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Complementary and Alternative Therapy of Rare Inflammatory/Autoimmune Diseases

Lead Guest Editor: Young-Su Yi
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Editorial Board

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Editorial

Complementary and Alternative Therapy of Rare Inflammatory/Autoimmune Diseases

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Inflammation is a series of biological processes to protect our body from harmful stimuli, including pathogens, damaged cells, and a variety of irritants. Although inflammation is the protective response, prolonged inflammation, known as chronic inflammation triggered and sustained by unknown reasons, leads to a progressive recruitment and activation of the inflammatory immune cells present at the inflammatory sites and is characterized by "stuck" in the active sites and simultaneous destruction of the tissues, finally resulting in inflammatory/autoimmune diseases.

Inflammatory/autoimmune diseases have been intensively studied for a long time, and recently, some inflammatory/autoimmune diseases which are very common in worldwide, including rheumatoid arthritis and psoriasis, have achieved an extraordinary progress in scientific understanding as well as drug development for a therapeutic purpose. However, a large subset of inflammatory/autoimmune diseases that are less common but more serious have not been focused on and poorly explored. These are categorized as "rare inflammatory/autoimmune diseases," indicating that they affect small number of patients. There are over 150 rare inflammatory/autoimmune diseases, many of which are incurable and terminal.

In this special issue, we invited investigators to contribute original research articles and review articles that will help understand the basic mechanisms as well as the development of new and promising complementary and alternative strategies to diagnose and treat the rare inflammatory/autoimmune diseases. Seven studies were published regarding the complementary and alternative therapy of rare inflammatory/autoimmune diseases.

The research article by Q. Xu et al. demonstrated the therapeutic effects and mechanisms of Qi-Wu Rheumatism Granule (QWRG), a Chinese herbal compound on adjuvant-induced RA in rats, and suggested that the antiarthritic properties of QWRG may be due to immunodepression and downregulation of inflammatory cytokines, which may be a potential candidate for the treatment of rheumatoid arthritis.

The research article by X. Deng et al. demonstrated that the effect and possible mechanism of icariin, a prenylated flavonol glycoside derived from the Chinese herb, *Epimedium sagittatum*, on the IL-1β pretreated human nucleus pulposus cells and suggested that icariin might be a protective traditional Chinese medicine, which prevent inflammation-induced degeneration of intervertebral discs partly through the PI3K/AKT pathway.

The research article by C. Lv et al. demonstrated the antibacterial function of Liu-He-Dan (LHD), a traditional Chinese medicine used to treat infective wounds by clinical and biomolecular researches, and suggested that LHD could specifically suppress the expression of IL-1β and upregulate the expression of basic fibroblast growth factor (bFGF) in the wounds, promoting the healing of infective wounds by decreasing the release of inflammatory factors from the infective wounds.

The research article by B. Ji et al. demonstrated the effects and underlying mechanism of Jinkui Shenqi pills (JKSQP), a popular formula with kidney warming and yang enhancing effects in a rat model of asthma with kidney-yang deficiency (KYD), and provided a basis for the development of JKSQP as a novel therapeutic agent to treat asthma.

The research article by J. Li et al. demonstrated the pharmacological effect of Sheng-jiang powder (SJP), a classic representative Chinese medicine formula to treat "ascending and descending dysfunction of spleens" that has been considered the primary cause of obesity on the pathogenesis of obesity and obesity-mediated multiorgan injuries in high fat diet-induced obese rats and suggested that SJP ameliorates inflammatory response in tissues of obese rats and mitigates obesity-induced multiple organ injuries.

Finally, the review article by X.-Q. Wang et al. described the Chinese medical theories relating to the pathogenesis of refractory nephrotic syndrome (RNS) and discussed the strategies and treatment options using Chinese herbal medicines. This review provided an insight and great potential of traditional Chinese medicines for the prevention and treatment of RNS.

We hope that readers will be interested in rare inflammatory/autoimmune diseases and that this special issue could attract the interest of scientific community in order to improve further investigations leading to the discovery of novel biomarkers in the field of rare inflammatory/autoimmune diseases.

Young-Su Yi

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Research Article

Jinkui Shenqi Pills Ameliorate Asthma with "Kidney Yang Deficiency" by Enhancing the Function of the Hypothalamic-Pituitary-Adrenal Axis to Regulate T Helper 1/2 Imbalance

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The aim of the study was to investigate the effects and underlying mechanism of JKSQP in a rat model of asthma with kidney-yang deficiency (KYD). *Materials and Methods*. Hydrocortisone (HYD) was used to establish the rat model of KYD; rats were then sensitized and challenged with ovalbumin (OVA). JKSQP was administered to OVA-challenged rats, and the changes in signs and symptoms of KYD were observed. The leukocyte number and subpopulations in bronchoalveolar lavage fluid (BALF) were counted and the cells were stained with Wright–Giemsa dye. Serum adrenocorticotropic hormone (ACTH), corticosterone (CORT), corticotropin-releasing hormone (CRH), total immunoglobulin E (IgE), and OVA-specific IgE levels were determined using relevant enzyme-linked immunosorbent assays (ELISA) kits. *Results*. JKSQP not only reversed the phenomenon of KYD but also significantly inhibited the number of leukocyte and eosinophils in the BALF, increasing the level of interferon (IFN)-γ and decreasing the levels of interleukin-4 (IL-4) and IgE in the serum compared with the OVA-challenged groups. *Conclusions*. Taken together, the antiasthma effects of JKSQP were likely mediated by the enhancement of the function of the hypothalamic-pituitary-adrenal axis and the reversal of T helper 1/2 imbalance.

1. Introduction

Asthma, which has numerous clinical manifestations, is characterized by chronic eosinophilic inflammation [1, 2]. There are 300 million patients with asthma globally, and China has one of the highest asthma-related death rates. Practitioners of traditional Chinese medicine (TCM) believe that recurrent asthma and its inherent features are closely related to kidney-yang deficiency (KYD). Moreover, poor function of the hypothalamic-pituitary-adrenal axis (HPAA) is commonly reported in patients with allergies, and HPAA dysfunction is a key feature of KYD [3]. The pathogenesis of asthma has not yet been clearly elucidated, but the imbalance in T cell-mediated immune regulation and chronic airway inflammation are deemed the most important mechanisms of asthma development [4, 5]. β-2 receptor agonists, glucocorticoids, leukotriene receptor blockers, and combination therapies are currently the main treatments for asthma.

Asthma can be traced back to almost 2000 years ago in ancient China and was first mentioned in Huangdi Neijing (黄帝内经), which also recorded the use of TCM to cure asthma [6]. The Jinkui Shenqi pill (JKSQP, 金匮肾气丸) is an important representative formula for the treatment of asthma dispensed by *Zhang Zhongjing* in the Synopsis of

Prescriptions of the Golden Chamber (also named *Jin Kui Yao Lue*) and has been widely used to treat KYD syndrome [7–9]. Modern pharmacology research has indicated its effects including lowering blood pressure [7], improving sexual dysfunction [10] and lipid profile, and protecting renal function [11], however these were mainly related to the characteristics of KYD syndrome. Although currently there are only few studies on the antiasthmatic effects of JKSQ pill (Jiang et al., 2015), the mechanism underlying the action of JKSQP in treating asthma has not been understood clearly. Therefore, in this experiment, we explored the relationship between asthma and KYD syndrome as well as the mechanism mediating the curative effects of JKSQP on asthma.

2. Material and Methods

2.1. Experimental Animals and Groups. Sixty pathogen-free female Wistar rats (10–12-week-old) were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences [production permit: (Hu) 2007-0005]. The animals were acclimatized for 1 week under constant temperature (22°C), humidity (72%), and a 12-h light/dark cycle. The rats had free access to a standard laboratory diet and were provided water ad libitum. Then, the rats were randomly assigned to six groups ($n = 10$ per group): control, ovalbumin (OVA), OVA + JKSQP, hydrocortisone (HYD), HYD + OVA, and HYD + OVA + JKSQP groups.

2.2. Drug Test and Treatment. JKSQP (金匮肾气丸, batch number 20140112) was produced by Henan Wan-West Pharmaceutical Co., Ltd., (Henan, China). The raw materials consisted of eight herbs: processed aconite (Fuzi, Radix Lateralis Preparata Aconiti Carmichaeli, 9.0 g), Cassia twig (Guizhi, Ramulus Cinnamomi Cassiae, 3.0 g), Rehmannia (Dihuang, Radix Rehmanniae Glutinosae, 24.0 g), Dioscorea root (Shanyao, Dioscoreae Rhizoma, 10.0 g), Cornus fruit (Shanzhuyu, Corni Fructus, 12.0 g), Alisma (Zexie, Rhizoma Alismatis, 9.0 g), Poria (Fuling, Scierotium Poreae Cocos, 9.0 g), and Cortex of the Peony Tree Rote (Danpi, Cortex Radicis Moutan, 9.0 g). The pills were dissolved in water to prepare a solution before the experiment, and the animals were orally given 14 g/kg, which is 10 times the human dose.

The fingerprint of the JKSQP was further analyzed using a high-performance liquid chromatography (HPLC) system. The fingerprints of the mixed standard compounds and JKSQP are shown in Figure 1. The HPLC analyses of JKSQP were performed using the Agilent XDB-ODS column (250 × 4.6 mm, 5-μm diameter). The mobile phase was 0.5% phosphoric acid solution (A) and acetonitrile (B). The following gradient elution mode was used: 0–20 min, 98–89% A; 20–38 min, 89–83% A; 38–43 min, 83% A; 43-44 min, 83–62% A; and 44–62 min, 62% A. The detection wavelengths were 234 nm and 274 nm, the flow rate was 1 mL/min, and the column temperature was 30°C.

2.3. KYD Rat Model. The rat KYD model was established by the administration of 15 mg/kg HYD for subcutaneous injections for 20 consecutive days.

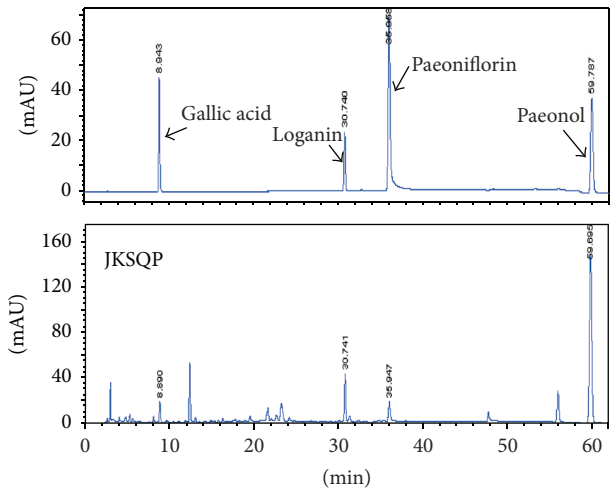

Figure 1: Fingerprints of Jinkui Shenqi pills (JKSQP) detected using high-performance liquid chromatography (HPLC).

2.4. Ovalbumin- (OVA-) Induced Asthmatic Model. Seven days after the last HYD injection, the rats were sensitized and challenged with 200 μg/100 μL OVA (grade V, Sigma-Aldrich, St. Louis, MO, USA) mixed with aluminum hydroxide via subcutaneous injections of 0.1 mL into six different parts: the bilateral groin, notum, and hind vola. In addition, they received intraperitoneal injections of 0.4 mL, and the treatments were repeated after 7 days. Two weeks after the sensitization, the rats were given 10 g/L OVA via inhalation for 30 min over the next 7 days.

2.5. Observation of Signs and Symptoms. During model establishment, the general state of the animals including body weight, anal temperature, food and water intake, urine volume and color, stool condition, hogback, curl-up, and irritability responses was recorded every 4 days. In the late stage of the experiment, all the rats were kept in a quiet, dark environment for 1 min, and then a multifunction event recorder was used to count their autonomic activity for 5 min.

2.6. Measurement of Serum Cytokines. After intraperitoneal injections of sodium pentobarbital, heart blood samples were collected, and the serum was subsequently separated using a refrigerated centrifuge at 4°C and 3000 rpm for 10 min. Levels of adrenocorticotropic hormone (ACTH), corticosterone (CORT), corticotropin-releasing hormone (CRH), total immunoglobulin E (IgE), and OVA-specific IgE were detected using enzyme-linked immunosorbent assay (ELISA) kits.

2.7. Bronchoalveolar Lavage and White Blood Cell Count and Classification. Anesthetized rats had undergone endotracheal intubation and bronchial ligation of the right lung preventing them from entering the lavage and then they were injected with Hanks solution containing heparin 1 mL for 3 times from the trachea intubation to the airway. The rinse was collected in the test tube. White blood cell count and classification count: the perfusate was diluted with 1% acetic

acid and the total number of white blood cells was counted using a microscope. The perfusate was smeared onto the glass slide, stained with Wright–Giemsa dye, then classified, and counted under high power magnification; calculate the ratio and number of white blood cell subgroup.

2.8. Cytokine Detection in Lung Tissue. Lung tissues were weighed, and an equal volume of saline solution was added; the tissues were homogenized and centrifuged at 10000 rpm/min for 10 min. The supernatant was used for relative cytokine detection performed according to the manual of interleukin (IL)-4 and interferon (IFN)-γ ELISA kit (R&D System, Minneapolis, MN).

2.9. Lung Histology. The right lungs were dissected, fixed in 10% paraformaldehyde overnight at 4°C, and followed by embedment in paraffin and then the tissue samples were cut into 4-μm sections. The sections were heated at 60°C for 2 h and then stained after conventional dewaxing with xylene and washing with ethanol followed by water. Then, the sections were stained with hematoxylin and eosin (H&E) for general morphological analysis and examination of cell infiltration. Both stains were subsequently observed using power field microscopy.

2.10. Statistical Analysis. All the data were expressed as means ± standard deviation (SD). Differences between mean values of normally distributed data were assessed using a one-way analysis of variance (ANOVA) using the statistical package for the social sciences (SPSS) 17.0 software. For comparison of two groups, Student's t-test and χ^2 test were used and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Detection of Body Weight, Autonomic Activity, and Body Temperature of Different Groups. First, we determined the differences in body weight among the groups, and as shown in Figure 2(a), the body weight of the HYD group decreased significantly compared with that of the control group ($P < 0.01$); the body weight of OVA-challenged rats with KYD decreased significantly compared with that of the OVA and HYD groups ($P < 0.01$). Second, the autonomic activity duration of the HYD group significantly decreased compared with that of the control group ($P < 0.05$ and $P < 0.01$); the HYD and OVA group also showed a marked decrease in autonomic activity duration compared with the OVA group (Figure 2(b)). Moreover, the anal temperature of the HYD group with or without OVA challenge obviously decreased compared with that of the control group and OVA group, respectively, while JKSQP markedly reversed the decreased body temperature induced by HYD and OVA ($P < 0.01$, see Figure 2(c)).

3.2. Detection of Numbers of BALF Leukocyte and Its Subpopulation among Groups. The results showed that the OVA challenge significantly increased the number of leukocytes, neutrophils, and eosinophils in the BALF of the OVA group compared with that of the control group ($P < 0.01$), as well as in the HYD + OVA group compared with that of the HYD group (Figure 3). Moreover, the number of eosinophils in the BALF of the HYD + OVA group significantly increased compared with that of the only OVA-challenged group (Figure 3(d)). While JKSQP obviously inhibited the increase in leukocyte and eosinophils numbers in the BALF of OVA-challenged rats with HYD, no effects were observed in the normal OVA-challenged rats (Figures 3(a) and 3(d)).

3.3. Evaluation of Serum CORT, ACTH, and ACTH Levels of Rats. ACTH, CORT, and CRH are widely used as dynamic criteria for evaluating KYD in TCM clinics, and their levels in the serum from the HYD group rats significantly decreased compared with those from the control group rats ($P < 0.01$ and $P < 0.05$). Moreover, OVA challenge more obviously decreased the levels of CORT and ACTH in rats with KYD (Figures 4(a) and 4(b)), but no distinct changes occurred in CORT levels of the normal rats. However, JKSQP reversed these symptoms in OVA-challenged rats with HYD (Figures 4(a) and 4(b)).

3.4. Evaluation of T Helper 1 (Th1) and Th2 Cytokines in Rat BALF and Serum. The total IgE levels of the various groups are shown in Figure 5 A, which distinctly revealed that the total IgE content in OVA-induced asthmatic rat serum significantly increased compared with that in the normal group ($P < 0.01$), as well as in the HYD + OVA group compared with that of the HYD group. Furthermore, allergen-specific IgE is believed to be inextricably associated with the induction of allergic airway symptoms and, therefore, is used as a guide for environmental modification and immunotherapy. The results shown in Figure 5(b) indicate that rats without or with KYD sensitized with OVA had a significantly increased IgE level compared with that of the normal and HYD groups, respectively ($P < 0.01$). However, JKSQP inhibited the increase in total IgE and their specific IgE levels in OVA-challenged rats with KYD (Figures 5(a) and 5(b)).

Interferon (IFN)-γ and interleukin (IL)-4, which have been shown to be the crucial cytokines in the serum and involved in the pathogenesis of asthma, are reported in patients with atopy. Compared with that in the normal group, the concentration of IFN-γ in the OVA group significantly decreased following the induction of asthma ($P < 0.01$). In addition, the IL-4 concentration was increased by OVA exposure compared with that in the unexposed normal group ($P < 0.01$), as well as in the HYD + OVA group compared with that in the HYD group. JKSQP reversed these changes by increasing the level of IFN-γ and decreasing that of IL-4 (Figures 5(c) and 5(d)).

