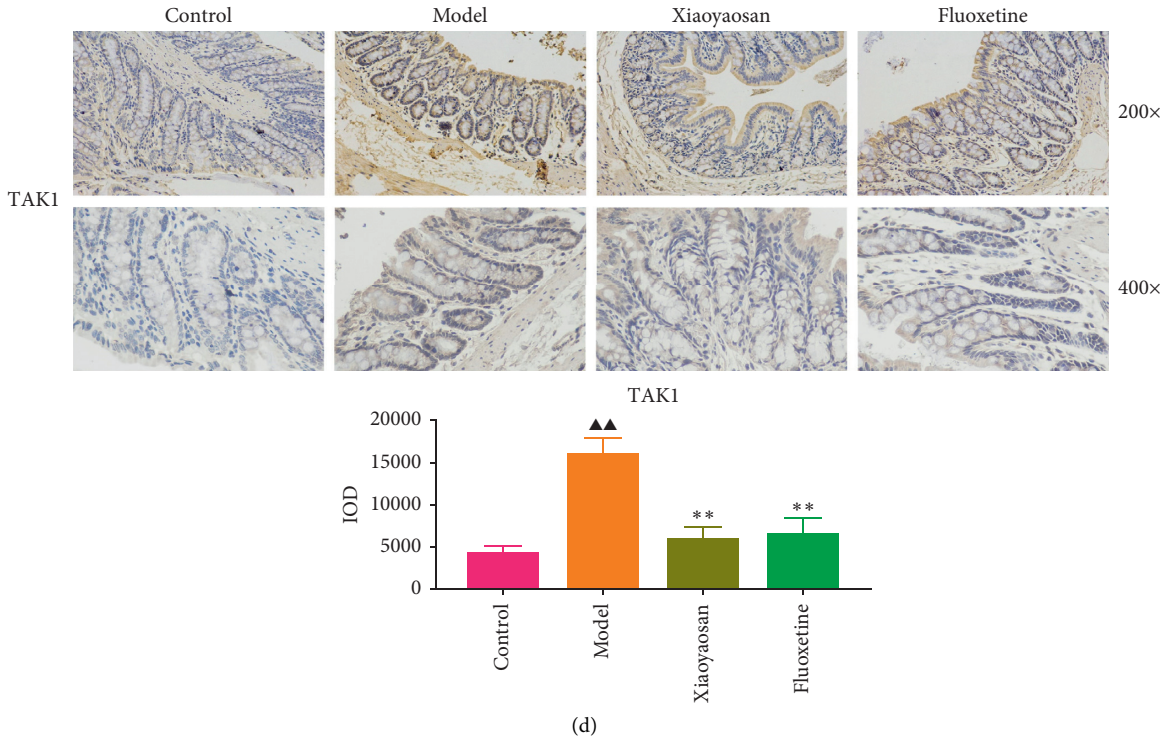


(b)

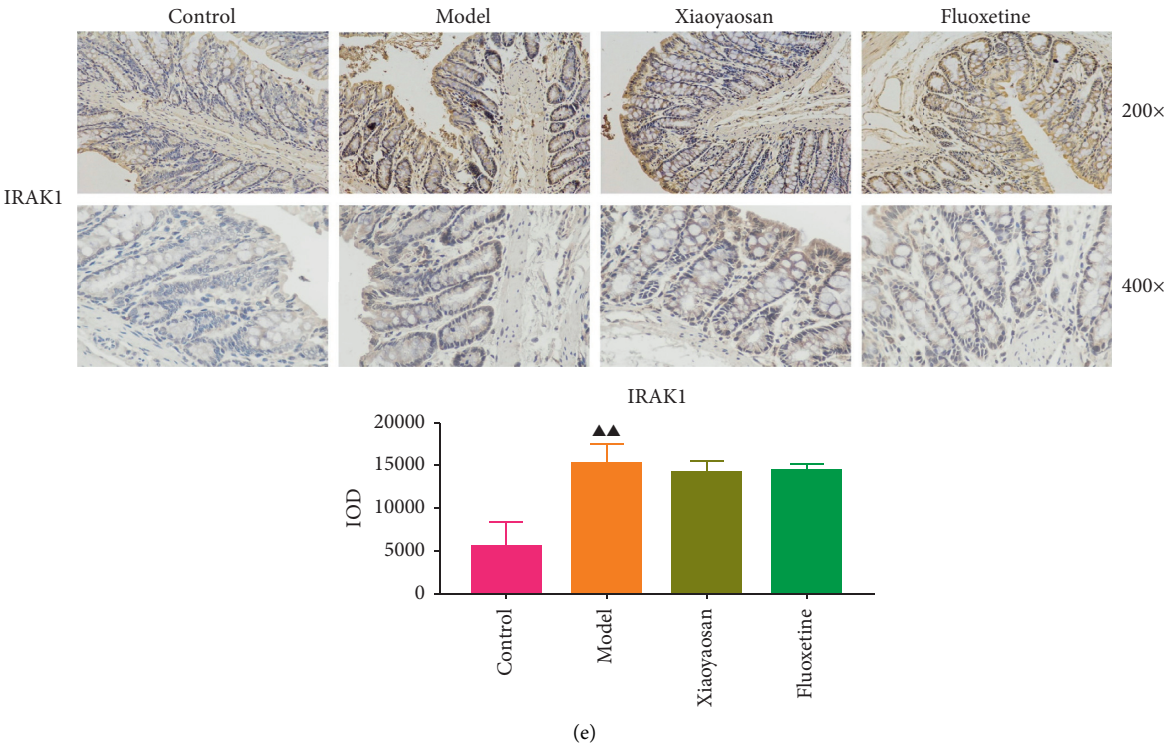


(c)

Figure 6: Continued.



(d)



(e)

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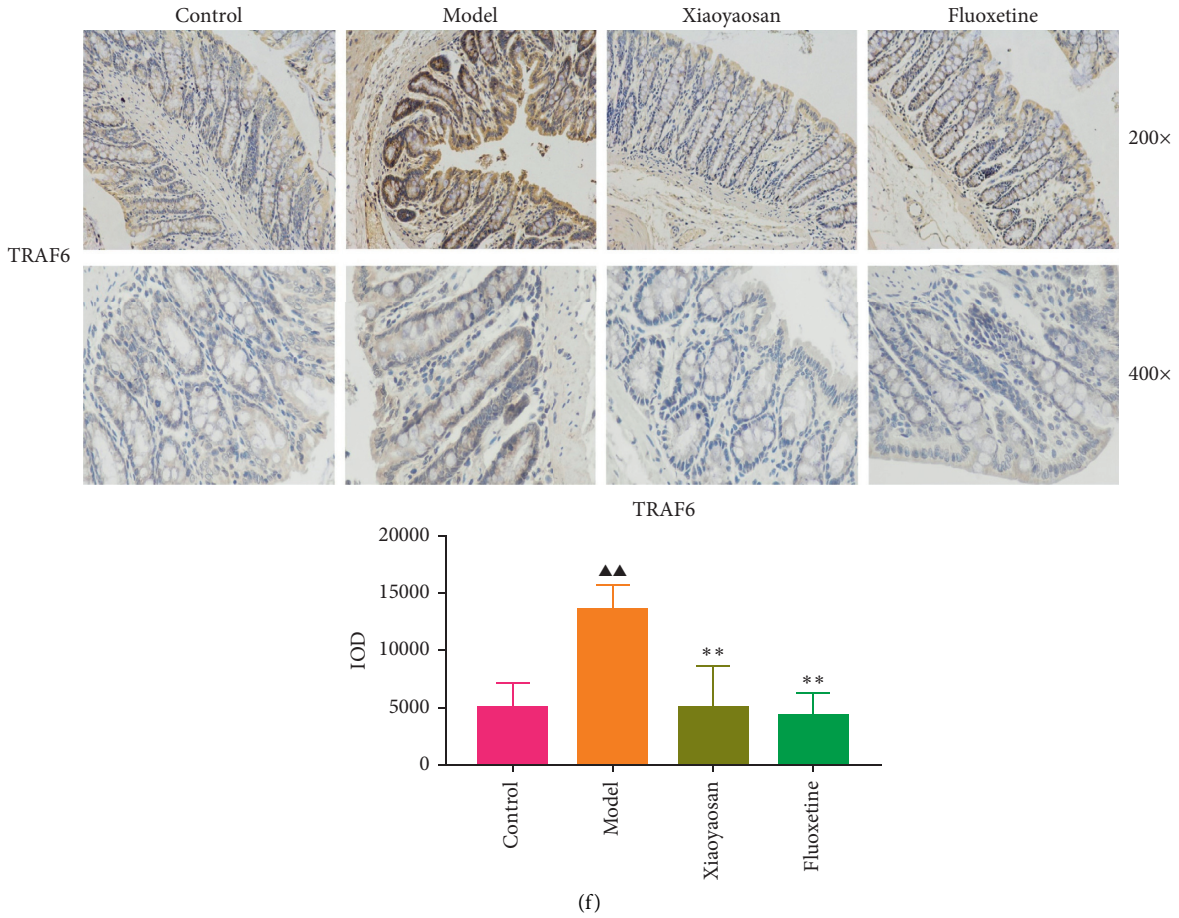


Figure 6: The effect of Xiaoyaosan on the immunohistochemistry of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 in the colon. (a)–(f) Immunohistochemical analysis of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 expression in colonic tissue (×200 and ×400 magnification) and quantification of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 immunohistochemical staining. Bars represent the means ± SD of 5 rats per group. $^{▲}p < 0.05$ and $^{▲▲}p < 0.01$ vs. the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the model group. TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response protein; IRAK1, interleukin 1 receptor-associated kinase1; TAK1, transforming growth factor beta-activated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; NF-κB-p65, nuclear factor kappa beta-p65.

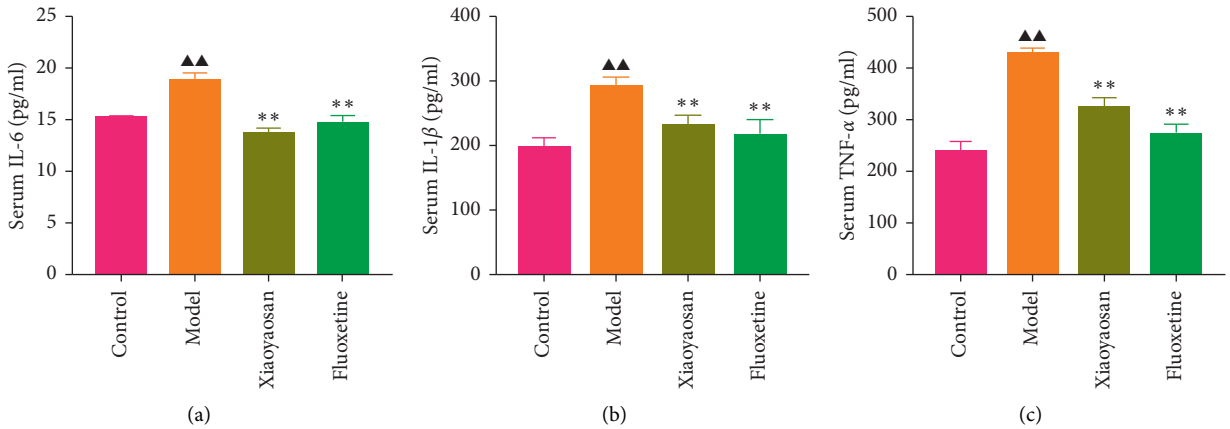


Figure 7: Continued.

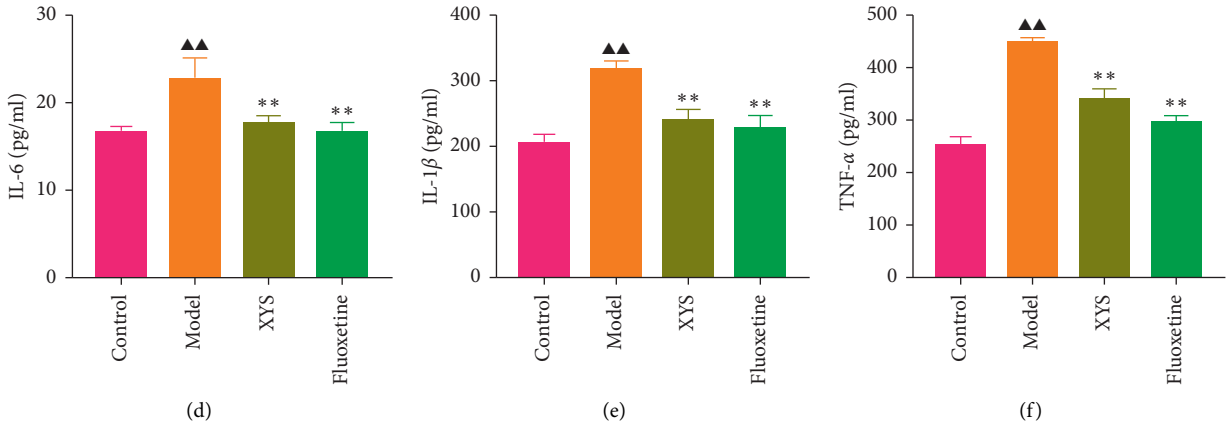


Figure 7: Xiaoyaosan (XYS) treatment reduced serum (a)–(c) and colon (d)–(f) levels of inflammatory factors (IL-6, IL-1β, and TNF-α). Bars represent the means ± SD of 5 rats per group. $^{\blacktriangle}p < 0.05$ vs. the control group; $^{\blacktriangle\blacktriangle}p < 0.01$ vs. the control group; $^{*}p < 0.05$ vs. the model group; $^{**}p < 0.01$ vs. the model group. TNF-α, tumor necrosis factor-α; IL-1β, interleukin 1 beta; IL-6, interleukin 6.

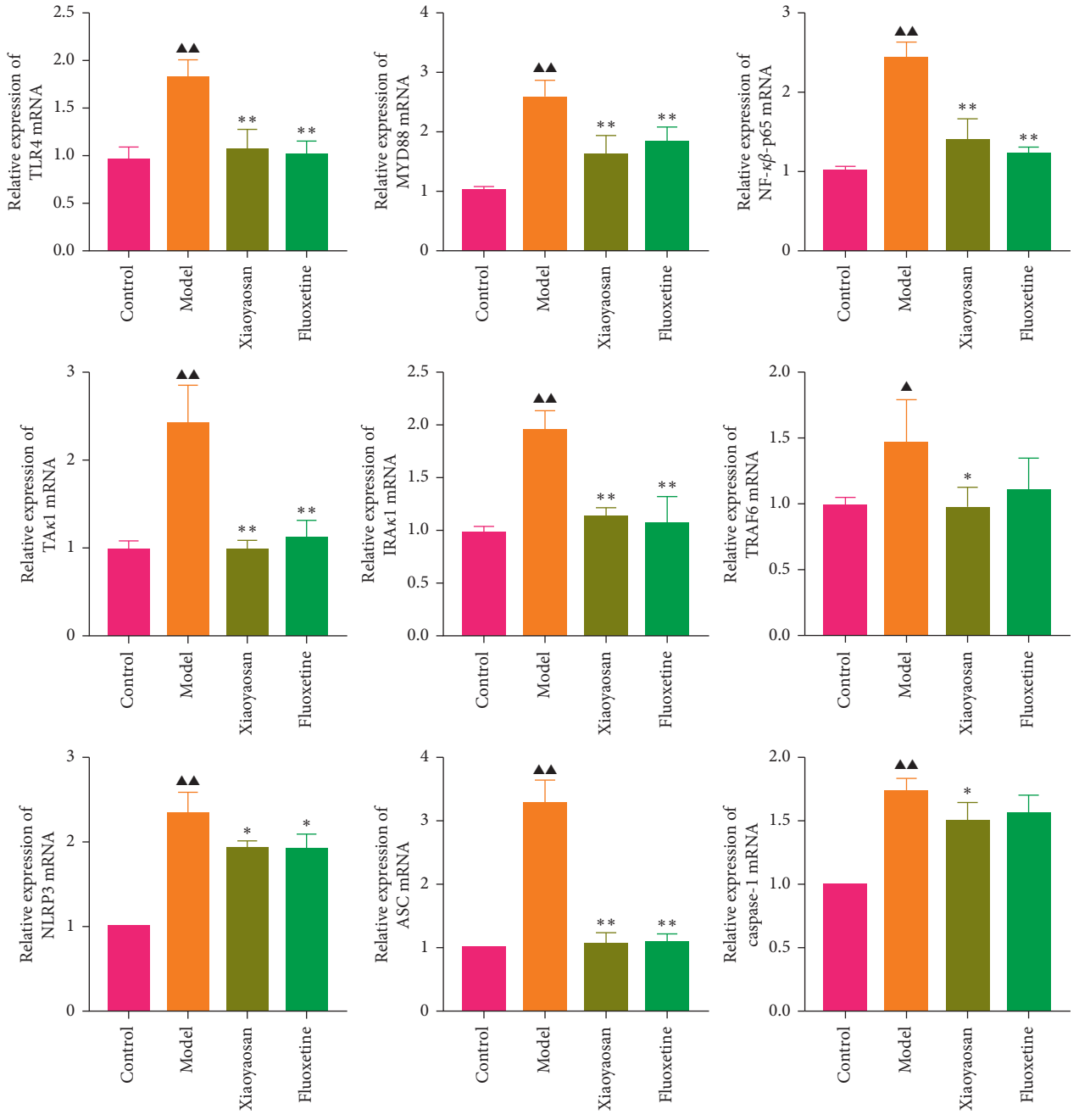
inflammatory responses, leading to an increase in the levels of inflammatory factors.

Depression may represent a chronic low-level inflammatory response involving neuroimmune-endocrine factors. Recent well-designed studies have confirmed that adverse life events, chronic stress, and depression can increase the likelihood of recurrence in patients with static inflammatory bowel disease (IBD). Experimental stress studies using animal models of colitis increasingly support this evidence. With the development of neuroimmunology, it is increasingly possible to unravel the mechanisms underlying how the nervous system affects immune function at the systemic and intestinal mucosal levels. The latest data show that chronic stress causes gastrointestinal immune inflammation [23]. Chronic stress may not only lead to intestinal barrier damage, immune cells activated, and to produce proinflammatory cytokines but also by the neural adrenergic nerve immune pathway to act on molecules expression on immune cells, such as the Toll receptor to produce inflammation. These inflammatory cytokines can not only affect the enteric nervous-smooth muscle, increased visceral sensitivity, intestinal motility, and mucosal secretion but also act on the central nervous system to produce changes in structure and function of mental symptoms of depression [24].

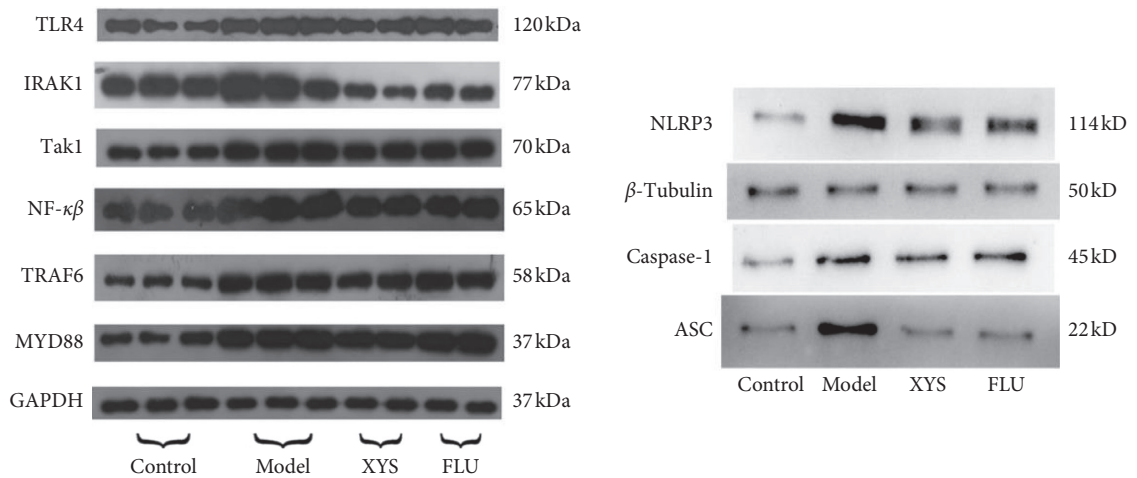
A large number of studies have shown that there is a complex interaction between the immune system and the CNS [25]. The immune system can affect the structure and function of the CNS, which can lead to changes in mood and behavior. The mechanisms involved in this interaction remain unclear. Nevertheless, inflammatory responses are considered to be an important link in the interaction between the immune system and the body, and there is substantial evidence to support that inflammatory mediators contribute to the interaction between the immune system and the CNS [26–29]. It is now known that inflammatory signals can be transmitted into the brain through humoral and neural pathways, and that changes in the brain immune environment and the production of inflammatory mediators are involved in the regulation of brain functions related to behavior, such as neurotransmitter metabolism, neuroendocrine function, and synaptic plasticity. Additionally, the intestinal mucosal immune response is also involved in the regulation of immune and inflammatory responses [30]. A "brain–gut axis" dialog pathway exists between the peripheral immune system and the CNS and is closely related to the occurrence and development of CNS diseases. The brain–gut axis-mediated transmission of inflammatory signals from the periphery to the CNS is considered to be an important link between CNS diseases with peripheral inflammation. The transmission of peripheral inflammatory signals to the CNS can lead to changes in neuronal signal transmission and neural circuit function. Under long-term chronic stimulation, alterations in mood and behavior can occur [31].

In humans, pattern recognition receptors play a critical role in innate immunity. The TLR4 signaling pathway and the NLRP3 inflammasome are closely related to depression and inflammatory bowel disease. How does the TLR4/NLRP3 inflammasome signaling pathway induce the inflammation-associated immune response in depression? TLR4 activation is induced by chronic restraint stress. TLR4 can recognize and bind lipopolysaccharide (LPS), leading to the activation of the death domain of MyD88, which recruits and activates the linker proteins IRAK4, IRAK1, and TRAF6, among others. TRAF6 can form a complex with UBC1/3UEV1A and function as an E3 ubiquitin ligase that activates TAK1 at the membrane; TAK1 then activates the downstream inhibitory IκB kinase and mitogen-activated protein kinase (MAPK) pathways, ultimately leading to the activation of an inflammatory reaction [32, 33]. NLRP3 binds to ASC, resulting in the enhanced production of procaspase-1. Procaspase-1 is subsequently converted to activated caspase-1, which further promotes the secretion of IL-1β, thereby generating a corresponding inflammatory response and promoting the pathogenesis of depression [11, 12]. TLR4 activation leads to the release of NF-κB, its translocation into the nucleus, and activation of the NLRP3 inflammasome.

TLR4/NLRP3 plays key roles in regulating intestinal homeostasis, maintaining intestinal epithelial barrier integrity, and reducing mortality during experimental colitis and can also affect the composition of the intestinal biota [34–37]. How would the peripheral inflammatory response induced by TLR4/NLRP3 in the colon affect CNS behavior? The effects of TLR4/



(a)



(b)

Figure 8: Continued.

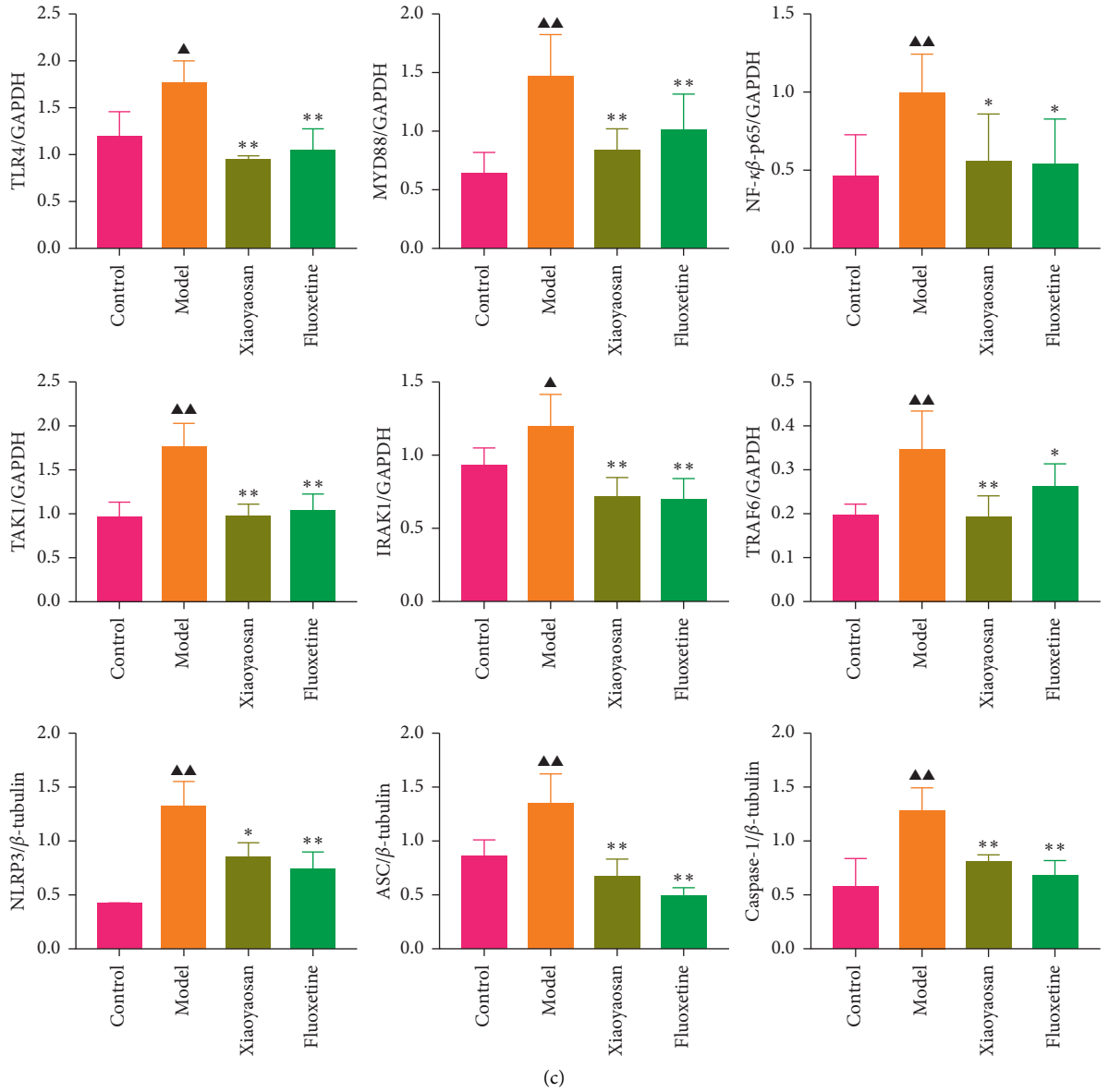


Figure 8: The effects of XYS on the mRNA and protein expression of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, NLRP3, caspase-1, and ASC in the rat colon. (a) The effects of XYS on the mRNA expression levels of *Tlr4, Myd88, NF-κB-p65, Tak1, Irak1, Traf6, Nlrp3,* caspase-1, and *Asc* in the rat colon. For *Tlr4, Myd88, NF-κB-p65, Tak1, Irak1,* and *Traf6,* data are expressed as the means ± SD of 5 rats per group. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the model group; $^{\blacktriangle}p < 0.05$ and $^{\blacktriangle\blacktriangle}p < 0.01$ vs. the control group. For *Nlrp3*, caspase-1, and *Asc*, data are expressed as the means ± SD of 3 rats per group. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the model group; $^{\blacktriangle}p < 0.05$ and $^{\blacktriangle\blacktriangle}p < 0.01$ vs. the control group. (b)-(c) Semiquantitative Western blot analysis of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, and TRAF6 expression in colonic tissue; data are expressed as the means ± SD of 5 rats per group. $^{\blacktriangle}p < 0.05$ and $^{\blacktriangle\blacktriangle}p < 0.01$ vs. the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the model group. Semiquantitative Western blot analysis of NLRP3, ASC, and caspase-1 expression in colonic tissue; data are expressed as the means ± SD of 3 rats per group. $^{\blacktriangle}p < 0.05$ and $^{\blacktriangle\blacktriangle}p < 0.01$ vs. the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the model group. TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response protein; IRAK1, interleukin 1 receptor-associated kinase 1; TAK1, transforming growth factor beta-activated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; NF-κB-p65, nuclear factor kappa beta-p65; NLRP3, NOD-like receptor protein 3; ASC, apoptosis-associated speck-like protein; XYS, Xiaoyaosan; FLU, fluoxetine.

NLRP3-mediated peripheral immune activation in the colon on behavior are mainly mediated via three mechanisms: (1) the release of proinflammatory cytokines at the periphery can directly increase the signal transduction of neuroregulatory cytokines through the blood-brain barrier (BBB) via "leakage" through the circumventricular organs; (2) activated immune cells can also directly cross the BBB as a form of neuroimmune signal transduction; and (3) peripheral cytokines can stimulate afferent pathways, such as the vagus nerve, and promote behavioral changes via neurological mechanisms [38]. Immune inflammatory response is one of the pathogenesis of depression. The cytokines produced by it can induce depression mainly

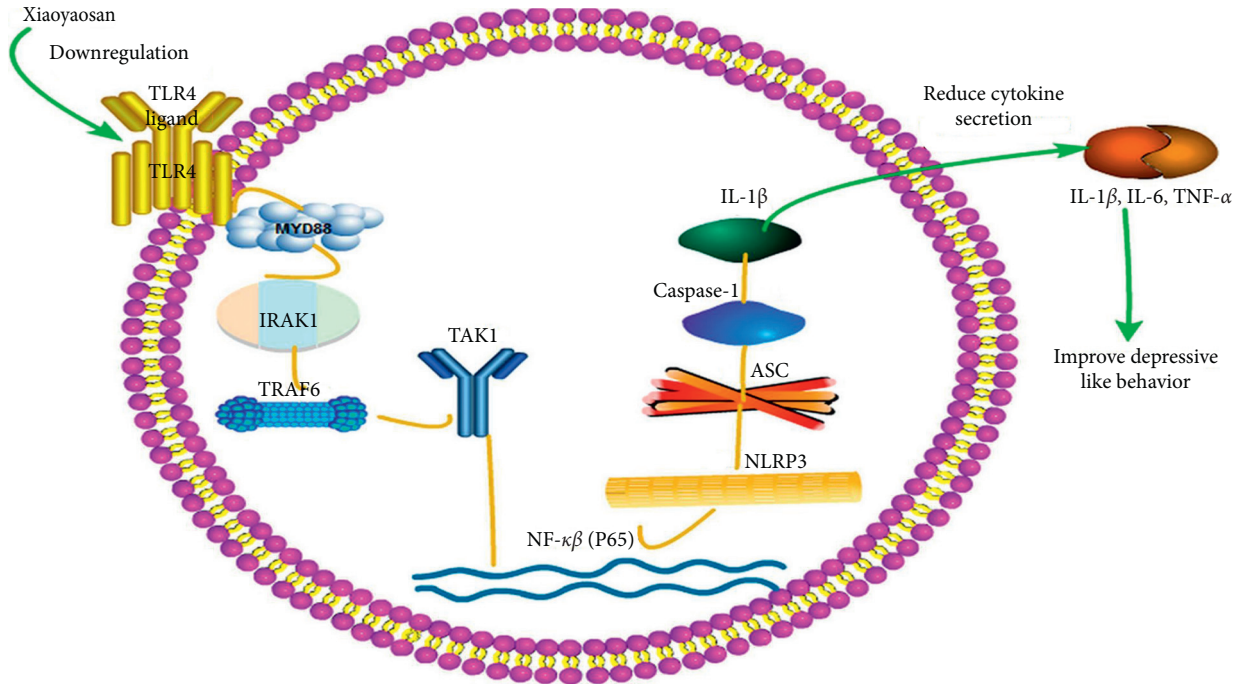


Figure 9: Xiaoyaosan exerts its antidepressive effects in rats with chronic restraint-induced stress via regulating the activation of the immunoinflammatory response induced by the TLR4/NLRP3 inflammasome signaling pathway in the colon. Xiaoyaosan treatment reduced the expression levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, NLRP3, ASC, and caspase-1 in colonic tissue of depressed rats; reduced the serum levels of the inflammatory factors IL-6, IL-1β, and TNF-α; and ameliorated colonic inflammation. TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response protein; IRAK1, interleukin 1 receptor-associated kinase 1; TAK1, transforming growth factor beta-activated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; NF-κB-p65, nuclear factor kappa beta-p65. NLRP3, NOD-like receptor protein 3; ASC, apoptosis-associated speck-like protein.

through the following ways after passing through the blood-brain barrier: intensifying the brain immune cell response; activating the neuroendocrine axis; inhibiting monoamine neurotransmitters; and changing the structure and function of brain regions related to emotion regulation to induce depression. [39].

In summary, chronic restraint stress can cause a gastrointestinal immune response, which is primarily mediated through the TLR4/NLRP3 inflammasome signaling pathway. Activation of this pathway may represent a common pathological process underlying the development of depression and gastrointestinal disease. Inflammatory cytokines are markers of immune system activation and also mediators of the CNS and immune system activity. Inflammatory cytokines passing through blood-brain barrier may intensify the brain immune cell response; activate the neuroendocrine axis; inhibit monoamine neurotransmitters; and change the structure and function of brain regions related to emotion regulation and induce depression.

Studies have shown that tetramethylpyrazine (TMP) can reverse increases in the levels of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 and inhibit the upregulation of the levels of TLR4/p38/NF-κB/NLRP3 signaling-associated proteins in the prefrontal cortex and hippocampus [40]. These results suggest that TMP may exert a potential antidepressive-like effect in a model of CUMS-induced depression. Using the same model, a different study [41] showed that Chaihu Shugan San treatment can reduce the serum levels of IL-1β. This formula can also inhibit hepatic and prefrontal cortical inflammatory responses by suppressing the TLR4/MyD88/NF-κB pathway and activating the NLRP3 inflammasome and can also ameliorate depressive-like behaviors by inhibiting the liver-brain inflammation axis. He et al. [42] reported that muscone can ameliorate LPS-induced depressive-like behaviors by repressing neuroinflammation in the prefrontal cortex of mice through suppressing microglial activation and the production of inflammatory cytokines via the TLR4 pathway. Overall, our results are consistent with those of the abovedescribed studies. Inhibiting the TLR4/NLRP3 inflammasome signaling pathway and reducing inflammatory cytokine levels are commonly used as a means of exploring whether drugs can suppress depressive-like behavior. However, the above studies are based on the prefrontal cortex, hippocampus, and liver-brain axis, among others, whereas we investigated the mechanism of action of XYS in reducing depressive-like behaviors with respect to an intestinal inflammatory immune response.