3.5. Lung Histopathological Analysis. The characteristic features of asthmatic airways are cell inflammation, the presence of hyperplastic goblet cells, mucus secretion, and collagen deposition [12]. The results in Figure 6 clearly show that the histological sections of lung tissue from the normal group had no detectable inflammatory response in the alveolar,

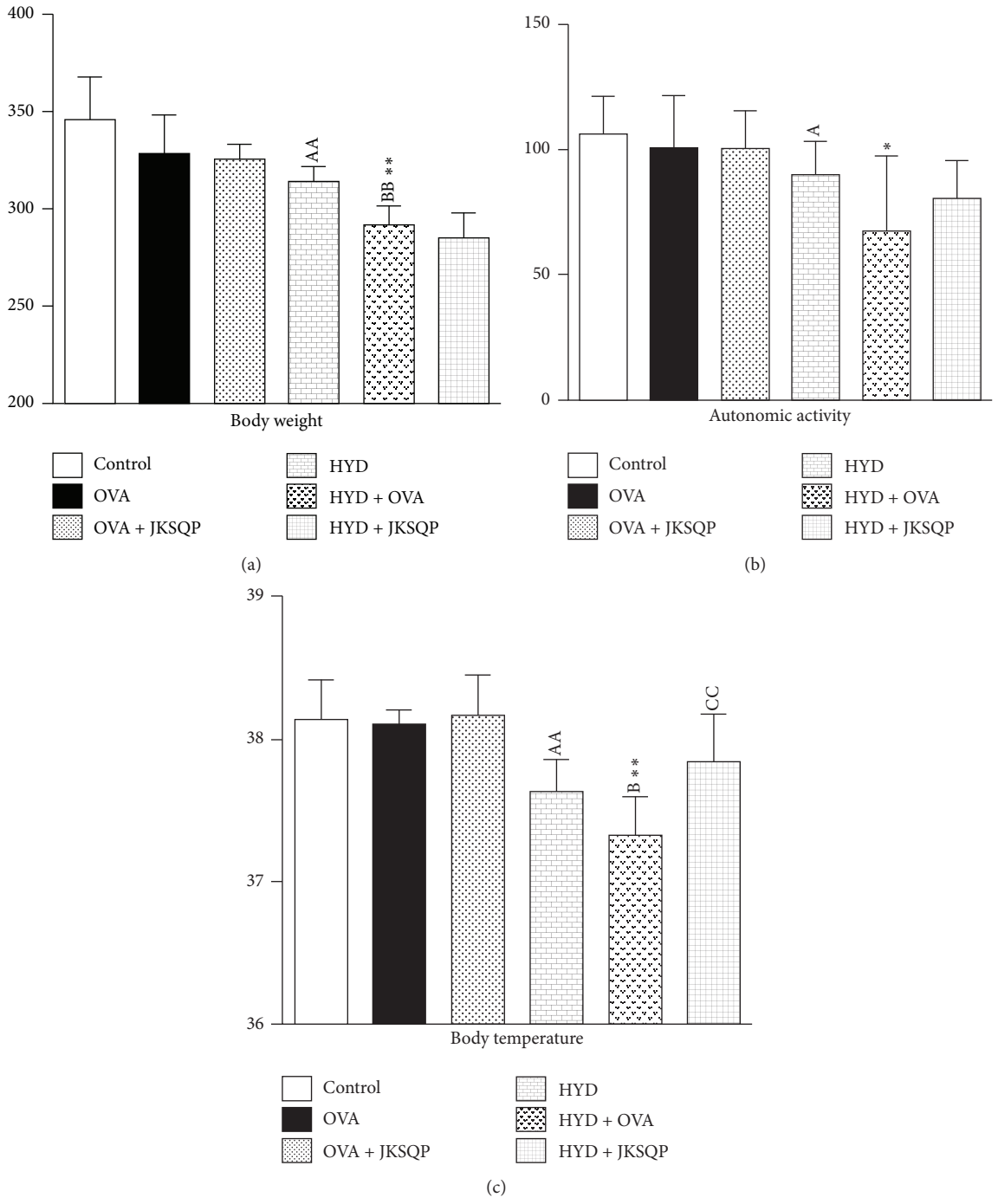

Figure 2: *Symptoms recorded during entire experiment.* No significant difference was reported in body weight, autonomic activity, and body temperature among the six groups before the experiment. (a) Body weight changes of OVA-challenged rats with or without JKSQP treatment. (b) Autonomic activity duration of OVA-challenged rats with or without JKSQP treatment. (c) Body temperature of OVA-challenged rats with or without JKSQP treatment. Data are mean ± standard deviation (SD) of 10 rats per group. $^{AA}P < 0.01$ and $^{A}P < 0.05$ versus control group; $^{BB}P < 0.01$ and $^{B}P < 0.05$ versus HYD group; and $^{CC}P < 0.01$ versus HYD + OVA group; $^{**}P < 0.01$ and $^{*}P < 0.05$ versus OVA group. OVA: ovalbumin, HYD: hydrocortisone, and JKSQP: Jinkui Shenqi pills.

bronchial, or vascular walls. However, lung tissue from the asthma and KYD asthmatic groups exhibited increased mucous plug obstruction and inflammatory secretions in the bronchial lumen and extensive infiltration of inflammatory cells around the airways and blood vessels, especially eosinophils and lymphocytes.

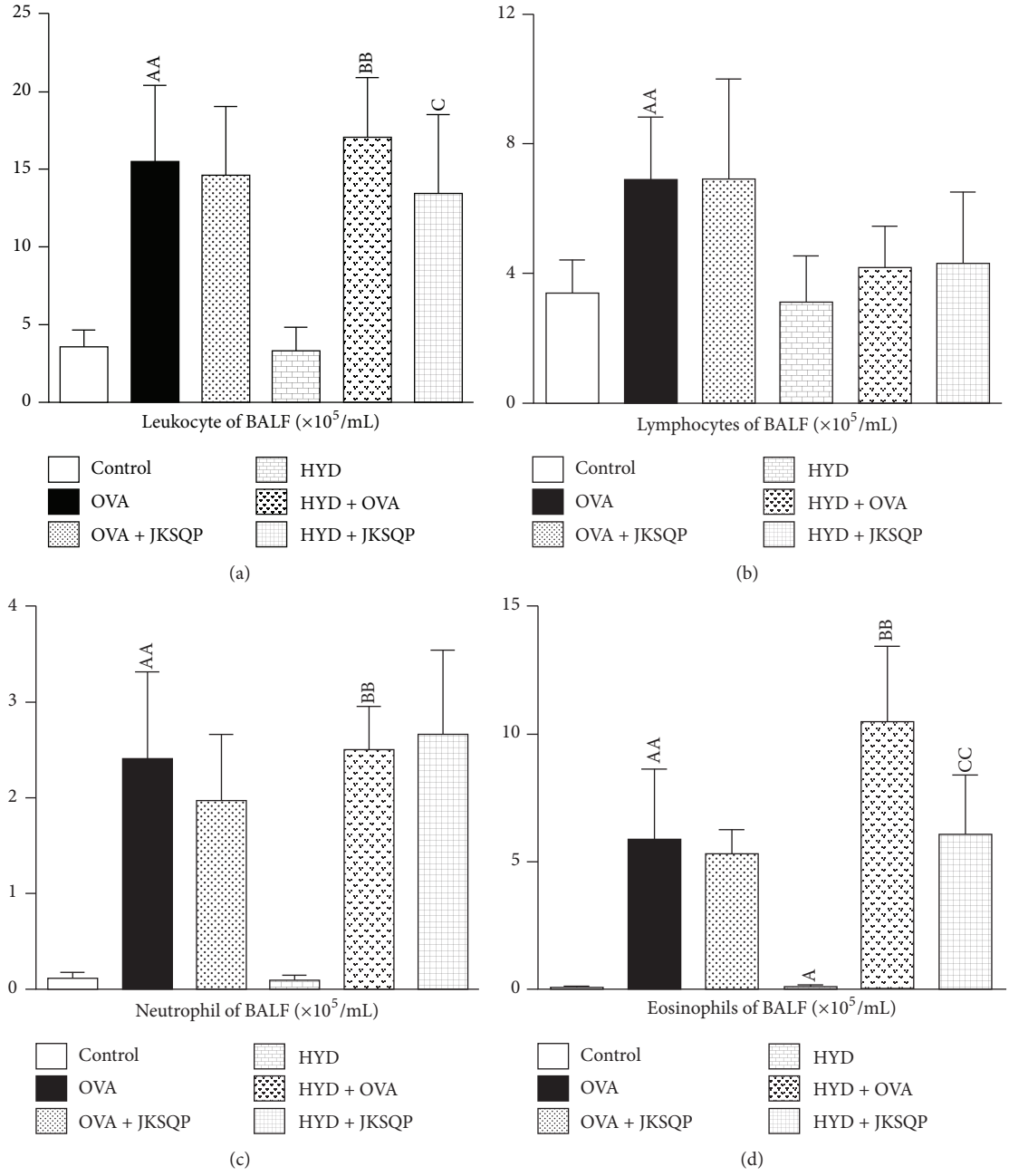

Figure 3: *(a) Leukocyte, (b) lymphocyte, (c) neutrophil, and (d) eosinophil levels in bronchoalveolar lavage fluid (BALF) evaluated at the end of the experiment using enzyme-linked immunosorbent assay (ELISA).* ELISA was performed according to the manufacturer's protocol. Data are mean ± standard deviation (SD) of 10 rats per group. $^{AA}P < 0.01$ and $^{A}P < 0.05$ versus control group; $^{BB}P < 0.01$ versus HYD group; $^{CC}P < 0.01$ and $^{C}P < 0.05$ versus HYD + OVA group. OVA: ovalbumin, HYD: hydrocortisone, and JKSQP: Jinkui Shenqi pills.

Moreover, hyperplastic bronchi, vascular smooth muscle, and a wide variability in alveolar interval were observed in the OVA-challenged rat lungs. This evidence indicates that the KYD model rats were more susceptible to developing OVA-induced asthma than the normal rats. Conversely, airway inflammation was inhibited more in the histological sections of lung tissue from the JKSQP-treated rats than it was in sections from the KYD plus OVA groups. These results were consistent with the score of HE staining (Table 1); for scoring criteria, please see Ji et al., 2005.

4. Discussion

The theory of TCM proposes that the physiological function of the body would be at a low level in KYD syndrome [13]. In this study, we observed that the KYD rat model exhibited lower autonomous activities and anal temperature than the normal group. The levels of CRH, ACTH, and CORT more significantly decreased in the KYD groups than they did in the normal group. Furthermore, HPAA hypofunction was observed in the KYD rat model. These results were similar

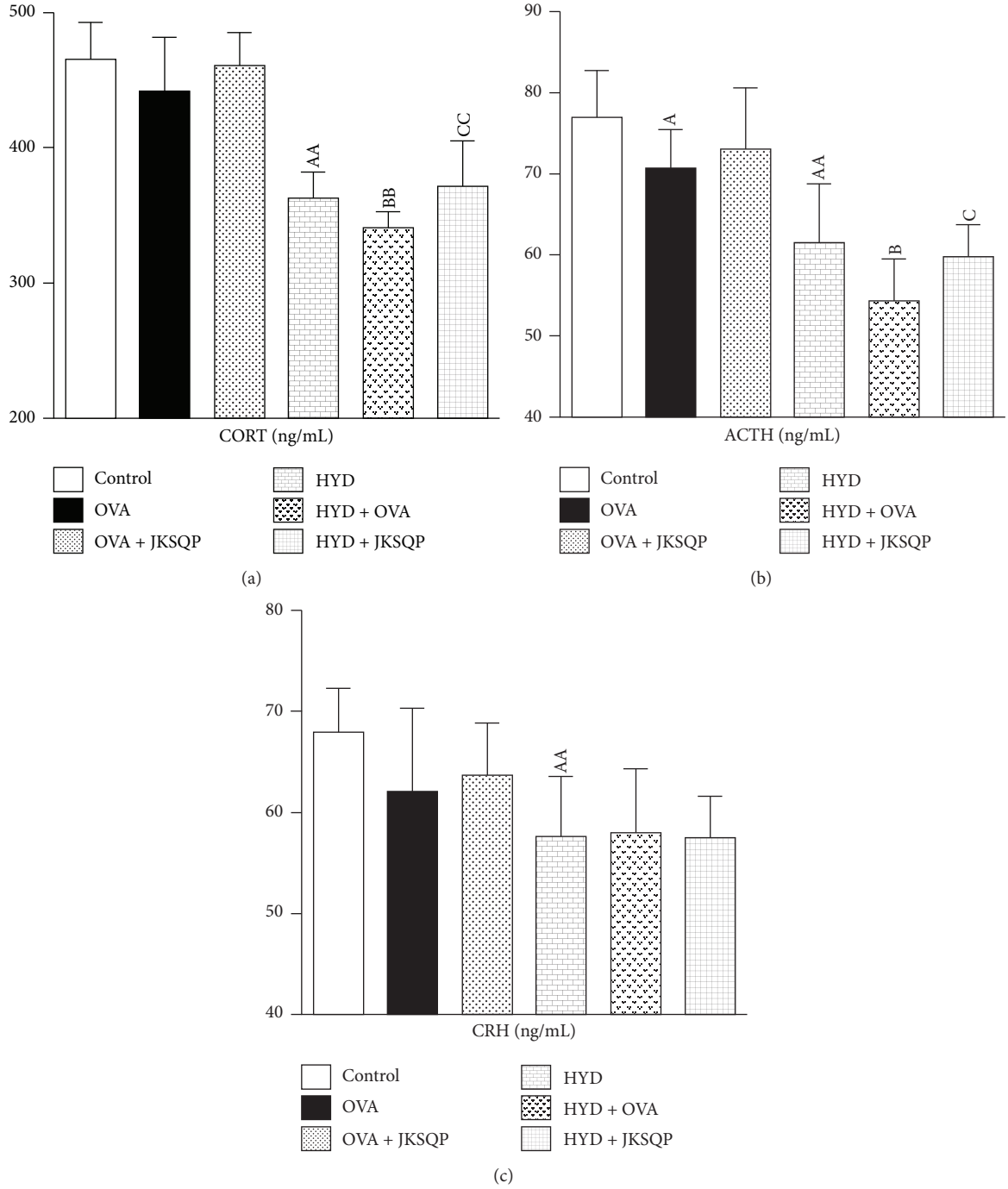

Figure 4: *(a) Serum corticosterone (CORT), (b) adrenocorticotropic hormone (ACTH), and (c) corticotropin-releasing hormone (CRH) evaluated at the end of the experiment using enzyme-linked immunosorbent assay (ELISA).* ELISA was performed according to the kit manufacturer's protocol. Data are mean ± standard deviation (SD) of 10 rats per group. $^{AA}P < 0.01$ and $^{A}P < 0.05$ versus control group; $^{BB}P < 0.01$ and $^{B}P < 0.05$ versus HYD group; and $^{CC}P < 0.01$ and $^{C}P < 0.05$ versus HYD + OVA group. OVA: ovalbumin, HYD: hydrocortisone, and JKSQP: Jinkui Shenqi pills.

to those observed in a specific KYD animal model in a previously reported study [3, 14].

Th1/Th2 imbalance, especially the excessive expression of Th2 cytokines, is a critical mechanism involved in mediating asthma attacks. The function of Th1 cytokines decreases, while that of Th2 markedly increases in patients with asthma [15]. The Th2 cytokine IL-4 induces B cells to differentiate into plasma cells, which then produce IgE [16, 17]. Eventually, the development of allergy is mainly associated with eosinophil infiltration and chronic airway inflammation, which rely on

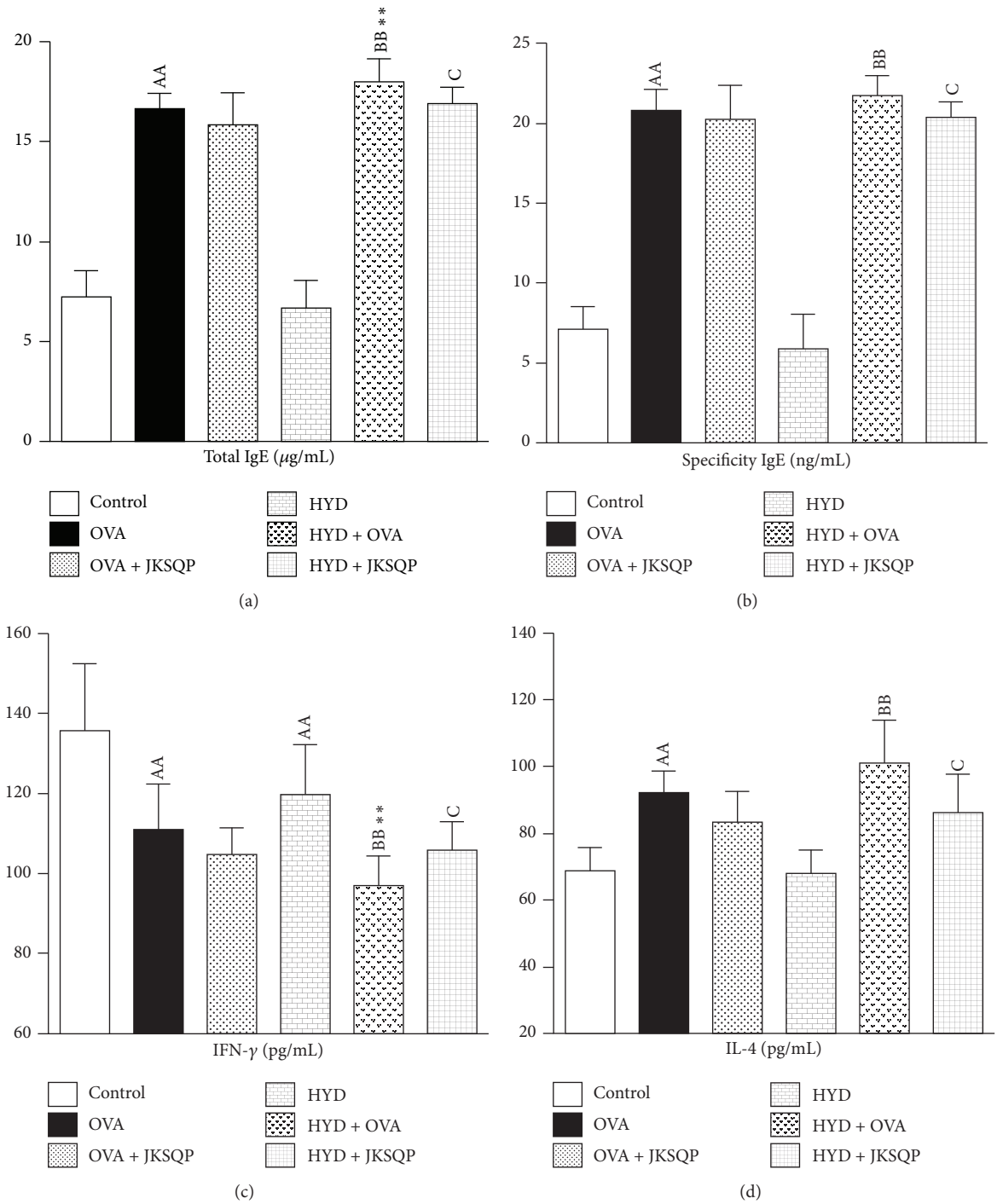

Figure 5: *(a) Serum total immunoglobulin E (IgE) and (b) its specificity, (c) lung interferon (IFN)-γ, and (d) interleukin (IL)-4 levels evaluated at the end of the experiment using enzyme-linked immunosorbent assay (ELISA).* ELISA kits were used according to the manufacturer's protocol. Data are mean ± standard deviation (SD) of 10 rats per group. $^{AA}P < 0.01$ versus control group; $^{BB}P < 0.01$ versus HYD group; $^{C}P < 0.05$ versus HYD + OVA group; $^{**}P < 0.01$ versus OVA group. OVA: ovalbumin, HYD: hydrocortisone, and JKSQP: Jinkui Shenqi pills.

IgE. Moreover, IL-4 not only promotes the differentiation of Th0 cells to Th2 cells but also inhibits the effect of Th1 cells. In contrast, IFN-γ inhibits the synthesis of IgE by B cells while it suppresses Th0 cell differentiation into Th2 cells. Furthermore, IFN-γ inhibits the agglomeration of eosinophils in the airway [18, 19].

From the test data, the level of the Th1 cytokine, IFN-γ, in the KYD groups decreased more than that in the normal group, which subsequently increased the level of the Th2 cytokine, IL-4. The Th1/Th2 ratio was altered in the KYD groups [20], while the KYD + OVA group showed a more significant increase in the levels of leukocytes, eosinophils,

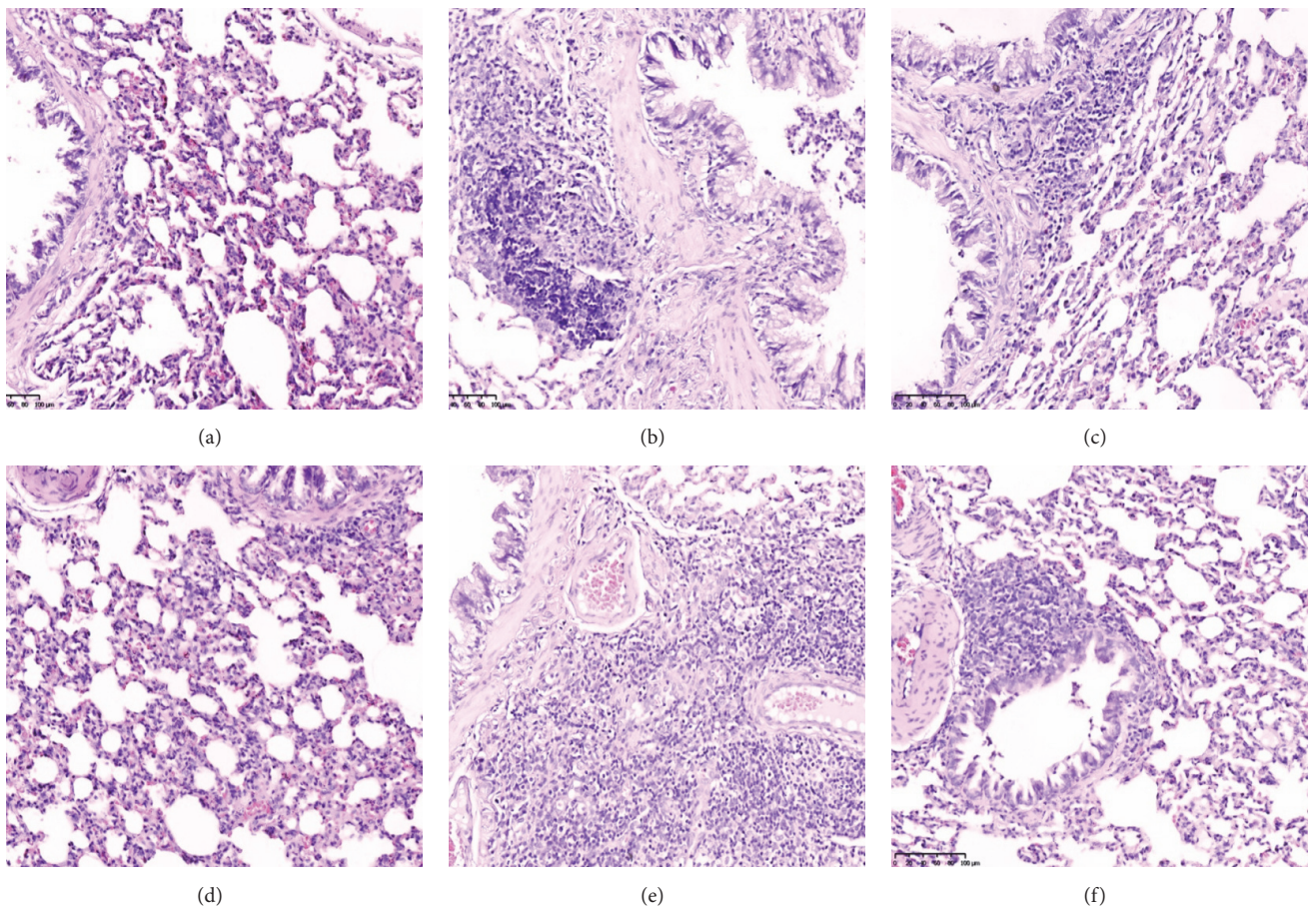

Figure 6: *Histological examinations of lung tissues for inflammatory cell infiltration.* Lung tissues obtained at the end of the experiment were stained with hematoxylin and eosin (H&E, 200x magnification). Groups: (a) Control, (b) OVA, (c) OVA + JKSQP, (d) HYD, (e) HYD + OVA, and (f) HYD + OVA + JKSQP. OVA: ovalbumin; HYD: hydrocortisone; JKSQP: Jinkui Shenqi pills.

Table 1: The score of HE staining for all groups.

	Peripheral blood vessels and bronchial EOS increase	Edema	Epithelial cell injury
Control	0.16 ± 0.40	0	0
OVA	3.33 ± 0.51^{AA}	3.00 ± 0.63^{AA}	2.66 ± 0.51^{AA}
OVA + JKSQP	3.1 ± 0.75	2.83 ± 0.75	2.50 ± 0.54
HYD	0.33 ± 0.51	0	0
HYD + OVA	4.83 ± 0.41^{BBB}	4.50 ± 0.83^{BBB}	4.33 ± 0.81^{BBB}
HYD + OVA + JKSQP	3.50 ± 0.83^{CC}	3.33 ± 0.51^{CC}	3.16 ± 0.41^{CC}

$^{AA}P < 0.01$ versus control group; $^{CC}P < 0.01$ versus HYD + OVA group; $^{BBB}P < 0.001$ versus HYD group. OVA: ovalbumin, HYD: hydrocortisone, and JKSQP: Jinkui Shenqi pills.

and total and specific IgE than the OVA group did. Moreover, the HPAA was hypofunctional in the KYD model, which decreased the physiological secretion of glucocorticoid. This suggests that a patient with KYD would likely experience asthma attacks more easily than a normal individual with the same risk factor. These results prove a close relationship between KYD and asthma and provide evidence supporting the "warming" of KYD in treating asthma.

JKSQP was recorded in the *Jingui Yaolue* (*Synopsis of Golden Chamber*, 金匮要略), which was written by Zhang Zhongjing at the end of the Eastern Han Dynasty 1800 years ago. JKSQP was considered one of the best kidney-yang warming drugs and has been widely used to treat numerous diseases affecting various body systems such as chronic diarrhea, edema, and especially asthma, which TCM doctors have treated with this agent for thousands of years. More than 200 years ago, *Huangdi Neijing* (黄帝内经), the earliest and greatest medical classic extant work on the physiology and pathophysiology of TCM in China, reported a close relationship between kidney-yang and asthma. Furthermore, Zhu Danxi considered phlegm an important factor in the pathogenesis of asthma. KYD is an important mechanism

involved in the formation of phlegm [21, 22] and, therefore, JKSQP is widely used to treat asthma.

As mentioned above, HPAA hypofunction and Th1/Th2 imbalance were observed in all OVA groups, while JKSQP treatment showed improvements in these parameters. Furthermore, the leukocyte, eosinophil, and total and specific IgE levels, which are biomarkers of airway inflammation, were lower in the asthma and drug groups than in the two OVA groups. In addition, the lesions in the asthmatic drug-treated lung tissues improved more than those in the two OVA groups did. These effects may be attributable to the JKSQP-induced improvement of HPAA function, which also increased glucocorticoid secretion.

Furthermore, the increased glucocorticoid secretion inhibited the expression of Th2 cytokine subpopulations. Then, the inflammation induced downstream by Th2 cytokines would reduce, thereby improving the airway inflammation and reducing the hyperreaction, which would control the asthmatic attack. However, other mechanisms might be involved in the antiasthmatic effect induced by treatment with JKSQP, in addition to the pathway associated with improved HPAA function, which reverses the imbalance in Th1/Th2. These speculations are worth further investigation in future studies.

5. Conclusion

KYD is associated with HPAA hypofunction and Th1/Th2 imbalance and is a risk factor that affects and exacerbates asthma. JKSQP cures and controls asthma by improving the HPAA function and reversing the imbalance between Th1 and Th2 cytokines, which inhibits or reduces the airway inflammation. Therefore, this study provides evidence to support the effectiveness of JKSQP in the treatment of asthma with KYD.

Abbreviations

ACTH: Adrenocorticotropic hormone
BALF: Bronchoalveolar lavage fluid
CORT: Corticosterone
CRH: Corticotropin-releasing hormone
ELISA: Enzyme-linked immunosorbent assays
H&E: Hematoxylin and eosin
HYD: Hydrocortisone
HPAA: Hypothalamic-pituitary-adrenal axis
IgE: Immunoglobulin E
IFN: Interferon
IL: Interleukin
JKSQP: Jinkui Shenqi pills
KYD: Kidney-yang deficiency
OVA: Ovalbumin
TCM: Traditional Chinese medicine.

Disclosure

Bing Ji and Yuan-yuan Li contributed equally to this study and should be regarded as co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Qi-yang Shou, Hui-ying Fu, and Bing Ji conceived and designed the experiments. Qi-yang Shou, Yuan-yuan Li, Li-zong Zhang, and Ming-sun Fang performed the experiments. Qi-yang Shou and Wei-ji Yang analyzed the data. Qi-yang Shou and Bing Ji contributed reagents/materials/analysis tools. Hui-ying Fu and Qi-yang Shou wrote the paper. Bing Ji and Yuan-yuan Li contributed equally to this study.

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References

[1] S. Abdureyim, N. Amat, A. Umar, H. Umar, B. Berke, and N. Moore, "Anti-inflammatory, immunomodulatory, and heme oxygenase-1 inhibitory activities of Ravan Napas, a formulation of Uighur traditional medicine, in a rat model of allergic asthma," *Evidence-Based Complementary and Alternative Medicine*, vol. 2011, Article ID 725926, 13 pages, 2011.

[2] S.-P. Fang, T. Tanaka, F. Tago, T. Okamoto, and S. Kojima, "Immunomodulatory effects of gyokuheifusan on INF-γ/IL-4 (Th1/Th2) balance in ovalbumin (OVA)-induced asthma model mice," *Biological & Pharmaceutical Bulletin*, vol. 28, no. 5, pp. 829–833, 2005.

[3] L. Zhao, H. Wu, M. Qiu et al., "Metabolic signatures of kidney Yang deficiency syndrome and protective effects of two herbal extracts in rats using GC/TOF MS," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 540957, 10 pages, 2013.

[4] T. Chen, L. Xiao, L. Zhu, S. Ma, T. Yan, and H. Ji, "Anti-asthmatic effects of ginsenoside Rb1 in a mouse model of allergic asthma through relegating Th1/Th2," *Inflammation*, vol. 38, no. 5, pp. 1814–1822, 2015.

[5] D. Huang, J. Yang, X. Lu, Y. Deng, Z. Xiong, and F. Li, "An integrated plasma and urinary metabonomic study using UHPLC-MS: intervention effects of *Epimedium koreanum* on 'Kidney-Yang Deficiency syndrome' rats," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 76, pp. 200–206, 2013.

[6] H.-Y. Chen, Y.-H. Lin, P.-F. Thien et al., "Identifying core herbal treatments for children with asthma: implication from a Chinese Herbal medicine database in Taiwan," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 125943, 10 pages, 2013.

[7] L. Y. Wang, K. W. Chan, Y. Yuwen, N. N. Shi, X. J. Han, and A. Lu, "Expert consensus on the treatment of hypertension with Chinese patent medicines," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 510146, 8 pages, 2013.

[8] J. Yang, Y. Wang, Y. Bao, and J. Guo, "The total flavones from *Semen cuscutae* reverse the reduction of testosterone level and the expression of androgen receptor gene in kidney-yang

deficient mice," *Journal of Ethnopharmacology*, vol. 119, no. 1, pp. 166–171, 2008.

[9] L. Zhang, X. Han, Z. Li et al., "Metabolomics research on time-selected combination of Liuwei Dihuang and Jinkui Shenqi pills in treating kidney deficiency and aging by chemometric methods," *Chemometrics and Intelligent Laboratory Systems*, vol. 130, pp. 50–57, 2014.

[10] J. Y. Chen, Y. Y. Zhang, and Q. Wu, "Effect of jingui shenqi pills on sex hormone in aged rats," *Zhongguo Zhong Yao Za Zhi*, vol. 18, no. 10, article 640, pp. 619-620, 1993.

[11] X. Xiong, P. Wang, X. Li, and Y. Zhang, "Shenqi pill, a traditional Chinese herbal formula, for the treatment of hypertension: a systematic review," *Complementary Therapies in Medicine*, vol. 23, no. 3, pp. 484–493, 2015.

[12] X. J. Yao, K. W. Huang, Y. Li et al., "Direct comparison of the dynamics of IL-25- and 'allergen'-induced airways inflammation, remodelling and hypersensitivity in a murine asthma model," *Clinical & Experimental Allergy*, vol. 44, no. 5, pp. 765–777, 2014.

[13] X. Lu, Z. Xiong, J. Li, S. Zheng, T. Huo, and F. Li, "Metabonomic study on 'Kidney-Yang Deficiency syndrome' and intervention effects of Rhizoma Drynariae extracts in rats using ultra performance liquid chromatography coupled with mass spectrometry," *Talanta*, vol. 83, no. 3, pp. 700–708, 2011.

[14] Y. Nan, X. Zhou, Q. Liu et al., "Serum metabolomics strategy for understanding pharmacological effects of ShenQi pill acting on kidney yang deficiency syndrome," *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences*, vol. 1026, pp. 217–226, 2016.

[15] E. Truyen, L. Coteur, E. Dilissen et al., "Evaluation of airway inflammation by quantitative Th1/Th2 cytokine mRNA measurement in sputum of asthma patients," *Thorax*, vol. 61, no. 3, pp. 202–208, 2006.

[16] L. J. Berglund, D. T. Avery, C. S. Ma et al., "IL-21 signalling via STAT3 primes human naive B cells to respond to IL-2 to enhance their differentiation into plasmablasts," *Blood*, vol. 122, no. 24, pp. 3940–3950, 2013.

[17] T. H. Caven, J. L. Sturgill, and D. H. Conrad, "BCR ligation antagonizes the IL-21 enhancement of anti-CD40/IL-4 plasma cell differentiation and IgE production found in low density human B cell cultures," *Cellular Immunology*, vol. 247, no. 1, pp. 49–58, 2007.

[18] E. Kurimoto, A. Kanehiro, N. Miyahara et al., "The effect of trehalose for the allergen-induced airway hyperresponsiveness and airway inflamation in mice," *European Respiratory Journal*, vol. 42, 2013.

[19] J. Schulze, S. Voss, U. Zissler, M. A. Rose, S. Zielen, and R. Schubert, "Airway responses and inflammation in subjects with asthma after four days of repeated high-single-dose allergen challenge," *Respiratory Research*, vol. 13, article no. 78, 2012.

[20] D. Fu, W. Yu, M. Li et al., "MicroRNA-138 regulates the balance of Th1/Th2 via targeting RUNX3 in psoriasis," *Immunology Letters*, vol. 166, no. 1, pp. 55–62, 2015.

[21] C.-Y. Li, J. Peng, L.-P. Ren et al., "Roles of histone hypoacetylation in LAT expression on T cells and Th2 polarization in allergic asthma," *Journal of Translational Medicine*, vol. 11, no. 1, article no. 26, 2013.

[22] B. Rogala, A. Bozek, J. Gluck, and J. Jarzab, "Original paper Prevalence of IgE-mediated allergy and evaluation of Th1/Th2 cytokine profiles in patients with severe bronchial asthma," *Postepy Dermatologii I Alergologii*, vol. 23, no. 4, pp. 274–280, 2015.

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Review Article

Traditional Chinese Medicine for Refractory Nephrotic Syndrome: Strategies and Promising Treatments

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Refractory nephrotic syndrome (RNS) is an immune-related kidney disease with poor clinical outcomes. Standard treatments include corticosteroids as the initial therapy and other immunosuppressants as second-line options. A substantial proportion of patients with RNS are resistant to or dependent on immunosuppressive drugs and often experience unremitting edema and proteinuria, cycles of remission and relapse, and/or serious adverse events due to long-term immunosuppression. Traditional Chinese medicine has a long history of treating complicated kidney diseases and holds great potential for providing effective treatments for RNS. This review describes the Chinese medical theories relating to the pathogenesis of RNS and discusses the strategies and treatment options using Chinese herbal medicine. Available preclinical and clinical evidence strongly supports the integration of traditional Chinese medicine and Western medicine for improving the outcome of RNS. Herbal medicine such as *Astragalus membranaceus, Stephania tetrandra* S. Moore, and *Tripterygium wilfordii* Hook F can serve as the alternative therapy when patients fail to respond to immunosuppression or as the complementary therapy to improve therapeutic efficacy and reduce side effects of immunosuppressive agents. Wuzhi capsules (*Schisandra sphenanthera* extract) with tacrolimus and tetrandrine with corticosteroids are two herb-drug combinations that have shown great promise and warrant further studies.

1. Refractory Nephrotic Syndrome—A Rare Immune Disease with Serious Consequences

Nephrotic syndrome is a rare but serious kidney disease that affects children and adults worldwide. Its clinical presentations include peripheral edema, heavy proteinuria, and hypoalbuminemia, often with hyperlipidemia. The reported annual incidence is 2–7 per 100,000 children and 3 per 100,000 adults [1, 2]. Although occurring at a low rate, it is responsible for approximately 12% of all causes of end stage renal disease (ESRD) and up to 20% of ESRD in children [3]. The etiology of nephrotic syndrome ranges from primary glomerulonephritis to secondary diseases associated with drugs, infections, and neoplasia. The cause of primary nephrotic syndrome is complex and not well understood; however ample evidence indicates that it is an immune-mediated disorder leading to glomerular podocyte injury and increased glomerular permeability [4].

Primary nephrotic syndrome consists of three major pathophysiological subtypes—idiopathic membranous nephropathy (IMN), minimal change disease (MCD), and focal segmental glomerulosclerosis (FSGS). Their pathogenic mechanisms, albeit different in aspects, all involve immune damage of glomerular podocytes with low inflammatory nature.

IMN is generally considered an autoimmune disease, characterized by immune complex deposition and complement activation in the subepithelial space between glomerular podocytes and the glomerular basement membrane, which together contribute to functional disruption of the glomerular capillary wall [5]. The identification of two important podocyte autoantigens—secretory phospholipase

A2 receptor (PLA2R1) and thrombospondin type 1 domain containing 7A protein (THSD7A)—and detection of their autoantibodies in 60–80% and 5–10% of patients with membranous nephropathy, respectively [6, 7], were regarded as landmark discoveries in understanding the molecular pathomechanism of IMN.

MCD and FSGS were traditionally described as separate entities. However, convincing evidence now suggests that they are in fact different manifestations of the same progressive disease, with FSGS being the more advanced stage than MCD [8]. Their immune pathogenesis is considered to arise from a systemic disturbance of T-cell function leading to the production of cytokines or other circulating permeability factors that cause direct or indirect impairment of glomerular function [9].

IMN is the leading cause of nephrotic syndrome in adults, but it is rare in children [1]. MCD and FSGS are the most common causes of childhood nephrotic syndrome. In adults, they each account for 10–15% and 40% of nephrotic syndrome cases, respectively [10].

The standard first-line treatment for nephrotic syndrome is corticosteroids. Although most children with nephrotic syndrome are sensitive to steroids, approximately 20% of children are steroid-resistant. Moreover, 80–90% of pediatric patients who respond initially to steroids experience relapse, and many develop steroid dependency following frequent relapses and repeated courses of steroid administration [11]. Refractory nephrotic syndrome (RNS) thus refers to the subset of patients with nephrotic syndrome who are steroid-resistant, or steroid-dependent, and experience frequent relapses [12]. RNS comprises 25–40% of nephrotic syndrome cases in children and adolescents [13], and its incidence is even greater (up to 70%) in adults [10]. For instance, MCD has over 50% relapse rate in adults [10], and 10–20% of the adult patients with MCD are steroid-resistant [14]. Due to the high incidence of RNS in adult nephrotic syndrome, the terms of nephrotic syndrome and RNS are often used interchangeably in medical practice and literature.

In recent years, second-line immunosuppressive therapies including cytotoxic agents, calcineurin inhibitors, mycophenolate mofetil, and rituximab have been widely used in RNS treatment with promising results. However, long-term use of steroids and some of these immunosuppressants causes serious adverse effects such as nephrotoxicity, hyperglycemia, dyslipidemia, osteoporosis, hypoimmunity, and high risk of infection. Moreover, the risk/benefit profile of these treatments is poor. Most patients with RNS still present severe nonremitting edema, heavy proteinuria, hypoalbuminemia, and sometimes decreased kidney function.

The limited success of currently available treatments for RNS has prompted active research into safer and more effective alternative therapies. Traditional Chinese medicine (TCM) has a long history of treating symptoms of kidney diseases such as edema and proteinuria. Through persistent trial and error, theorization, and retheorization, TCM has developed unique perspectives to explain nephrotic syndrome and accumulated rich clinical experience in treating this disease. Therefore, it makes sense to explore TCM for better RNS treatment.