In TCM theory, dysfunction of the spleen and stomach can lead to dysfunction of transportation, lack of metaplasia of Qi and blood, and dysfunction of Qi elevation and descent, thus leading to depression. In the 1990s, dysfunction of Qi caused by the spleen and stomach dysfunction was proposed to be the main pathogenic mechanism of depression [43]. TCM is commonly used to regulate the spleen and stomach in the treatment of depression. Shi et al. [44] used

a TCM approach to regulate the spleen and stomach to treat depression and showed that the short-term efficacy of this method was similar to that of fluoxetine hydrochloride capsules. XYS has been demonstrated to effectively interfere with CNP/NPR-B signaling pathway activity in the rectum of depressed rats [45]. XYS can improve the gastrointestinal and thus help with treating depression, through its ability to regulate effects at multiple levels, via multiple channels, and directed at multiple targets. Gao et al. [46] identified saikosaponin A, saikosaponin C, saikosaponin D, ferulic acid, Z-ligustrazine, atrazine enol I, atrazine acetenol II, atrazine acetenol III, paeoniflorin, leucaenanthoside, glycyrrhizic acid, and chlorpyruvic acid as the main antidepressive components of XYS, reflecting the multicomponent, multitarget, and multichannel characteristics of TCM.

In conclusion, the results of this study demonstrated that XYS can improve depressive-like behavior in rats by suppressing the activation of the TLR4/NLRP3 inflammasome signaling pathway, thereby inhibiting immunoinflammatory activation and reducing the levels of inflammatory cytokines in the colon. The observed improvement in depressive symptoms may have resulted from the downregulation of the levels of TLR4, MyD88, NF-κB-p65, TAK1, IRAK1, TRAF6, and NLRP3 inflammasome-related NLRP3, ASC, and caspase-1 proteins, leading to the subsequent downregulation of the levels of the inflammatory cytokines IL-6, IL-1β, and TNF-α (Figure 9).

5. Conclusions

XYS can improve depressive-like behavior by suppressing the TLR4/NLRP3 inflammasome signaling pathway, thereby inhibiting the activation of immunoinflammatory responses and, consequently, reducing the levels of inflammatory cytokines in the colon. This study offers new insights into the mechanism underlying the mode of action of XYS in vivo and provides a basis for the clinical use of XYS as an antidepressant.

Data Availability

The data used to support the findings of this study are available from the first author (zhuhuizheng@jnu.edu.cn) upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Huizheng Zhu and Yudan Liang contributed equally to this work. Huizheng Zhu, Jiaxu Chen, and Yuming Li conceived and designed the experiments. Huizheng Zhu, Yudan Liang, Qingyu Ma, and Wenzhi Hao performed the animal experiments. Xiaojuan Li analyzed the data. Huizheng Zhu and Yudan Liang wrote and revised the manuscript. All the authors read and approved the final version of the manuscript.

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Review Article

Insights from the Perspective of Traditional Chinese Medicine to Elucidate Association of Lily Disease and Yin Deficiency and Internal Heat of Depression

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Lily disease was first recorded in *Synopsis of the Golden Chamber* by Zhang Zhongjing. It is a disease of heart and lung internal heat by Yin deficiency, which belongs to the category of emotion disease in Chinese medicine. In recent years, researchers believe that lily disease and depression syndrome of Yin deficiency and internal heat have many similarities in etiology, pathogenesis, and clinical manifestations. This review summarizes the clinical symptoms, etiology, pathogenesis, and therapeutic medication of lily disease and modern Yin-deficient internal heat depression and discusses the relationship between them. Furthermore, the relationship between coronavirus disease 2019 (COVID-19) and lily disease was discussed from the etiology, pathogenesis, and treatment. It provides new ideas for the treatment of COVID-19 and the treatment of psychological problems after recovery.

1. Introduction

Major depression disorder (MDD) is a mood disorder characterized by sentimentality, despair, anhedonia, and sensitivity for social rejection [1]. These symptoms severely debilitated the patient's physical and mental homeostasis and brought a significant reduction in productivity and negative effects on overall health [2]. In terms of treatment and care costs for depression, the World Health Organization (WHO) has predicted it as a leading disease burden worldwide by 2030 [3].

Emerging evidences from human postmortem and animal studies point out depressive disorders may instigate from human body system imbalance affected by genetic and environmental factors [4]. However, the specific pathogenesis underlying the development of depression remains unclear. Currently, psychotherapy and antidepressant medications are the gold-standard treatment for depression in clinical practice [5]. Although antidepressant drugs are convenient treatments for depression, long-term side effects and drug dependence make patients less compliant with them. Hence, it is necessary to find novel therapies which may avert or impede the development of depression to replace conventional Western medicine.

As a critical component of complementary and alternative medicine, traditional Chinese medicine (TCM) is a multicomponent, multitarget, and multipathway therapy, which can achieve its unique therapeutic effect by adjusting the biological network of the human body system [6, 7]. TCM has been used to treat depressive disorders for more than two thousands of years and now has extensive scientific evidences supporting its efficacy [8]. Based on the TCM theory, depression can be divided into positive syndromes and deficiency syndromes, including Qi stagnation, fire

stagnation, phlegm stagnation, blood stagnation, heart and spirit loss nourish, deficiency of both heart and spleen, Yin deficiency, and fire excess [9].

Most of Yin deficiency and internal heat of depression is the deficiency syndrome, and its main clinical manifestations are anxiety, depression, tension, suspicion, irritated fever, night sweat and zygomatic red, palpitation and insomnia, red tongue with little moss, and thready and rapid pulse [10].

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2. Lily Disease

Based on the TCM theory, many disease occurrences are related to personal physical and emotional injury [12]. It will cause visceral dysfunction and the imbalance of Yin, Yang, Qi, and blood, when people are in long-term emotional stimulation or mental stimulation beyond the range of physical regulation can bear, leading to the occurrence of diseases [13]. According to the Chinese medicine principle, viscera are closely related to emotional activities. The vital substance of viscera is the material basis of emotional activity, and abnormal emotional activities can damage the vital substance of viscera, resulting in the disorder of Qi and blood, and the imbalance of Yin and Yang [14].

The "lily disease" is initially reported in *Synopsis of the Golden Chamber*: *Lily Disease, Huhuo and Yin Yang Toxin: Pulses, Syndromes and Treatment*. The manifestations of "lily disease" patients are marked by unclear consciousness, fluctuated appetite, frequent silence, restlessness, confused cold and heat body sensation, bitterness in the mouth and dark urine, red tongue body and less tongue coating, and weak and thready pulse. These characteristics are similar to the manifestations of depressive disorders [11]. Based on the TCM theory, "lily disease" results from Yin-Yang imbalance on the whole body, which causes heat and lung disorder. Endogenous heat (heart and lungs) caused by Yin deficiency, resulting in the abnormality of blood vessels and meridians, can be attributed to the pathophysiology of "lily disease" [15].

Emerging evidences from the ancient Chinese medical text suggest that the etiology factor of lily disease is mainly internal and external injury [16]. The internal cause is excessive thinking and emotional failure, so that the spleen, lung, liver, and other visceral functions are damaged, and then the whole body with diseases; the external factor is exogenous heat resulting in body residual heat, which leads to the lack of blood supply and mental confusion [17].

With regard to pathogenesis, there are many TCM hypothesis mechanisms underlying internal and external factors inducing lily disease (Figure 1). The most mainstream view is the theory of heart and lung. Scholars believe that the lung is in charge of all the meridians, the heart governs the blood, and the meridians hold the blood [18]. Thus, lily disease is the disease of the heart and lung. Heart and lung function in the human body is very important, if it is normal, then Qi and blood will be harmonized, and meridians will be nourished; if the heart, lung, Qi, and blood are damaged, the meridians will lose nourishment, and the invasion of exogenous pathogens factors will disturb the stomach, gallbladder, liver, kidney, and other viscera, leading to systemic diseases; as a result, many viscera show symptoms of disease and the position is not fixed [19].

The second view is the theory of gallbladder. For example, Liang et al. believe that "bitterness in the mouth" is a syndrome of Shaoyang gallbladder meridian disease [20]. *Plain questions* said that "all eleven dirty depends on the gallbladder." Therefore, if the gallbladder has heat, all veins are affected by Yin deficiency. The gallbladder controls the decision, so the deficiency of gallbladder Qi showed the symptoms of trance. The third view is the theory of liver. Some scholars believe that the location of this disease is the liver and that the failure of sentiment leads to liver Qi stagnation, endogenous heat deficiency, and Yin injury [21]. The fourth view is the theory of spleen and stomach. According to the symptoms that *Golden Chamber* described, TCM experts considered that its pathogenesis lies in the damage of coke due to irregular diet [22].

In summary, the cause of lily disease is the damage of viscera by external or internal injury, leading to Yin deficiency of viscera and disorder of Qi and blood; then, the veins will lose nourishment, and hence, it will lead to the symptoms of mental disorder. The Yin deficiency of viscera will lead to the virtual heat coming from the inside of the body, as well as bitterness in the mouth, red-colored urine, and other symptoms.

3. Yin Deficiency and Internal Heat of Depression

According to the TCM theory, depression can be divided into positive syndromes and deficiency syndromes, including Qi stagnation, fire stagnation, phlegm stagnation, blood stagnation, heart and spirit loss nourish, deficiency of both heart and spleen, and hyperactivity of fire due to Yin deficiency. Most of the Yin deficiency internal heat depression is menopausal depression or depression in old age, and its etiology is plant nerve dysfunction and endocrine dysfunction [23].

Contemporary scholars believe that depression belongs to the category of "no disease" in TCM. Most patients have no obvious organic lesions. The pathogenesis is the imbalance of Qi, blood, Yin, Yang, and the dysfunction of viscera, which makes the body in a state of excessive Yin and Yang or partial decline. Disorders of Qi and blood in the viscera will lead to abnormal mind, and changes in mind will also affect Qi and blood in the five viscera [24].

TCM experts generally considered that depression is closely related to Qi and blood blockage and liver unreel distributing [25, 26]. All kinds of negative emotional stimulation, such as mental panic, physical and mental exhaustion, and failure to achieve one's goals, can cause dysfunction of viscera, Qi, and blood and lead to depression. This disease was sufficiency syndrome at first and can be triggered by the internal heat due to Qi stagnation, then pathogenic factors will enter the blood from Qi, and the disease changed from sufficiency syndrome to deficiency syndrome, so there will be a mixture of deficiency and excess syndromes such as the deficiency of mind, deficiency of Qi and Yin, Yin deficiency, and fire excess [27].

The intrinsic mechanism of Yin deficiency and internal heat of depression is the imbalance of the kidney, lung, and spleen and other viscera. The clinical manifestation of Yin deficiency and internal heat of depression is a mixture of deficiency and excess syndromes [28]. Vital Qi deficiency is due to the lack of Yin and fluid, and after a while, it will become Qi and Yin deficiency. Thus, pathogenic factor excess is because of the body heat and blood stasis. The syndrome of Yin deficiency and internal heat of depression is deficiency in origin and excess in superficiality [29]. It consumes Yin and fluid at first, then feeling the pathogenic factor outside or modern internal injuries, and producing internal heat. The heat transmission will increase the loss of body fluid, then the Yin will not gather the Yang, and the empty fire is burning more. The lack of dirty Yin and spirit will lead to anxiety, depression, tension, and suspicion, and the internal heat due to Yin deficiency disturbance heart will lead to palpitations and insomnia [30].

4. Association of Yin Deficiency and Internal Heat of Depression and Lily Disease

Based on the TCM theory, lily disease is a kind of emotional disease, which is closely related to emotional and psychological factors. Lily disease occurs because of the disorder of seven emotions, which will hurt viscera and lead to the imbalance of Qi, blood, Yin, and Yang. In addition, it may be the sequela of exogenous fever or other diseases [31]. With regard to etiology, the pathogenesis of lily disease due to modern factors was similar to that of depression due to personality or negative emotional experience. In addition to emotional factors, lily disease is also related to exogenous febrile disease.

From the pathogenesis, the basic pathogenesis of lily disease is internal heat due to Yin deficiency, and its pathogenesis is the result of the interaction of various pathogenic factors [32]. From the clinical symptoms, lily disease showed trance, sleep, anxiety, insomnia, and other symptoms similar to the clinical diagnostic standard of modern depression. Besides, bitterness in the mouth, red-colored urine, and rapid and thready pulse are the typical symptoms of internal heat due to Yin deficiency [33]. Therefore, we can infer that internal heat depression due to Yin deficiency is major syndrome of lily disease from etiology and pathogenesis.

5. Traditional Chinese Formulas for Treatment of Lily Disease

Zhang Zhongjing believed that the treatment of emotional diseases should first improve the physical symptoms combined with Chinese herb treatment based on syndrome differentiation [34]. The main prescription for treating lily disease is Lily Bulb and Rehmannia Decoction (LBRD). The classical herbal formula LBRD is combined with the lily bulb and fresh Romanian root juice. According to TCM perspective, lily bulb is deemed cool and sweet in properties. The lily bulb is also associated to the lung and heart meridians and help to relieve cough and dry throat, clear heat and moisten the lung. *Rehmannia* root is naturally sweet with bitter flavor, and mostly shows its curative effects in the kidney, liver, and heart. It has effect on promoting body fluid production, nourishing Yin, and controlling heat [11]. When two ingredients are combined, LBRD will bring about the maximum of therapeutic efficacy on the mental instability, anhedonia, anxiety, absent mindedness, and insomnia.

Lily Zhimu Decoction (BZD) was another traditional Chinese formula for treatment of lily disease [35]. Zhimu (*Anemarrhena*) is bitter and sweet in properties, and it is also associated to the lung, stomach, and kidney meridian. It has the effect of clearing away heat, eliminating annoyance, draining the lungs, and nourishing the kidneys. Because "blood and sweat are homologous" and "sweat and fluid are homologous," excessive sweating will lead to more deficiency of body fluid, Qi and blood, and obvious symptoms of dry thirst [36].

According to the TCM theory, Talc Hematite Decoction (THD) was used to treat lily disease mistreated with purgative. Talc tastes sweet and is cold in nature. It was used to treat vexation, hot, and thirsty. Hematite tastes bitter and is flat in nature, into Stomach Channel of Foot-Yang Ming. It can reduce stomach gas to stop hiccups, relieve cough, and clear heat and have the characteristic of inducing astringency without hurting vital energy. After the wrong purgation, stomach Qi and body fluid were injured, and internal heat increased. Because the main disease has not changed, lily is still the monarch medicine. Talc is used as minister medicine to diuretic and purgation heat, and hematite is used as adjuvant drug to induce astringency and astringing Yin [37].

Lily disease mistreated with the vomiting method should be treated with Lily Yolk Decoction (LYD) underlying syndrome differentiation [21]. Yolk tastes sweat and is warm in nature, it can nourish the spleen and stomach and adjust heart gas to nourish Yin, and it has the effect of clearing heat to cool blood and detoxification. The erroneously treatment of the vomiting method will injure stomach Yin and disturbs lung and stomach Qi, leading to restlessness due to deficiency.

Lily disease patients with lasting for one month without alleviation can use lily to wash the body. If thirst is severe, it should be treated with Gualou oyster powder (GOP) [38]. Trichosanthin tastes bitter and is cold in nature, mainly used to treat thirst, except for body heat and irritation, and it can nourish body fluid without damaging the Yang of the spleen

and stomach; oyster tastes salty and is acerbity, cold in nature, and heavy in quality, which has the effect of reducing virtual heat and avoid draining the body fluid. Both of these two medicines can remove heat and supplement Yin-Jin to relieve thirst. Lily disease with thirst by GOP and the oyster in the formula did not act directly to regenerate body fluid and quench thirst, but to check exuberance of Yang, so that the Yin would rest the Yang, and the thirst would be quenched.

6. Traditional Chinese Herb Prescription for Treatment of Yin Deficiency and Internal Heat of Depression

TCM believes that the pathogenesis of depression is related to the deficiency of Qi and Yin in several viscera. Therefore, the main principles for treating Yin deficiency and internal heat of depression are nourishing Yin to clear heat in five viscera (Figure 2). However, the pathogenesis of Yin deficiency and internal heat of depression is complex and the viscera interact with each other. Thus, when treating from one viscus, other viscera should be considered at the same time.

Heart Qi deficiency can lead to poor blood flow and blockage of heart vessel. According to *the Canon of Internal Medicine*, people's spiritual consciousness and thinking activities are coordinated by the five viscera. The heart is the master of all the viscera recorded in *Lingjiu*. It means that the heart has a controlling effect on the five viscera, and the malfunction of the heart can also lead to the malfunction of other viscera. The treatment of Yin deficiency and internal heat of depression from the heart mainly adopts the method of replenishing Qi and nourishing the heart. Major Heart Supplementing Decoction was clinically used to treat heart Qi deficiency-type depression [31]. Cortex Moutan acts on blood, *Inula japonica* acts on Qi, and the two drugs used mutual promotion can benefit heart Qi and enhance heart function. Bamboo leaves can clear the mind and purge fire to remove irritation. Pulp of *Cornus* can ease the emergency of the heart and prevent dredge too much, leading to the consumption of Qi. Ginseng and dried ginger can benefit heart Qi to enhance heart function. This prescription has the effect of supplementing heart Qi. It is an effective prescription for treating depression due to heart Qi deficiency.

Yin deficiency and hot of the lung can lead to the failure of the lung to disperse and descend. The treatment of Yin deficiency and internal heat of depression from the lung is mainly based on the method of invigorating Qi and nourishing Yin. Zhang et al. considered that LBRD has a good therapeutic effect in clinical practice [15]. In this prescription, lily can moisten the lung to benefit Qi and clear heat to calm the mind. *Rehmannia* can nourish the heart and Yin, as well as cool blood to clear heat, and the two drugs can nourish the heart and lung and clear heat to calm nerves when they are used together.

Lack of body fluid in the spleen makes it become hot and dry and leads to Yin deficiency, which will cause the maladjustment of transport and transformation of the spleen. The spleen is the most negative viscera in the human body. And spleen Yin deficiency will affect the lung, liver, and kidney functions and result in the dry heat performance of the body [39]. Therefore, the treatment of Yin deficiency and internal heat of depression from the spleen mainly adopts the method of nourishing spleen Yin. For example, professor Liu found that decoction for invigorating the spleen has a good therapeutic effect on depression through experiments [40]. In this prescription, *Angelica sinensis*, *Astragalus*, and Arillus Longan have the effect of invigorating Qi, activating blood, and calming spirits. Lanceolata can nourish Qi and enhance the function of the spleen and lungs. *Atractylodes*, *Poria cocos*, and spine date seed have the function of calming the mind and eliminating dampness to strengthening the spleen. This prescription has the function of strengthening the spleen and blood to calm the spirit, and it is an effective prescription for the treatment of depression due to spleen deficiency.

Liver Qi stagnation will make the liver hot and then damage the liver Yin. If the dredge function of the liver is abnormal, the operation of Qi in the viscera and meridians will be obstructed, which will lead to the damage of liver Yin and the appearance of internal heat. The treatment of Yin deficiency and internal heat of depression from the liver is mainly based on the methods of invigorating liver Qi and nourishing Yin of the liver. Common formulations include Ease Powder, Bupleurum Decoction, and so on [41]. In addition, *Astragalus* is commonly used in clinical practice to replenish liver Qi. For example, Wang et al. [21] treat depression due to liver Qi deficiency with self-designed empirical formula "Danqi Powder." *Astragalus* can replenish Qi. Salvia can activate and nourish blood. *Pinellia ternata* tastes spicy and can resuscitate and dissipate the lump, and it is an adjuvant drug to inhibit the disadvantage of a large amount of *Astragalus*. The combination of these three drugs can invigorate Qi and activate blood circulation and has the advantage of supplementing without stagnation.

The kidney essence exhausted will make the kidney dry and hot and lead to the injury of kidney Yin. The kidney is the origin of congenital constitution and holds essence, and the essence of the kidney is the material basis of kidney Yin. It shows that the reason of kidney Yin deficiency is the insufficieny of kidney essence. The treatment of Yin deficiency and internal heat depression from the kidney is mainly based on the method of reinforcing the kidney [42]. Epicedium can warm the kidney to strengthen Yang and remove depression to anchore mind [43]. *Schisandra chinensis* has many functions, including tonifying the lung to collect Qi, nourishing the heart to remove annoyance and tranquility, strengthening the spleen and consolidating the base, regulating the rise and fall, softening the liver to relieve depression, and invigorating Qi to stabilize kidney essence [44]. These two drugs can be used according to the symptoms of depression caused by the essence insufficiency of the kidney.

7. Coronavirus Disease 2019 and Yin Deficiency and Internal Heat of Depression

Coronavirus disease 2019 (COVID-19) has the characteristics of fast transmission, wide transmission, strong infection, general population susceptibility, and no specific

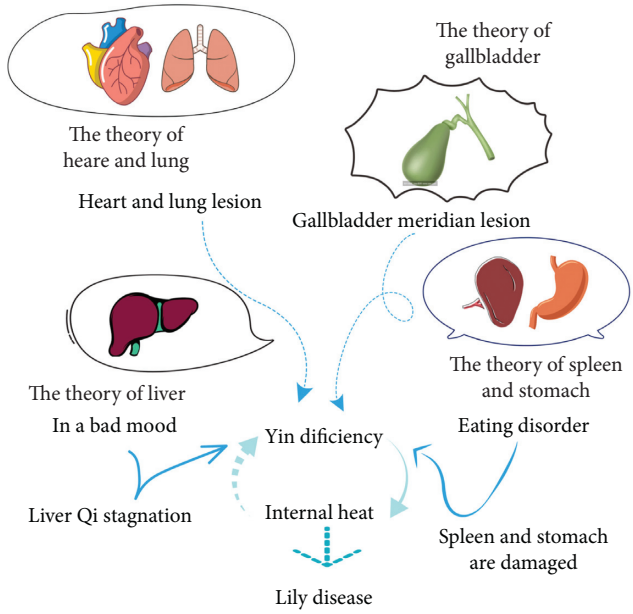

Figure 1: TCM hypothesis mechanism underlying internal and external factors inducing lily disease.

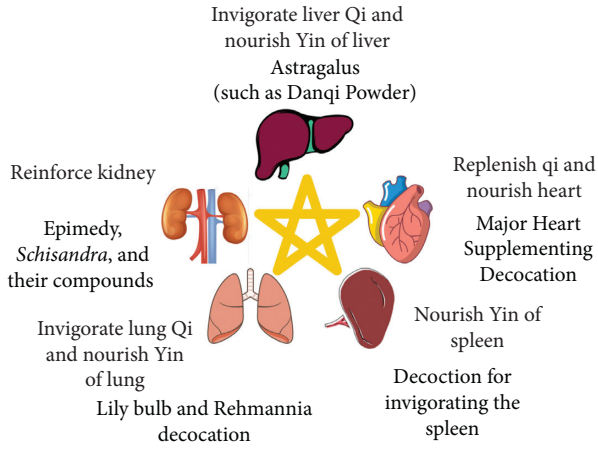

Figure 2: Traditional Chinese medicine for treatment of Yin deficiency and internal heat of depression.

therapeutic agents [45]. It is an emergent public health security event, and it not only causes physical damage to the body but also stimulates the psychological balance of the public. Recent research shows that anxiety is the highest incidence of psychological problems among the public during the epidemic, followed by depression and insomnia [46, 47].

TCM experts believe that COVID-19 was classified as "epidemic disease," with the lung and spleen as the main disease sites [48, 49]. Qi, blood, and body fluid syndrome differentiation can be divided into Qi deficiency, Qi and blood deficiency, Qi inverse, and body fluid loss; the differentiation of viscera is related to the five viscera. It can be seen that novel coronavirus pneumonia and lily disease are all located in the lung, which is closely related to the deficiency of Qi and Yin in the lung and dysfunction of the viscera [50]. Therefore, we guess COVID-19 may cause lily disease. In addition, lily disease belongs to "depression syndrome," which is closely related to emotion. TCM believes that grief damages the spleen, and it is also one of the main diseases of COVIN-19. When the novel coronavirus pneumonia causes lily disease in the later stage, it may aggravate the disease and is not conducive to the treatment of the disease.

The pathogenesis of COVID-19 varies from mild to severe, from surface to entrant, and from solid to deficiency [51, 52]. The pathogenesis is characterized by heat and located in the lung. At the beginning of the disease, the stomach accepts the pathogenic factor, so there are nasal congestion, runny nose, sore throat, and other symptoms. Pathogenic virus infected the lung and decreased its dysfunction of controlling dispersing outwards and inwards. Pathogenic virus depleted vital Qi and caused muscles to become tired and sore. Wind chill bounded body surface,

which leads to tongue coating with thin white or slightly greasy and pulse with floating tight. In the middle of the disease, pathogens block the lung, and depression causes internal heat and consumes body fluid, so the symptoms of high fever and thirst appear. Phlegm is obstructed, and Qi is stagnant, so bosom frowsty wheeze and breath are difficult [53]. Hot phlegm fills the lungs, so phlegm is sticky and mood is agitated, and at the same time, it is accompanied by short urine and constipation symptoms. In this period, some patients also have a series of typical Shaoyang syndrome such as depressed, upset, chest tightness, fever, bitterness in the mouth , poor appetite, nausea, and vomiting. In the later stage of the disease, many patients show cough, little sputum, mental exhaustion, lack of speech, thirst, poor absorption, dark red tongue with less moss, and thready or weak pulse. This is because in the later stage of the disease, the residual heat is not solved, and the spleen and stomach are damaged [54].

A recent study indicates that exogenous febrile disease and pulmonary diseases are related to the occurrence of lily disease [55]. From the perspective of TCM, COVID-19 patients are prone to lily disease in the middle and later stages of the disease. It is because that lung damage causes the malfunction of spreading and declining, and deficiency of Qi and Yin in the lungs will lead to hot and dry. If the residual heat in the late period of febrile disease has not been solved, it will consume Yin-Jin and lead to lily disease. From the perspective of sociology, COVID-19 patients often have feelings of longing for family ties, fearing of disease and the stigma of infection, loneliness, and guilt. Then, it will cause symptoms of anxiety and depression. Emotional failure will damage the Qi and blood functions of viscera and lead to the occurrence of lily disease.

TCM has been widely recognized and widely applied in the treatment of COVID-19 during the epidemic. COVID-19 prevention and treatment program and clinical prescription data in various regions of China suggested that the common high-frequency drugs of the prevention and treatment plan and clinical medical records included *Glycyrrhiza*, *Pogostemon cablin*, bitter almond, honeysuckle, *Poria cocos*, weeping forsythia, tangerine peel, and *Coix* seed [56]. Currently, Chinese patent medicine for treatment of COVID-19 is mainly included Jinhua Qinggan granule, Lianhuaqingwen capsule, etc. In addition, some researchers believe that moxibustion [57], fragrant medicines, and scraping can be applied to the prevention and treatment of COVID-19.

We believe that the treatment prescription of lily disease can be considered as a supplementary treatment for pneumonia in the middle and later stages, to clearing away heat and nourishing Yin, strengthening spleen and benefiting vital Qi, and regulating the function of viscera, Qi, and blood, which is beneficial for novel coronavirus pneumonia rehabilitation.

COVID-19 patient medical therapy should be paid not only to the treatment of pneumonia itself but also to the psychological state of patients. The patients' mood changes will affect the treatment and prognosis of the disease. So, it is necessary to carry out psychological intervention and drug therapy for emotional diseases. In addition, COVID-19 patients will still have different psychological problems after recovery and discharge from hospital, and follow-up and psychological intervention after discharge is still an urgent task.

8. Conclusion and Perspective

We systematically summarize the research status of lily disease and Yin deficiency and internal heat of depression and came to the conclusion that the cause of lily disease is the damage of viscera by external or internal injury, which will lead to Yin deficiency of viscera, and the disorder of Qi and blood; then, the veins will lose nourishment, and hence, it will lead to the symptoms of mental disorder. The Yin deficiency of viscera will lead to the virtual heat coming from the inside of the body, as well as bitterness in the mouth, red-colored urine, and other symptoms.

From the clinical symptoms, lily disease showed trance, sleep, anxiety, insomnia, and other symptoms similar to the clinical diagnostic standard of modern depression. And bitterness in the mouth, red-colored urine, and rapid and thready pulse are the typical symptoms of internal heat due to Yin deficiency. Therefore, we can infer that internal heat depression due to Yin deficiency is a major syndrome of lily disease from etiology and pathogenesis.

For COVID-19 patients, lung damage can cause spread and decline malfunction, and deficiency of Qi and Yin in the lungs will make it hot and dry. If the residual heat in the late period of febrile disease has not been solved, it will consume Yin-Jin and result in lily disease. Therefore, the treatment of lily disease can also be referenced when treating COVID-19 patients.

Data Availability

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

Disclosure

The authors strictly disclosed that all funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

BX Shang and HX Zhang contributed to literature review and data analyses. XY Zhou and Y Wang contributed to figures. K Ma contributed to the project design and paper writing. All authors have read and approved the final version of the manuscript. BX Shang and HX Zhang contributed equally to this work.

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Research Article

A Comparison Study of Chaihu Shugan San and Fluoxetine on Antidepression and Regulating Blood Rheology Effects with Chronic Restrained Stress Rats

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Chaihu Shugan San (CHSGS) is a traditional Chinese herbal formula that is often used in clinical practice to treat live Qi stagnation syndrome and depression. Fluoxetine is one of the commonly used drugs for the clinical treatment of depression. This study involved a comparison of CHSGS and fluoxetine on antidepression and regulating blood rheology effects with chronic restraint stress- (CRS-) induced depression rat models. Rats were induced depression models by CRS for 4 weeks. Upon successful induction of depression in the rats, the animal was administered CHSGS at 0.6 g/kg/d, 1.2 g/kg/d, or fluoxetine 1.8 mg/kg/d to corresponding groups by gavage for 2 weeks. The changes of CRS rats were determined by behavior observations and sucrose preference test and hypothalamic-pituitary-adrenal cortex (HPA) axis functional status. The changes in monoamine neurotransmitters and related indicators of blood status were detected by enzyme-linked immunosorbent assay (ELISA), blood rheometer, and other methods. The outcome shows that CHSGS is superior to fluoxetine in regulating the appearance and HPA axis function of model rats. In addition, CHSGS and fluoxetine have similar effects in improving blood rheology, and both can alleviate the hypercoagulable state of blood via the platelet 5-hydroxytryptamine receptor 2A (5-HT2A) pathway in rats of depression. It was also observed that CHSGS can improve the blood state of depressed rats by restoring liver coagulation-anticoagulation balance and endothelium-related functions.

1. Introduction

Depression is a mental disorder, which is closely related to chronic diseases such as stroke [1], cardiovascular disease [2], and diabetes [3]. Studies have pointed out that depression and adverse cardiac events have the same underlying pathological mechanism, including neuroendocrine dysfunction, cardiac autonomic control disorder, endothelial dysfunction, inflammation, and enhanced platelet reactivity [4]. In addition, the American Heart Association issued in 2014 stated that depression is closely related to the incidence rate and mortality of acute coronary syndrome [5].

Clinical studies show that the activation and aggregation rates of fibrinogen and platelet in patients with depression are significantly increased [6–8], and the levels of fibrinogen and platelet activation are related to the severity of depression [6, 7]. Among the patients with severe anxiety and depression, the levels of blood factor VII, von Willebrand factor, prothrombin fragment 1 + 2, and plasminogen activator inhibitor-1 (PAI-1) are significantly higher than those of the healthy control group [9]. Experimental studies also show that CRS enhances the platelet agonist thrombin levels and the ability of adenosine diphosphate (ADP) to stimulate platelet aggregation in mice [10]. Previous research indicated

that depression has hemorheological changes characterized by hypercoagulability, which is easy to form thrombus, and is an important factor inducing cardiovascular and cerebrovascular diseases. Therefore, it is of great significance for the rational use of drugs and the prevention and treatment of cardiovascular diseases to comprehensively assess the pharmacological actions of antidepressants, especially the effects on blood rheology.

Liver Qi stagnation syndrome is one of the most common clinical syndromes in traditional Chinese medicine (TCM). Its formation is related to the negative psychological stress states such as emotional depression [11]. The clinical symptoms include depression, moodiness, chest distention and stuffiness, irritability, and the tendency of crying. It is very similar to modern depression [12]. It is also observed that the animal model of liver Qi stagnation syndrome in TCM established by CRS has behavioral characteristics similar to depression [13]. It is suggested that liver Qi stagnation syndrome in TCM might have the same pathophysiological connotation as depression in western medicine.

CHSGS is composed of *Chaihu*, *Chenpi*, *Chuanxiong*, *Xiangfu*, *Zhiqiao*, *Shaoyao*, and *Gancao*, and is the representative formula in the treatment of liver Qi stagnation syndrome by improving the depression state of liver Qi stagnation syndrome [14]. Fluoxetine is a selective serotonin reuptake inhibitor, which is widely used in the treatment of depression [15]. However, there are few studies about the effect of fluoxetine on the blood rheology of depression.

In summary, depression has the hemorheological basis of cardiovascular disease, and both Chinese herbal formula CHSGS and fluoxetine have a certain effect on treating liver Qi stagnation syndrome and depression. However, there is no report about the efficacy characteristics of the two drugs in the treatment of depression. Based on this, we made a hypothesis that "CHSGS can regulate blood rheology while improving depression." To validate this hypothesis, this study intends to use a CRS rat model to compare CHSGS and fluoxetine on antidepression and improving blood rheology. This study provides a certain experimental basis for understanding the pathophysiological basis of the liver Qi depression syndrome in TCM and depression in western medicine, by rationally assessing the antidepression effects of the two drugs as well as their potential application values in preventing cardiovascular diseases.

2. Materials and Methods

2.1. Ethical Approval. Wistar male rats, purchased from the Beijing Vital River Laboratory Animal Technologies Co. Ltd., were maintained in a specific pathogen-free (SPF) laboratory. The experiments performed herein were approved by the Ethics Committee of Beijing University of Chinese Medicine (No. BUCM-4-2018060415-2019). In order to abide by the 3R Principle of Animal Experiments, the number of rats was minimized so that the smallest number was experimentally used while still retaining statistical significance. Thus, we chose $n = 10$ for each group.

2.2. Materials. CHSGS (*Chaihu*, *Chenpi*, *Chuanxiong*, *Xiangfu*, *Zhiqiao*, *Shaoyao*, and *Gancao*) was purchased from Beijing Tongrentang Technology Development Co. (Beijing, China). Fluoxetine hydrochloride capsules were purchased from Eli Lilly Suzhou Pharmaceutical Co., Ltd. (Jiangsu, China). The dopamine (DA), 5-hydroxytryptamine (5-HT), corticotropin-releasing hormone (CRH), noradrenaline (NE), corticotropin (CORT), 6-keto-protagiandinF1α (6-Keto-PGF1α), protein C (PC), free protein S (FPS), antithrombin III (AT-III), tissue-type plasminogen activator (t-PA), PAI-I, P-selectin (Ps), and thromboxane B2 (TXB2) ELISA kit were purchased from Beijing Rigorbio Science Development Co., Ltd. (Beijing, China). 5-HT2A, serotonin transporter (SERT), and glutamine transaminase (tGase) ELISA kit were purchased from Beijing Sino-UK Institute of Technology (Beijing, China). Rat calcium fluorescent probe Fluo-3 was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China).