In this article, we briefly review the current status of RNS treatment by conventional medicine. We describe the TCM theory for understanding the etiology and pathogenesis of RNS. We also discuss the TCM strategies and treatment options as alternative therapies or in a supportive role to improve the efficacy and reduce side effects of immunosuppressive drugs. Finally, we present two herb-drug combinations with promising clinical results and discuss possible mechanisms of action in the treatment of RNS.

2. Current Status of RNS Treatment

For over 40 years, empirical treatment with immunosuppressive agents including corticosteroids has been the mainstay of therapy for nephrotic syndrome [15]. This is largely due to insufficient knowledge of the detailed molecular and cellular mechanisms underlying nephrotic syndrome and thus lack of specific therapeutic targets and treatment guidelines. Steroids remain the standard initial therapy. Depending on the response to steroids, patients fall into steroid-sensitive and steroid-resistant groups. Steroid-resistant patients have a high risk of developing renal failure, while a substantial portion of steroid-sensitive patients experience frequent relapses, develop steroid dependency, and suffer a myriad of serious adverse effects from repeated steroid use. These patients are difficult to treat and face a long battle with RNS.

In order to improve clinical outcomes in patients with RNS, alternative immunosuppressive treatments have been introduced in recent decades, including cytotoxic drugs such as cyclophosphamide, lymphocyte DNA synthesis inhibitors such as mycophenolate mofetil, and calcineurin inhibitors such as cyclosporin and tacrolimus. These agents can be used alone or in combination with steroids. However, the rate of remission induced by different regimens has been highly variable in clinical trials, resulting in a lack of clear consensus regarding the comparative advantages of various combinations. Almost all of the immunosuppressive drugs produce toxic adverse effects that counter their therapeutic benefits. Furthermore, some long-term studies (follow-up for over 10 years) have failed to demonstrate greater remission rates with immunosuppressive therapy than with conservative therapy without immunosuppression [16]. Therefore, the overall risk-benefit profile of immunosuppressive therapy for RNS remains poor.

Considerable progress over the past decade in understanding the molecular mechanisms of nephrotic syndrome has enabled development and testing of novel immunotherapies [17]. Of particular importance is the use of specific B-cell targeting monoclonal antibodies such as rituximab [18]. In several studies, rituximab successfully improved remission rates in patients that had become dependent on other immunosuppressive drugs [18–21]. Based on the hypothesis of circulating permeability factors underlying FSGS and MCD, plasmapheresis has also been attempted in FSGS patients as an alternative approach for treating recurrent nephrotic syndrome following kidney transplantation [22].

Despite the promise that rituximab offers in the treatment of RNS, the available evidence of its efficacy is derived primarily from a few short-term small case studies which

showed mixed results [23, 24]. More research is warranted to confirm its efficacy and to observe its long-term safety profile. The prohibitive unit cost of rituximab is also an obstacle to its wide use [25]. Irrespective of different types of conventional therapies and new treatments, a majority of RNS patients still face frequent relapses. Moreover, toxic adverse effects associated with long-term use of immunosuppressive agents continue to present a serious challenge in the clinical management of RNS.

3. TCM Views and Treatments for RNS

Because severe edema is the most direct manifestation of RNS, the understanding of RNS in TCM has been based primarily on the theory and clinical experience in treating edema. The pathogenesis of edema was first described in *Yellow Emperor's Inner Classics*, a book written before 100 BC that established the theoretical foundation of TCM [26]. Edema was thought to arise from functional disturbance of three organ systems including Lungs, Spleen, and Kidneys, and the consequent disruption of fluid metabolism. In order to better understand TCM theories, it is important to point out that the definition of organs in TCM is different from that of Western medicine. Organs in the TCM context often constitute functional organ systems with both anatomical and functional meanings. Lungs represent the respiratory system that controls breath and perspiration, Spleen represents the digestive system that controls food assimilation and feces elimination, and Kidneys represent the urinary system that controls urine excretion. A myriad of factors can lead to disorders of these three systems, which separately or collectively result in disruption of fluid homeostasis leading to edema. Therefore, the fundamental TCM treatment strategy for edema is to regulate the functions of Lungs, Spleen, and Kidneys in order to promote fluid elimination through sweat, feces, and urine. When edema is not quickly resolved and becomes chronic, it can block energy flow and blood circulation and thus cause pathological patterns such as Damp Heat, Heat Toxins, Qi Stagnation, and Blood Stasis, leading to secondary complications such as localized or systemic inflammation, ischemia, swelling, pain, stiffness, and skin ulcers. When the root causes of edema and the associated complications are treated accordingly, better therapeutic effects can be achieved [27].

What determines the prognosis, remission, and relapse of nephrotic syndrome? Why are some patients more difficult to treat than others? From the TCM perspective, autoimmune diseases are largely considered to be constitutional diseases. Constitutional characteristics are the result of multiple factors, among which genetics is the decisive factor conferring relative stability and predictability to the constitution. Meanwhile, a person's constitution can be affected by other factors leading to its changeability. This concept is the basis for individuality of disease pathogenesis and responses to treatments. According to the TCM principles first described in *Yellow Emperor's Inner Classics*, personalized treatments must be based on duration of diseases, severity of symptoms, and body constitutions. One of the most important principles is avoiding damaging the patient's Upright Qi, which means a person's internal energy and immunity. In other words, Upright Qi determines the likelihood of remission and relapse of difficult-to-treat diseases. This notion is consistent with the knowledge of disease prognosis in modern medicine, for instance, in cancer treatment [28].

Why is RNS difficult to treat? The reason is its complexity. In the scenario of RNS, symptoms such as severe edema, heavy proteinuria, and hypoalbuminemia have not resolved; serious adverse effects of steroids and other immunosuppressants have persisted; and Upright Qi has been damaged which makes the disease resolution even more difficult. From the perspective of Yin-Yang balance, nephrotic syndrome is mainly characterized as Yang Deficiency and Yin Excess. When nephrotic syndrome becomes RNS, Yang often becomes more deficient, thus worsening the imbalance of Yin-Yang. Other pathological factors such as accumulation of toxins and Blood Stasis (i.e., poor blood circulation) further complicate treatments. Therefore, the crucial TCM concept in treating RNS is to regulate Yin-Yang and Qi-Blood, stabilize the internal environment, and avoid damaging Upright Qi by overtreatment. The comprehensive goal is to maintain biological homeostasis, improve blood circulation and eliminate toxins, minimize the doses and adverse effects of immunosuppressants, improve clinical symptoms, reduce proteinuria, and protect kidney functions in order to improve the overall survival and quality of life of patients with RNS [27].

The TCM strategy for treating edema was first established by Zhang Zhong-Jing (150–219 AD), the most eminent physician in the Chinese history [29]. For readers unfamiliar with Chinese medicine, the significance of Zhang Zhong-Jing and his work can be exemplified by a simple fact that Kampo medicine widely practiced in Japan still uses his original herb formulations with only the slightest changes. The one hundred forty-eight Kampo formulations approved by Japan's national healthcare system are mostly based on his work. These ancient formulations continue to prove valuable for treating modern diseases.

Zhang Zhong-Jing designed many herb formulas to treat edema with varying severities and symptoms (Table 1). Due to the exceptional efficacy of these formulas, they have served as the base formulas for treating edema with subsequent modifications by later generations of physicians. A review of these traditional formulas reveals that *Stephania tetrandra* S. Moore (Fang Ji) and *Astragalus membranaceus* (Huang Qi) are the most important herbs for treating edema.

The clinical effectiveness of *Astragalus membranaceus* in nephrotic syndrome has been consistently demonstrated in many clinical studies [30]. *Astragalus* is commonly used in clinical management of proteinuria of various etiologies and is especially effective in glomerulonephritis and nephrotic syndrome [31]. In one study conducted in China, 30 patients with chronic glomerulonephritis, among whom nine were diagnosed with RNS including eight with FSGS and one with IMN, received *Astragalus* therapy [32]. Following 3 weeks of intravenous injections of *Astragalus* extract at 80 g/day, marked reductions in proteinuria were observed in 6 of 8 patients with FSGS, but not in the one patient with IMN. In two separate case reports, patients with

Table 1: Traditional herb formulas and ingredients for treating edema.

Indications	Formulas	Herbs
Mild edema	*Stephania & Astragalus Decoction* (Fang Ji Huang Qi Tang)	*Stephania tetrandra* (Fang Ji), *Astragalus membranaceus* (Huang Qi), *Atractylodes macrocephala* (Bai Zhu), *Glycyrrhiza uralensis* (Gan Cao)
Moderate edema	*Stephania & Poria decoction* (Fang Ji Fu Ling Tang)	*Stephania tetrandra* (Fang Ji), *Astragalus membranaceus* (Huang Qi), *Glycyrrhiza uralensis* (Gan Cao), *Poria cocus* (Fu Ling), *Cinnamomum cassia* (Gui Zhi)
Severe edema	*Cocculus decoction* (Mu Fang Ji Tang)	*Cocculus orbiculatus* (Mu Fang Ji), *Panax ginseng* (Ren Shen), *Cinnamomum cassia* (Gui Zhi), *Gypsum* (Shi Gao)
Recurrent edema	*Cocculus minus Gypsum plus Poria plus Mirabilite decoction* (Mu Fang Ji Qu Shi Gao Jia Fu Ling Mang Xiao Tang)	*Stephania tetrandra* (Fang Ji), *Panax ginseng* (Ren Shen), *Cinnamomum cassia* (Gui Zhi), *Mirabilite* (Mang Xiao), *Poria cocos* (Fu Ling)
Severe edema with constipation and urinary retention	*Stephania Zanthoxylum Descurainia Rhubarb Pill* (Ji Jiao Li Huang Wan)	*Stephania tetrandra* (Fang Ji), *Zanthoxylum bungeanum* (Jiao Mu), *Descurainia sophia* (Ting Li Zi), *Rheum officinale* (Da Huang)

RNS due to IMN who had previously failed to respond to immunosuppressive therapy and supportive care were able to achieve complete remission after taking *Astragalus* [33, 34]. For example, a 77-year old patient was treated with supportive therapy (angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, statin, and diuretics) and immunosuppressive agents (cyclosporin and mycophenolate mofetil) for 1 year without any response. After 2 years of unremitting nephrosis, she began oral administration of a Chinese herbal medicine *Nephritis Four-ingredient Pill* (Shen Yan Si Wei Pian) with the active ingredient of *Astragalus* at a dose of 15 g/day. Proteinuria, hypoalbuminemia, hypercholesterolemia, and edema all resolved after approximately 1 year of therapy, and the remission persisted for the next 4 years [33].

Likewise, *Stephania tetrandra* has been widely used for treating nephrotic syndrome and chronic kidney disease, usually in combination with *Astragalus*. In a double-blinded randomized clinical trial (RCT) involving 578 patients with glomerulonephritis in stage 3 chronic kidney disease, we treated patients for 24 weeks with benazepril, a herbal medicine *Stephania*, *and Astragalus Decoction (Fang Ji Huang Qi Tang)*, or the combination of these two [35]. Results demonstrated that the herbal medicine improved renal function shown by significant increases in estimated glomerular filtration rate (eGFR) and hemoglobin with the lowest incidence of side effects; benazepril decreased proteinuria; and the benazepril-herb combination had synergistic effects on reducing proteinuria and protecting kidney functions. Many other studies evaluating *Stephania and Astragalus Decoction* in nephrotic syndrome have reported similar benefits in reducing proteinuria and hyperlipidemia and increasing serum albumin [36–38]. Circulating cytokine levels were affected by the herbal medicine with significant decreases in TNF- and IL-6 and increases in IL-10 [36, 37].

Shenqi particle is a Chinese herbal medicine consisting of 13 herbs including *Astragalus*. Since the 1980s, it has been successfully used to treat various immune-related kidney diseases. Previous small clinical studies showed that Shenqi particle and its components reduced proteinuria in patients with membranous nephropathy [39, 40]. A prospective RCT was conducted in 190 adult patients with membranous nephropathy to compare the efficacy and safety of Shenqi particle with standard therapy of prednisone and cyclophosphamide [41]. After 48 weeks of treatment, both groups showed comparable levels of reduction in proteinuria and similar improvement in serum albumin. However, patients treated with Shenqi particle had significantly higher eGFR compared with baseline, while eGFR was slightly decreased in patients receiving standard therapy. Furthermore, severe adverse events occurred only with standard therapy. These results indicate that Shenqi particle has similar efficacy but fewer side effects compared to standard therapy. It also protects kidney function, while standard therapy does not. Therefore, Shenqi particle is a promising alternative therapy for RNS.

There are more ongoing trials of Chinese herbal medicine in RNS. A double-blinded RCT is currently evaluating the effectiveness of QingReMoShen granules in combination with angiotensin II receptor blocker in the reduction of proteinuria and T lymphocytes in IMN (clinicaltrials.gov, NCT01845688).

4. TCM to Support Immunosuppressive Therapy for RNS

As mentioned previously, in TCM theories the basic body constitution for nephrotic syndrome is primarily Yang Deficiency leading to fluid retention with secondary complications such as Blood Stasis. The standard therapy for nephrotic syndrome in conventional medicine is corticosteroids combined with other immunosuppressive drugs. These immunosuppressive therapies often shift the pathology of nephrotic syndrome toward further disequilibrium of Yin-Yang. In the TCM perspective, corticosteroids are pure Yang agents with hot nature. Accumulated fluids in the body, when

Table 2: Herb formulas and ingredients commonly used for reducing side effects of immunosuppressive agents.

Indications	Formulas	Herbs
Side effects of steroids	*Six-Ingredient Rehmannia Decoction* (Liu Wei Di Huang Tang) & derivatives	*Rehmannia glutinosa* (Sheng Di Huang), *Dioscorea opposita* (Shan Yao), *Cornus officinalis* (Shan Zhu Yu), *Poria cocos* (Fu Ling), *Alisma orientale* (Ze Xie), *Paeonia suffruticosa* (Mu Dan Pi), *Glehnia littoralis* (Bei Sha Shen), *Ophiopogon japonicus* (Mai Men Dong), *Paeonia lactiflora* (Bai Shao), *Lycium barbarum* (Gou Qi Zi), *Anemarrhena asphodeloides* (Zhi Mu)
Side effects of other immunosuppressants	*Bolster the Spleen Decoction* (Shi Pi Yin) *Kidney Qi Pill* (Jin Gui Shen Qi Wan) & derivatives	*Aconitum carmichaelii* (Fu Zi), *Epimedium* (Yin Yang Huo), *Morinda officinalis* (Ba Ji Tian), *Rehmannia glutinosa* (Shu Di Huang), *Atractylodes Macrocephala* (Bai Zhu), *Codonopsis pilosula* (Dang Shen), *Astragalus membranaceus* (Huang Qi), *Zingiber officinale* (Gan Jiang), *Cinnamomum cassia* (Gui Zhi), *Alpinia Katsumadai* (Cao Dou Kou), *Amomum tsao-ko* (Cao Guo), *Cuscuta australis* (Tu Si Zi), *Cistanche deserticola* (Rou Cong Rong)
Hypoimmunity and frequent infections	*Eight-Treasure Decoction* (Ba Zhen Tang) *Jade Windscreen Powder* (Yu Ping Feng San)	*Astragalus membranaceus* (Huang Qi), *Panax ginseng* (Ren Shen), *Atractylodes Macrocephala* (Bai Zhu), *Glycyrrhiza uralensis* (Gan Cao), *Angelica sinensis* (Dang Gui), *Cordycepssinensis* (Dong Chong Xia Cao), *Ganoderma lucidum* (Ling Zhi)

heated by such Yang agents, inevitably generate Damp Heat leading to Yin Deficiency and Yang Excess. This in turn increases the risk of diabetes, hypertension, coronary heart disease, and osteoporosis, with symptoms such as facial flushing, obesity, acne, insomnia, polydipsia, polyphagia, hyperhidrosis, nocturnal emission, and premature ejaculation. On the other hand, immunosuppressive agents such as cyclophosphamide, mycophenolate mofetil, cyclosporin, tacrolimus, and rituximab are Yin agents with cold nature. Long-term use of these drugs damages Yang Qi and further aggravates Yang Deficiency and Yin Excess, manifested as poor appetite, abdominal bloating, nausea and vomiting, shortness of breath and fatigue, cold intolerance, frequent infection, bone marrow suppression, and reproductive suppression, and so on. Severe Yin-Yang imbalance is therefore the core problem facing patients with RNS, contributing to the chronic recurrent pattern of remissions and relapses as well as serious adverse events.

Based on this notion, the TCM strategy for difficult-to-treat RNS patients who are experiencing serious toxic effects is to restore the Yin-Yang balance and minimize the doses of immunosuppressive drugs in order to reduce adverse effects, improve therapeutic efficacy, and stabilize the internal environment. For patients receiving steroids, herbal formulas that nourish Yin and suppress Yang are used, with *Six-Ingredient Rehmannia Decoction* (Liu Wei Di Huang Tang) as the representative formula in this category. For patients receiving other immunosuppressive agents, herbal formulas that nourish Yang and boost Qi are used, with *Bolster the Spleen Decoction* (Shi Pi Yin) as the representative formula. For patients with low immunity and frequent infections, herbal formulas that support Upright Qi are used, represented by *Eight-Treasure Decoction* (Ba Zhen Tang) and *Jade Windscreen Powder* (Yu Ping Feng San). Depending on the patient's condition, these formulas can be combined and modified to best serve each patient in different phases of disease progression. The commonly used formulas and herb ingredients are listed in Table 2.

Tripterygium wilfordii Hook F (TWHF), a traditional Chinese herb with potent anti-inflammatory and immunosuppressive properties, has been used since the 1980s to treat nephrotic syndrome [42]. Several prospective RCTs reported the effectiveness of Tripterygium glycosides (TG), a fat-soluble extract from the root of TWHF, in supporting the use of steroids for IMN treatment [43–45]. When TG monotherapy and TG-steroid combination therapy were evaluated in 84 patients with IMN, following 12 months of treatment, TG alone improved proteinuria, but the combination therapy achieved much higher remission rate than TG alone (76.7% versus 43.9%) [43]. Two subsequent studies compared the efficacy of TG-steroid combination and tacrolimus-steroid combination in IMN [44, 45]. Zuo et al. [44] reported that the two treatments achieved comparable effective rate (76.9% versus 79.9%) at 12 months, but TG-steroid group had lower relapse rate than tacrolimus-steroid group (30.6% versus 52.5%) 6 months after discontinuation of treatment. TG-steroid group also had lower serum creatinine doubling rate, indicating less deterioration of renal functions. A recent meta-analysis of 18 studies analyzing 1,236 adult patients with primary nephrotic syndrome demonstrated greater efficacy of TG combined with steroid than steroid monotherapy [46]. Two earlier meta-analysis reviews also showed beneficial effects of TWHF in inducing remission of nephrotic syndrome and RNS [47, 48].

The chemical constituents of TWHF and its extracts are complex. Among multiple constituents isolated from TWHF, triptolide is a main active ingredient with important anti-inflammatory and immunosuppressive properties [49]. Triptolide can exert anti-inflammatory functions by reducing the release of many proinflammatory cytokines and mediators such as TNFα, IL-6, IL-8, and PGE2 [49]. Suppression of T-lymphocyte functions by triptolide is deemed largely responsible for its immunosuppressive properties, ranging from inducing T-cell apoptosis, inhibiting lymphocyte proliferation, regulating $CD4^+$ and $CD8^+$ cells, and reducing IL-2 and interferon-γ production [49–53].

Its immunosuppressive effects have also been attributed to inhibition of dendritic cell maturation and trafficking [54]. In an experimental model of membranous nephropathy, triptolide significantly reduced proteinuria, protected podocytes from C5b-9-mediated injury, and decreased the expression of desmin, a marker of podocyte injury [55].