2.3. Experimental Design and Tissue Collection. Male Wistar rats ($n = 50$, 9 weeks old), with an initial weight of 210–220 g, were randomly divided into 5 groups, namely, the control group, the model group, the CHSGS + 0.6 g/kg group, the CHSGS + 1.2 g/kg group, and the fluoxetine group. Except for the control group, the other groups were induced depression models by CRS [16], which is placing the rats in a cylinder of 25 cm length, 7 cm outer diameter, and 5 cm inner diameter, with a plexiglass restraint of adjustable length, for 3 h (8:00–11:00 am) daily for 28 days. During the last 2 weeks of CRS, the CHSGS + 0.6 g/kg group and the CHSGS + 1.2 g/kg group underwent intragastric administration of 0.6 g/kg/d and 1.2 g/kg/d CHSGS (CHSGS was made into granules by water extraction and alcohol precipitation, making sure each gram of granules contains 5 g crude medicine. The associated dose was determined using the "dose conversion coefficient table for animal and human body weight," which were 2 times and 1 time of the equivalent dose, respectively). The fluoxetine group underwent an intragastric administration of 1.8 mg/kg/d. The associated dose was determined using the "dose conversion coefficient table for animal and human body weight". The control group and the model group were given normal saline gavage during those 2 weeks. After 4 weeks, rats were sacrificed by intraperitoneal injection of 1% pentobarbital, blood and hypothalamus were taken, plasma and platelets were separated from the blood, and the hypothalamus was preserved at 80°C for detection.

2.4. Detection of Indicators

2.4.1. Behavioral Observations [17]. The rats in each group were monitored twice a week (Tuesday and Saturday) over body weight and general behaviors (behavioral state, activity level, emotional response, and sleep state). The scores of the changes in appearance and behavior of rats in each group were counted every week, and the mean of absolute values of observations twice a week was taken. Finally, the appearance representations of similar nature were combined into two

indicator groups (active state, emotional-sleep), and the scores of each indicator group were counted separately. See Table 1 for specific scoring criteria.

2.4.2. Sucrose Preference Test [18]. For this test, rats were trained to consume 1% sucrose solution for 2 days prior to the start of the experiment. On the first day, the rats were trained to adapt to two bottles of 1% sucrose solution, and 24 hours later, one of the bottles was replaced with water for 24 hours. After the adaption, the rats were deprived of water and food for 24 hours. The sucrose preference test was conducted at 9:00 a.m. in which rats were individually housed, and then each was given one bottle of water and one bottle of sucrose for 1 h. On the 28th day of the modeling, the volumes of consumed sucrose solution and water were measured, and the sucrose preference was calculated by the following formula: sucrose preference = sucrose consumption/(water consumption + sucrose consumption) × 100% and was corrected for body weight.

2.4.3. ELISA. The plasma samples were tested on the levels of 5-HT, CORT, Ps, TXB2, 6-keto-PGF1α, PC, FPS, AT-III, t-PA, and PAI-I, using specific ELISA kits according to the manufacturer's instructions. The hypothalamus samples were tested on the levels of CRH, DA, and NE, using specific ELISA kits according to the manufacturer's instructions. The platelet samples were tested on the levels of 5-HT2A, SERT, and tGase, using specific ELISA kits according to the manufacturer's instructions.

2.4.4. Ca^{2+} Concentration Detection. First, an equal volume of 20% Pluronic F127 solution was added to Fluo-3, AM/DMSO solution. Second, the sample was added to Fluo-3, AM working solution, and incubated at 37°C for 20 minutes. Then, 5 times the volume of HBSS containing 1% fetal bovine serum was added and continued to incubate for 40 minutes. Next, the cells were washed 3 times with HEPES buffer saline. Then, the cells were resuspended with HEPES buffer saline to make a solution of 1×10^5 cells/mL, incubated at 37°C for 10 minutes, and then used for detection. The excitation wavelength was 506 nm, and the emission wavelength was 526 nm.

2.4.5. Other Indicators. Whole blood viscosity, whole blood reduced viscosity, plasma viscosity, erythrocyte sedimentation rate, and erythrocyte sedimentation equation K value were detected by a blood rheometer. Platelet aggregation rate and the level of plasma coagulated with four items, prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen (FIB), were detected by an automatic coagulation instrument.

2.5. Statistical Analysis. SPSS statistics 20.0 software was used for statistical analyses. All of the data presented were the mean ± standard deviation (SD) and were compared by one-way ANOVA with data at a normal distribution. Nonnormal distribution data were analyzed using nonparametric statistics. p value <0.05 was considered to be statistically significant.

3. Results

3.1. Effects of CHSGS and Fluoxetine on the Appearance and Behavior in CRS Rats. There was no significant change in the activity status scores and the emotional-sleep scores of the control group at different time points in the experiment (Figures 1(a) and 1(b)). The activity status scores were significantly increased in the model group at 1–4 weeks compared with those of the control group ($p < 0.01$) (Figure 1(a)). The activity status scores were significantly decreased in the CHSGS + 0.6 g/kg group and the CHSGS + 1.2 g/kg group at the 4th week compared with those of the model group ($p < 0.01$) (Figure 1(a)). The emotional-sleep scores were significantly increased in the model group at 1–4 weeks compared with those of the control group ($p < 0.01$) (Figure 1(b)). The emotional-sleep scores were significantly decreased in the CHSGS + 1.2 g/kg group and the fluoxetine group in the 3rd week and significantly decreased in the CHSGS + 0.6 g/kg group, the CHSGS + 1.2 g/kg group, and the fluoxetine group in the 4th week compared with those of the model group ($p < 0.01$) (Figure 1(b)). The body weight was decreased significantly from week 1 to week 4 in the model group compared with that of the control group ($p < 0.01$) (Figure 1(c)). The body weight was increased significantly in the 3rd week and 4th week in the CHSGS + 1.2 g/kg group and significantly increased in the 3rd week in the CHSGS + 0.6 g/kg group compared with that of the model group ($p < 0.01$ or < 0.05) (Figure 1(c)). The body weight was increased significantly in the 3rd week in the CHSGS + 0.6 g/kg group and significantly increased in the 3rd week and 4th week in the CHSGS + 1.2 g/kg group compared with that of the fluoxetine group ($p < 0.01$ or < 0.05) (Figure 1(c)). The body weight was increased significantly in the 3rd week in the CHSGS + 1.2 g/kg group compared with that of the CHSGS + 0.6 g/kg group ($p < 0.01$) (Figure 1(c)). The sucrose consumption was significantly decreased in the model group compared with that of the control group ($p < 0.05$) (Figure 1(d)). The sucrose consumption was significantly increased in the CHSGS + 1.2 g/kg group and the fluoxetine group compared with that of the model group ($p < 0.05$) (Figure 1(d)).

3.2. Effects of CHSGS and Fluoxetine on the HPA Axis in CRS Rats. The CRH levels in the hypothalamus and CORT levels in plasma were significantly increased in the model group compared with those of the control group ($p < 0.01$) (Figures 2(a) and 2(b)). The CRH levels in the hypothalamus and CORT levels in plasma were significantly decreased in the CHSGS + 1.2 g/kg group compared with those of the model group ($p < 0.01$ or 0.05) (Figures 2(a) and 2(b)). The CORT levels in plasma were significantly decreased in the fluoxetine group compared with those of the model group ($p < 0.01$ or 0.05) (Figure 2(b)). The CRH levels in the

Table 1: General appearance behavior scoring standards in rats.

Observed indicators	Scoring standards (score)				
Behavioral state	Getting together 2	Less moving 1	Normal 0	Much moving 1	Restlessness 2
Activity level	Sluggish 2	Weaken 1	Normal 0	Much moving 1	Excited 2
Emotional response	Sluggish 2	Slower 1	Normal 0	Irritable 1	Angry 2
Sleep state	Lethargic 2	Tired 1	Normal 0	Light sleep 1	Easily awakened 2

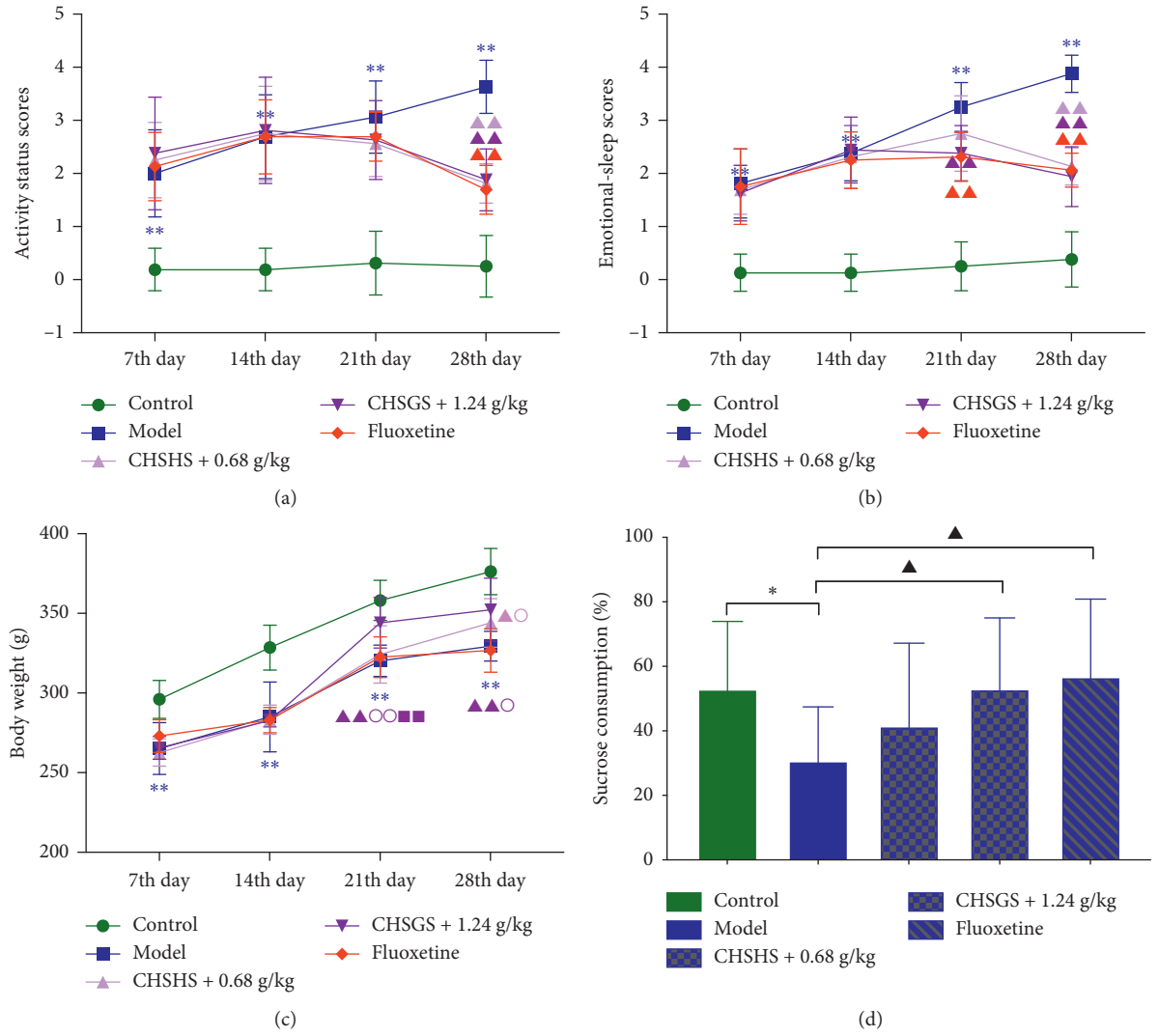

Figure 1: Effects of CHSGS and fluoxetine on the appearance and behavior in CRS rats. (a) Activity status scores. (b) Emotional-sleep scores. (c) Body weight. (d) Sucrose consumption. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, $^{*}p < 0.05$, compared with the control group; $^{\blacktriangle\blacktriangle}p < 0.01$, $^{\blacktriangle}p < 0.05$, compared with the model group; $^{\circ\circ}p < 0.01$, $^{\circ}p < 0.05$, compared with the fluoxetine group; $^{\blacksquare\blacksquare}p < 0.01$, $^{\blacksquare}p < 0.05$, compared with the CHSGS + 0.6 g/kg group.

hypothalamus and CORT levels in plasma were significantly decreased in the CHSGS + 1.2 g/kg group compared with those of the CHSGS + 0.6 g/kg group ($p < 0.01$ or 0.05) (Figures 2(a) and 2(b)).

3.3. Effects of CHSGS and Fluoxetine on the Monoamine Neurotransmitter in CRS Rats. The NE levels in the hypothalamus and 5-HT levels in plasma were significantly decreased in the model group compared with those of the control group ($p < 0.01$) (Figures 3(b) and 3(c)). The DA levels in the hypothalamus were significantly increased in the CHSGS + 0.6 g/kg group compared with those of the model group ($p < 0.05$) (Figure 3(a)). The NE levels in the hypothalamus were significantly increased in the CHSGS + 1.2 g/kg group compared with those of the model group ($p < 0.05$) (Figure 3(b)). The 5-HT levels in plasma were significantly increased in the CHSGS + 0.6 g/kg group, the CHSGS + 1.2 g/kg group, and the fluoxetine group compared with those of the model group ($p < 0.01$ or 0.05) (Figure 3(c)).

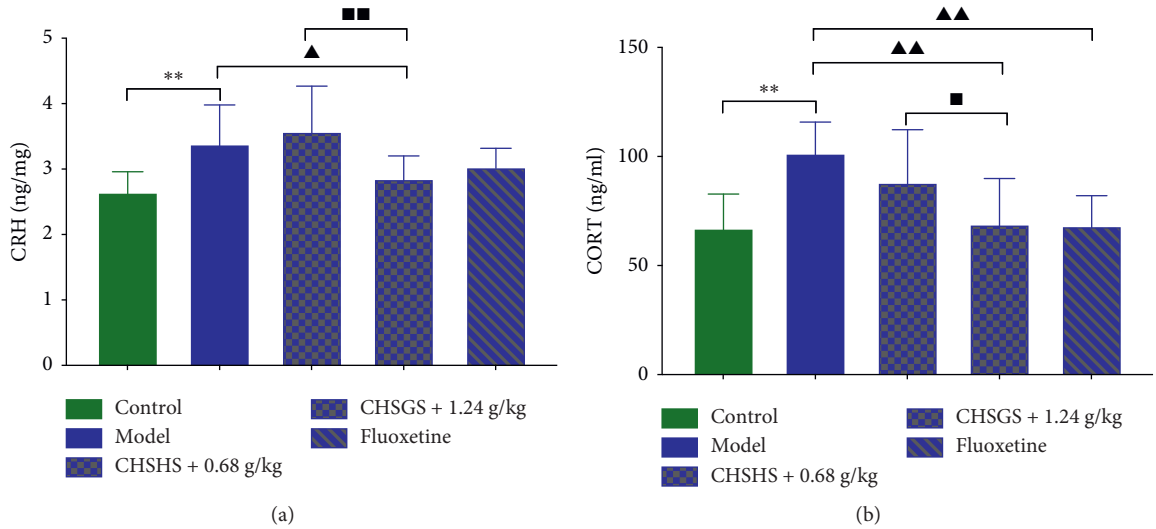

FIGURE 2: Effects of CHSGS and fluoxetine on the HPA axis in CRS rats. (a) The CRH levels in the hypothalamus. (b) The CORT levels in plasma. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, compared with the control group; $^{▲▲}p < 0.01$, $^{▲}p < 0.05$, compared with the model group; $^{■■}p < 0.01$, $^{■}p < 0.05$, compared with the CHSGS + 0.6 g/kg group.

3.4. Effects of CHSGS and Fluoxetine on Blood Rheology in CRS Rats. The plasma viscosity, the whole blood viscosity of low cut, medium cut, and high cut, the whole blood reduced viscosity of low cut, medium cut, and high cut, the blood sedimentation rate, and the blood sedimentation equation K value were significantly increased in the model group compared with those of the control group ($p < 0.01$ or 0.05) (Figure 4). The plasma viscosity, the whole blood viscosity of low cut and medium cut, the whole blood reduced viscosity of low cut, medium cut, and high cut, the blood sedimentation rate, and the blood sedimentation equation K value were significantly decreased in the CHSGS + 0.6 g/kg group compared with those of the model group ($p < 0.01$ or 0.05) (Figures 4(a), 4(b), and 4(d)–4(i)). The plasma viscosity, the whole blood viscosity of low cut, medium cut, and high cut, the whole blood reduced viscosity of low cut and high cut, the blood sedimentation rate, and the blood sedimentation equation K value were significantly decreased in the CHSGS + 1.2 g/kg group compared with those of the model group ($p < 0.01$ or 0.05) (Figures 4(a)–4(e) and 4(g)–4(i)). The plasma viscosity, the whole blood viscosity of low cut, medium cut, and high cut, the whole blood reduced viscosity of low cut, medium cut, and high cut, the blood sedimentation rate, and the blood sedimentation equation K value were significantly decreased in the fluoxetine group compared with those of the model group ($p < 0.01$ or 0.05) (Figure 4). The whole blood viscosity of low cut and the whole blood reduced viscosity of medium cut were significantly increased and the erythrocyte sedimentation rate was significantly decreased in the CHSGS + 1.2 g/kg group compared with those of the fluoxetine group ($p < 0.01$ or 0.05) (Figures 4(a), 4(f), and 4(h)). The erythrocyte sedimentation rate was significantly decreased in the CHSGS + 0.6 g/kg group compared with that of the fluoxetine group ($p < 0.01$) (Figure 4(h)). The plasma viscosity, the whole blood viscosity of low cut and high cut, and the whole blood reduced viscosity of low cut, medium cut, and high cut were significantly increased compared with those of the CHSGS + 0.6 g/kg group ($p < 0.05$) (Figures 4(b)–4(g)).

3.5. Effects of CHSGS and Fluoxetine on Four Items of Coagulation in CRS Rats. The PT and APTT were significantly decreased and the FIB was significantly increased in the model group compared with those of the control group ($p < 0.01$ or 0.05) (Figures 5(a), 5(b), and 5(d)). The PT and APTT were significantly increased and the FIB was significantly decreased in the CHSGS + 0.6 g/kg group, the CHSGS + 1.2 g/kg group, and the fluoxetine group compared with those of the model group ($p < 0.01$) (Figures 5(a), 5(b), and 5(d)). The TT was significantly increased in the fluoxetine group compared with that of the model group ($p < 0.05$) (Figure 5(c)).

3.6. Effects of CHSGS and Fluoxetine on Platelet Aggregation Rate in CRS Rats. The platelet aggregation rates at various time points were significantly increased in the model group compared with those of the control group ($p < 0.01$) (Figure 6). The platelet aggregation rates at various time points were significantly decreased in the CHSGS + 0.6 g/kg group, the CHSGS + 1.2 g/kg group, and the fluoxetine group compared with those of the model group ($p < 0.01$) (Figure 6). The platelet aggregation rates at 5 min were significantly decreased in the CHSGS + 1.2 g/kg group compared with those of the CHSGS + 0.6 g/kg group ($p < 0.05$) (Figure 6(c)).

3.7. Effects of CHSGS and Fluoxetine on the 5-HT2A Signaling Pathway in CRS Rats. The 5-HT2A, SERT, tGase, and Ca^{2+} levels in platelets and the Ps and TXB2 levels in plasma were significantly increased in the model group compared with those of the control group ($p < 0.01$) (Figure 7). The 5-HT2A, SERT, tGase, and Ca^{2+} levels in platelets and the Ps and TXB2 levels in

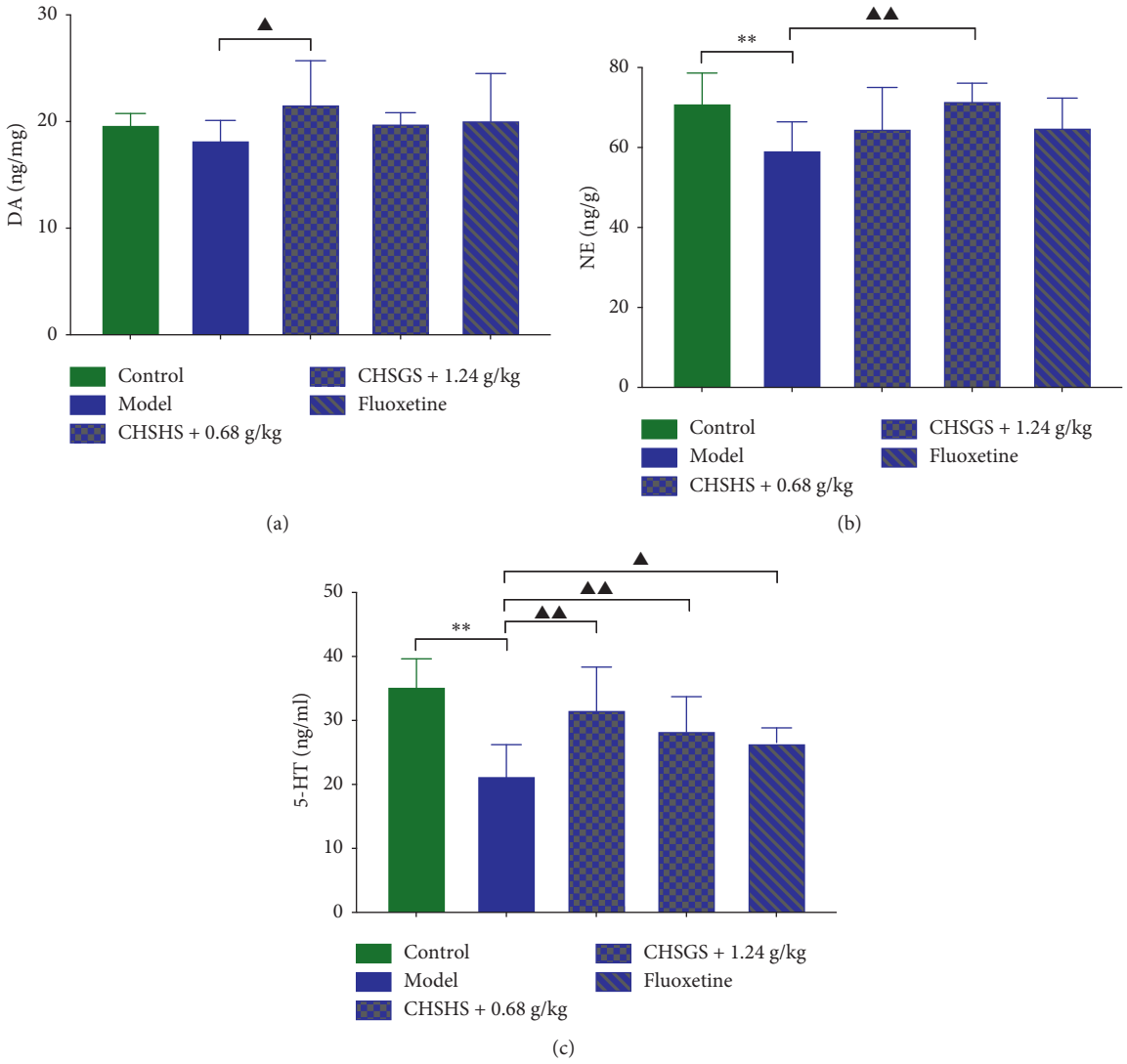

Figure 3: Effects of CHSGS and fluoxetine on the monoamine neurotransmitter in CRS rats. (a) The DA levels in the hypothalamus. (b) The NE levels in the hypothalamus. (c) The 5-HT levels in plasma. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, compared with the control group; $^{▲▲}p < 0.01$, $^{▲}p < 0.05$, compared with the model group.

plasma were significantly decreased in the CHSGS + 1.2 g/kg group and the fluoxetine group compared with those of the model group ($p < 0.01$ or 0.05) (Figure 7). The SERT tGase levels in platelets and the TXB2 levels in plasma were significantly decreased in the CHSGS + 0.6 g/kg group compared with those of the model group ($p < 0.05$) (Figures 7(b), 7(c), and 7(f)). The 5-HT2A, SERT, and tGase levels in platelets and the Ps and TXB2 levels in plasma were significantly increased in the CHSGS + 0.6 g/kg group compared with those of the fluoxetine group ($p < 0.01$ or 0.05) (Figures 7(a)–7(c), 7(e), and 7(f)). The SERT tGase levels in platelets and the Ps and TXB2 levels in plasma were significantly decreased in the CHSGS + 1.2 g/kg group compared with those of the CHSGS + 0.6 g/kg group ($p < 0.01$ or 0.05) (Figures 7(a), 7(b), 7(e), and 7(f)).

3.8. Effects of CHSGS and Fluoxetine on the Anticoagulant Cofactor in CRS Rats. The PC, FPS, and AT-III levels in plasma were significantly decreased in the model group compared with those of the control group ($p < 0.01$) (Figure 8). The PC, FPS, and AT-III levels in plasma were significantly increased in the CHSGS + 0.6 g/kg group compared with those of the model group ($p < 0.01$) (Figure 8). The FPS levels in plasma were significantly increased in the CHSGS + 1.2 g/kg group and the fluoxetine group compared with those of the model group ($p < 0.01$ or 0.05) (Figure 8(b)).

3.9. Effects of CHSGS and Fluoxetine on the Fibrinolysis Cofactor in CRS Rats. The t-PA and 6-keto-PGF1α levels in plasma were significantly decreased in the model group compared with those of the control group ($p < 0.01$) (Figures 9(a) and 9(c)). The t-PA and 6-keto-PGF1α levels in plasma were significantly increased in the CHSGS + 0.6 g/kg group compared with those of the model group ($p < 0.01$ or 0.05) (Figures 9(a) and 9(c)). The 6-keto-PGF1α levels in plasma were significantly increased in the

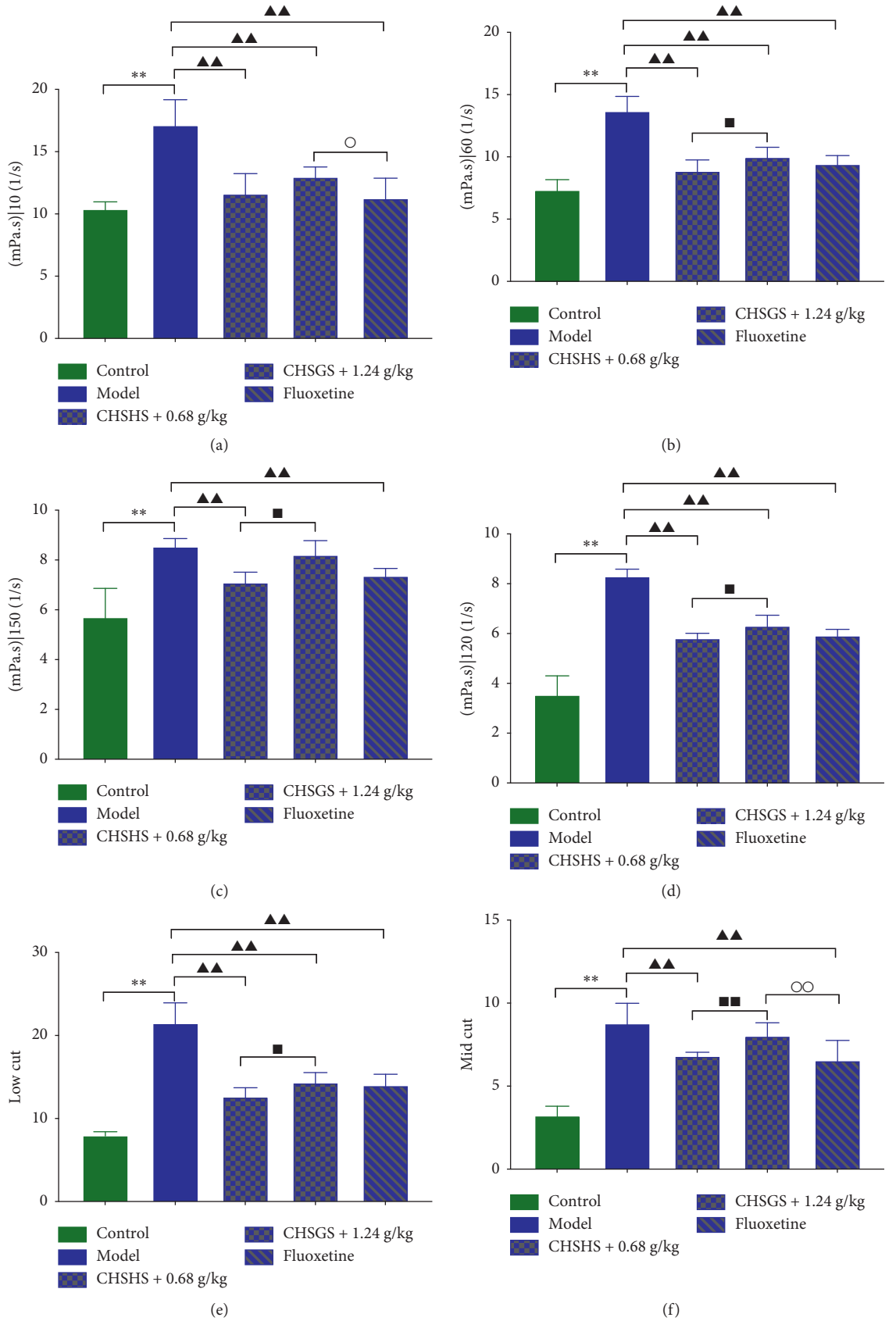

Figure 4: Continued.

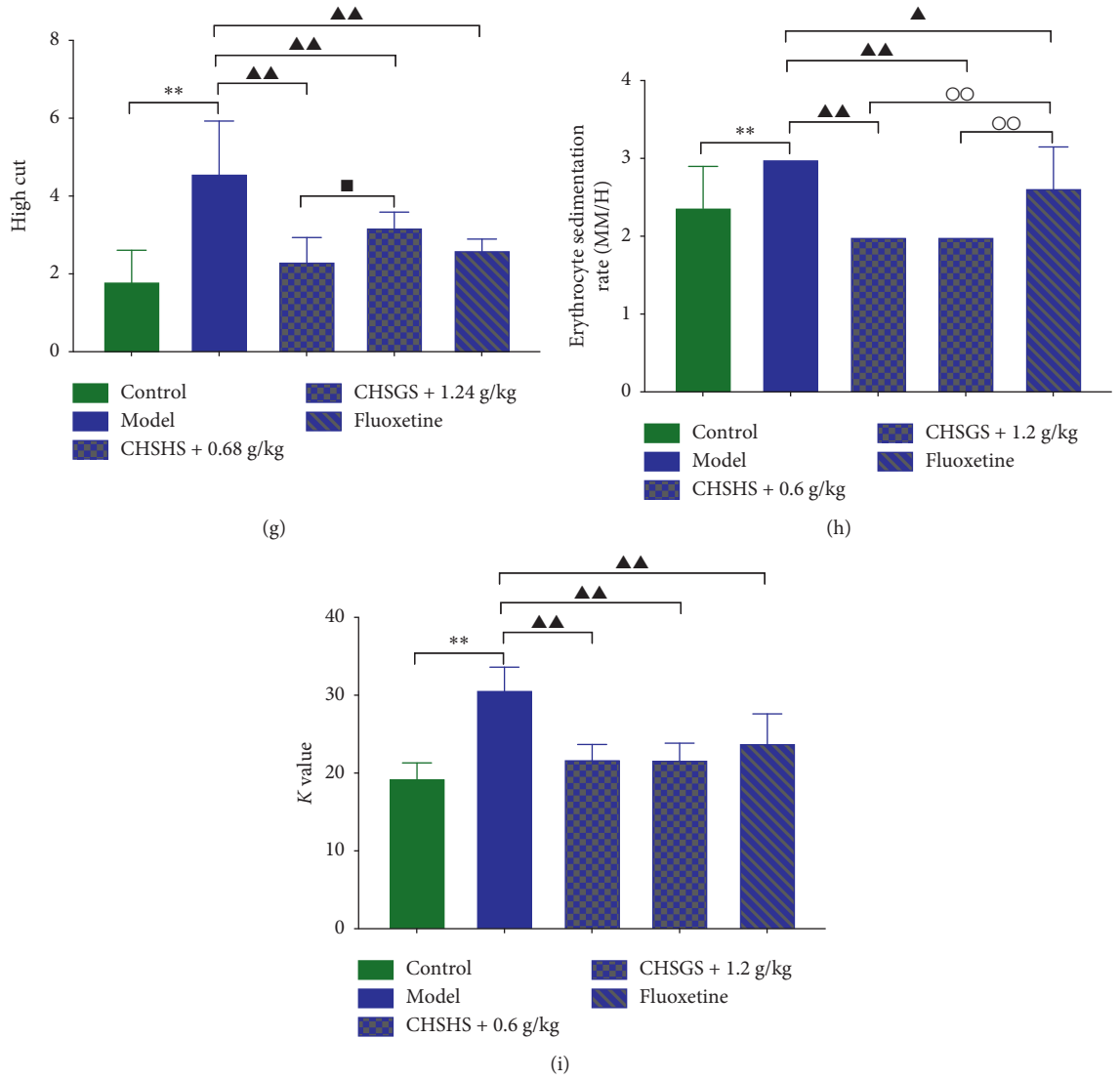

Figure 4: Effects of CHSGS and fluoxetine on blood rheology in CRS rats. (a) Low-cut whole blood viscosity. (b) Middle-cut whole blood viscosity. (c) High-cut whole blood viscosity. (d) The plasma viscosity. (e) Low-cut whole blood reduced viscosity. (f) Middle-cut whole blood reduced viscosity. (g) High-cut whole blood reduced viscosity. (h) Erythrocyte sedimentation rate. (i) Blood sedimentation equation K value. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, compared with the control group; $^{\blacktriangle\blacktriangle}p < 0.01$, $^{\blacktriangle}p < 0.05$, compared with the model group; $^{\circ\circ}p < 0.01$, $^{\circ}p < 0.05$, compared with the fluoxetine group; $^{\blacksquare\blacksquare}p < 0.01$, $^{\blacksquare}p < 0.05$, compared with the CHSGS + 0.6 g/kg group.

CHSGS + 1.2 g/kg group compared with those of the model group ($p < 0.05$) (Figure 9(c)). The 6-keto-PGF1α levels in plasma were significantly increased in the CHSGS + 0.6 g/kg group compared with those of the fluoxetine group ($p < 0.01$) (Figure 9(c)).

4. Discussion

4.1. Establishment of Liver Qi Stagnation Syndrome/Depression Rat Model. Although depression and liver Qi stagnation syndrome belong to different medical systems, clinical and experimental studies have shown that both of them have monoamine neurotransmitter disturbance [19, 20], HPA axis dysfunction [21, 22], and abnormal blood rheology [23, 24]. It is suggested that depression and liver Qi stagnation syndrome have a common pathophysiological basis. At present, there are 9 replication types of depression animal models, including learned helplessness (LH), unpredictable chronic mild stress (UCMS), early-life stress model, and CRS model [25]. Among them, the CRS model is relatively simple and has been widely used in the research of depression. It is also a recognized model of liver Qi stagnation syndrome in TCM [26]. Therefore, this study chose CRS to establish the rat model of liver Qi stagnation syndrome and depression.

In this experiment, we observed the appearance changes of model rats after two weeks of modeling, such as less movement, slow response, and loss of appetite and weight. After four weeks of modeling, sucrose consumption

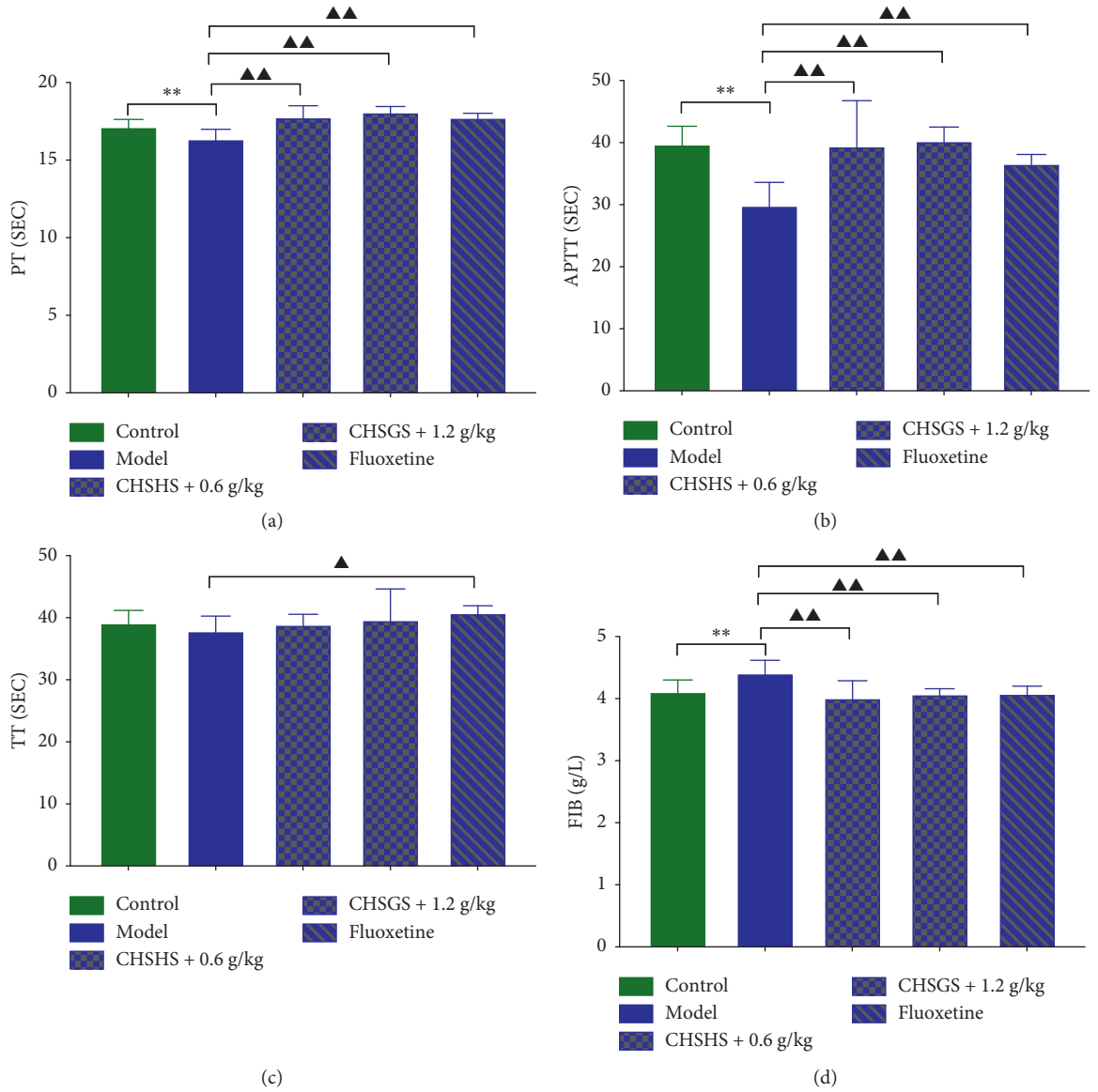

Figure 5: Effects of CHSGS and fluoxetine on four items of coagulation in CRS rats: (a) PT; (b) APTT; (c) TT; (d) FIB level in blood. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, compared with the control group; $^{▲▲}p < 0.01$, $^{▲}p < 0.05$, compared with the model group.

decreased significantly. It was consistent with previous experimental results [27], indicating that the model rats were in a depressed state. It was also observed that the levels of CRH in the hypothalamus and CORT in plasma were significantly increased, and the levels of NE in the hypothalamus and 5-HT in plasma were significantly decreased in model rats. It indicates that the model rats have peripheral monoamine neurotransmitter reduction and HPA axis hyperfunction, which is consistent with the neuroendocrine changes of depression [28].

4.2. The Antidepressant Effect of CHSGS and Fluoxetine. CHSGS is an effective compound formula for clinical treatment of liver Qi stagnation syndrome [14] and depression [29] in TCM. Fluoxetine is one of the commonly used medicines for depression in western medicine [17]. Pharmacological studies have shown that, as a nontricyclic antidepressant, it mainly inhibits the reuptake of 5-HT in the central nervous system and prolongs and improves the effect of 5-HT in order to treat depression [16]. This study observed the effect of the two drugs on the appearance behavior and neuroendocrine system of the model and compared the utility results of them. See Tables 2 and 3.

Both Tables 2 and 3 illustrate that CHSGS and fluoxetine improved the appearance behavior and laboratory indicators of CRS rats in varying degrees after two weeks of administration. Among them, the CHSGS + 1.2 g/kg group had a more obvious effect than the CHSGS + 0.6 g/kg group. Both CHSGS and fluoxetine had a positive effect on the appearance behavior

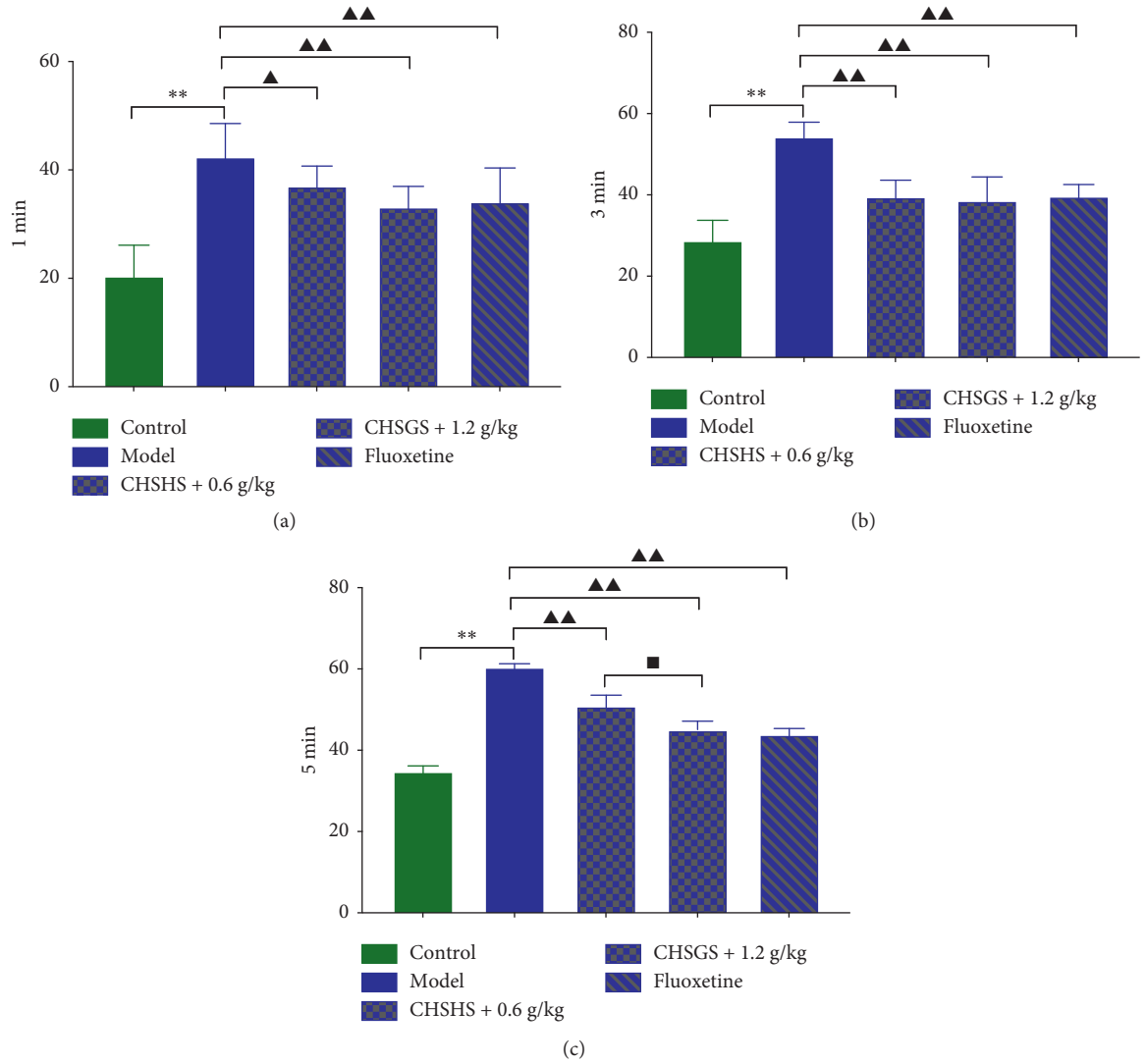

Figure 6: Effects of CHSGS and fluoxetine on platelet aggregation rate in CRS rats: (a) platelet aggregation rate at 1 min; (b) platelet aggregation rate at 3 min; (c) platelet aggregation rate at 5 min. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, compared with the control group; $^{▲▲}p < 0.01$, $^{▲}p < 0.05$, compared with the model group; $^{■}p < 0.05$, compared with the CHSGS + 0.6 g/kg group.

score, sucrose consumption, and monoamine transmitter of model rats. CHSGS could also antagonize the weight loss and HPA axis hyperfunction of model rats, while fluoxetine had no obvious effect. It is suggested that CHSGS is superior to fluoxetine in the comprehensive neuroendocrine regulation of the depression model rats.

4.3. Changes of Blood Coagulation and Rheology in CRS Rats. This experiment explored the blood state of depression model rats from three aspects: blood rheology, changes in blood coagulation time, and platelet activation and aggregation. Blood rheology examination mainly reflects the changes of blood fluidity, stagnant, and viscosity [30]. In four items of coagulation, PT, APTT, TT, and FIB are included. PT and APTT reflect the level of the coagulation factor in plasma. TT is the time required for fibrinogen to be converted into fibrin, reflecting the presence of anticoagulants and fibrinolysis in plasma. FIB is the precursor of fibrin, which reflects the content of fibrinogen in the blood. Fibrinogen can be enzymatically converted into fibrin by thrombin, which blocks blood vessels and prevents excessive bleeding. The content of FIB indicates whether the body is in a hypercoagulable state [31]. The platelet aggregation test mainly reflects the aggregation of platelets, and its intensity is closely related to blood hypercoagulability.

In this experiment, it was observed that the low-cut, middle-cut, and high-cut whole blood viscosity and whole blood reduced viscosity, plasma viscosity, erythrocyte sedimentation rate, and blood sedimentation equation K value of CRS rats were significantly increased. PT and APTT were significantly decreased, while FIB and platelet aggregation rate at 1 min/3 min/5 min were significantly increased. This indicates that the blood rheology disorder of model rats involved the enhancement of coagulation, the decrease of fibrinolysis, and the enhancement of platelet aggregation,

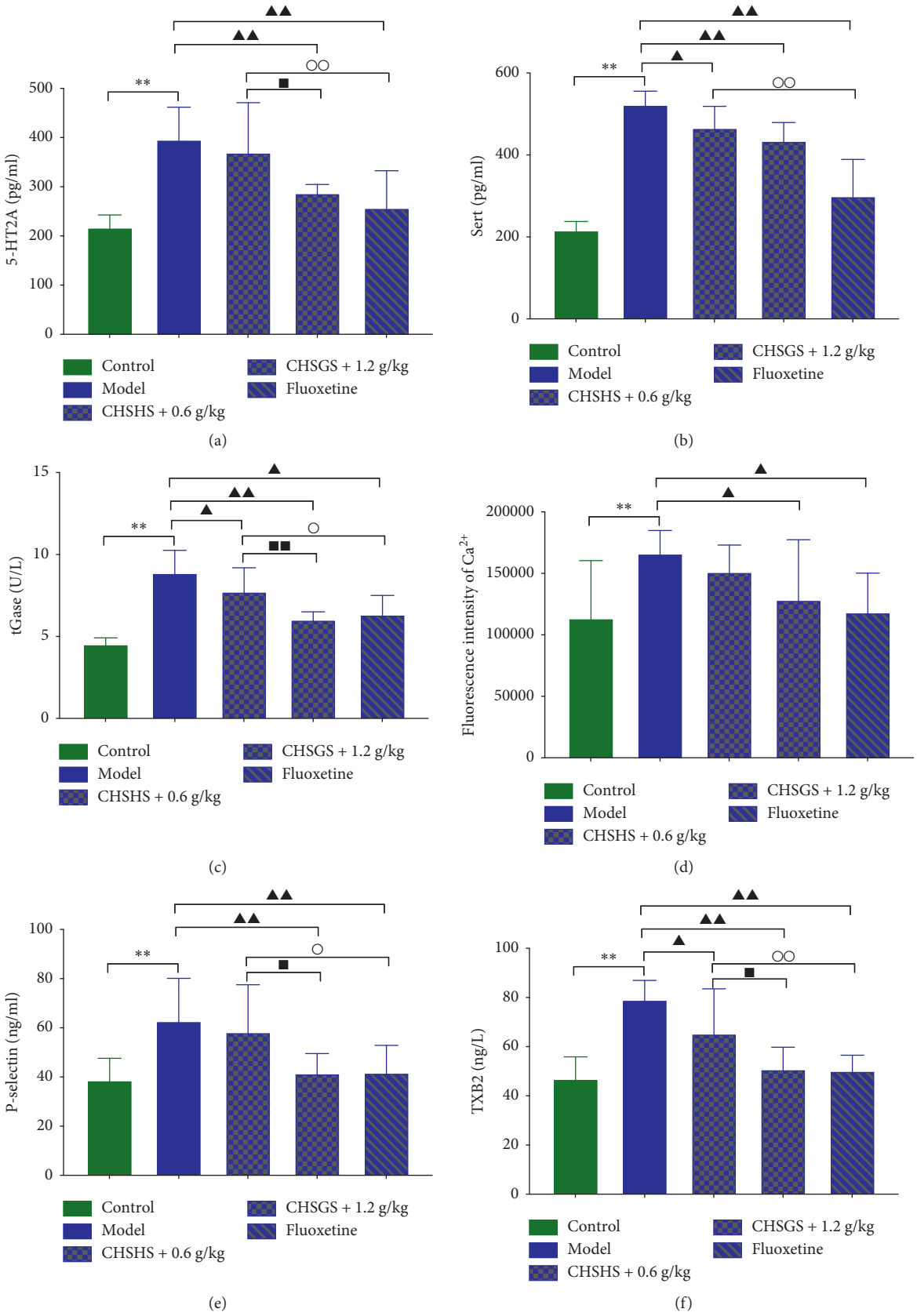

FIGURE 7: Effects of CHSGS and fluoxetine on the 5-HT2A signaling pathway in CRS rats: (a) 5-HT2A levels in platelets; (b) SERT levels in platelets; (c) tGase levels in platelets; (d) fluorescence intensity of Ca^{2+}; (e) Ps levels in plasma; (f) TXB2 levels in plasma. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, compared with the control group; $^{▲▲}p < 0.01$, $^{▲}p < 0.05$, compared with the model group; $^{○○}p < 0.01$, $^{○}p < 0.05$, compared with the fluoxetine group; $^{■■}p < 0.01$, $^{■}p < 0.05$, compared with the CHSGS + 0.6 g/kg group.

speculating that the blood is in a hypercoagulable state and has a tendency of thrombosis.

4.4. Regulatory Effect of CHSGS and Fluoxetine on Blood Rheology in CRS Rats

4.4.1. Positive Effect on Blood Rheology of Model Rats. The effect of CHSGS and fluoxetine on blood rheology of model rats was compared. See Tables 4 and 5.

Both Tables 4 and 5 illustrate that both CHSGS and fluoxetine groups have a significant improvement in blood state-related indicators. The utility spectrum of CHSGS and fluoxetine was similar. For example, they could improve blood rheology indicators, prolong blood coagulation time, reduce blood fibrinogen content, and inhibit platelet activation and aggregation. The results showed that both of them could improve the hypercoagulable state of blood in CRS rats. Apart from it, CHSGS seemed to be superior to fluoxetine in reducing erythrocyte sedimentation rate, while fluoxetine could prolong TT, suggesting that the anticoagulant mechanism of fluoxetine might involve the activation of fibrinogen.

Previous studies showed that CHSGS significantly reduced the blood viscosity and platelet aggregation rate of the liver Qi stagnation syndrome rat model by CRS [13]. Fluoxetine could significantly reduce the whole blood viscosity [32] and platelet aggregation and activity [33] in patients with depression. This study found that CHSGS and fluoxetine could not only improve blood rheology and inhibit platelet aggregation but also regulate the abnormalities of the coagulation/fibrinolysis system associated with depression. It is suggested that CHSGS and fluoxetine could improve blood rheology and reduce thrombosis of depressed patients in multiple ways.

4.4.2. Mechanism of CHSGS and Fluoxetine on Blood Rheology in CRS Rats

(1) Mechanism of Abnormal Blood Rheology in Depression Model Rats. Platelet activation plays an important role in the coagulation process. 5-HT can promote platelet aggregation as a weak platelet agonist [34]. 99% of 5-HT in the body is stored in the dense granules of platelets. When the body is depressed, the 5-HT receptor on the surface of platelets is upregulated [35]. It causes excessive 5-HT and SERT transported into platelets, activates tGase, increases Ca^{2+} concentration, and then induces the release of a large number of coagulation cofactors, such as Ps and TXA2 (TXB2 as stable metabolite), which further leads to the enhancement of platelet activation and aggregation [36–38].

Liver and vascular endothelium play a very important role in maintaining the balance of the coagulation state. The liver is not only the organ producing and secreting most clotting factors (reflected by four items of coagulation) but also can regulate the balance of coagulation and anticoagulant by producing anticoagulant auxiliary factors such as AT-III and PC and inhibit blood hypercoagulability and abnormal thrombosis. AT-III binds with activated coagulation factors and then hydrolyzes, thereby blocking the coagulation cascade. After PC activation, it combines with FPS, degrades coagulation factors Va and VIIIa, prevents their activation to form thrombin, and inhibits platelet activation. In addition, the combination of PC and FPS can inhibit the action of PAI-1, an inhibitor of plasminogen activator [39]. It is beneficial to the activation of plasminogen and inhibits thrombosis.

Endothelial cells can regulate the blood rheology through secreting factors such as anticoagulant cofactor prostacyclin (PGI2), fibrinolysis cofactor t-PA, and PAI-1. PGI2 (6-keto-PGF1α as its stable metabolite) can activate adenylate cyclase, synthesize cAMP, and reduce cytosolic Ca^{2+} level to inhibit platelet activation. Through activating plasminogen, t-PA is converted into plasmin and breaks down blood clots [40]. PAI-1 can inhibit the effect of t-PA, inhibit fibrinolysis, and make blood clots difficult to degrade [41].

In a word, liver and endothelial cells can maintain the coagulation-anticoagulation balance by producing coagulation, anticoagulation, and fibrinolysis cofactors, inhibiting platelet activity, and degrading and inactivating thrombin, coagulation factors, and fibrin in blood hypercoagulability.

In this experiment, 5-HT in the plasma of CRS rats was significantly decreased, expressions of platelet 5-HT2A and SERT were significantly increased, and tGase, Ca^{2+}, Ps, and TXB2 in the platelets were significantly increased. It is suggested that 5-HT in the peripheral circulation of depression model rats might be overtransported and enriched in platelets. On the one hand, it reduced the level of 5-HT in the blood. On the other hand, it activated the tGase-Ca^{2+} pathway in platelets, which led to the release of a large number of coagulation cofactors, promoted further activation and aggregation of platelets, and finally led to blood hypercoagulation. It was also found that the levels of the anticoagulant cofactor (PC, FPS, and AT-III), 6-keto-PGF1α, and t-PA in the plasma were significantly reduced. Combined with the results of four items of coagulation (PT, APTT shortened, FIB increased), it is speculated that abnormal blood rheology of depression model rats may be related to the abnormal accumulation of 5-HT in platelets and the further activation of platelets, as well as the abnormal regulation of liver and endothelium on blood coagulation and anticoagulation/fibrinolysis cofactors.

(2) Comparison of the Mechanism of CHSGS and Fluoxetine on Hemorheology in CRS Rats. The mechanism of CHSGS and fluoxetine on blood rheology of CRS rats was compared. See Tables 6 and 7.

Table 6 illustrates that the high dose of CHSGS and fluoxetine could increase 5-HT in plasma, downregulate the expression of platelet 5-HT2A and SERT, and reduce the level of tGase and Ca^{2+} in platelets and the secretion of coagulation cofactors (Ps and TXB2). The results showed that both CHSGS and fluoxetine could inhibit the activation of platelets by inhibiting the accumulation of 5-HT in platelets, thereby improving the hypercoagulability of the model rats. It was found by comparison that the low dose of CHSGS had no obvious effect on platelet 5-HT2A, Ca^{2+}, and Ps-selectin, or it was inferior to the high dose group. It is

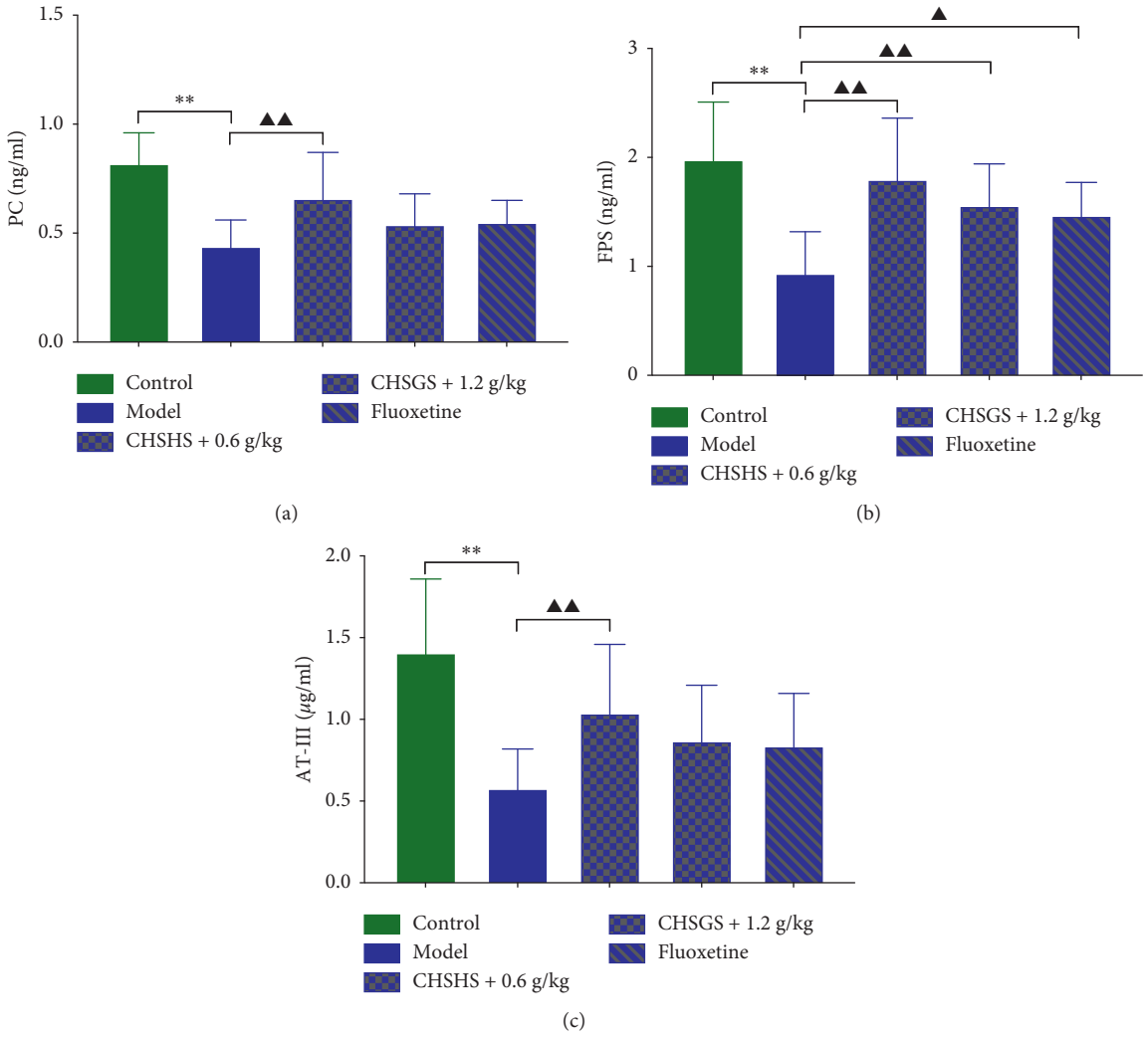

Figure 8: Effects of CHSGS and fluoxetine on the anticoagulant cofactor in CRS rats. (a) PC levels in plasma. (b) FPS levels in plasma. (c) AT-III levels in plasma. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, compared with the control group; $^{▲▲}p < 0.01$, $^{▲}p < 0.05$, compared with the model group.

suggested that CHSGS might have a dosing advantage in this link. In addition, the effect of the high dose of CHSGS on platelet 5-HT2A and tGase appears to be more significant than that of fluoxetine.

Table 7 illustrates that CHSGS + 0.6 g/kg group had a regulating effect on multiple indicators of fibrinolysis/anticoagulation cofactors in CRS rats. High dose of CHSGS could increase 6-keto-PGF1α and FPS, while the fluoxetine group only increased FPS. The results suggested that the high and low doses of CHSGS might have selectivity while improving the blood rheology state. High dose mainly regulated the platelet system, while low dose focused on the regulation of liver and endothelium. Compared with fluoxetine, CHSGS had advantages in regulating the balance of coagulation/fibrinolysis cofactors in model rats. It is speculated that the effect of CHSGS on the blood rheology of depression might involve the regulation of the liver and endothelium. It is suggested that this formula could regulate the coagulation-anticoagulation balance and improve blood hypercoagulability, so as to reduce the risk of cardiovascular diseases in patients with depression.

4.5. The Effect Material Basis of CHSGS. The ingredients of CHSGS are relatively complex and include saikosaponin, paeoniflorin, hesperidin, ferulic acid, tetramethylpyrazine, and albiflorin [42]. Among them, saikosaponin, paeoniflorin, hesperidin, and albiflorin have obvious antidepressant effects [43, 44], and ferulic acid and ligustrazine have the pharmacological activity of inhibiting platelet aggregation [45, 46]. Therefore, it is speculated that the antidepressant, anticoagulant, and blood rheological effects of this formula may be related to the ingredients such as saikosaponin, hesperidin, ferulic acid, and ligustrazine in this formula.

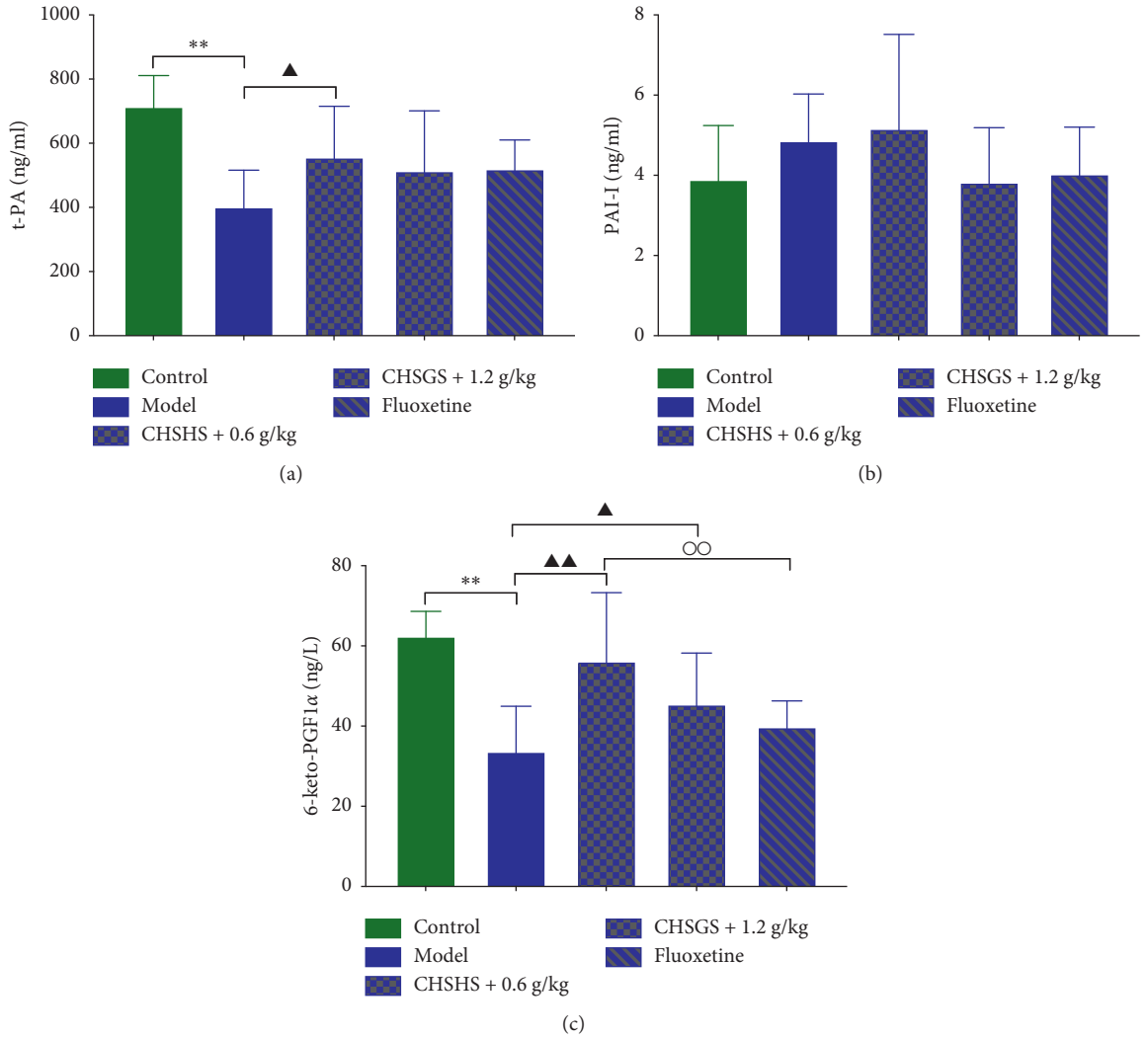

Figure 9: Effects of CHSGS and fluoxetine on the fibrinolysis cofactor in CRS rats. (a) t-PA levels in plasma. (b) 6-keto-PGF1α levels in plasma. (c) PAI-I levels in plasma. All data were expressed as the mean ± SD, $n = 8$, $^{**}p < 0.01$, compared with the control group; $^{▲▲}p < 0.01$, $^{▲}p < 0.05$, compared with the model group; $^{○○}p < 0.01$, compared with the fluoxetine group.

Table 2: Comparison of the effect of CHSGS and fluoxetine on the appearance behavior of CRS rats.

Group	Body weight				Activity status scores				Emotional-sleep scores				Sucrose consumption
	1 w	2 w	3 w	4 w	1 w	2 w	3 w	4 w	1 w	2 w	3 w	4 w	
Model	↓↓	↓↓	↓↓	↓↓	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↓
CHSGS + 0.6 g/kg	—	—	—	↑	—	—	—	↓↓	—	—	—	↓↓	—
CHSGS + 1.2 g/kg	—	—	↑↑	↑↑	—	—	—	↓↓	—	—	↓↓	↓↓	↑
Fluoxetine	—	—	—	—	—	—	—	↓↓	—	—	↓↓	↓↓	↑

Note. In the table, the model group was compared with the normal group, and others were compared with the model group. ↑ or ↓, $p < 0.05$;↑↑ or ↓↓, $p < 0.01$; —, no significant difference.

Table 3: Comparison of the effect of CHSGS and fluoxetine on laboratory indicators of CRS rats.

Group	Monoamine neurotransmitter			HPA axis	
	DA	NE	5-HT	CRH	CORT
Model	—	↓↓	↓↓	↑↑	↑↑
CHSGS + 0.6 g/kg	↑	—	↑↑	—	—
CHSGS + 1.2 g/kg	—	↑	↑↑	↓	↓↓
Fluoxetine	—	—	↑	—	↓↓

Note. In the table, the model group was compared with the normal group, and others were compared with the model group. ↑ or ↓, $p < 0.05$; ↑↑ or ↓↓, $p < 0.01$; —, no significant difference.

Table 4: Comparison of the effect of CHSGS and fluoxetine on blood rheology of CRS rats.

Group	Whole blood viscosity			Plasma viscosity	Erythrocyte sedimentation rate	K value	Whole blood reduced viscosity		
	Low cut	Middle cut	High cut				Low cut	Middle cut	High cut
Model	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑
CHSGS + 0.6 g/kg	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓
CHSGS + 1.2 g/kg	↓↓	↓↓	—	↓↓	↓↓	↓↓	↓↓	—	↓↓
Fluoxetine	↓↓	↓↓	↓↓	↓↓	↓	↓↓	↓↓	↓↓	↓↓

Note. In the table, the model group was compared with the normal group, and others were compared with the model group. ↑↑ or ↓↓, $p < 0.01$; —, no significant difference.

Table 5: Comparison of the effect of CHSGS and fluoxetine on blood coagulation of CRS rats.