Great efforts have been dedicated to exploring the potential of integrating Chinese and Western medicine in improving RNS treatment. From 2001 to 2010, more than 150 RCT studies were published that combined Chinese and Western medicine for RNS treatment, most of which were conducted in China and published in Chinese language journals. A meta-analysis selected 11 of the highest quality trials to evaluate the therapeutic effects of combined Chinese herbal medicine and immunosuppressive therapy in the treatment of RNS [56]. The results indicated that patients receiving herbal medicine in combination with immunosuppressive agents had significantly higher complete or partial remission rates and fewer serious adverse events than those receiving immunosuppressive agents alone.

It is important to point out that despite large numbers of TCM clinical trials in this field, high-quality RCT data are limited. Most studies so far were not double-blinded and had small sample sizes (fewer than 100 participants). Many lack details of cointervention and randomization methods. Overall there is a paucity of strong clinical evidence by the standards of modern medicine. In fact, how to design and conduct RCTs to evaluate the efficacy and safety of TCM in accordance with evidence-based medicine is a challenging task facing TCM researchers worldwide (a comprehensive discussion and review of this topic was undertaken by Fung and Linn [57]). Nevertheless, the large body of clinical data and real-world experience available to date has supported the benefits of integrating TCM and conventional medicine for the treatment of RNS.

5. Promising Herb-Drug Combinations for RNS

Extensive research in Chinese herbal medicine has identified many active ingredients that have immunosuppressive activity or can improve existing immunosuppressive therapies for treating autoimmune kidney diseases. Of special note are two herbal ingredients and the herb-drug combinations that have been investigated extensively in preclinical and clinical studies. Convincing evidence indicates that they can improve the therapeutic effects and reduce adverse effects of immunosuppressive agents. These two combinations are Wuzhi capsule with tacrolimus and tetrandrine with glucocorticoid.

5.1. Wuzhi-Tacrolimus Combination. Of calcineurin inhibitors, tacrolimus is preferred over cyclosporine for treating many types of autoimmune diseases and for preventing graft rejection after organ transplantation. This is largely owing to the fact that tacrolimus has consistently demonstrated better therapeutic effects and fewer adverse effects than cyclosporin in most comparative studies [58–62]. However, narrow therapeutic index and unpredictable bioavailability in humans (5%–67% with average of 27%) have made tacrolimus administration challenging for clinical practice [63]. The high cost of tacrolimus also limits its use.

Wuzhi capsule is an ethanol extract preparation of *Schisandra sphenanthera* (Nan-Wuweizi), a Chinese herb traditionally used for treating hepatitis, liver/kidney deficiency, and neurasthenia. The main active ingredients are schisandrin, schizandrol B, schisantherin A, schisanhenol, and deoxyschizandrin. Owing to its liver-protective, detoxifying, antioxidant, and antitumor activities [64], Wuzhi has been approved for coadministration with tacrolimus for the treatment of drug-induced hepatitis in organ transplant recipients in China [65, 66].

Clinical observations first recognized higher blood levels of tacrolimus when used in combination with Wuzhi. Further studies in humans [67] and animals [68] have confirmed this finding. A meta-analysis [67] evaluating the effects of Wuzhi on tacrolimus pharmacokinetics examined ten RCTs published between 2004 and 2014 that involved 491 patients including mostly kidney or liver transplant recipients and a small number of healthy subjects. Compared with tacrolimus alone, the Tacrolimus-Wuzhi combination significantly increased the plasma concentration of tacrolimus and decreased the dosage of tacrolimus required to maintain the desirable blood concentrations following intervention for one month and three months. These results indicate that Wuzhi capsule can increase the plasma concentration and bioavailability of tacrolimus. The effect appears to be mediated by inhibiting two pathways involved in tacrolimus metabolism, CYP3A and P-glycoprotein, expressed on the intestinal epithelial cells [69, 70].

Based on its successful use in organ transplantation, the use of Tacrolimus-Wuzhi combination was explored for the treatment of autoimmune kidney diseases. In a randomized trial of RNS due to IMN, 60 patients were treated for more than six months with tacrolimus-corticosteroids or tacrolimus-corticosteroids-Wuzhi [71]. The two groups showed similar remission rates, while the group containing Wuzhi used lower doses of tacrolimus and therefore had a higher cost-effectiveness. A recent single-center retrospective study compared tacrolimus alone with the Tacrolimus-Wuzhi combination in 60 patients with autoimmune glomerular disease [72]. The results demonstrated that Wuzhi capsule not only significantly reduced the dosage of tacrolimus required to maintain an effective blood concentration, but also resulted in a higher remission rate (86.7% versus 70.0%) and shorter time to achieve partial remission (2.60 months versus 5.22 months).

All studies to date [65–67, 71, 72] showed that Wuzhi does not increase adverse effects of tacrolimus. In fact, some studies reported improvement in liver functions when Wuzhi was added [67, 72], a finding that is consistent with its known liver-protective activity. Therefore, Wuzhi capsule is a promising tacrolimus-sparing agent for RNS treatment to improve tacrolimus bioavailability, clinical outcome, and pharmacoeconomics.

5.2. Tetrandrine-Glucocorticoid Combination. Tetrandrine is another herb ingredient that has received considerable

attention with increasing numbers of studies dedicated to understanding its therapeutic effects and biological activities [73]. Tetrandrine is the main active ingredient of *Stephania tetrandra* S. Moore, a key herb known for its effectiveness in reducing edema. Historically it has been used in the treatment of a wide range of inflammatory and autoimmune diseases. Since 1981 it has been approved in China for treating silicosis and rheumatoid arthritis [74]. The purported immunomodulatory activity of tetrandrine has presumably qualified it as a candidate and as an alternative disease-modifying antirheumatic drug (DMARD). Synergistic effects have been observed between tetrandrine and DMARDs such as tacrolimus and cyclosporin in patients with rheumatoid arthritis [75].

Despite abundant clinical evidence showing the benefit of tetrandrine in supporting immunosuppressive treatment of autoimmune diseases, mechanistic studies have been largely lacking. Of note is one earlier report by Seow et al. that demonstrated *in vitro* suppression of mitogen-induced lymphoproliferative responses and antibody production by tetrandrine [76].

Based on our clinical success using herbal formulas containing *Stephania tetrandra* in the treatment of edema and chronic kidney disease [35], we proposed that tetrandrine possesses direct immunosuppressive activity or can potentiate the effect of corticosteroids. We therefore carried out studies to investigate the potential synergistic interaction between tetrandrine and methylprednisolone and the possible mechanisms of action.

Mitogen-activated human peripheral blood mononuclear cells (PBMCs) are preferred over isolated T cells as an *in vitro* model for studying the human immune network. We first tested the immunosuppressive effect of methylprednisolone combined with tetrandrine in PBMCs from healthy human subjects [77]. Tetrandrine significantly decreased the half maximal inhibitory concentration (IC_{50}) value of methylprednisolone even at the lowest concentration of 0.3 nM, and no toxic effect was observed with tetrandrine even at the high concentration of 300 nM. Tetrandrine and methylprednisolone both suppressed the production of proinflammatory cytokines TNF-α and IL-6, and the combination showed stronger inhibitory effect. Tetrandrine appears to potentiate the immunosuppressive activity of methylprednisolone via at least two mechanisms. First, it inhibits the function of drug efflux pump P-glycoprotein 170 in T cells and thus increased the intracellular methylprednisolone concentration. Second, tetrandrine in combination with methylprednisolone synergistically inhibits the phosphorylation of mitogen-activated protein kinase family, specifically ERK1/2. However, $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells targeted by some other immunosuppressive drugs such as methotrexate are not affected by tetrandrine and methylprednisolone.

Subsequently, we evaluated the immunosuppressive pharmacodynamics of tetrandrine alone and in combination with methylprednisolone in PBMCs of hemodialysis patients [78]. Tetrandrine alone inhibited the proliferation of PBMCs, with a median (range) IC_{50} value of 1.61 (1.04–4.79) μM. At lower concentrations (0.3–300 nM), tetrandrine significantly decreased the IC_{50} value of methylprednisolone. According to a study on the pharmacokinetics of tetrandrine, the maximum blood concentration of tetrandrine after ingesting 40 mg tetrandrine could reach 17 μM in healthy volunteers [79]. Thus tetrandrine can augment the immunosuppressive effect of methylprednisolone at clinically relevant doses without risks of side effects.

Collectively, our results demonstrate that tetrandrine is not only an immune inhibitor by itself, but also synergistically potentiates the immunosuppressive efficacy of glucocorticoids in healthy subjects and hemodialysis patients. This finding provides a mechanistic explanation to the observed clinical benefits of *Stephania tetrandra* in autoimmune diseases. It also offers a clear rationale for using the combination of tetrandrine and glucocorticoids to reduce steroid resistance and attenuate toxic side effects of steroids.

6. Limitations and Future Studies

It is worth noting that some Chinese herbs mentioned above are associated with potential side effects. *Stephania tetrandra* S. Moore (Fang Ji) is a safe herb. However, historically *Aristolochia fangchi* (Guang Fang Ji) was sometimes mistakenly used as *Stephania tetrandra* S. Moore, partly due to their similar Chinese names. This contributed to aristolochic acid nephropathy events first discovered in Belgium in 1993 [80] and later found widely in China and other Asian regions [81]. Although most countries have banned *Aristolochia fangchi*, we should remain cautious because herbs containing aristolochic acid are still used in traditional herbal remedies [82]. *Tripterygium wilfordii* Hook F. and its extract triptolide, as potent immunosuppressive agents, share features common to immunosuppressants including toxicity. Short-term administration of the low doses traditionally used in TCM practice does not appear to have toxic effects. However, long-term high-dose treatments can cause liver dysfunction, leukopenia, and damage of the reproductive system [83]. Moreover, TCM emphasizes that herb medicine be prescribed based on the comprehensive differential diagnosis of a patient's conditions guided by TCM theories. Otherwise, if herbs are used inappropriately, even nontoxic herbs can cause adverse effects. Therefore, to avoid and minimize side effects, Chinese herbal prescription should follow the diagnosis and treatment principles of TCM.

Current research in this field is limited in several aspects. First, from the perspective of evidence-based medicine, many clinical trials have been poorly designed and have yet to provide robust evidence of efficacy. Therefore, more high-quality clinical studies with large sample sizes are needed to validate the efficacy and safety of Chinese medicine for RNS treatment. A prevailing methodologic drawback in most clinical trials in TCM has been the exclusion of TCM principles in the study design [57]. When Chinese herbal medicine is used to treat a Western medical diagnosis without following TCM principles, any lack of efficacy can arguably result from the mismatch of treatments and diseases in the TCM framework. Therefore, study designs incorporating TCM theories are necessary to improve future RCTs in TCM. Second, there is a lack of understanding of the molecular mechanisms for herbs that have shown promising results

in treating RNS. This applies to multiherb formulations, for example, *Stephania and Astragalus Decoction*, and purified components from single herbs, for example, tetrandrine from *Stephania tetrandra* S. Moore. Not only is detailed and in-depth basic research important to elucidate the mechanisms underlying the therapeutic effects of herbal medicine, but it also has the potential to reveal novel signaling pathways and treatment targets for RNS. Finally, investigations of empirical herbal prescriptions that have been well-tested in clinical practice will likely lead to identification of effective herbs and active compounds not previously recognized.

7. Summary

Recent advances in understanding the pathogenesis of RNS have led to development and testing of new immunosuppressive agents. Although this has resulted in higher remission rates in some RNS patients, poor clinical outcomes persist due to its chronic relapsing nature as well as unsatisfactory efficacy and toxic adverse effects of available immunosuppressive regimens. The urgent need for alternative treatments has prompted renewed interests in exploring traditional Chinese medicine for safer and more effective therapies for RNS. These efforts have provided evidence supporting the integration of Chinese herbal medicine and conventional therapies. Chinese herbal medicine can improve clinical symptoms, reduce proteinuria, and protect kidney functions of RNS patients through regulation of the internal environment. Some herbs can modulate immune function by affecting immune cells directly and thus can be used as alternative treatments when existing therapies have failed. Other herbs can be coadministered with steroids and other immunosuppressants and have synergistic effects in improving therapeutic efficacy and decreasing adverse events of existing therapies. Current research in this field has been limited by relatively low quality of clinical trials and lack of mechanistic studies. In the era of evidence-based precision medicine, more high-quality clinical studies with large sample sizes and incorporation of TCM principles in the study design are necessary to validate the efficacy and safety of Chinese medicine. More basic research is needed to elucidate the mechanisms of action underlying the therapeutic effects of herbal medicine for treating RNS. The great potential that traditional Chinese medicine holds for RNS warrants such efforts.

Conflicts of Interest

The authors have declared that they have no conflicts of interest.

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References

[1] A. A. Eddy and J. M. Symons, "Nephrotic syndrome in childhood," *The Lancet*, vol. 362, no. 9384, pp. 629–639, 2003.

[2] C. Kodner, "Diagnosis and management of nephrotic syndrome in adults," *American Family Physician*, vol. 93, no. 6, pp. 479–485, 2016.

[3] J. Sedor, M. Kretzler, and D. Taylor-Moon, The Nephrotic Syndrome Study Network: A Rare Disease Network for Precision Medicine in Nephrotic Syndrome, 2014, http://www.checkorphan.org/clinical-trials/detail?nctid=NCT0120900.

[4] V. Tesar, T. Zima, and M. Kalousová, "Pathobiochemistry of nephrotic syndrome," *Advances in Clinical Chemistry*, vol. 37, pp. 173–218, 2003.

[5] P. Ronco and H. Debiec, "Pathogenesis of membranous nephropathy: Recent advances and future challenges," *Nature Reviews Nephrology*, vol. 8, no. 4, pp. 203–213, 2012.

[6] A. Kuroki, "M-Type Phospholipase A2 Receptor as Target Antigen in Idiopathic Membranous Nephropathy NEJM," *New England Journal of Medicine*, vol. 361, no. 1, pp. 11–21, 2009.

[7] N. M. Tomas, B. L. C. Meyer-Schwesinger Jr. et al., "Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy," *New England Journal of Medicine*, vol. 372, no. 11, Article ID 2287, 2014.

[8] R. J. Maas, J. K. Deegens, B. Smeets, M. J. Moeller, and J. F. Wetzels, "Minimal change disease and idiopathic FSGS: Manifestations of the same disease," *Nature Reviews Nephrology*, vol. 12, no. 12, pp. 768–776, 2016.

[9] A. B. Fogo, "Causes and pathogenesis of focal segmental glomerulosclerosis," *Nature Reviews Nephrology*, vol. 11, no. 2, pp. 76–87, 2015.

[10] Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group, "KDIGO clinical practice guideline for glomerulonephritis," *Kidney International Supplements*, vol. 2, no. 2, pp. 139–274, 2012.

[11] Z. Zhao, G. Liao, Y. Li, S. Zhou, and H. Zou, "The efficacy and safety of rituximab in treating childhood refractory nephrotic syndrome: A meta-analysis," *Scientific Reports*, vol. 5, article no. 8219, 2015.

[12] R. K. Chandra, "Refractory nephrotic syndrome," *Nihon Rinsho Japanese Journal of Clinical Medicine*, vol. 62, no. 10, pp. 1794–1799, 2004.

[13] K. L. Gibson, P. Hansrivijit, and M. E. Ferris, "Emerging Agents for the Management of Nephrotic Syndrome: Progress to Date," *Pediatric Drugs*, vol. 18, no. 1, pp. 25–29, 2016.

[14] M. Vivarelli, L. Massella, B. Ruggiero, and F. Emma, "Minimal change disease," *Clinical Journal of the American Society of Nephrology*, vol. 12, no. 2, pp. 332–345, 2017.

[15] L. A. Greenbaum, R. Benndorf, and W. E. Smoyer, "Childhood nephrotic syndrome-current and future therapies," *Nature Reviews Nephrology*, vol. 8, no. 8, pp. 445–458, 2012.

[16] V. D. B. Ja, P. R. van Dijk, J. M. Hofstra, and J. F. Wetzels, "Long-term outcomes in idiopathic membranous nephropathy using a restrictive treatment strategy," *Journal of the American Society of Nephrology*, vol. 25, no. 1, pp. 150–158, 2014.

[17] P. Ruggenenti, F. C. Fervenza, and G. Remuzzi, "Treatment of membranous nephropathy: time for a paradigm shift," *Nature Reviews Nephrology*, vol. 13, no. 9, pp. 563–579, 2017.

[18] G. Remuzzi, C. Chiurchiu, M. Abbate, V. Brusegan, M. Bontempelli, and P. Ruggenenti, "Rituximab for idiopathic membranous nephropathy," *The Lancet*, vol. 360, no. 9337, pp. 923-924, 2002.

[19] A. Segarra, M. Praga, N. Ramos et al., "Successful treatment of membranous glomerulonephritis with rituximab in calcineurin inhibitor-dependent patients," *Clinical Journal of the American Society of Nephrology*, vol. 4, no. 6, pp. 1083–1088, 2009.

[20] K. Iijima, D. M. Sako, K. Nozu et al., "Rituximab for childhood-onset, complicated, frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome: a multicentre, double-blind, randomised, placebo-controlled trial," *The Lancet*, vol. 384, no. 9950, pp. 1273–1281, 2014.

[21] P. Ruggenenti, B. Ruggiero, P. Cravedi et al., "Rituximab in steroid-dependent or frequently relapsing idiopathic nephrotic syndrome," *Journal of the American Society of Nephrology*, vol. 25, no. 4, pp. 850–863, 2014.

[22] C. D. Garcia, V. B. Bittencourt, A. Tumelero, J. S. Antonello, D. Malheiros, and V. D. Garcia, "Plasmapheresis for Recurrent Posttransplant Focal Segmental Glomerulosclerosis," *Transplantation Proceedings*, vol. 38, no. 6, pp. 1904-1905, 2006.

[23] T. P. Andolino and J. Reid-Adam, "Nephrotic syndrome," *Pediatrics in Review*, vol. 36, no. 3, pp. 117–126, 2015.

[24] S. Yin, T. He, Y. Li et al., "Rituximab shows no effect on remission in patients with refractory nephrotic syndrome: A MOOSE-compliant meta-analysis," *Medicine (United States)*, vol. 95, no. 50, p. e5320, 2016.

[25] F. Iorember, D. Aviles, M. Kallash, and O. Bamgbola, "Cost analysis on the use of rituximab and calcineurin inhibitors in children and adolescents with steroid-dependent nephrotic syndrome," *Pediatric Nephrology*.

[26] Q. Q. Wang, *Selected Readings from the Yellow Emperor's Inner Classic*, China Press of Traditional Chinese Medicine, Beijing, China, 2nd edition, 2007.

[27] X. Q. Wang, C. J. Wang, and J. Yuan, "Immune glomerular diseases difficulties and Chinese medicine treatment strategies," *Chinese Journal of Integrated Traditional and Western Nephrology*, vol. 11, no. 2, pp. 169–171, 2010.

[28] V. T. Devita, T. S. Lawrence, and S. A. Rosenberg, *Cancer: principles practice of oncology : primer of the molecular biology of cancer*, Wolters Kluwer Health, 2nd edition, 2015.

[29] Z. J. Zhang, *Synopsis of prescriptions of the golden chamber (Jinkui yaolue fanglun)*, Beijing Science and Technology, Beijing, China, 2010.

[30] A. Peng, Y. Gu, and S. Y. Lin, "Herbal treatment for renal diseases," *Annals, Academy of Medicine, Singapore*, vol. 34, no. 1, pp. 44–51, 2005.

[31] J. C. Meng, "Astragalus is effective medicine for chronic proteinuria," *Jiangsu Chinese Medicine Journal*, vol. 16, no. 1, pp. 316-316, 1995.