Group	PT	APTT	TT	FIB	Platelet aggregation rate		
					1 min	3 min	5 min
Model	↓↓	↓↓	—	↑↑	↑↑	↑↑	↑↑
CHSGS + 0.6 g/kg	↑↑	↑↑	—	↓↓	↓	↓↓	↓↓
CHSGS + 1.2 g/kg	↑↑	↑↑	—	↓↓	↓↓	↓↓	↓↓
Fluoxetine	↑↑	↑↑	↑	↓↓	↓↓	↓↓	↓↓

Note. In the table, the model group was compared with the normal group, and others were compared with the model group. ↑ or ↓, $p < 0.05$; ↑↑ or ↓↓, $p < 0.01$; —, no significant difference.

Table 6: Comparison of CHSGS and fluoxetine on the regulation of platelet activity in CRS rats.

Group	5-HT	5-HT2A	SERT	tGase	Ca^{2+}	Ps	TXB2
Model	↓↓	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑
CHSGS + 0.6 g/kg	↑↑	—	↓	↓	—	—	↓
CHSGS + 1.2 g/kg	↑↑	↓↓	↓↓	↓↓	↓	↓↓	↓↓
Fluoxetine	↑	↓↓	↓↓	↓	↓	↓↓	↓↓

Note. In the table, the model group was compared with the normal group, and others were compared with the model group. ↑ or ↓, $p < 0.05$; ↑↑ or ↓↓, $p < 0.01$; —, no significant difference.

Table 7: Comparison of CHSGS and fluoxetine on the effect of the coagulation system in CRS rats.

Group	Anticoagulant				Fibrinolysis	
	6-keto-PGF1α	PC	FPS	AT-III	t-PA	PAI-1
Model	↓↓	↓↓	↓↓	↓↓	↓↓	—
CHSGS + 0.6 g/kg	↑↑	↑↑	↑↑	↑↑	↑	—
CHSGS + 1.2 g/kg	↑	—	↑↑	—	—	—
Fluoxetine	—	—	↑	—	—	—

Note. In the table, the model group was compared with the normal group, and others were compared with the model group. ↑ or ↓, $p < 0.05$; ↑↑ or ↓↓, ; —, no significant difference.

5. Conclusion

The CRS rats not only showed depression-related appearance behaviors and changes in laboratory indicators but also had abnormalities in blood rheology. Further exploration showed that the CRS rats had the abnormal regulation of platelet 5-HT2A signal pathway, the abnormal expression of blood coagulation and anticoagulant/fibrinolytic cofactor which was related to liver and endothelium, and the imbalance of liver coagulation-anticoagulant relationship. It is suggested that the depression model induced by CRS could be used in mechanism research and drug evaluation of depression with the risk of cardiovascular diseases.

CHSGS in TCM and fluoxetine in modern medicine could not only improve the appearance behaviors and neuroendocrine abnormalities of the depression model rats but also improve the hypercoagulable state of blood to different degrees. The regulation effects of CHSGS on behavior observation and HPA axis hyperfunction in CRS rats

were more obvious than those in fluoxetine. They had the same effect on improving blood rheology. The mechanism involved the regulation of the platelet 5-HT2A signaling pathway, but CHSGS was also related to the recovery of liver coagulation-anticoagulation balance and the regulation of endothelial function.

This study provided further evidence for the common pathophysiology basis between liver Qi stagnation syndrome in TCM and depression in modern medicine. It also provided pharmacological evidence over the potential advantages of CHSGS and fluoxetine in the treatment of depression accompanied with the risk of cardiovascular diseases.

Abbreviations

CHSGS:	Chaihu Shugan San
CRS:	Chronic restraint stress
DA:	Dopamine
5-HT:	5-Hydroxytryptamine
CRH:	Corticotropin-releasing hormone
NE:	Norepinephrine
CORT:	Corticotropin
6-Keto-PGF1α:	6-keto-protagiandinF1α
PC:	Protein C
FPS:	Free protein S
AT-III:	Antithrombin III
t-PA:	Tissue-type plasminogen activator
PAI-I:	Plasminogen activator inhibitor type 1
Ps:	P-selectin
TXB2:	Thromboxane B2
5-HT2A:	5-Hydroxytryptamine receptor 2A
SERT:	Serotonin transporter
tGase:	Glutamine transaminase
PT:	Prothrombin time
APTT:	Activated partial thromboplastin time
TT:	Thrombin time
FIB:	Fibrinogen.

Data Availability

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

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Meng Qian performed the experiments and wrote the manuscript. Rongyan Peng contributed to the manuscript writing. Chen Yue performed the experiments and data analysis. Zongchun Yang, Haoru Zhu, and Biyuan Liu performed the experiments. Ming Xie designed the experiment and submitted the manuscript.

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Research Article

So-Ochim-Tang-Gamibang, a Traditional Herbal Formula, Ameliorates Depression by Regulating Hyperactive Glucocorticoid Signaling *In Vitro* and *In Vivo*

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So-ochim-tang-gamibang (SOCG) is a Korean traditional medicine; it has previously been shown to be safe and effective against depression. Persistently increased levels of circulating glucocorticoids have been considered as a pathological mechanism for depression and associated with decreased neurotrophic factors in the hippocampus. This study investigated whether SOCG controls the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and the molecular mechanisms underlying its effects *in vivo* and *in vitro*. Wistar Kyoto (WKY) rats were subjected to restraint stress, where SOCG was orally administered to the animals for 2 weeks. An open field test (OFT), forced swimming test (FST), and sucrose preference test (SPT) were performed to explore the antidepressant activity of SOCG in WKY rats. Plasma levels of HPA axis hormones were measured by ELISA or western blotting analysis. The expression levels or activation of HPA axis-related signaling molecules such as brain-derived neurotrophic factor (BDNF), cAMP response element-binding protein (CREB), extracellular regulated kinase (ERK), and glucocorticoid receptors (GRs) in the brain were determined by real-time PCR and western blotting analysis. Furthermore, a corticosterone- (CORT-) induced cell injury model was established using SH-SY5Y cells to explore the antidepressive effects of SOCG *in vitro*. The results of the OFT, FST, and SPT revealed that SOCG ameliorated depressive-like behaviors in the WKY rats. The blood plasma levels of HPA axis hormones such as CORT, CORT-releasing hormone (CRH), and adrenocorticotrophic hormone were downregulated by SOCG. On the other hand, SOCG upregulated the phosphorylation of CREB and ERK in both the rat hippocampus and CORT-treated SH-SY5Y cells. Moreover, it also increased the GR expression. These results suggested that SOCG may improve depression by controlling hyperactive glucocorticoid signaling *via* the downregulation of HPA axis hormones and upregulation of GR.

1. Introduction

Depression is a highly prevalent psychiatric ailment characterized by repetitive events of sadness and a loss of interest in normal activities; the incidence of depression has been increasing worldwide. Although the pathogenesis of depression is complex and has not been understood completely, it has been accepted that excessive circulating levels of glucocorticoids (GCs) are associated with not only the onset of depression but also depression-induced deterioration of nerve regeneration [1]. In healthy subjects, acute stress induces the release of corticosterone- (CORT-) releasing hormone (CRH) from the hypothalamus, which subsequently induces the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. The ACTH reaches the adrenal gland, ultimately triggering the

production of GCs against the stress [2]. The activity of the hypothalamic-pituitary-adrenal (HPA) axis is driven by glucocorticoid receptors (GRs), whose balanced expression level is thought to be an important factor in regulating the activity of the HPA axis. Particularly, in depression, a high concentration of circulating cortisol is a major factor that alters the activity of GRs, thereby changing the functions of GRs in the hippocampus, ultimately leading to a situation where the activity of the ACTH and CRH cannot be controlled; this seems to be responsible for depressive symptoms [3]. The HPA axis is a liable factor for increased circulating GC-mediated stress response; thus, high GC levels are a signal for negative feedback mechanisms to turn off the HPA activity in the hippocampus.

Previous studies have reported that chronic stress downregulates the hippocampal GR expression; GRs play a critical role in affecting hippocampal function [3, 4]. Excessive circulating GC levels are associated with not only the loss of GR-containing cells in the paraventricular nucleus but also decreased GR gene expression [5, 6]. It is hypothesized that the application of different stressors and the specific durations of the stress may cause alterations in the GR expression in certain rodent strains [7]. Furthermore, previous studies have revealed that the levels of neuronal nitric oxide synthase (nNOS), an endogenous inhibitor of GR in the hippocampus, increase upon exposure to stress [8] and are involved in the regulation of the HPA axis activity via GRs [9]. Thus, the inhibition of nNOS in the hippocampus leads to the decrease of the CORT concentration in the plasma and reduced CRH expression in the hypothalamus. Growing evidence has shown that a persistent increase in the circulating GC levels negatively affects neurogenesis [10]. In subjects with depression, the expression of brain-derived neurotrophic factor (BDNF), which is required for neurogenesis, eventually decreases; this is associated with high GC levels, and signaling for the promotion of neurogenesis, including the phosphorylation of the signaling molecules cAMP response element-binding protein (CREB) and extracellular regulated kinase (ERK), in the hippocampus is altered, which may negatively affect the HPA axis activity. Presently, an increasing number of herbal medicines with potent antidepressant effects have become the major focus of attention to treat depressive moods, as these natural herbs, which may include plants such as *Piper methysticum* and St John's wort, are associated with a higher level of safety [11].

We have been reported that *So-ochim-tang-gamibang* (SOCG) is a therapeutic agent for stress-related disorders such as depression [12]. SOCG is based on So-ochim-tang, which had described in the qi chapter of Donguibogam, a Korean traditional medical book [13]. After reformulation by replacing Aquilariae Resinatum Lignum to Aucklandia Radix (*Aucklandia lappa* DC.) and adding Aurantii Fructus (*Citrus aurantium* L.) and Platycodi Radix (*Platycodon grandiflorus* Jacq. A.DC) according to Bang-yak-hap-pyeon [14], SOCG has been prescribed for treating mental activity- and depression-associated somatoform pain [15]. We have reported that SOCG has therapeutic effects against stress-induced depression and is safe for use [16, 17]; the antidepressive effects of SOCG may be mediated *via* its neuroprotective activity and ability to lower the circulating GC concentration [12]. However, the mechanisms underlying the SOCG-mediated regulation of HPA hyperactivity have not yet been investigated at a molecular level. Wistar Kyoto (WKY) rats represent a convenient rodent model of depression; in these rats, depressive episodes can be reversed by antidepressants [18]. By utilizing *in vitro* bioassays and *in vivo* models, the effects of SOCG on the hyperactivity of the HPA axis were evaluated with a focus on stress hormone regulation, GRs, and nNOS, as well as the BDNF/CREB/ERK signaling pathways.

2. Materials and Methods

2.1. Animals. Male Wistar Kyoto rats (10 weeks old, 180–200 g) were provided by Dr. Tae Wan Kim from the Kyungpook National University, Daegu, the Republic of Korea, for our experiments. The animals were maintained at standard conditions (12 : 12 h light-dark cycle, $23 \pm 1^{\circ}$C), with free access to water and food. The rats were subjected to one week of acclimatization before the experiments were begun. All the experiments involving animals were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1985), and the Institutional Animal Care and Use Committee of the Daejeon University approved the experimental protocol (DJUARB2015-030).

2.2. Experimental Design and Drug Administration. The preparation of SOCG and the choice of its standard dose (300 mg/kg) were based on a recently published report [12]. Briefly, Cyperi Rhizoma (*Cyperus rotundus* L.), Lindera Radix (*Lindera strychnifolia* Fern.-Vill.), Aucklandiae Radix (*Aucklandia lappa* Decne.), Glycyrrhizae Radix (*Glycyrrhiza uralensis* Fisch.), Aurantii Fructus (*Citrus aurantium* L.), and Platycodi Radix (*Platycodon grandiflorus* Jacq. A.DC) (8 : 4 : 1 : 1 : 4 : 4, in the order given) were boiled together in distilled water at 100°C for 2 h. The extract was evaporated and freeze-dried. The SOCG powder was stored at −20°C. The drug-extract ratio (DER) was 6.8 (yield ratio, 14.6%). Voucher specimens (no. 194A079-85) were deposited in the herbarium of Han Kook Shin Yak Co., Ltd. (Nonsan, Korea). Naive rats were housed under identical conditions in a separate room and had no contact with the animals in the stressed (control) and treatment groups. The rats in the chronic restraint stress group were placed in acrylic cylinders (250 mm long × 75 mm diameter, with air vents at the nasal end of the cylinder) for 3 h (09:00 to 12:00 h) every day from the age of 8 or 9 weeks for 2 weeks. The rats were assigned to four experimental groups: (a) naive, no restraint stress ($n = 6$); (b) con, restraint stress with vehicle ($n = 6$, saline, 0.9% NaCl); (c) AMI, restraint stress with 10 mg/kg amitriptyline ($n = 5$, per oral administration, Sigma-Aldrich Co., St. Louis, MO, USA); and (d) SOCG, restraint stress with 300 mg/kg SOCG ($n = 6$, per oral administration).

2.3. Depression-Like Behavior Tests. After the repeated administration of the restraint stress, the animals were subjected to the open field test (OFT), forced swimming test (FST), and sucrose preference test (SPT). All tests were performed between 09:00 and 14:00 h in a quiet room.

The locomotion behavior of rats was assessed via the OFT. The open-field arena ($100 \times 100 \times 50$ cm) was constructed using acrylic sheets. Their behavior was observed and videotaped for 10 min using a video tracking software (SMART 3.0; Panlab SI, Barcelona, Spain). Travel distance (m) was used as the parameter to measure locomotor activity.

In the FST, the rats were individually forced to swim in a cylinder (40 cm high × 18 cm diameter) containing tap water ($25 \pm 2^{\circ}$C), and the cylinder was long enough to not let the animals escape or rest by touching the bottom. The rats were forced to swim for 15 min (pretest) on the day before the session. After 24 h of the pretest, each session lasted 360 sec, and the duration of immobility was recorded. Mobility is defined as any movement beyond what is necessary to maintain the head above water. The total immobility time was measured during the last 4 min of each session using video tracking software (SMART 3.0; Panlab SI, Barcelona, Spain). The first 2 min of each session is acclimated time for immobility.

In the case of the SPT, prior to the testing, the animals were trained to adapt to a sucrose solution (1%, w/v); two bottles of sucrose solution were placed in each cage for 24 h, and one bottle of sucrose solution was then replaced with water for 24 h. After the adaptation, the rats were deprived of water and food for 24 h. During the test, the rats were housed in individual cages for 3 h and had free access to both sucrose solution and water. After this time period, the water and sucrose bottles were removed, and the consumed volumes were noted. Sucrose preference was calculated from the data obtained after 3 h of testing as follows: (volume of sucrose solution consumed/total volume of liquid consumed) × 100%.

2.4. Analysis of Plasma Stress Hormone Levels. One day after the behavioral tests, the animals were anesthetized via the inhalation of laboratory ether; the blood from the rats was collected into Vacutainer tubes containing EDTA (Becton Dickinson, NJ, USA) by heart puncture. The plasma was isolated by the centrifugation of the blood samples for 15 min at 3,000 rpm; the supernatants were collected. The plasma was stored at −80°C until further analysis. The plasma levels of CORT (Enzo Life Sciences, NY, USA), ACTH (Cloud-Clone Corp., TX, USA), and CRH (Cloud-Clone Corp., TX, USA) were measured using commercially available ELISA kits, according to the manufacturer's protocols.

2.5. SH-SY5Y Cell Culture. The neuroblastoma cell line SH-SY5Y (American Type Culture Collection, VA, USA) was maintained in DMEM supplemented with 10% heat-inactivated FBS and 1% (v/v) penicillin/streptomycin at 37°C in an atmosphere of 5% CO_2 and 95% air. The medium was replaced every 2–3 days.

2.6. Cell Viability Assay. The cells were seeded into a 96-well plate at a density of 2×10^5 cells/well and incubated for 24 h; then, they were exposed to various doses of SOCG (1, 10, 100, or 500 μg/ml) or CORT (100, 200, 300, and 400 μM) for another 24 h. To evaluate the protective effects of SOCG against CORT-induced cell injury, the cells were coincubated with SOCG (1, 10, 100, or 500 μg/ml) for 2 h and then with CORT (100 μM) for another 24 h. At the end of the treatment process, 10 μl of MTT (EZ cytox, DoGenBio Co., Ltd., Seoul, South Korea) was added to each well, followed by incubation for 4 h. The absorbance of the samples was measured at 450 nm using a microplate reader (Molecular Device, Sunnyvale, CA, USA). Cell viability was expressed as the percentage of viable cells relative to the nontreated control.

2.7. Western Blotting. After the whole brains were isolated, the hippocampus, hypothalamus, and pituitary gland were immediately dissected while the brains were placed on an ice surface. The tissues were stored at −80°C until further use. The SH-SY5Y cells were seeded into a six-well culture plate at a density of 2×10^5 cells/well for 24 h; they were pretreated with SOCG (1, 10, or 100 μg/ml) for 1 h and then treated with CORT (100 μM) for 24 h. They were then rinsed with ice-cold PBS and lysed in the RIPA buffer. Equal amounts of each protein sample were resolved on 8–18% sodium dodecyl sulfate-polyacrylamide gels; the resultant bands were transferred onto nitrocellulose membranes (Hybond ECL; Amersham Pharmacia Biotech, Piscataway, NJ, USA). The membranes were blocked in 5% skim milk solution for 1 h. Next, they were incubated with antibodies against BDNF, CREB, GR (1 : 1000; Santa Cruz Biotechnology Inc., CA, USA), ERK (1 : 1000; Cell Signaling Technology, MA, USA), and nNOS (1 : 1000; Merck, Darmstadt, Germany) overnight at 4°C and then with horseradish peroxidase-labeled IgG antibodies (1 : 2000; Santa Cruz Biotechnology Inc., CA, USA) for 2 h at room temperature. For the detection of the protein bands, the ECL Western Blotting Detection System (Amersham Biosciences, PA, USA) was used. The band intensities were measured using the ImageJ software (version 1.49).

2.8. Real-Time PCR. SH-SY5Y cells were seeded in a 6-well plate at a density of 2×10^5 cells/well. The cells were pretreated with SOCG for 1 h, followed by incubation with CORT for 24 h to measure the mRNA expression levels. Total RNA was isolated using the TRIzol reagent (Invitrogen) and then used for cDNA synthesis, which was performed with the PrimeScript™ RT reagent kit (TaKaRa, Shiga, Japan). The specific genes were quantified using a 7500 Real-Time PCR System (Applied Biosystems, CA, USA), with the Power SYBR® Green PCR Master Mix and TaqMan® Gene Expression Master Mix (Applied Biosystems, CA, USA). The sequences of the real-time PCR primers used were as follows:

rat BDNF, forward 5'-CAGCTGGGTAGGCCAAGTTG-3' and reverse 5'-CACAATGTTCCACCAGGTGAGA-3', and rat GR, forward 5'-GGCTGAGCAGATTACATAGGC-3' and reverse: 5'-GATGGAAAGGGGCCTTTTGG-3'. A rat GAPDH primer set (Endogenous Control, VIC®/MGB Probe, Primer Limited) was purchased. For the PCR analysis of the SH-SY5Y cells, the sequences of the primers used for the real-time PCR analysis included the following: human BDNF, forward 5'-CCAACGGATTTGTCCGAGGT-3' and reverse 5'-ATCTCAGTGTGAGCCGAACC-3'; human GR, forward 5'-GGACCACCTCCCAAACTCTG-3' and reverse 5'-GCTGTCCTTCCACTGCTCTT-3'; and human GAPDH (Endogenous Control), forward 5'-TGAA-GACGGGCGGAGAGAAAC-3' and reverse 5'-TGACTCCGACCTTCACCTTCC-3'. The PCR was run for 40 cycles at 95°C (15 sec) and 60°C (1 min). The relative expression levels of the target genes were calculated using the ΔΔCt method, where Ct is the threshold concentration.

2.9. Statistical Analysis. The data were analyzed using GraphPad Prism 5 and represented as the mean ± SEM in all experiments, except in the MTT test, in which they were represented as the mean ± SD. Comparisons between the data from different groups were performed using one-way ANOVA followed by Tukey's or post hoc analyses. P values < 0.05 were considered statistically significant [19].

3. Results

3.1. SOCG Decreased Depressive-Like Behaviors in WKY Rats. First, we evaluated the antidepressive effects of SOCG in WKY rats by performing behavioral tests including OFT, FST, and SPT (Figure 1). In the OFT, the control rats showed reduced locomotion activity (2.15 ± 0.44 m, $F[3, 19] = 0.911$, $P < 0.01$) compared to the naive rats (3.69 ± 0.41 m). The AMI- and SOCG-treated rats showed an increased locomotion distance (3.27 ± 0.98 m and 3.08 ± 0.87 m, respectively) compared to the control rats (Figure 2(a)). In the FST, the control rats exhibited an increased immobility duration (94.84 ± 7.60 s, $F[3, 19] = 5.876$, $P < 0.05$) than the naive rats, while SOCG significantly suppressed the immobility duration time of the rats (54.67 ± 6.41 s, $P < 0.05$), compared to the case for the control rats (Figure 2(b)). Next, in the SPT, we found that the sucrose consumption was reduced in the case of the control rats (39.06 ± 2.33%, $F[3, 19] = 9.393$, $P < 0.01$) compared to the naive rats (60.39 ± 5.54%). However, SOCG treatment increased the sucrose preference in the restraint stress-treated WKY rats (64.39 ± 2.79%, $P < 0.05$), compared to the case for the control rats (Figure 2(c)). These results indicate that SOCG alleviated depressive-like behaviors in WKY rats subjected to restraint stress. SOCG ameliorated the circulating stress hormone levels.

3.2. SOCG Ameliorated the Circulating Stress Hormone Levels. Since the HPA axis is strongly activated under conditions of uncontrollable stress, we investigated the plasma levels of the HPA axis hormones CORT, CRH, and ACTH by ELISA. The plasma levels of CORT (266.6 ± 15.72 ng/ml, $P < 0.001$), CRH (26.75 ± 1.95 ng/ml, $P < 0.01$), and ACTH (85.35 ± 21.76 pg/ml, $P < 0.01$) were significantly higher in the restraint stress-treated rats than in the naive rats (113.50 ± 27.41 ng/ml, 12.84 ± 0.60 ng/ml, and 28.91 ± 13.94 pg/ml, Figures 3(a)–3(c)). In contrast, SOCG treatment ameliorated the plasma CORT (102.30 ± 23.07 ng/ml, $P < 0.001$), CRH (16.85 ± 2.12 ng/ml, $P < 0.05$), and ACTH (36.90 ± 18.10 pg/ml) levels, compared to the case for the control rats. Collectively, these data indicate that SOCG treatment ameliorated the chronic stress-induced increase of the levels of circulating HPA axis hormones in a rat model of depression. SOCG downregulated the ACTH and CRH levels in the pituitary gland and hypothalamus, respectively.

3.3. SOCG Downregulated the ACTH and CRH Levels in the Pituitary Gland and Hypothalamus. Next, we investigated the levels of ACTH and CRH in the pituitary gland and hypothalamus, respectively, where the HPA axis hormones are produced. The protein levels of CRH in the hypothalamus increased following restraint stress; however, SOCG significantly reduced the CRH concentration (by approximately 60%) compared to the case for the samples from the control rats (Figure 4(a)). SOCG treatment downregulated the restraint stress-induced ACTH overexpression in the pituitary gland (Figure 4(b)). These results suggest that SOCG acted on specific sites in the brain to inhibit the excess production of HPA axis hormones.

3.4. Effects of SOCG on the Hippocampal Expression of HPA Axis-Related Signaling Molecules. We examined the hippocampal BDNF expression, which is controlled by HPA axis hormones, by western blotting analysis. SOCG strongly increased the BDNF levels in the hippocampus (Figure 5(a)). The expression of BNDF at the RNA level was confirmed by real-time PCR. SOCG treatment significantly reversed the decrease in the hippocampal BDNF mRNA levels in the stressed animals (Figure 5(b)). We also investigated the activation of CREB and ERK, which regulate the expression of BDNF following the stimuli of the HPA axis hormones. There were notable decreases in the levels of phosphorylated CREB (Figure 5(c)) and ERK (Figure 5(d)) in the control rats, compared to the case for the naive rats. However, SOCG treatment increased the levels of CREB and ERK phosphorylation (Figures 5(c) and 5(d)). These results suggest that SOCG improved depression-like behaviors *via* regulating the expression of HPA axis-related signaling molecules.

3.5. Effects of SOCG on the Hippocampal GR Expression. The GR, a receptor of CORT, mediates the activity of the GC hormone [19]. The GR level in the hippocampus increased significantly following SOCG treatment (by approximately 3-fold), compared to the case for the samples from the control rats, which showed lower GR levels than those from the naive rats, as revealed by the western blotting analysis (Figure 6(a)). Moreover, SOCG dramatically increased the

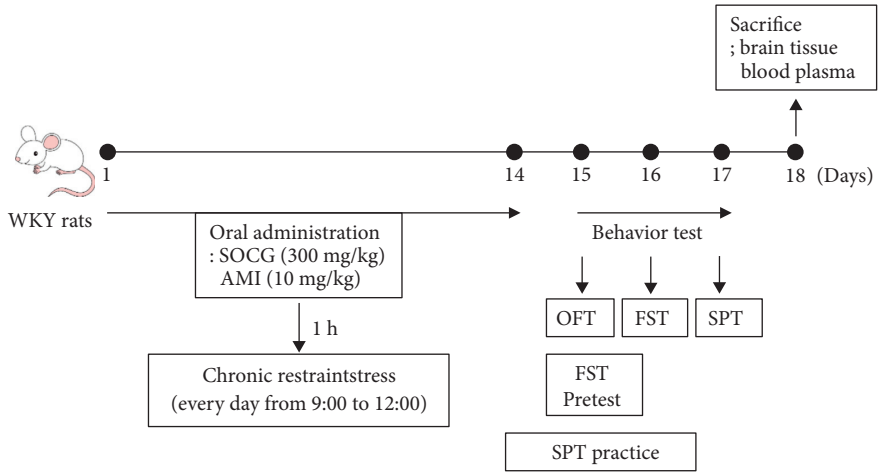

Figure 1: Schematic diagram of the animal experimental procedure. WKY: Wistar Kyoto, SOCG: *So-ochim-tang-gamibang*, AMI: amitriptyline, OFT: open field test, FST: forced swimming test, and SPT: sucrose preference test.

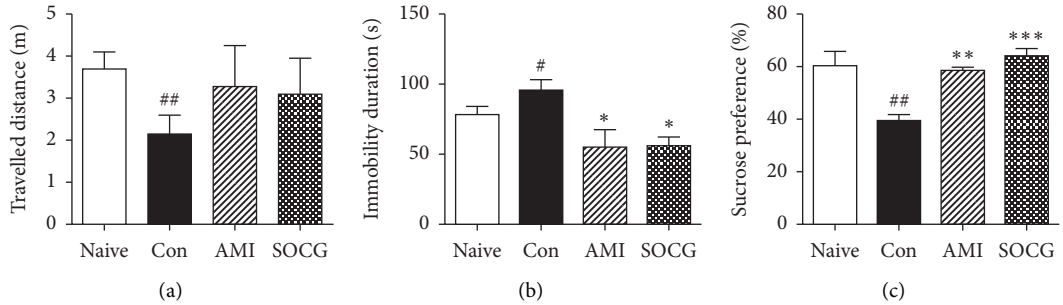

Figure 2: Effects of SOCG on depressive-like behavior in WKY rats. The animals were subjected to chronic restraint stress for 2 weeks, and SOCG was orally administered (300 mg/kg). (a) Travelled distance in the OFT, (b) immobility duration in the FST, and (c) sucrose preference in the SPT were measured. The data are presented as the mean ± SEM. $\#\#P < 0.01$, significant difference compared to the naive group. $P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, significant difference compared to the control group. AMI: amitriptyline (10 mg/kg).

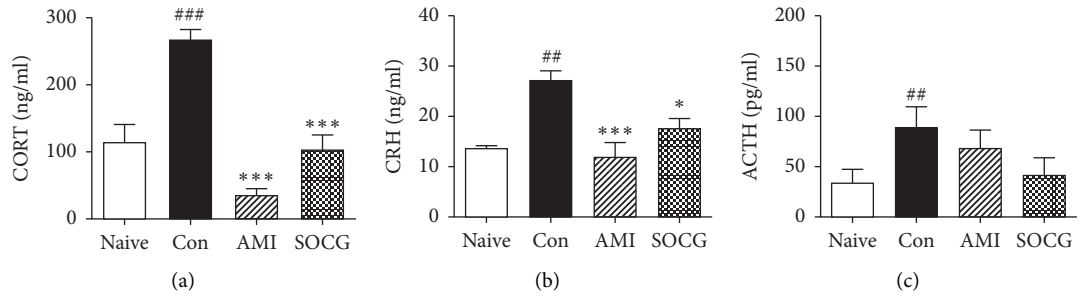

Figure 3: Effects of SOCG on the plasma levels of HPA axis hormones. WKY rats were subjected to chronic restraint stress for 2 weeks, and SOCG was orally administered (300 mg/kg). The plasma levels of (a) corticosterone, (b) corticosterone-releasing hormone, and (c) ACTH were measured by ELISA. The data are presented as the mean ± SEM. $\#\#P < 0.01$ and $\#\#\#P < 0.001$, significant difference compared to the naive group. $^{*}P < 0.05$ and $^{***}P < 0.001$, significant difference compared to the control group. AMI: amitriptyline (10 mg/kg).

mRNA expression levels of GR in the hippocampus, compared to the case for the samples from the control rats (Figure 6(b)). nNOS is known to regulate the activity of the HPA axis; it inhibits the activity of GRs in the hippocampus [20]. Thus, we measured the hippocampal nNOS expression in the depressed animals. Restraint stress increased the hippocampal nNOS levels in samples from the depressed rats, compared to the case for those from the naive rats. Notably, SOCG significantly suppressed the hippocampal nNOS expression to a level similar to that observed in the samples from the naive rats (Figure 6(c)). Thus, SOCG may control the hyperactivity of the HPA axis *via* the upregulation of hippocampal GR expression and inhibition of nNOS expression in depressed animals.

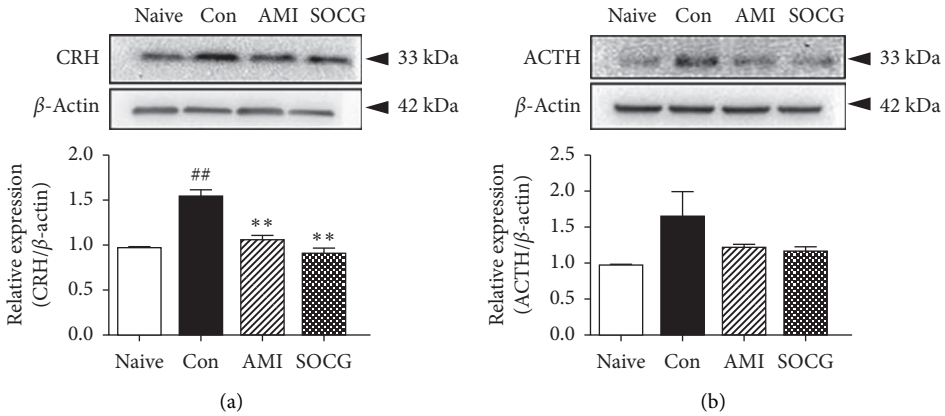

Figure 4: Effects of SOCG on the levels of HPA axis hormones in the brain. WKY rats were subjected to chronic restraint stress for 2 weeks, and SOCG was orally administered (300 mg/kg). The pituitary gland and hypothalamus were isolated from the rat brains and the levels of (a) CRH and (b) ACTH, respectively, were measured by western blotting. The data are presented as the mean ± SEM. $\#\#P < 0.01$, significant difference compared to the naive group. $^{**}P < 0.01$, significant difference compared to the control group. AMI: amitriptyline (10 mg/kg).

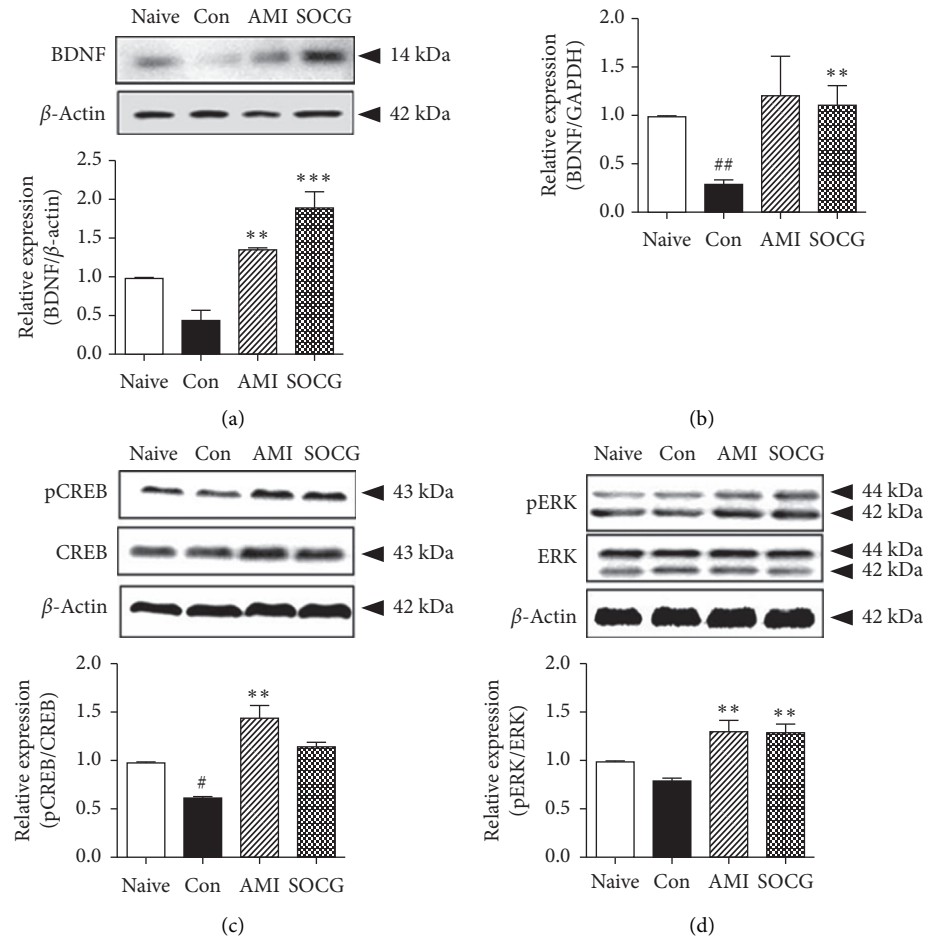

Figure 5: Effects of SOCG on the expression of neuronal factors in the hippocampus. WKY rats were subjected to chronic restraint stress for 2 weeks, and SOCG was orally administered (300 mg/kg). The hippocampus was dissected from the rat brains and used for protein and mRNA purification. The levels of BDNF were measured by (a) western blot analysis and (b) real-time PCR. The levels of (c) phosphorylated CREB and (d) phosphorylated ERK were measured by western blotting analysis. The data are presented as the mean ± SEM. $\#P < 0.05$ and $\#\#P < 0.005$, significant difference compared to the naive group. $^{**}P < 0.01$ and $^{***}P < 0.001$, significant difference compared to the control group. AMI: amitriptyline (10 mg/kg).