[32] J. F. Shi, H. W. Zhu, C. Zhang, F. Bian, P. J. Shan, and W. Lu, "Therapeutic effect of Astragalus in patients with chronic glomerulonephritis," *Acta Univ Medicinalis Secondae Shanghai*, vol. 22, no. 3, pp. 245–247, 2002.

[33] M. S. Ahmed, S. H. Hou, M. C. Battaglia, M. M. Picken, and D. J. Leehey, "Treatment of idiopathic membranous nephropathy with the herb *Astragalus membranaceus*," *American Journal of Kidney Diseases*, vol. 50, no. 6, pp. 1028–1032, 2007.

[34] D. J. Leehey, T. Casini, and D. Massey, "Remission of Membranous Nephropathy After Therapy With Astragalus Membranaceus," *American Journal of Kidney Diseases*, vol. 55, no. 4, p. 772, 2010.

[35] Y. J. Wang, L. Q. He, W. Sun, Y. Lu, and X. Q. Wang, "Optimized project of traditional Chinese medicine in treating chronic kidney disease stage 3: a multicenter double-blinded randomized controlled trial," *Journal of Ethnopharmacology*, vol. 139, no. 3, pp. 757–764, 2012.

[36] X. R. Chen, "Clinical observation on curative effect of primary nephrotic syndrome treated with JiaweiFangjiHuangqi Tang and its effects on serum IL-10 and TNF-α," *Traditional Chinese Medicine Technology*, vol. 23, no. 4, pp. 393–395, 2016.

[37] W. J. Du and A. J. Zhan, "Effect of FangjiHuangqi Tang on serum TNF-a and IL6 in primary nephrotic syndrome," *Henan Traditional Chinese Medicine*, vol. 34, no. 5, pp. 805-806, 2014.

[38] J. T. Nie, "Efficacy of fangjihuangqi decoction in treatment of patients with primary nephrotic syndrome in edema period and its influence on 24hr urinary protein, serum albumin and lipid," *Jounral of Clinical Medicine in Practice*, vol. 21, no. 1, pp. 48–51, 2017.

[39] L. Han, Y. Y. Deng, Y. P. Chen et al., "Clinical therapeutic research of idiopathic membranous nephropathy by YiqiHuoxueHuashi Project and analysis of correlative influencing factors," *Chinese Journal Integrated Traditional and Western Nephrolopgy*, vol. 11, no. 10, pp. 879–883, 2010.

[40] L. Wang, Y. P. Chen, Y. Y. Deng et al., "The treatment of membranous nephropathy by, YiqiHuoxueHuashi Project," *Chinese Journal Integrated Traditional and Western Nephrolopgy*, vol. 7, no. 8, pp. 393–396, 2006.

[41] Y. Chen, Y. Deng, Z. Ni et al., "Efficacy and safety of traditional chinese medicine (Shenqi particle) for patients with idiopathic membranous nephropathy: a multicenter randomized controlled clinical trial," *American Journal of Kidney Diseases*, vol. 62, no. 6, pp. 1068–1076, 2013.

[42] S. Y. Chen, "Effect of Tripterygium wilfordii on the remission of proteinuria in patients with nephrotic syndrome," *Chinese Journal of Modern Developments in Traditional Medicine*, vol. 5, no. 3, pp. 164–166, 1985.

[43] Z. H. Liu, S. J. Li, Y. Wu et al., "Treatment of membranous nephropathy with Tripterygium wilfordii and steroid: a prospective randomized control trial," *Chinese Journal of Nephrology Dialysis Transplantation*, vol. 18, no. 4, pp. 303–309, 2009.

[44] K. Zuo, S. J. Li, Y. Wu et al., "Treatment of idiopathic membranous nephropathy with tripterygium wilfordii hook F: a prospective randomized control trial," *Chinese Journal of Nephrology Dialysis Transplantation*, vol. 23, no. 6, pp. 507–511, 2014.

[45] S. Liu, X. Li, H. Li, Q. Liang, J. Chen, and J. Chen, "Comparison of tripterygium wilfordii multiglycosides and tacrolimus in the treatment of idiopathic membranous nephropathy: A prospective cohort study," *BMC Nephrology*, vol. 16, no. 1, Article ID 0199-x, 2015.

[46] Y. Y. Zhang, S. F. Zeng, B. Yan, D. Z. Chen, F. N. Liu, and L. H. Yin, "Tripterygium Glycosides Combined with Glucocorticoid for Primary Nephrotic Syndrome in Adults:a Meta-analysis," *Journal of Chinese General Practice*, vol. 20, no. 14, pp. 1742–1748, 2017.

[47] G. Xu, W. Tu, D. Jiang, and C. Xu, "Tripterygium wilfordii Hook F treatment for idiopathic refractory nephrotic syndrome in adults: a meta-analysis," *Nephron Clinical Practice*, vol. 111, no. 4, pp. c223–c228, 2009.

[48] Y. Chen, Z. Gong, X. Chen et al., "Tripterygium wilfordii Hook F (a traditional Chinese medicine) for primary nephrotic syndrome.," *Cochrane Database of Systematic Reviews*, vol. 8, p. CD008568, 2013.

[49] B. J. Chen, "Triptolide, a novel immunosuppressive and anti-inflammatory agent purified from a Chinese herb Tripterygium

Wilfordii Hook F," *Leukemia & Lymphoma*, vol. 42, no. 3, pp. 253–265, 2001.

[50] R. J. Pei, L. H. Qi, and X. J. Liu, "Mechanism of immunosuppressive effects of triptolide," *Chinese Pharmacological Bulletin*, vol. 9, no. 1, pp. 68–72, 1993.

[51] X. M. Guo, C. Z. Wang, and Zhangjiakou, "Effect of tripholide on peripheral blood lymphocytes IL-5, GM-CSF mRNA and CD4~+, CD8~+ expression in allergic guinea pigs," *Chinese Journal of Pathophysiology*, vol. 15, no. 11, pp. 1003–1005, 1999.

[52] Y. Yang, Z.-H. Liu, E. Tolosa, J.-W. Yang, and L.-S. Li, "Triptolide induces apoptotic death of T lymphocyte," *International Journal of immunopharmacology*, vol. 40, no. 2, pp. 139–149, 1998.

[53] M. A. Chan, J. E. Kohlmeier, M. Branden, M. Jung, and S. H. Benedict, "Triptolide is more effective in preventing T cell proliferation and interferon-gamma production than is FK506," *Phytotherapy Research*, vol. 13, no. 6, pp. 464–467, 1999.

[54] X. Chen, T. Murakami, J. J. Oppenheim, and O. M. Z. Howard, "Triptolide, a constituent of immunosuppressive Chinese herbal medicine, is a potent suppressor of dendritic-cell maturation and trafficking," *Blood*, vol. 106, no. 7, pp. 2409–2416, 2005.

[55] Z.-H. Chen, W.-S. Qin, C.-H. Zeng et al., "Triptolide reduces proteinuria in experimental membranous nephropathy and protects against C5b-9-induced podocyte injury in vitro," *Kidney International*, vol. 77, no. 11, pp. 974–988, 2010.

[56] P. J. Lv, L. B. Wei, L. Chen et al., "Meta-Analysis of combined Chinese and Western Medicine in Primary Refractory Nephritic Syndrome," *Journal of Mathematical Medicine*, vol. 25, no. 3, pp. 332–335, 2012.

[57] F. Y. Fung and Y. C. Linn, "Developing traditional Chinese medicine in the era of evidence-based medicine: current evidences and challenges," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 425037, 9 pages, 2015.

[58] H. Fu, G. Qian, and Z. Jiang, "Comparison of second-line immunosuppressants for childhood refractory nephrotic syndrome: a systematic review and network meta-analysis," *Journal of Investigative Medicine*, vol. 65, no. 1, pp. 65–71, 2016.

[59] E. Gathogo, M. Harber, S. Bhagani et al., "Impact of tacrolimus compared with cyclosporin on the incidence of acute allograft rejection in human immunodeficiency virus-positive kidney transplant recipients," *Transplantation*, vol. 100, no. 4, pp. 871–878, 2016.

[60] M. Kamel, M. Kadian, T. Srinivas, D. Taber, and M. A. Salas, "Tacrolimus confers lower acute rejection rates and better renal allograft survival compared to cyclosporine," *World Journal of Transplantation*, vol. 6, no. 4, p. 697, 2016.

[61] S. M. Goring, A. R. Levy, I. Ghement et al., "A network meta-analysis of the efficacy of belatacept, cyclosporine and tacrolimus for immunosuppression therapy in adult renal transplant recipients," *Current Medical Research and Opinion*, vol. 30, no. 8, pp. 1473–1487, 2014.

[62] M. Rodríguez-Perálvarez, M. Guerrero-Misas, D. Thorburn, B. R. Davidson, E. Tsochatzis, and K. S. Gurusamy, "Maintenance immunosuppression for adults undergoing liver transplantation: A network meta-analysis," *Cochrane Database of Systematic Reviews*, vol. 3, no. 3, Article ID CD011639, 2017.

[63] S. Tremblay and R. R. Alloway, "Clinical Evaluation of Modified Release and Immediate Release Tacrolimus Formulations," *The AAPS Journal*, vol. 19, no. 5, pp. 1332–1347, 2017.

[64] H. D. Chen, H. He, and Y. L. Zhang, "Baiying Decoction in the treatment of 43 cases of chronic hepatitis B," *Journal of Anhui Traditional Chinese Medical College*, vol. 21, no. 6, pp. 17–19, 2002 (Chinese).

[65] S. P. Xie, Q. Yan, and H. Z. Chen, "Study on the clinical application of wuzhi capsule after renal transplantation," *Chinese Journal of Integrated Traditional Western Medicine*, vol. 31, no. 9, pp. 1213–1215, 2011.

[66] K. Wang, Q. S. Qu, Y. X. Zhang, S. Z. Miao, and X. Jiang, "Effects of Wuzhi capsule on blood concentration of tacrolimus after renal transplantation," *J Biol Regul Homeost Agents*, vol. 30, no. 1, pp. 155–159, 2016.

[67] M. Zeng, D. I. Xiao-Hui, W. J. Zhao, Y. G. Yang, and D. O. Pharmacology, "Effect of Wuzhi capsules (or tablets) on blood concentration and pharmacokinetics of tacrolimus in recipients of liver (or kidney) transplantation:a meta-analysis," *Pharmaceutical Journal of Chinese Peoples Liberation Army*, vol. 30, no. 5, pp. 405–409, 2014.

[68] H. Wei, X. Tao, P. Di et al., "Effects of traditional chinese medicine wuzhi capsule on pharmacokinetics of tacrolimus in rats," *Drug Metabolism and Disposition*, vol. 41, no. 7, pp. 1398–1403, 2013.

[69] H. Iwata, Y. Tezuka, S. Kadota, A. Hiratsuka, and T. Watabe, "Identification and characterization of potent CYP3A4 inhibitors in Schisandra fruit extract," *Drug Metabolism and Disposition*, vol. 32, no. 12, pp. 1351–1358, 2004.

[70] W.-F. Fong, C.-K. Wan, C.-Y. Zhu et al., "Schisandrol A from Schisandra chinensis reverses P-glycoprotein-mediated multidrug resistance by affecting Pgp-substrate complexes," *Planta Medica*, vol. 73, no. 3, pp. 212–220, 2007.

[71] Z. Sun, M. Ren, Q. Wu, and X. Du, "Co-administration of Wuzhi capsules and tacrolimus in patients with idiopathic membranous nephropathy: Clinical efficacy and pharmacoeconomics," *International Urology and Nephrology*, vol. 46, no. 10, pp. 1977–1982, 2014.

[72] N. Yang, J. L. Chen, S. F. Liu, and H. Xie, "Tacrolimus alone or combined Wuzhi capsules for the treatment of autoimmune glomerular disease in a single-center retrospective study," *Journal of Medicine and Philosophy*, vol. 38, no. 10, pp. 34–36, 2017.

[73] N. Bhagya and K. R. Chandrashekar, "Tetrandrine—A molecule of wide bioactivity," *Phytochemistry*, vol. 125, pp. 5–13, 2016.

[74] Z. L. Gao, "A new drug to cure silicosis-Tetrandrine," *Radiation Protection Bulletin*, vol. 2, p. 56, 1981.

[75] L.-J. Ho and J.-H. Lai, "Chinese herbs as immunomodulators and potential disease-modifying antirheumatic drugs in autoimmune disorders," *Current Drug Metabolism*, vol. 5, no. 2, pp. 181–192, 2004.

[76] W. K. Seow, A. Ferrante, D. B. H. Goh, A. H. Chalmers, S.-Y. Li, and Y. H. Thong, "In vitro immunosuppressive properties of the plant alkaloid tetrandrine," *International Archives of Allergy and Applied Immunology*, vol. 85, no. 4, pp. 410–415, 1988.

[77] W. Xu, K. Meng, Y. Tu et al., "Tetrandrine potentiates the glucocorticoid pharmacodynamics via inhibiting P-glycoprotein and mitogen-activated protein kinase in mitogen-activated human peripheral blood mononuclear cells," *European Journal of Pharmacology*, vol. 807, pp. 102–108, 2017.

[78] W. C. Xu, K. Meng, J. Kusano et al., "Immunosuppressive efficacy of tetrandrine combined with methylprednisolone against mitogen-activated peripheral blood mononuclear cells of hemodialysis patients," *Clinical Experimental Pharmacology Physiology*, vol. 44, no. 9, pp. 924–931, 2017.

[79] Q. Dong, "Study on the pharmacokinetics of tetrandrine tablets in healthy volunteers," *Journal of Clinical Medicine in Practice*, vol. 15, no. 7, pp. 83–85, 2011.

[80] M. Vanhaelen, R. Vanhaelen-Fastre, P. But, and J. Vanherweghem, "Identification of aristolochic acid in Chinese herbs," *The Lancet*, vol. 343, article 174, 1994.

[81] H.-Y. Yang, P.-C. Chen, and J.-D. Wang, "Chinese herbs containing aristolochic acid associated with renal failure and urothelial carcinoma: A review from epidemiologic observations to causal inference," *BioMed Research International*, vol. 2014, Article ID 569325, 2014.

[82] N. Anandagoda and G. M. Lord, "Preventing aristolochic acid nephropathy," *Clinical Journal of the American Society of Nephrology*, vol. 10, no. 2, pp. 167-168, 2015.

[83] C. Xi, S. Peng, Z. Wu, Q. Zhou, and J. Zhou, "Toxicity of triptolide and the molecular mechanisms involved," *Biomedicine & Pharmacotherapy*, vol. 90, pp. 531–541, 2017.

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Research Article

Experimental Study on the Expression of IL-1β and bFGF in Wound Healing Process of Rabbit Cutaneous Infective Wound in Liu-He-Dan

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Objective. This study applied Liu-He-Dan (LHD) to treat the infective wounds of rabbits to explore the mechanism of LHD in promoting wound healing. *Method*. Five circular infective incisions were generated on the back of each rabbit. Wound dressings were performed every day since postoperative day 1. Ten rabbits were euthanized on days 3, 7, 14, and 21. Each specimen was divided into two parts, one was used for detecting interleukin-1 beta (IL-1β), and the other one was used for detecting basic fibroblast growth factor (bFGF). *Result*. The content of IL-1β in the model group was higher than those in the other groups ($P < 0.05$). The content of IL-1β in the treatment group was lower than the other groups on days 14 and 21. The expression of bFGF in treatment group is significant on days 3, 7, and 14, compared with traditional Chinese medicine group and model group. The expression of bFGF has no significant difference with Western group. *Conclusion*. The research approved that LHD could specifically suppress the expression of IL-1β and upregulate the expression of bFGF in the wound, decreasing the release of inflammatory factor of the infective wounds and promoting the healing of the infective wounds.

1. Introduction

The main etiology of anal fistula is the infection of anal glands, which locates in the internal sphincter. Inflammation, trauma, or fecalith causes the obstruction of the drain ducts of the gland; thus, abscess forms in the intersphincteric space, and 30%~40% of the cases may develop into the anal fistula. The mainly therapeutic modality in China is anal fistulectomy. Dressing change has to be performed after defecation every day at least for a month, which is considered an important therapeutic method in promoting wound healing, but this method increases patients' pain and lengthens the time of wound healing; both are problems in clinical treatment. Traditional Chinese medicine (TCM) has been adopted to accelerate postoperative wound healing for thousands of years. Liu-He-Dan (LHD) is a traditional herbal ointment, which has been proved to be effective in treating acute inflammation, such as acute pancreatitis. It will reduce inflammation and relieve pain. However, there were few reports on the research on controlling chronic inflammation reaction and curative effect on postoperative wound. This study applied LHD to treat the infective wounds of rabbits. We performed the biochemical and immunohistochemical evaluation to explore the mechanism of LHD in promoting wound healing.

2. Materials and Methods

2.1. Animals and Wounds Model. Forty healthy New Zealand white rabbits were purchased from West China Laboratory Animal Service Station, Sichuan University (Chengdu, China, Scxk (chuan) 2013-14). Forty rabbits were kept separately in clean cages and had equal amounts of standard food and water. They were housed in temperature-controlled (18~24°C)and humidity-controlled (40%–60%) rooms with 12-hour light/dark photoperiods and allowed to adapt to their environment for one week before the experiment.

First, rabbits were anesthetized with 0.7% pentobarbital sodium via auricular veins (6 ml/Kg). Then, their back hair was shaved (12 cm × 10 cm), and the wound sites were

disinfected with povidone iodine. Next, five full-thickness circular excisional skin wounds (20 mm in diameter and deep into the fascia) were created on the back of each rabbit with scissors and forceps. Each rabbit had two longitudinal wounds on the left side of the back and three longitudinal wounds on the right side. All the wounds were divided into five groups, control group, model group, treatment group (LDH), Western medicine group (calcium alginate), and traditional Chinese medicine group (shikonin oil), according to the counterclockwise order from the upper left. The wounds were covered with 2 cm diameter circular gauze and infected with 1 ml *Staphylococcus aureus*. The rabbits were kept in cages during the study period, with equal amounts of food and water.

The wounds dressings were changed for the first time on postoperative day 1, during the 21-day experiment period; the rabbits' wounds dressings were carefully changed every day. The calcium alginate and shikonin oil were adopted to the wound surface areas, and LHD was adopted to the surface areas around the wound, with dressing at 24-hour intervals with disposable applicators. Of note, rabbits were excluded from the experiment if dead.

2.2. Wound Healing Rate. On days 3, 7, 14, and 21, ten rabbits were selected randomly to be euthanized, and their wounds areas were drawn on transparent film and calculated by putting the transparent film on the electrocardiograph paper. The percentage of wound closure was calculated as follows: wound closure (%) = (area of original wound-area of actual wound)/area of original wound × 100.

2.3. Histopathological Evaluation of Wound Healing. Circular full-thickness skin from wounds sites of five groups was taken (the wound site with a margin of 2 mm) after the rabbits were euthanized. Each specimen was divided into two parts; one was immediately fixed in buffered formaldehyde (10% formalin) and then sent for histopathological assessments; the specimens were embedded in paraffin, sectioned into 5 μm slices by microtome (Leica, Germany, RM2155), and stained with hematoxylin and eosin. Histopathological evaluation of physiological parameters including the following criteria indicates the wound healing process: acute and chronic inflammatory infiltrates, granulation tissue, collagen deposition, neovascularization, and reepithelialization. Samples were assessed by microscope (Olympus, Tokyo, Japan, BX5), and images were analyzed by CCD (Olympus, Tokyo, Japan, DP73). Two investigators who assessed tissue samples and analyzed images in this study were blinded to the agents given.

2.4. Biochemical Evaluation of Wound Healing. The other part of specimen was irrigated with PBS, and the homogenate was centrifugated at 10,000 rpm at 4°C for 15 min. The content of IL-1β in the granulation tissue was determined by Elisa kit (Beijing Biosynthesis Biotechnology Co., Ltd.) according to the manufacturer's instructions.