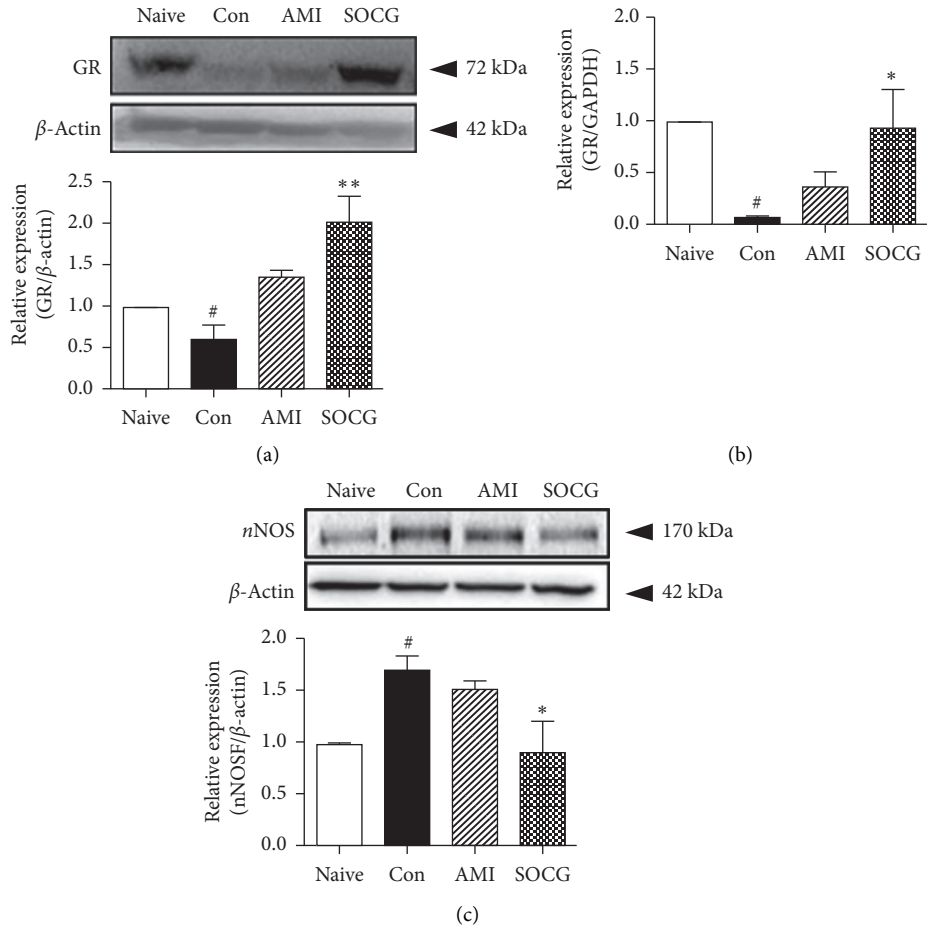

Figure 6: Effects of SOCG on the hippocampal GR and nNOS expression levels in WKY rats. The animals were subjected to chronic restraint stress for 2 weeks, and SOCG was orally administered (300 mg/kg). The hippocampal GR levels were measured by (a) western blotting analysis and (b) real-time PCR. (c) The hippocampal nNOS levels were measured by western blotting analysis. The data are presented as the mean ± SEM. GAPDH was used for the normalization of the expression levels of the target genes in the real-time PCR analysis. #$P < 0.001$, significant difference compared to the naive group. $^{*}P < 0.05$ and $^{**}P < 0.01$, significant difference compared to the control group. AMI: amitriptyline (10 mg/kg).

3.6. Effects of SOCG on the Expression of HPA Axis-Related Neuronal Factors in SH-SY5Y Cells. We confirmed the effects of SOCG on the HPA axis *in vitro*. First, we tested the cytotoxicity of SOCG in cells from a neuronal cell line, i.e., SH-SY5Y cells. Treatment with SOCG (1, 10, and 100 μg/ml) for 24 h did not show cytotoxicity, except in case of treatment with 500 μg/ml ($P < 0.001$) SOCG (Figure 7(a)). Therefore, we used SOCG concentrations of less than 100 μg/ml for the following tests. The BDNF expression in CORT-treated SH-SY5Y cells was assessed by real-time PCR. CORT treatment decreased the BDNF mRNA expression level; however, it strongly increased the BDNF expression (by more than 6-fold), compared to the samples from the control rats (Figure 7(b)). SOCG also increased the GR expression in a dose-dependent manner in CORT-treated SH-SY5Y cells (Figure 7(c)). On the other hand, SOCG downregulated the CORT-induced increase of the CRH mRNA expression levels in the SH-SY5Y cells (Figure 7(d)). These *in vitro* results confirmed the antidepressive effects of SOCG *via* its action on the HPA axis.

4. Discussion

It is well known that exposure to chronic stress affects HPA axis activity, thereby increasing the synthesis and secretion of GCs. The HPA axis is one of the major endocrinological systems that control stress. The increase in the circulatory levels of GC is the human body's response to stress. However, when the stress is persistent or uncontrollable, the HPA axis activity is abnormally regulated, resulting in depression [2]. Therefore, proper response to stress-induced alterations in the activity of the HPA axis can be a therapeutic target for depression. Our previous study has proposed the hypothesis that SOCG may control the hyperactivity of the HPA axis since SOCG downregulated the plasma levels of CORT, which is a major HPA axis-related stress-responding hormone, in a mouse model of stress-induced depression [12]. In this study, we showed that SOCG administration inhibited the increase in the plasma levels of stress-related HPA axis hormones including CORT, CRH, and ACTH. Since the hypothalamic CRH and

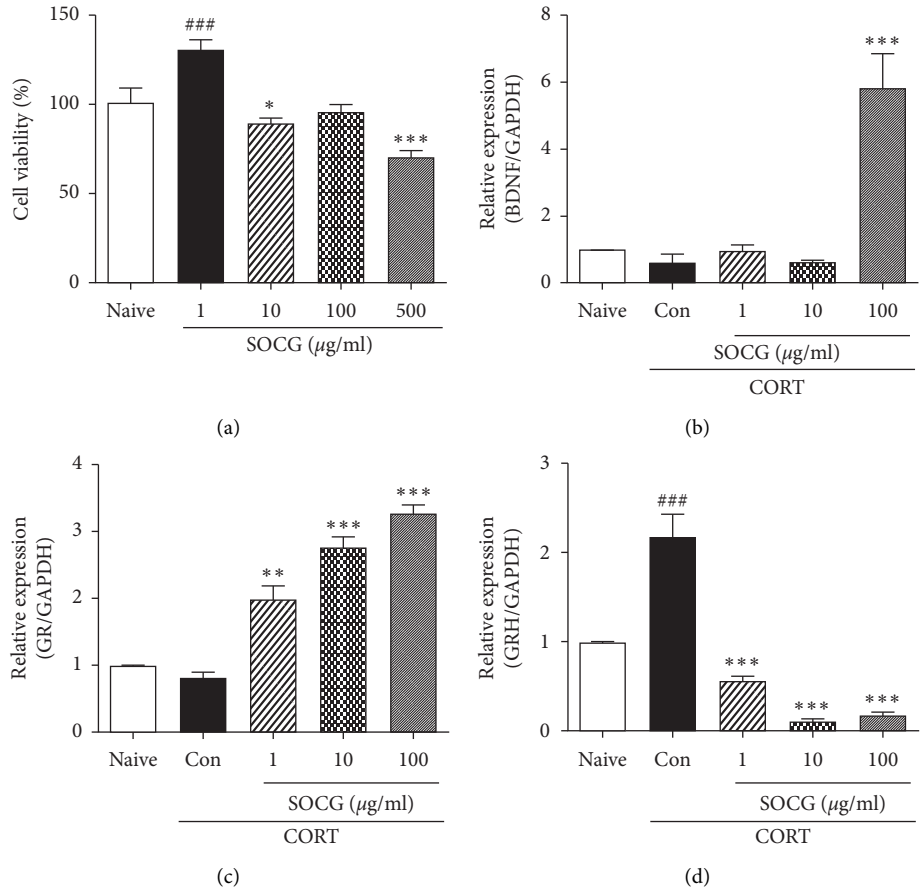

Figure 7: Effects of SOCG on the expression of HPA axis-related molecules in SH-SY5Y cells. (a) The viability of SH-SY5Y cells was determined by the MTT assay after they were cocultured with various concentrations of SOCG for 24 h. The cells were pretreated with SOCG for 1 h followed by incubation with corticosterone (100 μM) for 24 h for measuring the (b) BDNF, (c) GR, and (d) CRH mRNA expression levels. Total RNA was isolated and the levels of genes were measured by real-time PCR. The data are presented as the mean ± SEM. GAPDH was used for the normalization of the expression levels of the target genes in the real-time PCR analysis. ###$P < 0.001$, significant difference compared to the naive group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, significant difference compared to the control group. CORT: corticosterone.

pituitary ACTH levels were also downregulated by SOCG, it seems that SOCG controls the secretion of CRH and ACTH in the central nervous system. Our data strongly evidence that SOCG ameliorates depression-related symptoms by controlling the expression of HPA axis hormones.

The therapeutic effects of SOCG in the hippocampus appear to be elicited by its ability to increase the expression of neurotrophins *via* the control of the HPA axis activity. In accordance with our previous study using a mouse model, the BDNF expression, which was downregulated in the hippocampus of depressed rats, increased following SOCG treatment. Since the hippocampal level of BDNF is closely correlated with the severity of depressive symptoms, the increased expression of neurotrophins in the brain reflects the antidepressive effects of SOCG [21]. Further, our data suggest that SOCG increased the BDNF expression by modulating the phosphorylation of CREB and ERK. CREB is a transcription factor regulating the expression of neurotrophins such as BDNF, which is controlled by the activity of ERK [22, 23]. The activation of ERK signaling cascades, along with CREB signaling cascades, mediates neuronal activities such as neuronal cell differentiation, survival, and synaptic plasticity [24]. As BDNF plays a critical role in the therapeutic process of depression, the increase in the hippocampal BDNF levels may be an important therapeutic molecular mechanism underlying the antidepressant activity of SOCG [25]. The secretion of CORT in response to stress activates c-fos in the brain, leading to the suppression of the BDNF expression in the brain [26]. This suggests that the HPA axis is closely related to the BDNF expression. Our data demonstrated that SOCG suppressed the CORT levels in the blood but elevated the BDNF expression. The increase of the hippocampal BDNF levels may result from the SOCG-mediated modulation of the HPA axis activity. However, the detailed molecular mechanisms underlying the SOCG-mediated control of BDNF expression *via* the HPA axis remain to be elucidated.

It has been suggested that appropriate signaling by the GRs plays a critical role in the regulation of the HPA axis in the brain. Especially, hippocampal GR activation is important for GC-mediated negative feedback action of the HPA axis to control CORT release and hormonal

homeostasis [27]. Therefore, it has been suggested that GR may be a target of antidepressants [28]. Our studies examining the consequences of chronic stress revealed that the GR levels in the hippocampus were downregulated [4]. With respect to chronic stress, the decreased GR level reflects the insensitivity of CORT stimulation in the brain, which indicates a broken HPA axis circuit. However, SOCG was able to restore the GR levels in a rat model of depression. This may induce the recovery of the sensitivity of CORT and the activity of the HPA axis, resulting in the antidepressive effects of SOCG. Although the detailed molecular mechanisms underlying these phenomena must be investigated, the SOCG-mediated increase in the GR levels may contribute to the restoration of depressed rats.

It is well known that higher nNOS expression in the hippocampus leads to neuronal loss under chronic stress conditions. Our study revealed that nNOS was overexpressed in the hippocampus in depressed animals, while SOCG reversed this increase of nNOS expression [29]. This downregulation of hippocampal nNOS expression by SOCG is quite interesting because suppressed NO production in the hippocampus may promote hippocampal neurogenesis in patients with depression [30]. Therefore, SOCG may improve depression symptoms by modulating the NO production.

SOCG is formulated by composing 6 herbs. Some active compounds from each herb showing antidepressive effects were reported. Cycloartane and iridoid isolated from *Cyperus rotundus* were demonstrated to have beneficial effects on depression by animal experiments with FST and TST [31]. Glutamate-induced neuronal cell death was protected by platycodin from *Platycodon grandiflorum* [32]. Antidepressive effects of liquiritin from *Glycyrrhiza uralensis* were investigated in the chronic stress-induced depression rat model by SPT and FST [33]. Essential oil from *Citrus aurantium*, which contains 97.83% of limonene and 1.43% of myrcene, was reported to have anxiolytic effect [34]. Those compounds would play roles in antidepressive effects of SOCG, and there is a possibility that they may act synergistically; however, identification of the active compound of SOCG needs to be investigated.

5. Conclusion

In conclusion, SOCG controlled the levels of HPA axis hormones in a rat model of chronic stress-induced depression. SOCG suppressed the stress-induced increase of the circulating plasma levels of the HPA axis hormones, decreased the ACTH and CRH levels in the pituitary and hypothalamus, respectively, and increased the hippocampal expression of GR, which is an important receptor for the GC hormone. In SOCG-treated depressed rats, an upregulation of the hippocampal expression of the HPA axis-related signaling molecules CREB and ERK was observed; this may lead to the increase of BDNF expression. Furthermore, SOCG treatment increased the GR and BNDF mRNA expression levels and ameliorated the CORT-induced increase in the CRH mRNA expression levels in CORT-treated SH-SY5Y cells. The effects of SOCG on the expressions of CRH and GR were confirmed in SH-SY5Y cells, human neuroblastoma cells. SH-SY5Y cells are widely used as an *in vitro* model of neuronal diseases including depression, Alzheimer's disease, and Parkinson's disease. As the characteristics of the cells are not the same as that of CRH-releasing neurons in the hypothalamus, a further *in vitro* study is needed to verify the function of SOCG on the hypothalamus. Nevertheless, our *in vivo* and *in vitro* studies indicated that SOCG ameliorates depression-related symptoms by regulating the HPA axis. Further studies are warranted to explore the detailed molecular mechanisms underlying the endocrinological action of SOCG, which may serve as a promising antidepressive therapeutic agent.

Data Availability

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

MJ designed this study and wrote the paper. SYP, HJC, and YS performed experiments and analyzed data. EC discussed the data and wrote the paper. ICJ analyzed and discussed the data. JJC supervised research and wrote the paper.

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Review Article

The Therapeutic Prospects of Naturally Occurring and Synthetic Indole Alkaloids for Depression and Anxiety Disorders

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Depression and anxiety are the most common disorders among all age groups. Several antidepressant drugs including benzodiazepine, antidepressant tricyclics, azapirone, noradrenaline reuptake inhibitors, serotonin selective reuptake inhibitors, serotonin, noradrenaline reuptake inhibitors, and monoamine oxidase inhibitors have been used to treat these psychiatric disorders. However, these antidepressants are generally synthetic agents and can cause a wide range of side effects. The potential efficacy of plant-derived alkaloids has been reviewed against various neurodegenerative diseases including Alzheimer's disease, Huntington disease, Parkinson's disease, schizophrenia, and epilepsy. However, data correlating the indole alkaloids and antidepressant activity are limited. Natural products, especially plants and the marine environment, are rich sources of potential new drugs. Plants possess a variety of indole alkaloids, and compounds that have an indole moiety are related to serotonin, which is a neurotransmitter that regulates brain function and cognition, which in turn alleviates anxiety, and ensures a good mood and happiness. The present review is a summary of the bioactive compounds from plants and marine sources that contain the indole moiety, which can serve as potent antidepressants. The prospects of naturally occurring as well as synthetic indole alkaloids for the amelioration of anxiety and depression-related disorders, structure-activity relationship, and their therapeutic prospects have been discussed.

1. Introduction

Depression is a common mental problem that affects an estimated 264 million people globally [1]. The main symptoms include the inability to experience interest and pleasure, self-doubt, loss of concentration, social anxiety, appetite, and sleep disorder [2]. Certain chemicals or hormonal imbalances, for example, serotonin, in the brain is considered as the causal factor for depression. Besides, hormones such as dopamine and norepinephrine can also contribute to depression as the optimum concentrations of these hormones are essential for brain physiology and to control one's feelings [3].

For the treatment of depression, the choice of psychotherapy and medications depends on the severity of the symptoms. Antidepressant drugs are of various types; they differ in their mechanisms of action, side effects, and cost. The first-line treatment for depression may include either a selective serotonin reuptake inhibitor (SSRI) or a tricyclic antidepressant (TCA) [4]. Although several antidepressant

formulations are available in the market for the therapeutic management of depression, the majority of them have a wide range of side effects [5]. Therefore, the use of herbal extracts in their crude from or a semi-purified form is gaining ground among clinicians as well as patients as an alternative therapy for depressive disorders [6, 7].

The data regarding the mechanism of action of phytochemicals on the central nervous system (CNS) for the amelioration of depressive disorders are limited. The studies have discussed the relationship between the structure of the flavonoids isolated from natural and synthetic sources and antidepressant activity. The overall activity and the potential use of synthetic indole alkaloids (IAs) in medicine have also been described in various studies [8, 9]. However, studies on the antidepressant potential of indole alkaloids from plants or other natural sources are scarce. In this review, we have discussed the potential of indole alkaloids from plants and of marine origin as well as synthetic indole alkaloids for the treatment of depression and anxiety. Moreover, the structure, activity, potential targets, and sources of indole alkaloids have also been discussed.

2. Structure and Function of Indole Alkaloids

Indole alkaloids (IAs) are bicyclic compounds with a 6-membered benzene ring that is fused to a 5-membered pyrrole ring. The presence of nitrogen atom in the pyrrole ring leads to the basic characteristics of IAs making them pharmacologically active [9]. IAs are widely found in various plant families like Loganiaceae, Nyssaceae, Apocynaceae, and Rubiaceae. Major IAs that are extracted from plants involve the potent antitumor drugs, vincristine, and vinblastine, from the species *Catharanthus roseus*, and the antihypertensive agent, reserpine, from the species *Rauvolfia serpentine* [10, 11]. Studies reporting the use of IAs in the treatment of depression have been carried out since 1952; however, not enough attention has been paid by the researcher community toward the therapeutic benefits of plants, especially the antidepressant potential.

IAs are usually involved in the functioning of G protein-coupled receptors (GPCRs), particularly in neuronal transmission using the 5-hydroxytryptamine/HT (serotonin) receptors. In addition to donating hydrogen bond through free nitrogen-hydrogen, the density of pi-electrons also facilitates the highest energy molecular orbital of the indole skeleton, which enables the interaction with nitrogen bases, specifically target proteins and protonated atoms [8]. The N-atom present within the indole ring maintains the aromaticity and makes the NH binding acidic instead of nitrogen basic. This indole moiety is capable of forming H-bonds via pi-pi stacking, NH moiety, or cation-pi interactions, through the aromatic moieties [12]. The hydrophobicity of indole moieties is almost equal to the phenyl ring and less than the classical isosteric benzofuran and the benzothiophene group. The NH group of indole has a key role in interacting with the target bioreceptor, whereas the synthesized benzofuran and benzothiophene derivatives show mild-to-moderate affinity toward the targeted bioreceptor [8]. Reserpine is an example of IAs obtained in the past sixty years which exhibits a sedative effect on the central nervous system (CNS). Furthermore, two chemicals, i.e., serotonin and tryptamine are also derivatives of indole alkaloids and are found within the brain.

3. The Indole Ring in Drugs

The indole ring is found in many drugs that are available in the market. Many of these belong to a group of triptan drugs that are utilized for relieving migraine and headaches. All members of the triptan class are considered to be the agonists of the migraine-associated $5HT_{1D}$ and $5HT_{1B}$ serotonin receptors. Imitrex (Sumatriptan) was formulated by GlaxoSmithKline for treating migraine and was the first triptan member to be introduced in the market [13, 14]. In comparison to the 2nd generation triptans, Imitrex has a relatively short half-life and low oral bioavailability. Frovatriptan (Frova), formulated by Vernalis, is used for treating menstrual migraine headaches. The affinity of frovatriptan toward the migraine-specific $5HT_{1B}$ receptors is found to be greater than all triptans [15]. Moreover, frovatriptan can also bind to the receptors of $5HT_7$ and $5HT_{1D}$ subtypes [16, 17]. Zolmitriptan (Zomig) commercialized by AstraZeneca is used for the treatment of cluster headaches and acute migraine. Naratriptan has also been used for the management of migraine headaches, and its adverse reactions include tiredness, dry mouth, tingly feet or hands, and dizziness. All these available triptan drugs are highly effective and well-tolerated [18]. The highest prevalence of CNS-associated side effects, i.e., drowsiness and dizziness, have been reported for rizatriptan (10 mg), eletriptan (80 mg, 40 mg), and Zomig (5 mg) [19]. The differences observed in the adverse events for triptan drugs are probably not due to their different binding affinity toward neurological receptors or serotonin receptors within the CNS. There is a positive association between the adverse effects of CNS and the lipophilicity coefficient; these unwanted effects depend on the concentration and dosage.

The indole moiety is also called bioisosteres as it has physical and chemical properties akin to other biological molecules. The similarity has been used in prototype drug development, which aimed at improving the pharmacological activity as well as the pharmacokinetic (PK) profile. In a study, the pharmacological evaluation of thienopyrrole and benzo[b]furans resulted in bioisosteric molecules having dimethyltryptamine (DMT)-like effect. The initial work with 3-indenalalkylamines and benzo[b]thiophenes revealed that in the compounds that lack a ring substituent, their ability to act as agonists in rat fundus is almost like the tryptamines. The findings demonstrated that indole N-H is not essential for activating the serotonin (5-HT2) receptor in rat fundus [20].

4. Serotonin Receptors as Potential Targets for Neurologically Active IAs

Considering that depression affects almost 18 million Americans every year, it is critical to design new and effective medications to counter it. Intensive investigations have been

carried out in the field of novel target identification for antidepressant therapies; however, the majority of antidepressants still target different neurotransmitters, primarily dopamine, noradrenaline, and serotonin [21, 22].

Serotonin, a neurotransmitter that is found in the peripheral and central nervous system, plays a crucial role in the functioning of the normal brain as well as the regulation of mood, sleep, memory, appetite, anxiety, sexual function, and several others [23]. Serotonin functions through seven different receptor families, viz., 5-HT1-5-HT7, which are subdivided into various classes. Excluding the receptor 5-HT3, which belongs to a superfamily of ligand-gated ion channel (LGIC), all 5-HT receptors are a group of GPCR families. Owing to the absence of selective ligands, still little is known about numerous subclasses of serotonin receptors [24, 25]. However, structural similarity between the exogenous agonists of IAs and endogenous neurotransmitters, for example, serotonin, has driven the researchers to determine the probable neurological effect of these potent molecules.

5. Medicinal Plants: New Leads for the Development of Antidepressant Drugs

It seems that presently the patients are increasingly dependent on synthetic drugs for the amelioration of emotional disorders. However, the studies have indicated that the use of herbal products for the management of psychiatric disorders has also gained importance. Recent scientific studies have focused on the confirmation of the supposedly psychoactive properties of medicinal plants. The phytochemical screening of plants is a multistep process that includes fractionation, purification, isolation, chemical elucidation of phytoconstituents, and the pharmacological studies, as shown in Figure 1.

The researchers have isolated various compounds acting on the CNS from different plants, which have been used clinically in their natural or modified form or are being tested in preclinical and clinical trials, as shown in Table 1.

5.1. Passiflora incarnata. P. incarnata along with other species, e.g., *P. alata, P. edulis, and P. caerulea,* have been widely utilized in traditional treatments as a sedative in the United States and some European countries [49]. The chemical structure of benzos (benzodiazepines) drugs contains the fusion of a benzene ring and a diazepine ring, with a 7-membered heterocyclic ring having two N-atoms. IAs obtained from *Passiflora incarnata, viz.*, harmine, harmol, harmalol, harmaline, and harman, also contain a benzene ring that is fused to a 5-membered heterocyclic ring comprising one N-atom. Various studies have demonstrated that *Passiflora incarnata* possesses a pharmacological activity much like benzos and functions via receptors of γ-aminobutyric acid [50].

5.2. Mitragyna speciosa. The leaves or extracts of kratom (Mitragyna speciosa) have been commonly used in traditional medicines for the improvement of blood circulation and the treatment of diabetes and diarrhea [51]. Mitragynine is an important indole alkaloid found in kratom and its analogs, paynantheine, speciociliatine, and speciogynine [52]. Two experiments performed on the alkaloid and the aqueous extracts of kratom produced the effect of antidepressants during behavioral despair tests on animal models [53]. A study carried out on mitragynine exhibited an antidepressant-like effect in behavioral animal models of depression by interacting with the hypothalamic-pituitary-adrenal (HTPA) axis within the endocrine system [54].

5.3. Peganum harmala. P. harmala has been used in traditional medicine in different societies for the treatment of certain nervous system disorders and psychiatric conditions such as Parkinson's disease and nervousness and to relieve severe pain [55–58]. The alkaloids obtained from *P. harmala* were found to be psychoactive, and different studies in animal models have indicated a wide range of effects such as hallucination, analgesia, excitation, and antidepressant effect produced by the active alkaloids of P. harmala [59–62]. Harmine, harmaline, and norharmane are the alkaloids found in *P. harmala*, which are also present in the body. However, in certain patients and conditions such as Parkinson's disease, drug addicts, alcoholics, and smokers, the high concentration of these alkaloids have been found; therefore, it is believed that these alkaloids play an important role in various CNS problems [63].

Studies have shown that beta-carbolines derived from *P. harmala* can interact with dopamine, opioid, 5-hydroxytryptamine, Gamma-aminobutyric acid (GABA), imidazoline, and benzodiazepine receptors on the nervous system, thereby exerting various pharmacological effects [58, 62, 64, 65]. Furthermore, these alkaloids were found to inhibit monoamine oxidase and have shown neuroprotective activity. This significant feature makes them a choice for the treatment of anxiety and depressive disorders [60, 65, 66].

5.4. Piper methysticum. Piper methysticum is used as a beverage named kava, which gives a happy state of mind while decreasing anxiety and fatigue [67]. The investigation revealed that most pharmacological effects were associated with the use of kava resin (lipid extract) rather than the aqueous extract. Seven pyrones, known as kavalactone, are present in the kava resin. These kavalactones usually interact with the serotonin, glutamatergic neurotransmitters, GABA, dopaminergic pathways; inhibit monoamine oxidase (MAO)-B; and exert various effects on several ion channels [68]. Dihydromethysticin (DHM) is a major kavalactone present in the roots of the kava plant. The chemical structure of the DHM consists of arylethylene-alpha-pyrone, which is linked to an indole moiety containing two oxygen atoms instead of nitrogen atoms. This facilitates an anxiolytic effect and serves as an antidepressant medicine. Double-blind, placebo-controlled investigations revealed that kavalactone compounds exert antianxiety activities without decreasing

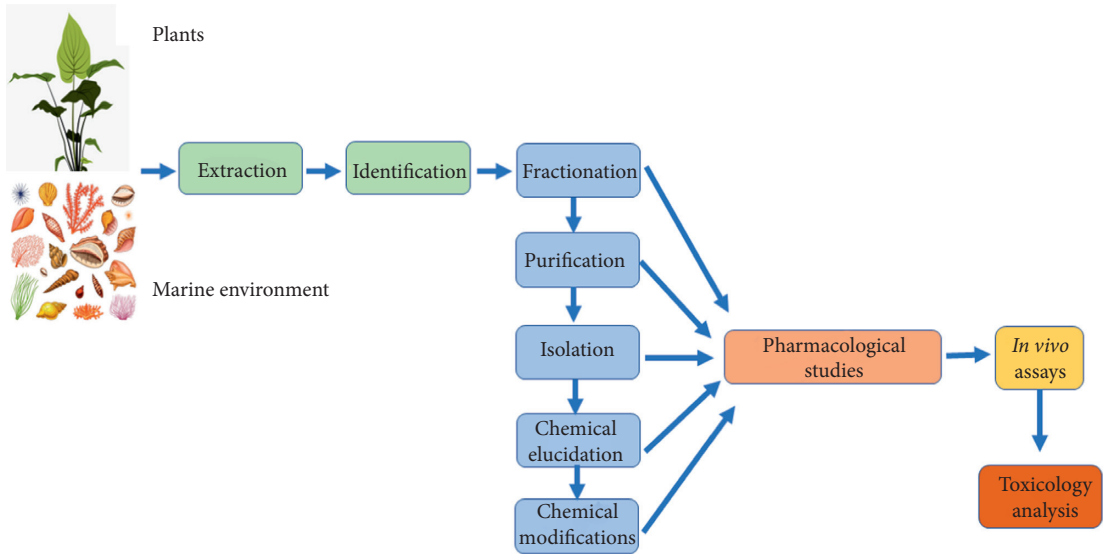

Figure 1: The model for the testing of alkaloids from plant and marine sources with antidepressant properties.

the motor and mental functions as well as by improving the sleep quality. Kavalactones have also been used as an alternative to the use of benzos in the treatment of depression [69].

5.5. Valeriana officinalis. *Valerian* is extensively used in various countries for its anticonvulsant, sedative, anxiolytic, and hypnotic-like effects [70]. Valepotriates and valerenic acid are active ingredients found in various pharmaceutical formulations. Furthermore, the crude extracts of valerian have been used in several countries [71]. Valepotriates containing triesters of otherwise unstable polyhydroxy cyclopenta-(c)-pyrans with carboxylic acids, namely, isovaleric, β-methylvaleric, valeric, β-hydroxyisovaleric, alpha-(isovaleroxy)-isovaleric, beta-acetoxy-beta-methylvaleric, beta-acetoxy-isovaleric, and acetic acid, have been utilized as sedatives. The most thermolabile, unstable valerian components are valepotriates that decompose quickly in alkaline or acidic water, and also in alcoholic solvents [71]. Valepotriates have also been used for the improvement of human and animal pathological conditions during the withdrawal of benzodiazepine [72].

The mode of action is described as the interaction between valerian and GABA receptors inside the brain via GABA-aminotransferase inhibition, interference in intake and uptake of functional GABA within synaptosomes, and through the interaction of the benzodiazepine/GABA receptor [73].

6. IAs of Marine Origin

An increasing number of IAs have been reported from several marine organisms. Because of the occurrence of enzymes, i.e., haloperoxidases, within the marine ecosystem, the largest alkaloids group isolated from seaweeds, mollusks, ascidians, and sponges are halogenated.

Mono-IAs from the marine environment possess structural similarities to serotonin and have facilitated a better understanding of 5-HT receptor (5-HTR) function to synthesize novel drug compounds for treating migraines, anxiety, depression, and other disorders associated with 5-HTR. Numerous compounds containing an indole ring possessing an affinity toward various serotonin receptors have been identified: 8,9 dihydrobarettin, sigma-conotoxin, barettin, and gelliusines A and B [74, 75]. Methylaplysinopsin obtained from the sponge *Aplysinopsis reticulata* has been found to inhibit the monoamine oxidases (Mao) and displace the serotonin from the receptor sites [76]. The other compounds of this group, N-3′ ethylaplysinopsin, 6-bromoaplysinopsin, and 6-bromo 2′-de-N-methylaplysinopsin, obtained from the sponge *Smenospongia aurea* have also been identified to displace the antagonist binding at $5HT_{2A}$ and $5HT_{2C}$ receptors [77]. N-3′ ethylaplysinopsin has not shown selectivity toward any of these receptors. 6-Bromoaplysinopsin exhibited only a little selectivity to 5-HT_{2C} receptors; conversely, 6-bromo 2′-de-N-methylaplysinopsin showed strong selectivity toward 5-HT_{2C} receptors. In addition to neural activity, 6-bromoaplysinopsin has also exhibited significant activity against the malarial parasite *Plasmodium falciparum*.

Studies have shown the antimicrobial activity of 5-bromo-DMT (5-bromo-*N*,*N*-dimethyltryptamine) and 5,6-dibromo-DMT [78, 79]. Both of these compounds have also been reported to have neurological activity: 5-bromo-DMT have shown powerful sedative activity in open field test; 5,6-dibromo-DMT have exhibited antidepressant effect in tail-suspension and behavioral despair tests [80]. 5,6-Dibromo-DMT compound was considerably more effective than monobromotryptamine as it has also been reported to exhibit considerable antitumor activity in the MTT assay with HCT116 colorectal cancer cell lines [81]. An interesting and novel marine metabolite, possessing structural similarities to cannabinoids and indoles, has been found with powerful antidepressant action in behavioral despair tests [82].

A lot of naturally occurring IAs have not yet been evaluated for their neurological activity. However, their

Table 1: The medicinal use of plants for the treatment of depression and/or anxiety.

Plants	Active ingredients	Mechanism of action	Therapeutic purposes	Study type (animal mode/ clinical)	References
Actaea racemosa L	Triterpenes and derivatives of flavones	Dopaminergic effect. Also, acts on the hypothalamus vasomotor center	Anxiety and depression	Randomized clinical trial	[26, 27]
Agastache mexicana subsp. Mexicana (Lamiaceae)	Tilianin	The ligand of $GABA_A$/BZDs receptor	Nerve tonic and tranquilizing	Mice	[28, 29]
Agastache mexicana subsp. Xolocotziana (Lamiaceae)	Tilianin	GABAergic activity	Nerve tonic and tranquilizing	Mice	[28, 30]
Annona cherimola Mill. (Annonaceae)	Liriodenine, nornuciferine, and anonaine	Increase in monoaminergic neurotransmission	Anxiety and tranquilizing	Mice	[31]
Hypericum perforatum L (Hypericaceae)	Hyperforin and hypericin	A selective inhibitor of MAO-A and MAO-B; inhibition of serotonin, dopamine, and norepinephrine uptake, the antagonist of N-methyl-D-aspartate receptor; interactions with the GABA-A receptor	Depression, anxiety, and insomnia	Clinical studies	[32, 33]
Lavandula angustifolia Mill. (Lamiacae)	Linalool and linalyl acetate	Serotonin neurotransmission through 5-HT_{1A} receptors	Depression	Mice Randomized clinical trial	[34, 35]
Litsea glaucescens (Lauraceae)	Linalool and b-pinene	Interaction with the serotonergic 5-HT_{1A} receptors, α2-adrenoceptor, and the β1-adrenoceptors D1 receptor	Sadness	Mice	[36, 37]
Melissa officinalis L. (Lamiaceae)	Rosmarinic acid and the triterpenoids, ursolic acid, and oleanolic acid	Inhibitor of GABA transaminase	Benign palpitations	Randomized clinical trial	[38]
Mimosa pudica (Fabaceae)	Norepinephrine, b-sitosterol, d-pinitol, mimosine	Mediated by the central serotonergic system	Depression and insomnia	Rats	[39]
Passiflora incarnata	Orientin, isoorientin, vitexin, isovitexin, and chrysin	$GABA_A$ and $GABA_B$ receptors agonist	Generalized anxiety disorder, insomnia, and depression	Clinical studies	[40, 41]
Pimenta pseudocaryophyllus (Gomes) L.R. Landrum	Dichloromethane fraction	Effect on monoamine biosynthesis	Nerve tonic, a calming agent	Mice	[42]
Piper methysticum G. Foster	Kavalactones, Kawain, dihydrokavain	Inhibition of MAO-B and blocked the uptake of noradrenaline	Anxiety	Clinical studies	[43, 44]
Tagetes lucida Cav. (Asteraceae)	Quercetin, caffeic acid, gallic acid	Mediated by 5-HT1A and 5-HT2A receptors	Anxiety and depression	Rats	[45, 46]
Valeriana officinalis L.	Valerenic acid and valerenol	Enhance the response to $GABA_A$ receptors	Sleep and anxiety disorders	Mice, clinical studies	[47, 48]

structures reveal a possible affinity toward dopamine, adrenergic, and serotonin receptors. A fraction comprising 6-bromotryptamine has been reported to exhibit in-vitro antifungal and antimicrobial activity [83]. One more tryptamine derivative, Nb-acetyltryptamine, has been obtained from an unclassified marine fungus that grows on the *Gracilaria verrucose* surface [84]. This compound along with the deacetylated derivative has been identified from a marine bacterial specie *Roseivirga echinicomitans* ($KMM6058^T$), which is associated with Strongylocentrotus intermedius (sea urchin) [85]. These compounds were observed to be slightly cytotoxic to Erlich carcinoma cells; *N*,*N*-diacetyltryptamine showed greater hemolytic activity causing 50% damage to egg and sperm cells membrane at 15 and 7.5 μg/ml concentrations, respectively. The dibrominated compounds 12 and 11 were initially reported as antimicrobial metabolites isolated from a *Polyfibrospongia maynardii* sponge [86]. Later on, these alkaloids were obtained from the

marine sponge *Hyrtios erecta* and observed to be involved in selective inhibition of nitric oxide synthases (neuronal isoform) [87].

Three bromoindoles obtained from the mid-intestinal gland of the gastropod mollusk *Drupella fragum* have been found to exhibit antioxidative properties [88]. 6-BroMo-5-hydroxyindole showed greater antioxidant activity than the alpha-tocopherol. Two more compounds, 6-bromoindole-3-carboxaldehyde along with its debromo analog, were extracted from a species of *Acinetobacter*, a bacteria obtained from the surface of *S. murrayi* [89]. This brominated alkaloid exhibited antibacterial activity as well as inhibited the larval settlement of the Barnacle, *Balanus Amphitrite*.

A novel indole derivative named 3-indoleacrylamide has been reported to have an in-vitro antihelminthic activity [90]. The heterocyclic compound was obtained from the *Chondria atropurpurea* (red alga) along with many other known indole and bisindole alkaloids. One more antimicrobial indole was obtained from the Palauan ascidian *Distaplia regina* [91]. Moreover, monoindole alkaloids were found regulating the plant growth mechanism: this kind of activity has also been reported for indole-3-acetamide as well as 3-(hydroxyacetyl)indole [92].

7. Synthetic IAs

In the literature, numerous studies have been focused toward synthesizing selective 5-HT receptor ligands. Several structures have been identified as selective and potent agents for 5-HTRs; some of them have structural similarities to compounds obtained from sponges. 2-Ethyl-5-methoxy-*N*,*N*-dimethyltryptamine (EMDT), a tryptamine derivative, was synthesized as a first selective agonist for the 5-HT_6 receptor [93].

Several structure-activity relationship (SAR) studies have reported the promising structures for both antagonists and agonists of 5-HTRs. Quantitative SAR (QSAR) and structure affinity relationship of numerous tryptamine derivatives have been investigated for a 5-HT_{1E}-like receptor subtype [94]. Findings revealed that the chain of two atoms involved in the separation of the indole ring from its amine functional group is important for the interaction of tryptamine analogs with receptors. Moreover, the branching of the chain results in decreased affinity. Therefore, the indole ring seems crucial for receptor affinity and changes in the benzene ring or substitution of the NH group with S will decrease the receptor affinity. However, the replacement of the amine functional group, only if the substituent groups remain smaller, will not affect the affinity.

Agents that bind to the 5-HT_6 receptors have also been extensively studied [95]. In a study, it was identified that *N*,*N*-dimethylation, or *N*-monomethylation, of 5-HT derivatives can result in a small increase in the binding affinity. In 5-HT derivatives, the primary (1°) amine can metabolize rapidly through oxidative deamination causing problems by decreasing the capability of a compound for crossing the BBB (blood-brain barrier). The substitution of a primary (1°) amine with secondary (2°) or tertiary (3°) amines can increase the molecular lipophilicity, making it less susceptible to metabolism, as well as increasing its probability of being used as an effective drug.

In a study, it was reported that oxindoles have an antidepressant-like effect, and SAR studies revealed that the best possible sidechain for these heterocyclic compounds could be (CH2)3NHCH3 or any group capable of metabolizing to this. The branching of the sidechain can result in a reduced effect similar to the replacement of the indolinone ring. The replacement at the nitrogen atom of a heterocycle must be a phenyl group, and the substitution of the group at 3-position on indolinone must be small for maintaining the activity [96]. It was reported that 2-substituted tryptamines possess pharmacological activity. Among the compounds tested in this study, 2(2-methyl-2-amino)-propylindole hydrochloride was involved in motor excitation, tail and limbs tremor, stereotyped head spasm in rats and mice [97].

The latest medicinal and synthetic chemistry-based research has concentrated on the synthesis of various types of specific ligands for 5-HTRs. In a study, 5-alkhyltryptamine analogs were evaluated for finding the substituents that are important for the binding affinity of the particular molecule toward 5-HT_{1D} receptors [98]. It was also revealed that the substituent at 5-position did not require properties of H-bonding for exhibiting a strong binding affinity to this receptor, as the size of a group determines the affinity. *N*-Methyl-5-tert-butyltryptamine exhibited a greater affinity toward 5-HT_{1D} receptor, and compound 56 was the most powerful agonist with a Ki value of 0.45 nM.

A research group investigated thieno [3,2-b]- and thieno [2,3-b]-pyrrole bioisosteric analogs of DMT and found that the thiophene compound cannot be used as a substituent of the phenyl group within the indole ring of tryptamine compounds that bind to the 5-HT_2 receptors [99]. Nevertheless, the thiophene compounds could be an appropriate bioisosteric replacement for compounds showing an activity toward the 5-HT_{1A} receptor. In another study, this research group investigated how fluorination affects the hallucinogen-like activity of tryptamines [100]. Their findings exhibited that fluorination of the tryptamine compounds at position, 5,4,7, and 6 reduces their hallucinogen-like activity. The introduction of fluorine at position 6 of the 5-methoxy-DMT (5-MeO-DMT) reduces the binding affinity toward the 5-HT_{1A} receptor. However, in the case of the DMT, the fluorination at 6-position led to a five-time decrease in binding affinity to the 5-HT_{1A} receptor.

Ring fluorination of 5-MeO-DMT at 4-position resulted in an increased affinity for the $5HT_{1A}$ receptor, which yielded a selective and potent 4-fluoro-5-MeO-DMT (Ki 0.23 nM). The fluorination of 5-HT_{2C} and 5-HT_{2A} receptors at the 6th position insignificantly affected the affinity toward these receptors. A study reported the process for the synthesis of *N*-(2-arylethyl)-benzamine compounds as potent 5-HT_6 antagonists. The researchers revealed that these antagonists can be used for the treatment of neurocognitive disorders and several other disorders that are related to 5-HT_6 receptors, such as schizophrenia, anxiety, migraine, epilepsy, sleep disorders, Parkinson's disease, convulsions, and cognitive disorders associated with the age.

The studies have found that several tryptamine-like intermediate compounds and 8-substituted-tetrahydro-β-carbolines possess a great affinity for all the 5-HT_2 receptor subtypes [101]. In another report, the compounds were synthesized with a high affinity toward 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{2A} receptors, which can be used for the management of several disorders related to these receptors, like dyspepsia, depression, tachygastria, schizophrenia, migraines, anxiety, and achalasia. The indole derivatives along with their affinity toward 5-HT_2 were reported to be effective for the treatment of mammals that suffer from 5-HT2-associated disorders, e.g., depression, anxiety, and hypertension. Moreover, few more indole compounds were revealed as potential inhibitors of the angiogenesis pathway, which has an important role in the development of cancer, inflammatory and immune disorders.

A novel subtype of 5-HTRs, i.e., the 5-HT_7 was recently found to be associated with psychiatric disorders, for example, schizophrenia and depression; however, their function is still not widely known. In a comprehensive investigation, the inverse agonists for these receptors were described, and it was revealed that derivatives of tryptamine, e.g., compound 111 with no added aromatic rings display only reduced affinity to these receptors [102].

Several enantiomers of alpha-methyltryptamines (AMTs) were investigated. The researchers examined the analogs of tryptamine in 5-HT2 and 5-HT1B receptor-binding assays, which revealed that both the binding sites have different enantioselectivity, depending on their aromatic substituents. In both the subtypes, the binding affinity for AMTs was in the order: 5-substituted > 4-substituted > unsubstituted > 6-substituted. For α-methtyl-serotonin, the *S*-isomer exhibited greater affinity toward both receptors than the *R*-isomer. In the case of compounds 118 and 115, the R-isomers displayed greater affinity to 5-HT_{1B} but not toward 5-HT_2 [103].

Three novel IAs were synthesized through microbial biotransformation using *S. staurosporeus*. The bacterium was fed with the 6-fluoro-tryptamine, 5-fluror-tryptamine hydrochloride, and tryptamine hydrochloride, obtained from the bacterial cultures and extracted with beta-hydroxy-Nb-acetyltrptamine along with its 6- and 5-fluoro derivatives [104]. Procedures for the synthesis and the insecticidal activities of numerous simple IAs have been explained in a study. Salts, stereoisomers, and drugs having the aryl-thioether tryptamine derivatives have been reported to be useful for central nervous system diseases that are associated with the 5-HT_6 receptors, e.g., depression, anxiety, and movement disorders.

8. New Indole and Tryptamine Derivatives

One novel compound studied in the past several years that possesses structural similarities to the IAs belongs to the Wyeth compounds, i.e., WAY 161503, which is a selective agonist of the 5-HT2C receptor, and which is involved in various aspects related to mood, appetite control, and reward-related behavior [105]. WAY 161503 is protected by multiple patents and is reported to be effective for the prevention and management of involuntary urination and clinical depression.

One more compound, WAY 163909, a potent and selective agonist of the 5-HT_{2C} receptor, has also been found to be useful for the treatment of obesity. This compound displayed antipsychotic and antidepressant activity in animal models. PD-6735 (TIK-301), a melatonin agonist that has just finished Phase II trials for blind individuals associated with sleep disorders, also contains a structural indole moiety. This drug not only proved to be effective in the reestablishment of the right day-night cycle but also exhibited a good safety profile.

9. Conclusion

The major limitations of the studies are the use of crude or semi-purified phytochemicals for the treatment of psychiatric diseases. Moreover, the results of the studies in animal models and/or clinical trials vary and lack reproducibility. This may be due to disparities in the metabolite contents in different geographical areas owing to the climate, the ecological conditions, and the availability of nutrients. Additionally, the bioactivity of phytochemicals may be attributed to the mixture of compounds; therefore, it is suggested to obtain the active ingredients followed by identification and metabolomics study for the better characterization of these phytochemicals.. Moreover, the chemical synthesis of indole alkaloids is also proposed as indole alkaloids from natural sources are quite complex. The synthetic IAs can be a better option as the structure of various receptors and enzyme inhibitors are available. However, some of the naturally occurring IAs may not be synthesized by the available methods. The marine IAs also have an incredible potential for the treatment of the different psychiatric disorders; therefore, further studies can offer better insights to the utility of the IAs for the amelioration of anxiety and depressive disorders. In conclusion, several IAs, especially from the plants, have been used as antianxiety medication and antidepressants. In the future, this reservoir of IAs from plants can be utilized as a valuable starting point to develop effective alternatives for the therapeutic management of depression and anxiety-related disorders.

Initially, the standard crude extracts are prepared, followed by the phytochemical studies including fractionation, purification, isolation, and chemical elucidation of phytoconstituents. These alkaloids can either be modified structurally or new compounds are synthesized based on the chemical structure of the alkaloids. The pharmacological studies of the crude extracts as well as the fractioned, isolated, and chemically modified compounds are performed for antianxiety and/or antidepressant properties. *In vitro* assays including the light-dark box test, open field, elevated plus-maze, tail suspension test, and forced swimming test are performed. Finally, the toxicological tests are performed in cell culture using animal models.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Use of Traditional Chinese Medicine for Patients Diagnosed with Postpartum Depression: A Nationwide Population-Based Study

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Postpartum depression (PPD) is one of most common postnatal complications, affecting approximately 10%–15% of women after childbirth annually. Traditional Chinese medicine (TCM) has been gaining popularity as the choice of treatment for PPD in Taiwan. Hence, our aim was to analyze the utilization of TCM among PPD patients in Taiwan. A cross-sectional study was conducted using a random sample of one million beneficiaries selected from the Taiwanese National Health Insurance Research Database. We identified patients with PPD who had received either TCM treatment or non-TCM treatment from the database during 2000–2012. Multivariate logistic regression analysis was used to identify the factors associated with the use of TCM. A total of 653 patients with PPD were enrolled. The majority of patients with PPD were 26–30 years old, lived in a highly urbanized area of Taipei, had a monthly income <20,000 NT$, and were private enterprise employees. Around 52.7% of PPD patients had the motivation to seek TCM services. Younger women, who resided in central and southern Taiwan and who had used TCM one year before PPD diagnosis, were more likely to use TCM services. PPD patients who underwent TCM treatment had a lower overall medical expenditure than non-TCM users. Most TCM users chose simple Chinese herbal medicine. The coexisting factors that made PPD patients to seek TCM services were respiratory or oral infections. We demonstrated the characteristics of those that seek TCM for PPD, which may provide useful insights to health care providers towards resource allocation.

1. Introduction

Childbirth is usually a unique and unforgettable experience for most women of childbearing age. However, some factors interfere with the positive aspects of mother-child interactions of new mothers, including the exhausting process of childbirth, postpartum physical uncomfortable conditions, breastfeeding stress, lifestyle changes, and relationship dynamic changes [1, 2]. Hence, some women suffer from fluctuating moods postpartum. Postpartum depression (PPD) is a depressive disorder often occurring within one year of delivery [3]. It is one of the most common postnatal complications, during which the person with PPD can experience sadness, worthlessness, or hopelessness, which could potentially result in maternal and familial negative consequences, disabilities, or life-threatening situations [4, 5]. PPD affects about 10%–15% of new, adult mothers annually, which often leads to a substantial humanistic burden on affected mothers, their partners, and their other children [6, 7].

Psychotherapy and antidepressant medications are the gold-standard treatment for PPD [8]. Although antidepressant medications are convenient treatments for PPD, their potentially troubling side effects can affect normal routine activities. These side effects include weight gain, sleep disturbances, and sexual dysfunction, which should be considered before taking long-term antidepressants [9]. Furthermore, some reports revealed that different levels of

antidepressant drugs may transfer into breast milk, which leads to the mother having to make the choice whether she should receive medication therapy at the risk of her infant's health [10, 11]. Hence, some patients consider other safe, effective, economic therapies to replace conventional western medicine (WM).

Traditional Chinese medicine (TCM), which includes Chinese herbal medicine (CHM), acupuncture, and manipulative therapies, has been used for depressive disorders for more than 5000 years [12] and now has extensive scientific evidence supporting its efficacy. A systematic review and meta-analysis report included 443 PPD patients who received acupuncture and 444 PPD patients who did not, with the results showing that acupuncture led to improvements on the Hamilton Depression Scale and had an effective rate [13]. Yang et al. concluded that CHM could significantly reduce PPD symptoms compared to antidepressants [14]. However, there is still little evidence about the factors involved in TCM use by the PPD population based on a large sample size.

Since 1995, the National Health Insurance (NHI) program has offered insurance coverage for TCM and WM for nearly 99.6% of the 23 million residents of Taiwan [15]. The Bureau of the NHI has cooperative agreements with nearly 92% of all clinics and 97% of all hospitals [16]. Intact clinical data of TCM use by women with PPD were collected from the Taiwanese National Health Insurance Research Database (NHIRD), which is considered to reflect real-world evidence of TCM clinical practice for research purposes [17]. Thus, this study aimed to apply the NHIRD information to explore the determinants of TCM use in patients with PPD.

2. Materials and Methods

2.1. Data Source. We conducted a nationwide, population-based, cross-sectional analysis by using the Longitudinal Health Insurance Database 2000 (LHID2000), which comprises a random sample of one million subjects from the NHIRD longitudinally linked data available from 1997 through 2013. This database contains sociodemographic data, dates of visits, International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnostic codes, complete prescription details, and expenditure incurred by the beneficiaries. Data of detailed diagnoses and treatments provided by physicians were included. These files were linked by using a scrambled, anonymous identification number for each subject to obtain the longitudinal medical history. This study was approved by the Institutional Review Board of China Medical University in central Taiwan (CMUH109-REC2-031).

2.2. Study Population. We selected participants covering the period from January 1, 2000, to December 31, 2012, by using the LHID2000. The flow chart is shown in Figure 1. First, we identified the postnatal women included in the LHID2000 ($n = 61{,}332$). Any diagnosis of depression, in which at least two ambulatory or inpatient claims were made by psychiatrists who prescribed antidepressants for treatment within one year after delivery was defined as PPD (ICD-9-CM code: 296.2, 296.3, 296.82, 300.4, 309.0, 309.1, 311). Among these, we excluded subjects that fell under the criteria of being younger than 20 years of age, who had a previous history of depression, or had incomplete medical records. Finally, a total of 653 women newly diagnosed with PPD that met our criteria were enrolled. Subjects who had visited a Chinese medicine clinic and received TCM treatment at least once after being newly diagnosed with PPD were deemed "TCM users," and the rest were deemed "non-TCM users." The date of first TCM usage after the diagnosis date of PPD was defined as the index date for the TCM cohort. The follow-up period of all participants was within one year from the date of initial diagnosis.

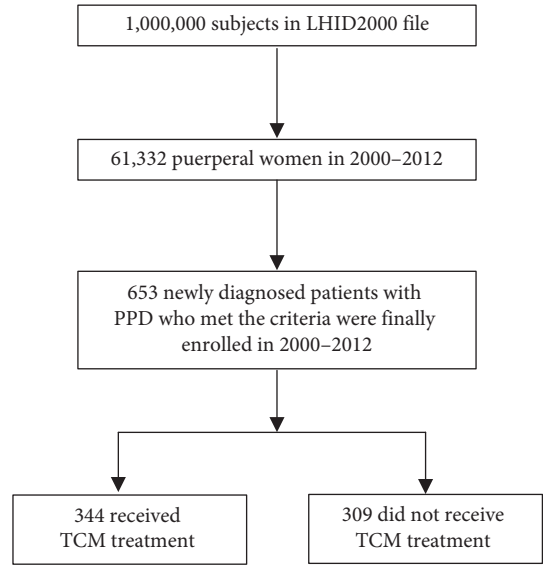

Figure 1: Flowchart for study subject enrolment. LHID2000: Longitudinal Health Insurance Database 2000. TCM: traditional Chinese medicine.

2.3. Sociodemographic Characteristics. The sociodemographic factors included age, insured amount, urbanization level, residential area, and insured unit categories. Adult patients were further divided into six subgroups: 20–25, 25–30, 30–35, 35–40, 40–45, and >45 years. The amount of insurance premium, determined from the individual working salary, was classified into four levels: < 20,000, 20,000–39,999, 40,000–59,999, and >60,000 NT$/month. Furthermore, the urbanization level of the townships in Taiwan was categorized according to educational level of the population, population density, ratio of elderly people, and occupation in general. The residential areas of the study population comprised of 6 areas: northern area, Taipei, central area, southern area, eastern area, and Kao-Ping area. The insured units included government, school, private enterprise, occupational members, farmers and fishermen, low-income household, and veterans. Furthermore, the season during which the delivery took place was included as a factor. We used the official definition of seasons of the Taiwanese Central Weather Bureau (CWB) as spring

(March–May), summer (June–August), autumn (September–November), and winter (December–February).

2.4. Medical Factors. TCM use one year prior to PPD diagnosis was considered. Baseline comorbidities with at least two ambulatory or inpatient claims were also considered in order to qualify for the present study, including diabetes mellitus (ICD-9-CM 250), hypertension (ICD-9-CM 401–405), hyperlipidemia (ICD-9-CM 272), stroke (ICD-9-CM 430–438), coronary artery disease (ICD-9-CM codes 410–414), cirrhosis (ICD-9-CM 571), and renal disease (ICD-9-CM 585).

2.5. Medical Expenditure. We analyzed the total number of medical expenditures within one year after the first diagnosis date of PPD, which took TCM and WM ambulatory care into consideration. Total medical expenditure included consultation fees, charges for treatment and medical supplies, diagnosis fees, and drug fees.

2.6. Coexisting Diseases of Outpatient Department Visits after PPD Diagnosis. We analyzed the frequency distribution of coexisting diseases of OPD (outpatient department) visits after PPD diagnosis in the TCM cohort and non-TCM cohort based on the ICD-9 codes.

2.7. Statistical Analysis. The continuous variables were evaluated using means and standard deviations (SD), whereas categorical variables were evaluated using numbers and percentages. To compare the differences in continuous variables between TCM users and non-TCM users, Student's t test was used, whereas the chi-squared test was used to analyze the categorical variables. Furthermore, the adjusted odds ratio (OR) and 95% confidence interval (CI), calculated using a multivariate logistic regression analysis, were used to explore the determinants of TCM use. A p value <0.05 was considered statistically significant. All statistical analyses and figures were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, U.S.A.).

3. Results

3.1. Factors Associated with TCM Use in Patients with PPD. We identified a total of 653 patients with newly diagnosed PPD who met the study criteria between 2000 and 2012 in the LHID2000 (Figure 1). Among the included subjects, 344 patients (52.7%) received TCM treatment and 309 patients (47.3%) did not receive TCM treatment.

Characteristics of the TCM user and non-TCM user cohorts of patients with PPD are presented in Table 1. The mean duration between the delivery dates and initial diagnosis of PPD was approximately 0.44 years. The mean age of PPD patients was 31.2 years. The highest proportion of PPD patients in both cohorts were in the 26-30-year age group, followed by the 31–35-year-old and 20–25-year-old groups. There were no substantial differences in the insured amounts, urbanization levels, or insured units between the two groups. The difference between the number of patients with PPD was not significantly different among spring, summer, autumn, and winter. The most common baseline comorbidity was cirrhosis, followed by hypertension and coronary artery disease. The prevalence of all baseline comorbidities was similar in both groups. Furthermore, using a multivariate-adjusted analysis, we observed that patients with PPD who were in the 20-35-year age group, TCM users one year prior to the PPD diagnosis, who resided in the central and southern area, were more likely to use TCM services.

3.2. TCM Visits and Medical Expenditure. The mean duration between the initial diagnosis of PPD and the first TCM treatment was approximately 0.31 years. The mean number of OPD visits to a TCM clinic within one year of PPD diagnosis was 5.58 (Table 1).

The total medical expenditure of the TCM users and non-TCM users with PPD within a one-year follow-up after the initial diagnosis of PPD is compared in Table 1. The total (average) cost in TCM users was lower than that of non-TCM users; nevertheless, the difference in average cost between these two groups was minimal (43,071 NT$ vs. 47,395 NT$, $p = 0.74$).

3.3. Types of TCM Use. We categorized the most frequent types of TCM use in Taiwanese patients into 3 categories: simple CHM treatment, simple acupuncture/manipulative treatment, and combination of CHM/acupuncture/manipulative treatment. As shown in Table 2, in the TCM user cohort, 71.5% of patients with PPD were treated with CHM treatment only, 2.3% were treated with acupuncture or manipulative treatment only, and 26.2% were treated with combination treatment within a one-year follow-up.

3.4. Distribution of Coexisting Diseases in TCM and Non-TCM Visits in Patients with PPD. We observed which infectious diseases commonly coexisted as the reason for OPD visits after the patients' diagnosis of PPD, irrespective of TCM use by the patients (Table 3). The top 5 coexisting diseases of PPD patients who used TCM were respiratory or oral infection-related conditions, including acute upper respiratory infections (5.83%), acute nasopharyngitis (3.79%), acute tonsillitis (3.5%), dental caries (3.21%), and acute pharyngitis (2.04%).

4. Discussion

The determinants for TCM use for PPD patients in a nationwide population have scarcely been investigated in the past. The NHIRD offered a sufficient population sample size and a great deal of information, eliminating the bias associated with a limited sample size. This means that the data can be considered close to real-world evidence and can be used as an appropriate source to assess the disease and treatment efficacy. In addition, it is worth noting that the TCM practice is only performed by well-trained, qualified

Table 1: Characteristics of patients with postpartum depression according to use of traditional Chinese medicine.

	Total ($N = 653$)		TCM used: No ($N = 309$)		TCM used: Yes ($N = 344$)		p value	Adjusted OR (95% CI)[‡]
	n	%	n	%	n	%		
Age mean ± SD (years)[&]	31.2 ± 8.48		31.7 ± 8.91		30.8 ± 8.06		0.22	
Age group							0.10	
20–25	123	18.8	53	17.2	70	20.4		3.98 (1.43, 11.1)***
26–30	211	32.3	102	33.0	109	31.7		3.05 (1.13, 8.21)***
31–35	186	28.5	82	26.5	104	30.2		3.28 (1.26, 8.57)***
36–40	80	12.3	41	13.3	39	11.3		2.30 (0.84, 6.30)
40–45	26	3.98	19	6.15	7	2.03		1.00 (0.32, 3.08)
>45	27	4.13	12	3.88	15	4.36		1.00
Insured amount (NT$/month)							0.49	
<20,000	581	89.0	275	89.0	306	89.0		1.00
20,000–39,999	51	7.81	27	8.74	24	6.98		1.14 (0.60, 2.18)
40,000–59,999	17	2.60	6	1.94	11	3.20		1.83 (0.59, 5.64)
≥60,000	4	0.61	1	0.32	3	0.87		1.40 (0.20, 9.89)
Urbanization[†]							0.45	
Level 1 (highest)	203	31.1	92	29.8	111	32.3		1.00
Level 2	214	32.8	111	35.9	103	29.9		0.81 (0.51, 1.29)
Level 3	101	15.5	47	15.2	54	15.7		0.98 (0.54, 1.78)
Level 4	88	13.5	41	13.3	47	13.7		1.03 (0.53, 1.99)
Level 5 (lowest)	47	7.20	18	5.83	29	8.43		1.86 (0.79, 4.38)
Residential area							0.002	
Northern	85	13.0	54	17.5	31	9.01		1.00
Taipei	256	39.2	126	40.8	130	37.8		1.68 (0.92, 3.07)
Central	113	17.3	46	14.9	67	19.5		2.05 (1.09, 3.86)***
Southern	84	12.9	27	8.74	57	16.6		3.17 (1.58, 6.33)***
Eastern	20	3.06	9	2.91	11	3.20		1.91 (0.61, 5.95)
Kao-Ping	95	14.6	47	15.2	48	14.0		1.71 (0.88, 3.32)
Insured unit							0.21	
Government, school employees	75	11.5	28	9.06	47	13.7		3.07 (1.34, 7.05)***
Private enterprise employees	279	42.8	131	42.4	148	43.2		2.98 (1.46, 6.10)***
Occupational member	159	24.4	75	24.3	84	24.5		3.36 (1.58, 7.14)***
Farmers, fishermen	58	8.90	33	10.7	25	7.29		1.00
Low-income households and veterans, other regional populations	81	12.4	42	13.6	39	11.4		2.26 (1.03, 4.95)**
Season of delivery							0.51	
Spring (March–May)	168	25.7	83	26.9	85	24.7		1.00
Summer (June–August)	180	27.6	87	28.2	93	27.0		1.09 (0.69, 1.74)
Autumn (September–November)	170	26.0	72	23.3	98	28.5		1.47 (0.92, 2.36)
Winter (December–February)	135	20.7	67	21.7	68	19.8		1.03 (0.63, 1.69)
TCM use one year prior to PPD diagnosis							<0.001	
Non-TCM users	369	56.5	230	74.4	139	40.4		1.00
TCM users	284	43.5	79	25.6	205	59.6		4.36 (3.08, 6.19)***
Baseline comorbidity								
Diabetes mellitus[#]	9	1.38	3	0.97	6	1.74	0.51	0.62 (0.18, 2.09)
Hypertension	49	7.50	21	6.80	28	8.14	0.52	0.99 (0.45, 2.18)
Hyperlipidaemia	41	6.28	18	5.83	23	6.69	0.65	1.45 (0.66, 3.21)
Stroke[#]	6	0.92	4	1.29	2	0.58	0.43	0.77 (0.15, 4.13)
Coronary artery disease	48	7.35	23	7.44	25	7.27	0.93	1.23 (0.61, 2.47)
Cirrhosis	107	16.4	47	15.2	60	17.4	0.44	1.10 (0.68, 1.76)
Renal disease[#]	10	1.53	4	1.29	6	1.74	0.76	0.81 (0.24, 2.73)
TCM visits, (mean, SD)					5.58	6.73		
Total medical expenditures (NT$) (mean, SD)	45118	164779	47395	217714	43071	95086	0.74	
Duration between delivery date and diagnosis date, years (mean, SD)	0.44 ± 0.30		0.45 ± 0.30		0.43 ± 0.30		0.71	
Duration between diagnosis date and index date, years (mean, SD)					0.31 ± 0.29			

TCM, traditional Chinese medicine; OR, odds ratio; SD, standard deviation; CI, confidence interval. [&]t test; [#]Fisher's exact test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. [†]The urbanization level was categorized by the population density of the residential area into 5 levels, with level 1 as the most urbanized and level 5 as the least urbanized. [‡]Adjusted ORs were from the model considering age, urbanization, TCM visit one year ago, insured amount, residential area, insured unit, season, and baseline comorbidity.

Table 2: Types of traditional Chinese medicine treatment received in patients with postpartum depression.

Total TCM users	Simple Chinese herbal medicine treatment	Simple acupuncture or manipulative treatment	Combined with both treatment
n (%)	*n* (%)	*n* (%)	*n* (%)
344 (100)	246 (71.5)	8 (2.3)	90 (26.2)

TCM, traditional Chinese medicine.

Table 3: Top 5 coexisting disease codes among patients after diagnosed postpartum depression for all outpatients visits.

	Non-TCM users			TCM users		
Ranking	Disease (code)	*n*	%	Disease (code)	*n*	%
1	Acute upper respiratory infections of unspecified site (465.9)	15	4.89	Acute upper respiratory infections of unspecified site (465.9)	20	5.83
2	Acute bronchitis (466.0)	10	3.26	Acute nasopharyngitis (common cold) (460)	13	3.79
3	Acute tonsillitis (463)	8	2.61	Acute tonsillitis (463)	12	3.5
4	Other and unspecified noninfectious gastroenteritis and colitis (558.9)	8	2.61	Dental caries (521.0)	11	3.21
5	Acute nasopharyngitis (common cold) (460)	7	2.28	Acute pharyngitis (462)	7	2.04

TCM: traditional Chinese medicine.

TCM doctors in Taiwan, which guarantee the most suitable treatment for patients, as well as accurate and valuable research results.

We reported that the incidence of PPD was approximately 1% retrieved from the NHIRD, which was lower compared with the previous studies [18, 19]. According to a previous study involving NHI data, around 25.5% and 26.8% of people sought out TCM treatment at least once in South Korea and Taiwan, respectively [20]. In the TCM user population, women have a higher proportion of use (adjusted OR 1.47–1.62) than men [21]. This could potentially be attributed to the consideration of the adverse effects of current WM therapy for PPD and the worry about the potential negative impact of chemicals affecting their baby through breastfeeding, leading some women to seek alternative therapy or natural therapy for treatment. The present findings revealed that more than fifty percent of patients with PPD had the desire to consult TCM services to help treat the disease in addition to WM. The proportion of TCM use for this disease is higher than the average use of TCM, which is why TCM use in PPD is noteworthy for TCM physicians and affects health resource allocation. In addition, the prevalence of TCM outpatient visits may be underestimated, because some patients visiting TCM doctors preferred to look for decoction formulations of Chinese medicine, which were self-paid and not covered by the NHI program (in Taiwan, the NHI program only supplies medicine in scientifically concentrated powder or granule form, which is more convenient than traditional decoction formulations of CHM).

Our findings revealed the largest proportion of TCM use was simple CHM therapy, followed by combination therapy, including CHM, acupuncture, and manipulative therapies. In the Chinese society, postpartum women undergo postpartum conditioning (also called "doing the month") with CHM therapy and follow the traditional Chinese diet and culture [22, 23]. For example, *Sheng-Hua-Tang* (a well-known Chinese formula) has been widely applied to resolve blood stasis, promote blood flow, and eliminate lochia, when used within the first week of the postpartum period). *Si-Wu-Tang*, also a widely known Chinese formula, is widely used to increase blood supply and regulate menstruation after delivery. Both have been proven to improve women's physical and mental health conditions after child birth [22, 24, 25].

Previous studies also showed that acupressure, acupuncture, and moxibustion therapy can be used to improve postpartum mental health, including anxiety, depression, and sleep quality [26–28]. Nevertheless, our present findings showed that only 2.3% patients with PPD received simple acupuncture therapy. This phenomenon merits further exploration.

Empirical TCM theories emphasized the relationship and interactions between the human body and the environment [29]. Interestingly, climate variations might lead to the incidence of different disease tendencies and different behaviors of TCM usage [30, 31]. Hence, we were curious about the association between seasonal variations of delivery times of PPD patients and how it is related to TCM use. A minority of enrolled PPD women in the present study had a winter delivery in both the TCM user group (21.7%) and the non-TCM group (19.8%). Although the present study did not reflect the real incidence rate of PPD development in different seasons, an earlier study revealed that the risk of PPD development for winter delivery was higher than for deliveries in other seasons [32]. Other studies, however, showed that there is still equivocal evidence in the relationship between the season of delivery and PPD [33–36]. An earlier study also showed that the OR of overall TCM use was highest in winter, in 1997–2013 in Taiwan [37]. However, the season in which delivery was done did not affect TCM use frequency, although patients with PPD that had an autumn delivery seemed potentially higher than those that had a delivery in other seasons (adjusted OR 1.47, 95% CI 0.92–2.36 compared with spring). The reason for this still merits further exploration. In addition, because Taiwan is a narrow subtropical island with limited differences in

latitude, such that the climate temperature or day-night variations are not distinct enough during the four seasons, different patterns of CHM of PPD with seasonal variations is still interesting, and further investigation is warranted.