2.5. Immunohistochemical Analysis of Wound Healing. The paraffin embedded specimens were sectioned into 5 μm slices, The expression of bFGF in the granulation tissue was determined by immunocytochemistry and rabbit anti-TNF alpha antibody (Beijing Biosynthesis Biotechnology Co., Ltd.) according to the manufacturer's instructions.

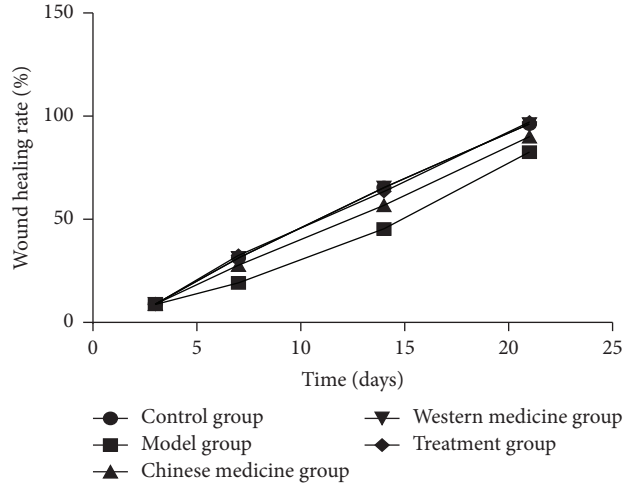


Figure 1: Wound healing rate.

3. Statistical Analysis

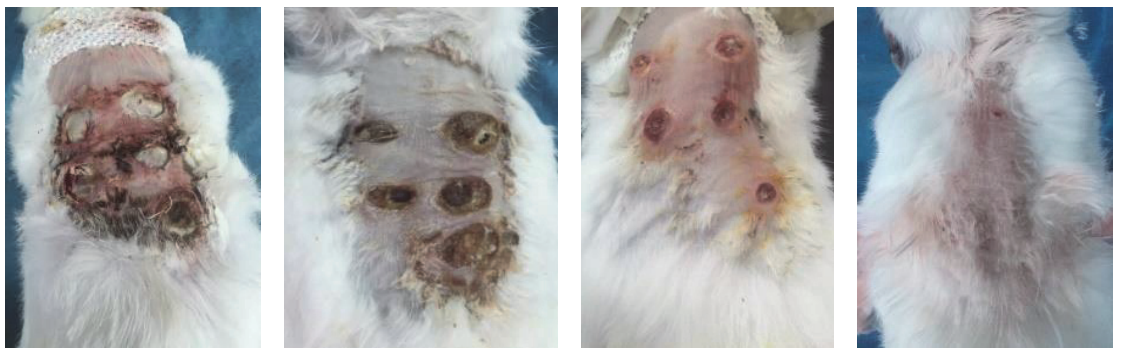
The results of expression of IL-1β were presented as mean ± standard deviation (SD). Statistical comparisons were made with SNK-q (SPSS Statistics software version 17; Chicago, Illinois, USA). The values of $P < 0.05$ were considered statistically significant.

4. Results

4.1. Wound Healing Rate. All rabbits survived during the whole time of the experiment. The results indicated no statistically significant difference among the five groups on day 3. The wound healing rate of treatment group, Western medicine group, and control group were significantly increased compared with model group and traditional Chinese medicine group on days 7, 14, and 21 ($P < 0.05$); the results indicated no statistically significant difference among the three groups ($P > 0.05$) (Figures 1 and 2).

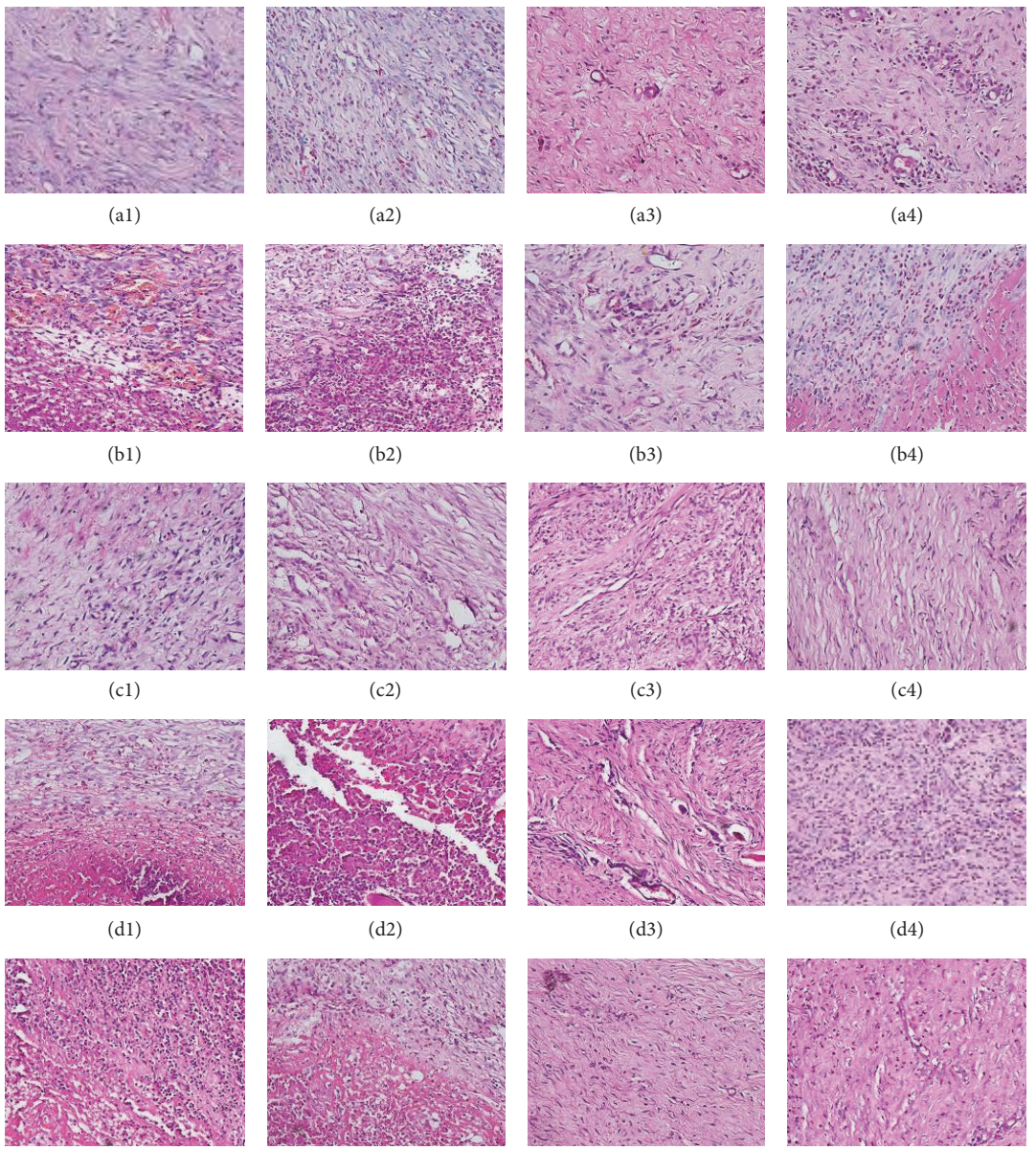
4.2. Histopathological Examinations. Swelling, acute inflammation infiltration, necrotic tissue, and mass inflammatory cell were observed in all the groups on day 3. The inflammatory cell and necrotic tissue were significantly reduced in the treatment group, and inflammatory infiltrates and granulation tissue were observed on day 7. On day 14, skin appeared; fibroblasts, granulation tissue, and new blood vessels were observed. On day 21, the complete wounds closures and collagen deposition, vascular maturation, and scar formation were observed in all groups (Figure 3).

4.3. Biochemical Examinations. The content of IL-1β in model group was higher than in the other groups during the experiment ($P < 0.05$). The content of IL-1β in control group



On day 3 On day 7 On day 14 On day 21

Figure 2: The pictures of wounds healing of days 3, 7, 14, and 21.



(a1) (a2) (a3) (a4)

(b1) (b2) (b3) (b4)

(c1) (c2) (c3) (c4)

(d1) (d2) (d3) (d4)

(e1) (e2) (e3) (e4)

Figure 3: The images of H&E slide of all groups on days 3, 7, 14, and 21. ((a1) control group on day 3, (a2) control group on day 7, (a3) control group on day 14, (a4) control group on day 21, (b1) model group on day 3, (b2) model group on day 7, (b3) model group on day 14, (b4) model group on day 21, (c1) traditional Chinese medicine group on day 3, (c2) traditional Chinese medicine group on day 7, (c3) traditional Chinese medicine group on day 14, (c4) traditional Chinese medicine group on day 21, (d1) treatment group on day 3, (d2) treatment group on day 7, (d3) treatment group on day 14, (d4) treatment group on day 21, (e1) Western medicine group on day 3, (e2) Western medicine group on day 7, (e3) Western medicine group on day 14, and (e4) Western medicine group on day 21).

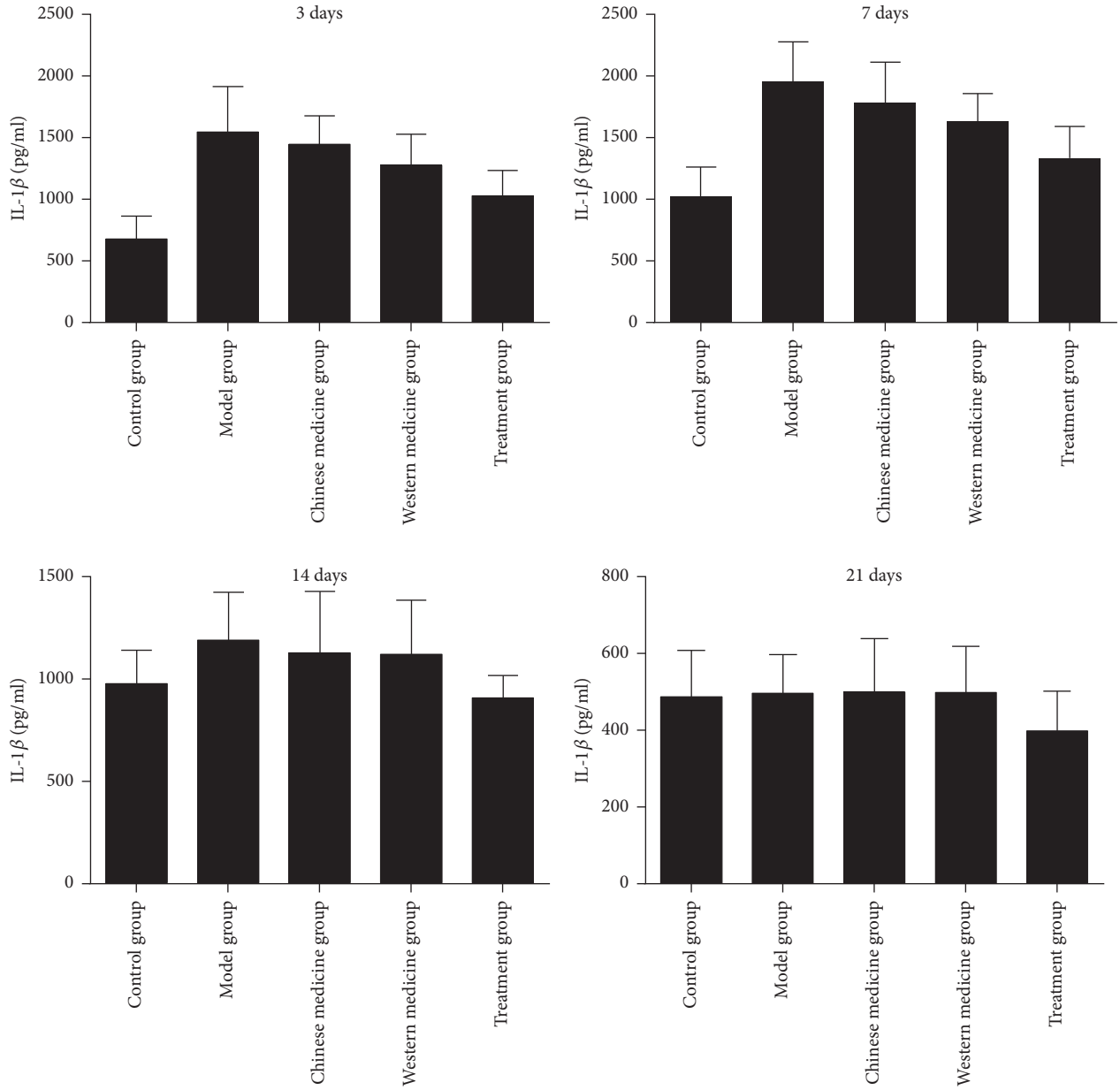


Figure 4: The content of IL-β in granulation tissues.

was lower than those in the other groups on day 3 and day 7 ($P < 0.05$), but the content of IL-1β in the treatment group was lower than the control group on day 14 and day 21 ($P < 0.05$). The content of IL-1β in the treatment group was lower than traditional Chinese medicine group, Western medicine group, and model group on days 3, 7, and 21 ($P < 0.05$). The content of IL-1β in the Western medicine group was lower than traditional Chinese medicine group on day 3 and day 7 ($P < 0.05$), but there was no significant difference between the two groups on day 14 and day 21 (Figure 4).

4.4. Immunohistochemical Analysis. The expression of bFGF in the treatment group has a significant expression on days 3, 7, and 14, compared with the traditional Chinese medicine group and the model group. The expression of bFGF has no significant difference when compared with Western group (Figure 5).

5. Discussion

Anal fistula is a common disease in clinical treatment; the main treatment in China is the anal fistulectomy, which is accepted by most clinical doctors. Wounds healing needs at least a month, which costed more, made time extension of the hospital stays, and reduced living quality. Traditional Chinese medicine has been adapted to postoperative wound and accelerating wound healing for thousands years. However, it lacked convincing evidence, and the mechanism is unclear. LHD is a kind of Chinese herbal ointment, consisting of *Rheum officinale* Baill., *Phellodendron chinense* Schneid.,

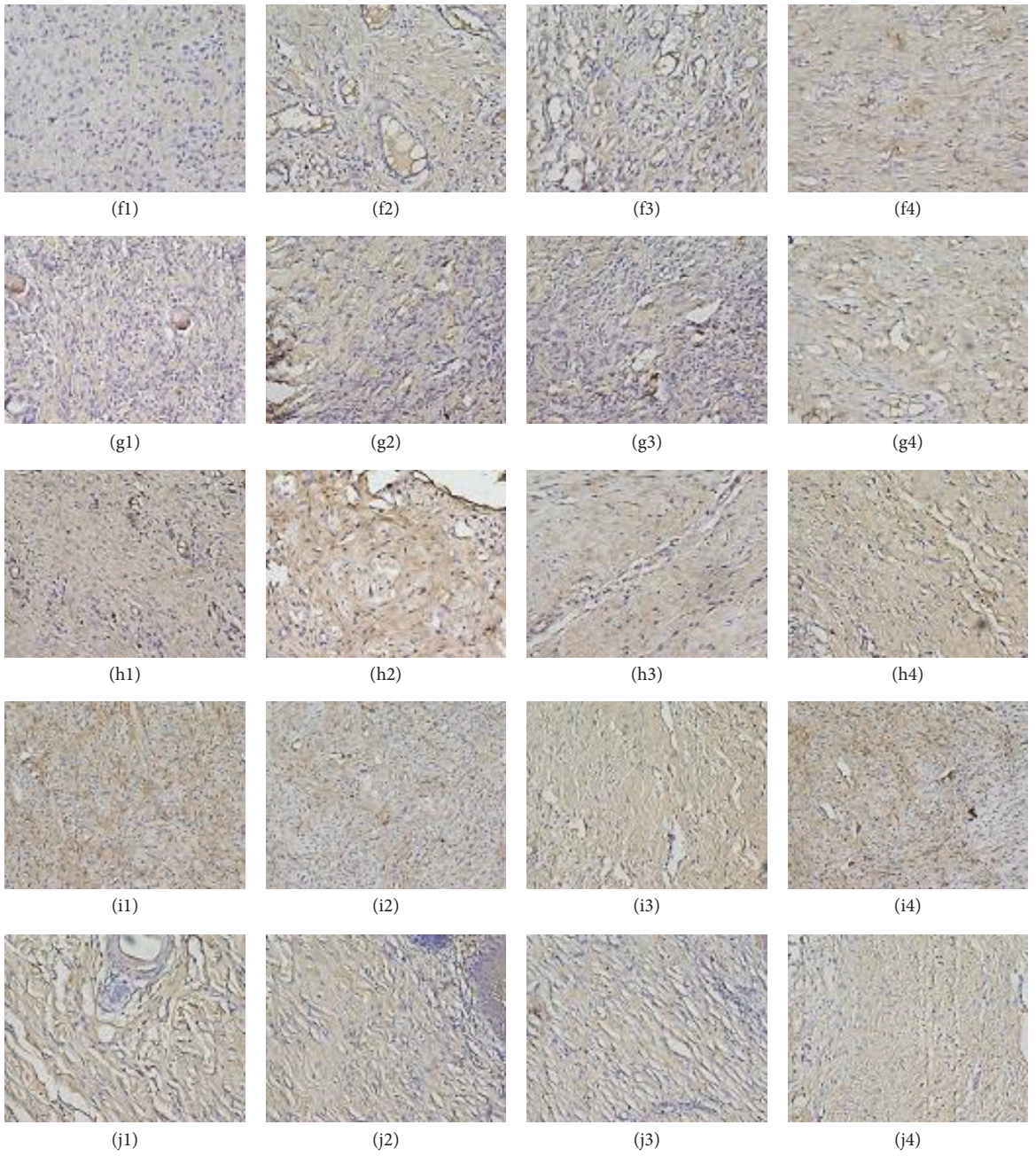


Figure 5: The images of immunohistochemical staining of all groups. ((f1) control group on day 3, (f2) control group on day 7, (f3) control group on day 14, (f4) control group on day 21, (g1) model group on day 3, (g2) model group on day 7, (g3) model group on day 14, (g4) model group on day 21, (h1) traditional Chinese medicine group on day 3, (h2) traditional Chinese medicine group on day 7, (h3) traditional Chinese medicine group on day 14, (h4) traditional Chinese medicine group on day 21, (i1) treatment group on day 3, (i2) treatment group on day 7, (i3) treatment group on day 14, (i4) treatment group on day 21, (j1) Western medicine group on day 3, (j2) Western medicine group on day 7, (j3) Western medicine group on day 14, and (j4) Western medicine group on day 21).

Bletilla striata, *Mentha haplocalyx* Briq., *Angelica dahurica* Benth., honey, flour, and other herbs. LHD is adapted to acute inflammation, pancreatitis, adhesive ileus, skin boil, sores, phlebitis, superficial infection, and acute gouty arthritis, which has been proved to be effective. In the previous study, LHD could reduce serum proinflammatory cytokines, such as IL-6 and CRP [1, 2]. Evidence on LHD applied to postoperative infective wound is insufficient; this study examined the effects of this product in the process of infective wound healing [3].