Compared with the residents in Taipei city, residents in central (adjusted OR: 2.05, 95% CI: 1.09–3.86) and southern (adjusted OR: 3.17, 95% CI: 1.58–6.33) Taiwan had a higher TCM use rate. The most historical and famous TCM doctor's education school, China Medical University, was in Taichung in central Taiwan. It offers intact baccalaureate TCM or postbaccalaureate TCM education programs, which delivers most of the qualified TCM doctors in Taiwan [38]. In addition, central Taiwan has the highest density of TCM doctors. The aforementioned university is probably why a particularly higher prevalence of TCM use was observed in central Taiwan. Furthermore, the reason for the higher prevalence of TCM use observed in southern Taiwan might be because southern Taiwan has a greater amount of Chinese herbal medicine pharmacies that provide convenient, traditional, and experienced prescriptions. Culture could be a factor driving residents to establish habits of taking Chinese herbal medicines or visiting TCM clinics.

Beyond expectations, our study shows that combination therapy using TCM and WM for patients with PPD not only caused extra financial burden, it even slightly lowered the burden compared to using simple WM. Even though we could not address direct therapeutic efficacy through the NHIRD files, we assumed the reason that patients use TCM could be to reduce WM clinic visits and western medication use, indirectly proving that TCM is empirically considered a "simple, convenient, cheap, and effective therapy" compared with WM in treating diseases.

However, there are still some limitations in this study. First, the NHIRD data did not contain a symptomatic or biochemical approach for the PPD cohorts. Hence, further studies are required to explain the substantial efficacy of TCM. Second, the database does not contain information on daily activity, dietary habits, and lifestyle, which may also be factors for TCM use and health care costs. Further studies considering the above information are warranted. Third, misclassification bias could be a concern in this study as TCM user classification was done only on the basis of reimbursement by the Taiwanese NHI program. The program only reimburses granular or powder forms of CHM; however, some patients purchase decoction formulations of Chinese medicine directly in TCM pharmacies, which are self-paid and not covered by the NHI program. Therefore, it may be possible that some TCM users were misclassified as non-TCM users and the prevalence of TCM use could be underestimated in the present study. Lastly, we only explored general TCM use, which showed that CHM is the most widely accepted type of TCM. In regard to the PPD and TCM core prescriptions, we are currently analyzing CHM patterns, using animal experiments, and performing clinical studies to discover the regulatory effects and mechanisms.

5. Conclusion

The present study is the first report to overview TCM use and prevalence among patients with PPD that underwent treatment with WM in Taiwan. Our study showed that TCM as a complementary therapy combined with WM was accepted by more than half of the patients with PPD, and it potentially could lead to a reduced medical expenditure in treating the disease. The study also discussed the associated sociodemographic and medical factors regarding TCM use of PPD patients. The results of our study may be helpful to clinical practitioners as well as health-policy decision-makers while considering the integration of TCM with WM in patients with PPD.

Abbreviations

CI:	Confidential interval
ICD-9-CM:	International classification of diseases, ninth revision
LHID 2000:	Longitudinal Health Insurance Database 2000
NHIRD:	National Health Insurance Research Database
OR:	Odds ratio
PPD:	Postpartum depression
TCM:	Traditional Chinese medicine
WM:	Conventional western medicine.

Data Availability

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

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

J-M Li interpreted the data and wrote the manuscript, C-L Lin helped to analyze data from the NHIRD in Taiwan, and K-R Liao and C-C Liao designed the protocol and revised the manuscript. All authors reviewed the manuscript and agreed to submission.

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Review Article

Effect of Acupuncture on Chronic Pain with Depression: A Systematic Review

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Background. Numerous studies suggested that chronic pain and depression were closely related and widespread in the population. When patients have symptoms of chronic pain and depression, the corresponding treatment will become difficult. Acupuncture, a unique therapeutic method of traditional Chinese medicine, has been reported to potentially serve as an alternative treatment for patients with comorbid chronic pain and depression by many research studies. *Methods*. A comprehensive search was conducted through the online database, including the Cochrane Library, PubMed, EMBASE, SinoMed, CNKI, and Wanfang database. Trials were RCTs published in the English or Chinese language, recruiting participants with chronic pain and depression comorbidity. The primary outcomes were the Visual Analogue Scale (VAS) and Hamilton Depression Scale (HAMD). Statistical analyses were conducted using Review Manager 5.3. Each trail was quality appraised with the five-point Jadad Score. *Results*. 7 eligible RCTs involving 535 patients were included. Better therapeutic effect and safety could be observed in the experimental group compared with the control group. There was a significant decrease in the VAS (mean difference (MD) = −0.68 (−1.24, −0.12), $P = 0.02$) and HAMD (MD = −2.18 (−3.09, −1.26), $P < 0.00001$) scores and the incidence of adverse events between two groups. *Conclusion*. In the treatment of chronic pain with depression, acupuncture could not only get better clinical efficacy, but also have higher security compared with medicine therapy, which can be used in patients with poorer response to the conventional medication or suffering from serious side effects.

1. Introduction

According to the definition of chronic or persistent pain given by the International Association for the Study of Pain, if the pain lasts longer than 3 months or beyond the time period when an acute insult would have been expected to heal, it becomes a chronic condition [1]. Chronic pain is considered one of the most prevalent physical conditions in developed countries, affecting approximately 1 in 10 adults [2]. Since pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage," depression and pain always co-occur [3]. Additionally, the physiological and emotional burden of chronic pain and a lack of efficient treatments might act as barriers to recovery and contribute to the development of persistent pain and major depression [4].

Depression is prevalent around the world, affecting more than 350 million people worldwide [5]. A recent up-to-date article noted that depression is the most common psychiatric disorder in the general population and the most common mental health condition in patients seen in primary care [6]. It is also a leading cause of disability and can cause high levels of distress and increased risk of suicide [7]. The World Health Organization (WHO) projected that depression will rank the largest burden of disease worldwide by 2030 [8]. In

practice, for the diagnosis of depression, the two main classificatory diagnostic systems, the Diagnostic and Statistical Manual of Mental Disorders and the International Classification of Diseases, rely on the identification of some key symptoms [9]. Therefore, depression is a disorder with symptoms forming a syndrome and causing functional impairment, which can lead to considerable loss of productivity and quality of life.

As two of the most widespread disorders, studies suggested that pain and depression were closely related [10]. The combination of these two disorders can exacerbate the experience of one's health state, interfere with people normal functioning, and decrease the quality of life seriously [11]. A substantial proportion (30% to 45%) of patients with chronic pain present with frank symptoms of depression [12], and also 52% to 65% of patients with depression suffer from chronic pain [13]. Mental distress contributes to more serious pain, at the same time to greater pain-related disability and poorer response to the pharmacological treatment [5, 14]. In other words, chronic pain and depression create a vicious cycle in which pain worsens symptoms of depression, and then the resulting depression worsens feelings of pain. Studies have shown that depression and chronic pain share some of the same neurotransmitters and nerve pathways [15]. However, the causal association between depression and chronic pain is yet unclear.

In view of the biopsychosocial aspects involved in chronic pain, a multimodal approach to management is essential [16]. This usually involves pharmacological or nonpharmacological therapy or both. Many guidelines [17, 18] recommended acetaminophen as the first-line agent for chronic pain due to its safety and tolerance. However, it is not an ideal choice for chronic inflammatory pain (such as rheumatoid arthritis and osteoarthritis) compared with nonsteroidal anti-inflammatory drugs (NSAIDs) due to the lack of anti-inflammatory activity [19]. COX2-selective and nonselective NSAIDs are particularly helpful in treating an inflammatory type of pain, which display both analgesic and anti-inflammatory properties [20]. However, the side effects also need to be vigilant, especially the gastrointestinal and cardiovascular events [21, 22]. Generally, from a pharmacological perspective, opioids are considered as the most powerful painkillers [23]. In addition, these medications have been increasingly used for the treatment of chronic nonmalignant pain which failed response to other medications [24]. However, previous reports also have indicated the prescription opioid misuse [2, 25] and death [26] in the comorbidities of the joint drug problem. Other medications, including anticonvulsants, serotonin-norepinephrine reuptake inhibitors (SNRIs), antidepressants, benzodiazepines, muscle relaxants, and topical lidocaine, all have their own indications and adverse reactions [27]. Thus, the points on the management of chronic pain have shifted to a multimodal and multidisciplinary therapy, and it also includes psychological (mindfulness meditation and cognitive behavioral therapy), rehabilitative, interventional, and complementary/alternative therapies [27–30].

Conventional treatment of depression mainly includes medication, psychotherapy, and physiotherapy. Taking antidepressants as the most preferred treatment of this disease, only a third of patients with depression respond fully to antidepressant medication [31]. Long-term side effects and drug dependence make patients less compliant with them. Although evidence-based studies confirmed that cognitive behavioral therapy (CBT) was effective [32], its effect was a gradual and cumulative process, which was slower than that of drugs. Therefore, patients are often more willing to accept drug treatment than to tolerate the gradual process in the early stage. Physical therapy includes electroconvulsive therapy (ECT), vagal nerve stimulation (VNS), and transcranial magnetic stimulation (TMS). Although the safety and efficacy of physical therapy have been verified by some studies [33–35], the effectiveness varies from person to person [36]. Therefore, for some special types of depression, such as chronic, refractory, severe, and adolescent, the combined treatment model has become a new trend of depression treatment [31, 34]. In brief, medication, psychotherapy, and physiotherapy have been shown to be effective, but the actual clinical effect is unsatisfactory.

In terms of management for chronic pain and depression comorbidity, there was a significant overlap in the pharmacological treatment [28, 37]. However, these medicine cotreatments may induce new clinical issues due to drug-to-drug interactions or drug-related adverse events. For instance, application of opioids can relieve chronic pain effectively, but it was agreed that it can also cause severe dependence and addiction in patients [38], and long-term use of opioids has been confirmed to increase the risk of depression [39]. Benzodiazepines, a kind of antidepressants, do not have analgesic effect; however, up to one-third of patients taking opioids for chronic pain have reported taking benzodiazepines simultaneously, which may increase the risk of sedation and respiratory depression [40]. Therefore, it is necessary to find another treatment method with good therapeutic effect and little side effects.

Acupuncture, a unique therapeutic method of traditional Chinese medicine with a history of thousands of years, has become a widely recognized alternative and complementary therapy in clinical practice [41]. Acupuncture uses needles to stimulate specified acupuncture points, which has the advantages of simple operation, economical cost and few side effects, and it has obvious curative effect on pain and depression separately. Many clinical research studies have verified that acupuncture is an effective treatment for patients with cancer pain, migraine, and low back pain [42–44]. There are also many randomized controlled trials having confirmed that acupuncture can alleviate depression and improve patients' quality of life [45, 46]. As the optimized comorbid chronic pain and depression management is to allow progress in restoring function while reducing long-term reliance on medical therapy [47], acupuncture may potentially serve as an alternative treatment for patients with comorbid chronic pain and depression.

To date, there have been several systematic reviews of acupuncture in the treatment for chronic pain or for depression [48, 49]. However, the effect of acupuncture as a treatment for chronic pain and depression comorbidity is questionable, and there is no systematic review of the use of

acupuncture for comorbid chronic pain and depression, so the experts may confront problems to conduct further research in the related field. Therefore, a comprehensive systematic review of acupuncture in the treatment for chronic pain with depression is needed.

2. Methods

2.1. Search Strategy. A comprehensive search for studies about the effectiveness of acupuncture for chronic pain with depression was conducted through the online database. The following electronic databases were searched from inception to March 17, 2020: the Cochrane Central Register of Controlled Trials, PubMed, EMBASE, Chinese Biomedical Database (SinoMed), China National Knowledge Infrastructure (CNKI), and Wanfang database. The following terms were used: "chronic pain" OR "pain, chronic" AND "depression" OR "depressive disease" OR "depressive disorder" AND "acupuncture therapy" AND "random." During searching Chinese databases, the similar search strategy with Chinese terms was adopted. The initial database search was done by 3 authors (Yan, Zhu, and Wang) independently to ensure reproducibility.

2.2. Inclusion and Exclusion Criteria

2.2.1. Type of Study. Trials were eligible if they were randomized controlled trials (RCTs) recruiting participants with chronic pain and depression comorbidity, regardless of whether there was single blind, double blind, or nonblind.

2.2.2. Type of Participant. Patients diagnosed with chronic pain combined with depression or depression combined with chronic pain will be included. The main concern of studies cited must be chronic pain and depression, that is to say, the author must explain the definition or diagnostic criteria for chronic pain and depression. There will be no limits on the age, sex, and source of cases.

2.2.3. Intervention. Patients in the experimental group were treated with acupuncture alone or in combination with other therapy, while those in the control group were subjected to other therapy without acupuncture for chronic pain with depression.

2.2.4. Outcome Measures. The primary outcomes of interest were the pain scores and depression severity, i.e., Visual Analog Scale (VAS): higher scores indicate more severe pain, and Hamilton Depression Scale (HAMD): higher scores indicate a greater degree of depression, and to observe the changes of indicators before and after intervention. The secondary outcomes included any adverse events, i.e., Treatment Emergent Symptom Scale (TESS) or Rating Scale for Side Effects (SERS).

2.2.5. Exclusion Criteria. In order to evaluate the independent effects of acupuncture, the following trials were excluded: (1) conference abstracts, review articles, animal studies, cadaveric studies, in vitro studies, or articles published in a form other than clinical trials; (2) any control group that included acupuncture therapies; (3) literatures without relevant indicators or quantitative data; (4) evaluation indicators include only chronic pain or only depression; (5) repeated published literature.

2.3. Selection of Studies. 4 authors (Yan, Zhu, Wang, and Da) independently screened all potential eligible studies. Titles and abstracts were first screened to exclude irrelevant papers. Full text of all articles of potentially relevant abstracts were retrieved and screened according to the study inclusion and exclusion criteria. Final article selection was done independently by all four reviewers, and disagreements were resolved by consensus.

2.4. Quality Assessment. 4 authors (Yan, Zhu, Wang, and Da) independently conducted the methodological quality of all included studies. Each article was quality appraised with the five-point Jadad Score [50]. Three factors associated with risk of bias were evaluated: randomization, blinding, and follow-up. The specific scoring criteria are as follows: when the study provides a detailed description of randomization, such as using a random number table, 2 points are obtained; if only a random method is used and there is no exact description, 1 point is obtained; if there is no random allocation, no score. 2 points were scored when the study used the appropriate placebo or a similar method; 1 point was scored when the trial involved blinding and no description; no blinding means no score. When describing the follow-up and the reasons for loss of follow-up, score 1 point, otherwise no score. If the total score is greater than or equal to three, the paper is considered to be of high quality.

2.5. Data Extraction. Data were extracted into a prespecified data extraction table, with items including the authors' names, the year of publication, total sample size, age, gender, detailed intervention information of two groups, outcome measures, and adverse reactions.

2.6. Statistical Methods. Review Manager 5.3, provided freely by the Cochrane cooperation net, was applied for statistical analysis. The primary outcomes, VAS and HAMD, were both continuous variables, and mean difference (MD) was used as effect values. The confidence internal was set as $\alpha = 0.05$.

3. Results

3.1. Results of the Search. A total of 521 references were retrieved after removing duplicates (Figure 1). 3 authors (Yan, Zhu, and Wang) independently screened these references. Based on the review of the title and abstract, 89 full-text papers were reviewed and 7 eligible RCTs [51–57] involving 535 patients were included. All 7 RCTs were conducted in China and were published between 2000

and 2018. There were a total of 265 participants receiving acupuncture alone or in combination with other therapy (experimental group) and 270 receiving other therapy without acupuncture (control group). Table 1 shows the distributions of sex, age, and time since diagnosis between the experimental and control groups, and the detailed intervention information is shown in Tables 2 and 3.

3.2. Comparison of the Pain-Related Score. A total of 6 studies assessed pain [51–56], and 5 of them used the VAS score [51, 53–56], while the other one used the comprehensive headache score [52]. The evaluation time point of each study was different, and most studies chose pretreatment and 4 weeks after treatment as time points for pain assessment (Table 4). There was no significant difference in VAS between the two groups before therapy. After 4 weeks of treatment, VAS decreased significantly in both groups, and the experimental group was more significant than the control group (MD = −0.68 (−1.24, −0.12), $P = 0.02$, $I^2 = 85\%$) (Figure 2). However, there was a high degree of heterogeneity in the study ($I^2 = 85\%$). Sensitivity analysis reduced heterogeneity ($I^2 = 20\%$) (Figure 3) after deleting a study whose data were apparently different from the others.

3.3. Comparison of the Depression-Related Score. In the eligible RCTs, all trials conducted the depression-related assessments, and HAMD was used in 6 of them [51, 53–57]. Similar to the VAS, most studies included the time point of pretreatment and 4 weeks after treatment (Table 5). There was no significant difference in HAMD between the two groups before therapy. After 4 weeks of treatment, HAMD decreased significantly in both groups, especially in the experimental group, which indicates that there was a significant difference between the two groups (MD = −2.18 (−3.09, −1.26), $P < 0.00001$, $I^2 = 52\%$) (Figure 4). Similarly, because of the high heterogeneity, after removing a research, the heterogeneity was significantly declined (Figure 5).

3.4. Security Assessment. All studies recorded adverse events during treatment in both groups. Incidence of adverse events was recorded in 5 studies [51–54, 56], and other security assessments included TESS [53, 57] and SERS [55]. 2 studies reported no difference in the incidence of adverse events between the two groups [53, 54], and other studies have shown that acupuncture therapy could significantly reduce the incidence of adverse events and the adverse event-related score (Table 6).

3.5. Quality Assessment. The quality assessment of the trials was performed using the five-point Jadad Score (Table 7). Randomization was mentioned in all included studies, but only one had a detailed description of it [51]. Blind method cannot be used in research due to the characteristic of acupuncture. Besides, only two trails describe the details of follow-up [51, 52].

4. Discussion

This study is a comprehensive systematic review of acupuncture in the treatment for chronic pain with depression. There were significant differences in the VAS score and HAMD, decreasing between the two groups after treatment, indicating that acupuncture used alone or in combination with medication therapy can relieve pain and depression better than medication. Patients in the experimental group had lower incidence of adverse events and side effect scores, which proved the safety of acupuncture. The above results suggested that acupuncture has not only a better clinical result on the treatment of chronic pain with depression but also has a higher security compared with medication therapy.

When it comes to acupuncture therapy, we found that different individual studies might utilize different acupoints, but from these prescriptions we could also find something in common. In practice, there are many ways to select acupoints during acupuncture treatment. Selection of local acupoints around the pain location was the main method to treat pain. In the treatment of depression, acupoints on the head were selected more frequently, such as Governor Vessel (GV) 20, GV 24, Extra-points of Head and Neck (EX-HN) 1, and EX-HN 3. In addition, some distal acupoints were also be selected, including Pericardium meridian of hand-jueyin (PC) 6, Heart meridian of hand-shaoyin (HT) 7, Spleen meridian of foot-taiyin (SP) 6, and Liver meridian of foot-queyin (LR) 3. According to traditional Chinese medicine theory, human emotional activities are closely related to the five internal organs, so this explains why acupoints on multiple meridians can be selected for the treatment of depression.

The treatment of chronic pain and depression comorbidity has always been a tough problem because of poorer response to the pharmacological treatment and long-term side effects of drugs. Acupuncture, as an effective, simple, and economical treatment, has been used in the treatment of many diseases in China. The results of the systematic review suggested that acupuncture has a promising application prospect due to its unique advantages for the treatment of chronic pain with depression, and it can be used in patients with poorer response to the medication or suffering from serious side effects.

Previous systematic reviews of acupuncture for chronic pain and depression comorbidity are not available. However, findings from other chronic pain reviews and depressive disorder reviews are consistent with this review and indicate that acupuncture used alone or in combination with other treatment measures has a better therapeutic effect to the control group [49, 58–61].

There are many patients suffering from chronic pain with depression at present, and the findings of this systematic review provided a valuable alternative to these patients. However, most of the included articles had a quality score of no more than 2 points [52–57], and only 1 article got 3 points [51], which meant that the quality of the majorities was low. Considering the characteristics of acupuncture therapy itself, it is difficult to implement the blind method.

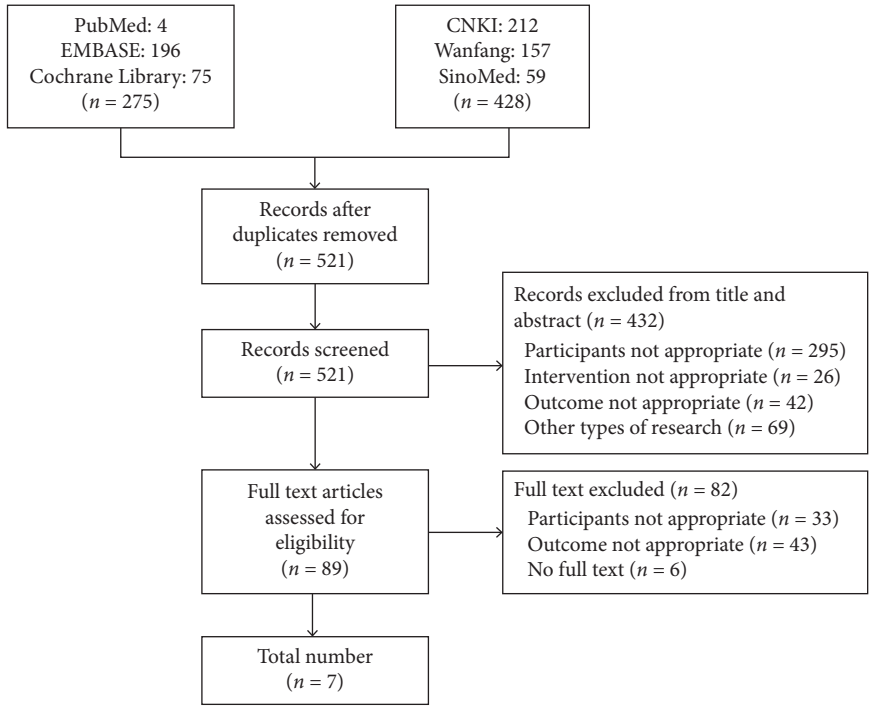

Figure 1: Study flow diagram.

Table 1: Basic information of 7 RCT studies.

No.	Author	Year	Sample (EG/CG)	Age (SD)		Gender (EG/CG)	Time since diagnosis (SD) (EG/CG)
				EG	CG		
1. [51]	Wang	2012	60 (30/30)	51.5 (4.1)	50.3 (4.7)	—	—
2. [52]	Xiao	2015	48 (24/24)	53.17 (9.89)	50.58 (8.80)	8/16 (M/F)/10/14 (M/F)	—
3. [53]	Ma	2015	12 8 (64/64)	39.93 (12.93)	38.69 (14.19)	27/37 (M/F)/29/35 (M/F)	20.33 (12.76) m/20.00 (12.12) m
4. [54]	Luo	2018	84 (42/42)	57.15 (11.26)	57.39 (11.58)	22/20 (M/F)/23/19 (M/F)	42.31 (8.75) m/41.92 (8.32) m
5. [55]	Liu	2013	90 (45/45)	47 (8)	48 (8)	15/30 (M/F)/16/29 (M/F)	3.5 (1.8) m/3.2 (1.7) m
6. [56]	Cao	2008	60 (30/30)	20–70		23/37 (M/F)	0.5–30 y
7. [57]	Huang	2000	65 (30/35)	30.39 (7.01)		25/40 (M/F)	—

EG, experimental group; CG, control group; —, not available; m, month; y, year.

Table 2: Detailed intervention information 1.

No.	Study type	Diagnosis	EG	CG	Duration (wks)
1	RCT	Depression with chronic pain	Abdominal acupuncture	Deanxit	4
2	RCT	Migraine with depression	Acupuncture	Deanxit combined rizatriptan benzoate tablets	4
3	RCT	Depression with chronic pain	Acupuncture combined duloxetine	Duloxetine	8
4	RCT	Recurrent chronic trigeminal neuralgia accompanied by depression	Acupuncture combined traditional Chinese medicine	Traditional Chinese medicine	4
5	RCT	Depression with chronic pain	Acupuncture combined SSRI antidepressants	SSRI antidepressants	4
6	RCT	Chronic pain with depression	Acupuncture	Deanxit	4
7	RCT	Depression with chronic pain	Acupuncture	Amitriptyline	6

EG, experimental group; CG, control group.

Table 3: Detailed intervention information 2.

No.	EG			CG		
	Intervention	Dose	Frequency	Intervention	Dose	Frequency
1	Acupuncture	—	Once a day for 3 days, then performed every 3 days	Deanxit	Flupirtine, 0.5 mg/ meritoxin 10 mg	Once a day
2	Acupuncture	—	Once a day for, 5 times a week	Deanxit	Flupirtine, 0.5 mg/ meritoxin 10 mg	Once a day
				Rizatriptan benzoate tablets	1 tablet	If necessary
3	Acupuncture	—	5 times a week	Duloxetine	60 mg/d	Once a day
	Duloxetine	60 mg/ d	Once a day			
4	Acupuncture	—	Once a day	TCM	—	Once dose a day
	TCM	—	Once dose a day			
5	Acupuncture	—	Once every 2 days	SSRI antidepressants	—	Once a day for 1 week, then adjust the dosage
	SSRI antidepressants	—	Once a day for 1 week, then adjust the dosage.			
6	Acupuncture	—	5 times a week	Deanxit	Flupirtine, 0.5 mg/ meritoxin 10 mg	Twice a day for 10 days, then once a day
7	Acupuncture	—	6 times a week	Amitriptyline	25–150 mg	Once a day

EG, experimental group; CG, control group; TCM, traditional Chinese medicine; —, not available.

Table 4: VAS scores in each study.

Author	Year	EG		CG	
		Before therapy mean (SD)	4 w mean (SD)	Before therapy mean (SD)	4 w mean (SD)
Wang	2012	7.0 (1.8)	2.9 (1.0)	6.8 (1.5)	3.2 (1.0)
Ma	2015	7.33 (1.22)	4.81 (1.25)	7.28 (1.19)	5.27 (1.32)
Luo	2018	7.92 (1.16)	2.29 (1.01)	7.86 (1.22)	3.12 (1.25)
Liu	2013	3.68 (1.15)	0.93 (0.78)	3.70 (1.12)	2.53 (1.09)
Cao	2008	7.50 (1.12)	2.34 (1.43)	7.48 (1.27)	2.40 (1.45)

EG, experimental group; CG, control group.

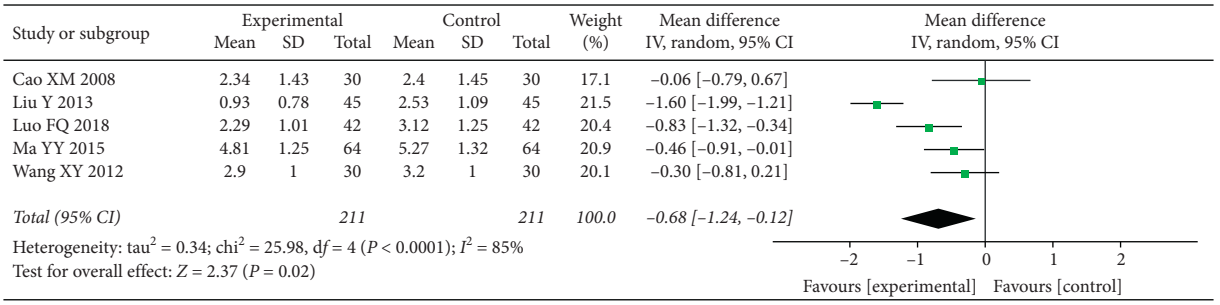

Figure 2: Forest plot depicting the VAS.

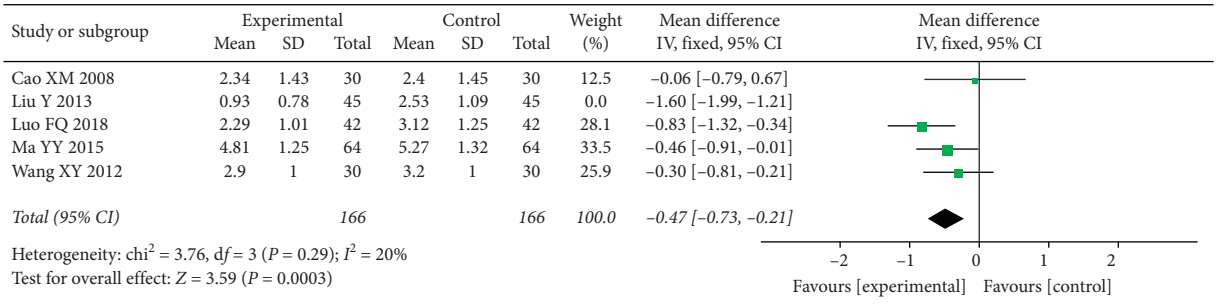

Figure 3: Forest plot depicting the VAS after sensitivity analysis.

Table 5: HAMD scores in each study.

Author	Year	EG		CG	
		Before therapy mean (SD)	4 w mean (SD)	Before therapy mean (SD)	4 w mean (SD)
Wang	2012	18.5 (3.8)	9.3 (3.9)	19.4 (3.4)	10.9 (4.9)
Ma	2015	23.88 (1.86)	16.20 (2.40)	23.91 (1.56)	17.60 (2.29)
Luo	2018	11.09 (2.79)	4.32 (1.41)	11.23 (2.70)	7.06 (2.59)
Liu	2013	24.08 (4.96)	10.84 (3.86)	25.13 (4.96)	14.33 (4.12)
Cao	2008	25.87 (7.76)	12.07 (6.92)	26.43 (9.00)	12.90 (6.01)
Huang	2000	26.71 (5.13)	—	26.87 (4.25)	—

EG, experimental group; CG, control group; —, not available.

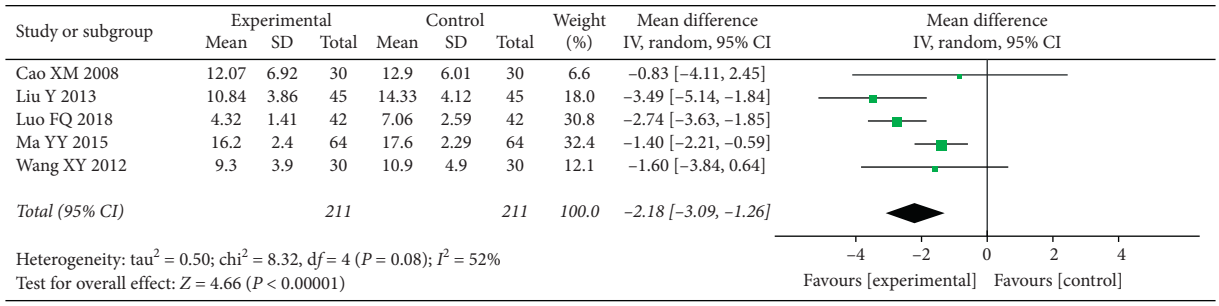

Figure 4: Forest plot depicting the HAMD.

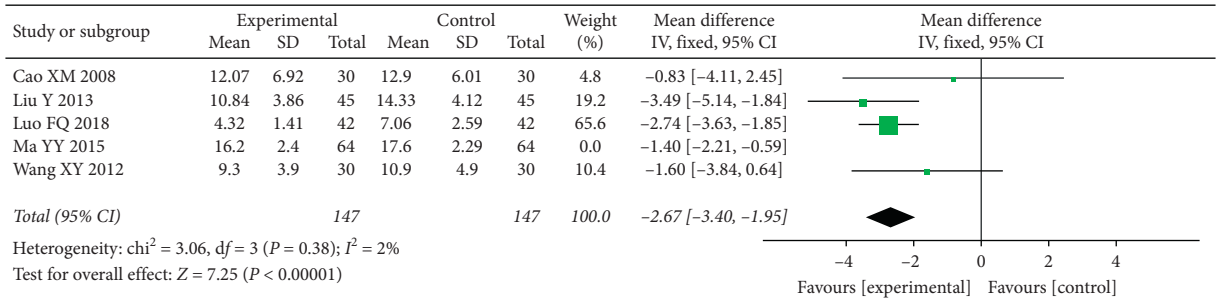

Figure 5: Forest plot depicting the HAMD after sensitivity analysis.

Table 6: Side effect scores in each study.

No.	Adverse events		TESS score		SERS score	
	EG (%)	CG (%)	EG mean (SD)	CG mean (SD)	EG mean (SD)	CG mean (SD)
1	3 (10%)	15 (50%)	—	—	—	—
2	0 (0%)	4 (16.67%)	—	—	—	—
3	23 (35.94%)	28 (43.75%)	3.25 (1.55)	3.77 (1.86)	—	—
4	7 (16.67%)	9 (21.43%)	—	—	—	—
5	—	—	—	—	3.78 (2.67)	6.48 (4.04)
6	0 (0%)	20 (66.67%)	—	—	—	—
7	—	—	0	10.8 (2.88)	—	—

EG, experimental group; CG, control group; —, not available.

The acupuncture points, frequency, and course of treatment selected in different studies were different, so there were difficulties in formulating a standardized treatment. Thus, more high-quality, rigorously designed, and well-controlled RCTs are still needed to support the clinical application of acupuncture for the treatment of chronic pain with depression.

The design of future studies in this area can play an important role in improving the quality of evidence and address the lack of evidence to support acupuncture for the

Table 7: Assessment of studies' quality.

No.	Randomization	Blinding	Follow-up	Score
1	2	0	1	3
2	1	0	1	2
3	1	0	0	1
4	1	0	0	1
5	1	0	0	1
6	1	0	0	1
7	1	0	0	1

management of chronic pain and depression comorbidity. Hence, there are some recommendations to guide future research: (i) improving methodological quality; (ii) extending follow-up periods to include intermediate- and long-term follow-up; (iii) increasing sample sizes. High-quality studies should also include costs, risks, and synergistic values of combining acupuncture with drugs compared with monotherapy otherwise.

5. Conclusion

Acupuncture has a promising application prospect due to its unique advantages for the treatment of chronic pain with depression comorbidity, which can be used in patients suffering from some certain chronic pain with depression comorbidity with poorer response to the conventional medication or suffering from serious side effects. High-quality RCTs are needed to support the current clinical application of acupuncture for the treatment of chronic pain with depression comorbidity and to broaden the clinical application.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors have no conflicts of interest.

Authors' Contributions

Bin Yan, Shibai Zhu, Yu Wang, and Gula Da contributed equally to this work.

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