Wound healing is a response to an injury, which is complex and dynamic sequence of event. The event requires constant communication among participating tissues. This process comprises four phases: hemostasis, inflammation, proliferation, and remodeling. The process includes vascular injury, fibrin-fibronectin clot formation, platelet recruitment,

migration, and proliferation of vascular endothelial cells, fibroblasts, and granulation tissue formation. In the inflammation phase, inflammatory cells migrate into the wound to promote the inflammation phase [4–6]. Inflammatory cells could communicate with each other through cell-cell contact, cytokines, and growth factors [7]. IL-1β is a major proinflammatory cytokine produced by a variety of immune cells including keratinocytes, monocytes, and other epithelial cells, involved in the inflammatory response [8, 9]. IL-1β upregulates adhesion molecule expression, activates neutrophils, promotes the secretion of other proinflammatory cytokines, and further contributes to local inflammatory response [10], which is thought to be deleterious in the wound repair.

In the proliferation phase, growth factors as bFGF and VEGF are effectors to stimulate tissue deposition and epithelialization, accelerating wound healing. bFGF is a member of a large FGF family and induces angiogenesis, endothelial cell migration, and fibroblast proliferation in the wound healing process [11–14].

Generally, the direct and effective parameters involved in wound healing effects are wound healing rate, wound healing duration, and pathological analysis of wounds [15]. Persistent inflammation in wound generates delay in wound healing and wound chronicity [16]. Our data showed that LHD would inhibit the content of IL-1β, IL-1β has a significant expression on day 7 in all the groups, and then the content of IL-1β was suppressed on days 14 and 21. However, the content of IL-1β in treatment group was lower than in the other groups on days 3, 7, 14, and 21. LHD would promote the expression of bFGF, on days 3, 7, and 14; the expression of bFGF in the treatment group was stronger than the traditional Chinese group and the model group; however, there was no significant difference when compared with Western group. Thus, the wound healing rate of treatment group is higher than the other groups on days 7 and 21.

Our result indicates that LHD can inhibit the content of IL-1β and upregulate the expression of bFGF. Thereby, LHD alleviates the duration of inflammation and increases neovascularization, vascular endothelial cells, and fibroblasts in the granulation tissue. LHD promotes the expression of growth factors, bFGF, accelerating proliferation of resident fibroblasts; thus the number of fibroblasts increases.

In conclusion, LHD can promote cutaneous excisional wound healing by inhibiting the expression of IL-1β and upregulating the expression of bFGF, revealing primary mechanism to accelerate wound healing, but further studies are still needed.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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References

[1] S. F. Zhu, W. W. Chen, and J. Xiang, "Pharmacokinetic and pharmacodynamic comparison of chinese herbal ointment liu-he-dan and micron liu-he-dan ointment in rats with acute pancreatitis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 389576, 7 pages, 2014.

[2] M.-H. Wan, X.-L. Zhao, W.-W. Chen et al., "Therapeutic effect of Chinese Herbal ointment Liu-He-Dan in patients with acute Pancreatitis," *Chinese Medical Journal*, vol. 129, no. 9, pp. 1128–1130, 2016.

[3] D. S. Lee, S. Sinno, and A. Khachemoune, "Honey and wound healing: an overview," *American Journal of Clinical Dermatology*, vol. 12, no. 3, pp. 181–190, 2011.

[4] M. Peppa, P. Stavroulakis, and S. A. Raptis, "Advanced glycoxidation products and impaired diabetic wound healing," *Wound Repair and Regeneration*, vol. 17, no. 4, pp. 461–472, 2009.

[5] G. C. Gurtner, S. Werner, Y. Barrandon, and M. T. Longaker, "Wound repair and regeneration," *Nature*, vol. 453, no. 7193, pp. 314–321, 2008.

[6] K. Dzobo, T. Turnley, A. Wishart et al., "Fibroblast-derived extracellular matrix induces chondrogenic differentiation in human adipose-derived mesenchymal stromal/stem cells in vitro," *International Journal of Molecular Sciences*, vol. 17, no. 8, article no. 1259, 2016.

[7] E. Engelhardt, A. Toksoy, M. Goebeler et al., "Chemokines IL-8, GROα, MCP-1, IP-10, and mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing," *The American Journal of Pathology*, vol. 153, no. 6, pp. 1849–1860, 1998.

[8] J. E. Park and A. Barbul, "Understanding the role of immune regulation in wound healing," *The American Journal of Surgery*, vol. 187, no. 5, supplement 1, pp. S11–S16, 2004.

[9] G. Rogler and T. Andus, "Cytokines in inflammatory bowel disease," *World Journal of Surgery*, vol. 22, no. 4, pp. 382–389, 1998.

[10] W. Wagner and M. Wehrmann, "Differential cytokine activity and morphology during wound healing in the neonatal and adult rat skin," *Journal of Cellular and Molecular Medicine*, vol. 11, no. 6, pp. 1342–1351, 2009.

[11] Y. H. Xuan, B. B. Huang, H. S. Tian et al., "High-glucose inhibits human fibroblast cell migration in wound healing via repression of bFGF-regulating JNK phosphorylation," *PLoS ONE*, vol. 9, no. 9, Article ID e108182, 2014.

[12] A. Bikfalvi, S. Klein, G. Pintucci, and D. B. Rifkin, "Biological roles of fibroblast growth factor-2," *Endocrine Reviews*, vol. 18, no. 1, pp. 26–45, 1997.

[13] L. Yan, W. Wu, Z. Wang et al., "Comparative study of the effects of recombinant human epidermal growth factor and basic fibroblast growth factor on corneal epithelial wound healing and neovascularization in vivo and in vitro," *Ophthalmic Research*, vol. 49, no. 3, pp. 150–160, 2013.

[14] S. Kaikai, S. Yuchen, J. Lili, and W. Zhengtao, "Critical role of c-Jun N-terminal kinase in regulating bFGF-induced angiogenesis in vitro," *The Journal of Biochemistry*, vol. 150, no. 2, pp. 189–197, 2011.

[15] A. Ali, D. N. Herndon, A. Mamachen et al., "Propranolol attenuates hemorrhage and accelerates wound healing in severely burned adults," *Critical Care*, vol. 19, no. 1, article no. 217, 2015.

[16] T. Dinh, F. Tecilazich, A. Kafanas et al., "Mechanisms involved in the development and healing of diabetic foot ulceration," *Diabetes*, vol. 61, no. 11, pp. 2937–2947, 2012.

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Research Article

Icariin Prevents IL-1β-Induced Apoptosis in Human Nucleus Pulposus via the PI3K/AKT Pathway

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Purpose. To explore the effect and possible mechanism of icariin, a prenylated flavonol glycoside derived from the Chinese herb *Epimedium sagittatum* that was applied to IL-1β pretreated human nucleus pulposus (NP) cells. *Methods*. Human NP cells were isolated from intervertebral discs of patients with scoliosis and lumbar spondylolisthesis. The cells were divided into five groups: A (blank control); B (20 ng/ml IL-1β); C (20 ng/ml IL-1β + 20 μM icariin); D (20 μM icariin + 20 ng/ml IL-1β + 25 μM LY294002); E (20 ng/ml IL-1β + 25 μM LY294002). For each of the five groups, the CCK8, apoptosis rates, ROS rates, and JC-1 rates were determined and an electron micrograph was performed. Different expression levels of apoptosis proteins and proteins in the PI3K/AKT pathway were detected via western blot. *Results*. We found that the damage effects on human nucleus pulposus cells from 20 ng/ml of IL-1β exposure were attenuated by icariin. When the PI3K/AKT pathway was blocked by LY294002, a specific inhibitor of this pathway, the protective effect of icariin was impaired. In summary, icariin might be a protective traditional Chinese medicine, which prevents inflammation-induced degeneration of intervertebral discs partly through the PI3K/AKT pathway.

1. Introduction

Inflammation is involved in many pathological processes and is associated with the degeneration of intervertebral disc [1]. Nucleus pulposus, located in the center of intervertebral discs, lacks a blood supply, oxygen, and nutrition [2]. It is often exposed to an inflammatory microenvironment. IL-1β, a proinflammatory cytokine involved in inflammatory processes and the induction of apoptosis in response to cell injury, has been reported to have a connection with the degeneration of intervertebral discs [3]. Low back pain (LBP) is a frequent musculoskeletal disorder worldwide that affects approximately 70% of the adult population, sometime during their lives, and frequently results in musculoskeletal disability. According to statistics [4–8], LBP can exert an enormous economic burden every year.

Icariin is the most frequently used medicinal herb in traditional Chinese medicine. This micromolecule steroid compound is extracted from herba epimedii and is mostly applied in the treatment of osteoporosis, especially in postmenopausal osteoporosis. What is more, icariin has also been reported to have protective effects against amyloid beta-induced apoptosis in PC-12 cells [9], attenuating LPS-induced acute inflammatory responses [10], and protecting against MPP(+)-induced toxicity in MES23.5 cells [11]. Additionally, several complex roles for icariin in a number of pathway activation instances have been proposed, such as the PI3K/AKT pathway [9, 10, 12–15], the ROS/JNK-dependent mitochondrial pathway [16], the Wnt/beta-catenin pathway [17], and NO/cGMP signaling [18].

In this work, we simulated an inflammatory activation microenvironment in the intervertebral disc through the use

of IL-1β at 20 ng/ml. We try to explore the protective effect of icariin on IL-1β-induced inflammatory intervertebral disc model. We also used LY294002, an inhibitor of the PI3K/AKT pathway, in order to verify the PI3K/AKT pathway participating in the anti-inflammatory function of icariin.

2. Materials and Methods

2.1. General Supplies. Instruments, reagents, and the experimental animals were provided by the animal center of Tongji Medical College and Huazhong University of Science and Technology. IL-1β was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Icariin (purity ≥ 98%) was purchased from Nanjing Zelang Pharmaceutical Technology (Nanjing, China). Fetal bovine serum was purchased from Gibco. F12-Dulbecco's modified Eagle medium was purchased from Hyclone (Logan, UT, USA). Cell counting kit-8 (CCK8) was purchased from Kaiji Bioengineering Institute (Jiangsu, China). LY294002 was purchased from Sigma-Aldrich (St. Louis, MO, USA). The reactive oxygen species (ROS) detection kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). JC-1 assay kit was purchased from Beyotime (Beijing, China). Annexin V-FITC/propidium iodide detection kit was purchased from Nanjing KeyGen Biotech (Nanjing, China). β-Actin, Bcl-2, bax, caspase-3, phospho(p)-AKT, rabbit monoclonal antibodies, and the p53 and AKT mouse monoclonal antibody were purchased from Abcam (Cambridge, UK). Goat antirabbit and goat antimouse IgG were purchased from Proteintech (Wuhan, China). Microplate reader was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The inverted fluorescence microscope used was manufactured by Olympus (Japan).

2.2. Culture and Synchronization of the NP Cells and the Detection of Cell Density and Morphology [19]. The density and morphology of NP cells under different treatments were observed and photographed with an inverted phase contrast microscope. NP cells were isolated from the nucleus pulposus tissue from a patient that underwent surgery for scoliosis. Briefly, NP tissue was aseptically removed, placed in a Petri dish containing 0.25% (w/v) type II collagenase, and cut into 0.1 mm × 0.1 mm pieces. Samples were digested with 0.25% (w/v) type II collagenase overnight and serum was used to stop the reaction. After centrifugation at 1200 rpm for 7 min, the supernatant was discarded and the pellet was resuspended in F12-Dulbecco's modified Eagle medium, supplemented with 20% fetal bovine serum, 100 U/mL penicillin, and 100 mg/L streptomycin. Cell cultures were maintained at 37°C and 5% CO_2. The medium was changed 3–5 days later, when the cells had been attached, and then changed every other day after that. When NP cells reached approximately 80% confluence, each primary culture was subcultured at a 1 : 3 ratio with a 0.25% (w/v) trypsin solution.

2.3. Experimental Protocols. Cells were tested for the ability of icariin to activate the PI3K/AKT pathway. Kinetics of the phosphorylation of AKT were estimated by western blot analysis at 0 h, 1 h, 2 h, 3 h, 4 h, and 5 h. The remaining cells were randomly separated into five groups with at least three replicates: A (blank control); B (20 ng/ml IL-1β); C (20 ng/ml IL-1β + 20 μM icariin); D (20 μM icariin + 20 ng/ml IL-1β + 25 μM LY294002); E (20 ng/ml IL-1β + 25 μM LY294002). Treatment with LY294002, icariin, and IL-1β was performed for 2 h, 24 h, and 48 h, respectively. Icariin and LY294002 were both preporcessed. That means we added LY294002 in the medium for 2 h and then took it out by transferring the medium. Icariin was then added for 24 h and then removed. In the end, IL-1β was added for 48 h and different detection was conducted.

2.4. Detection of Icariin Cytotoxicity, Cell Viability, and Proliferation. The cytotoxicity of cells exposed to icariin treatments was evaluated by measuring lactate dehydrogenase (LDH) release using a CytoTox96® Non-Radioactive Cytotoxicity Assay kit (Promega), according to the manufacturer's instructions.

When exposed to different concentration of icariin, the cell viability was detected by CCK8 assay. NP cells at passage 3 were replated in 96-well plates at a density 1×10^5 cells per well, and the culture medium was plated after synchronization. Cells were then treated with icariin for 24 h at various concentrations (0.1, 0.5, 1, 5, 10, 20, 40, and 50 μM) to evaluate the effect of icariin on cell's proliferation rate. Cell viability was detected according to the instructions of the CCK8 assay. Then cells were treated according to the aforementioned experimental groupings. Cell viability was again detected according to the manufacturer's instructions.

2.5. Apoptosis Assay [19]. Cells were harvested and washed with PBS twice at 4°C. Next, cells were resuspended in 200 μL of binding buffer and incubated with 10 μL of Annexin V-FITC solution (15 min, room temperature) in the dark. Then cells were incubated with 10 μL PI and 300 μL binding buffer and immediately analyzed in a BD FACSCalibur cytometer to separate living cells, apoptotic cells, and necrotic cells into different periods.

2.6. Observation by Transmission Electron Microscope. The cells were double-fixed by glutaraldehyde and osmic acid, dehydrated by gradient acetone, immersed in embedding medium, ultrathin-sectioned using an automatic microtome (LeicaRM2235, Leica, Germany), and stained with 1% uranyl acetate. The cells' sections were observed and filmed under a transmission electron microscope (Hitachi, Japan) to observe the status of mitochondria in the human NP cells.

2.7. Mitochondrial Membrane Potential. Changes in the mitochondrial membrane potential were monitored using a JC-1 assay kit (Beyotime, Beijing, China), according to the manufacturer's instructions. Purified mitochondrial pellets (0.1 mL), with a total protein content of 100 μg, were incubated with 0.9 mL of JC-1 dye working solution for 20 min, and the fluorescence intensity was immediately measured using a fluorescence spectrophotometer (Shimadzu RF 5301, Kyoto, Japan). In the mitochondria with high membrane potential, the JC-1 dye mainly existed in the mitochondrial

matrix with red fluorescent aggregates, while green fluorescence represented the monomeric form of JC-1 and the mitochondria with low membrane potential. The red/green fluorescence intensity ratio was used to denote the level of mitochondrial membrane potential depolarization (Ex = 525 nm and Em = 590 nm for aggregates; Ex = 490 nm and Em = 530 nm for JC-1 monomers).

2.8. Detection of Intracellular ROS Levels by Flow Cytometry. Cells were treated according to the aforementioned experiment grouping design. Then, 200 μL of culture medium from each group was collected to detect intracellular ROS levels. Experimental steps were strictly executed according to the manufacturer's instructions.

2.9. Expression of AKT, p-AKT, p53, Bcl-2, Bax, and Caspase-3 by Western Blot Analysis. Proteins were extracted according to the instructions of the Total Extraction Sample Kit. Equal amounts of the proteins (10 μg) were loaded onto 10% sodium dodecyl sulfate polyacrylamide gels, electrophoresed, and then transferred to polyvinylidene fluoride membranes. The membranes were incubated with 5% nonfat milk for 2 h followed by incubation with primary antibodies overnight at 4°C (0.5 μg/mL AKT, p53, p-AKT, Bcl-2, and caspase-3; 1 : 5000). After washing in TBST, membranes were incubated with the secondary antibody for 1.5 h at room temperature (rabbit anti-mouse or goat anti-rabbit, 1 : 5000). Bands were visualized by incubating with an enhanced chemiluminescent reagent for 2 min after the membranes were washed with TBST. Densitometry measurements of p-AKT, AKT, Bcl-2, bax, and caspase-3 levels were performed using Image J software (National Institutes of Health, Bethesda, MD, USA).

2.10. Statistical Analysis. Data are presented as means ± standard deviation. For group-wise comparisons, a one-way ANOVA with the LSD or Dunnett's T3 test was performed using SPSS 19.0 (IBM, Chicago, IL, USA). Values were considered significantly different at $^{*}P < 0.05$.

3. Results

3.1. Human Nucleus Pulposus Cells Were Successfully Separated and Cultured (The Cell Photograph Was Shown in Figure 1(a)). IL-1β at concentration of 10 ng/ml and 20 ng/ml both delayed the grow rate in human nucleus pulposus cells and as the concentration goes up, inhibiting effect has been strengthened. We used the concentration of 20 ng/ml for follow-up experiment for its strong effect of inhibition and damage. We found that, over time, nucleus pulposus cells treated with IL-1β grew slower compared to the control group. Results were shown as a growth curve in Figure 1(b). What is more, we found that icariin had no promoting or inhibiting effect in cell proliferation at the concentration of 0.1 uM to 50 uM but when concentration reached 50 uM, cytotoxicity could be detected by LDH release assay. Results were shown in Figures 1(c) and 1(d). What is more, LY294002 has no cytotoxicity at working concentration of 25 uM with the time. Results were shown in Figure 1(e).

3.2. Icariin Decreased IL-1β-Induced Apoptosis Rate in Human Nucleus Pulposus Cells. Apoptosis rate was detected by flow cytometry as shown in Figure 2. when IL-1β was added to the culture medium, human NP cells died at a significantly higher rate (Figures 2(a) and 2(b)) ($^{*}P < 0.05$). Pretreated with 20 μM icariin for 24 h, the apoptosis rate decreased significantly (Figure 2(c)) ($^{*}P < 0.05$). When the PI3K/AKT pathway was blocked by LY294002, this protective effect was attenuated (Figure 2(d)) ($^{*}P < 0.05$). What is more, we found that if LY294002 was pretreated alone, compared with group B (20 ng/mL IL-1β), group E (20 ng/ml IL-1β + 25 μM LY294002) showed higher apoptosis rates (Figure 2(e)) ($^{*}P < 0.05$). We thought LY294002 could be considered a risk factor alone.

3.3. Icariin Could Attenuate IL-1β-Induced Intercellular ROS Accumulation in Human Nucleus Pulposus Cells as Shown in Figure 3. Intercellular ROS rates could reflect an oxidative stress status and were closely related to intercellular inflammation. ROS plays a key role in the inflammatory and cell damage processes. We observed that IL-1β induced the increase of intercellular ROS rate (Figures 3(a) and 3(b)), while icariin attenuated the damage (Figure 3(c)). The PI3K/AKT pathway was involved in this process (Figures 3(d) and 3(e)) ($^{**}P < 0.01$ versus control group).

3.4. Icariin Attenuated IL-1β-Induced Mitochondrial Membrane Potential (MMP) Losses (Figure 4). The analysis of changes in MMP demonstrated that occur during apoptosis provides important information on the mechanisms and pathways of cell death. Changes in mitochondrial membrane potential were considered to be an early indicator of apoptosis. Variation in MMP could reflect the membrane state of cells.

3.5. Icariin Protects Human NP Cells from IL-1β-Induced Mitochondria Damage (Figure 5). Using transmission electron microscopy, we found IL-1β could induce mitochondrial swelling and membrane breakup (Figure 5(a)). In icariin pretreated cells, the damage was attenuated and protective effects were weakened by LY294002 (Figures 5(b)–5(d)). Compared to group B, group E sustained the most damage (Figure 5(e)), which indicated an independent injury function of LY294002.

3.6. Icariin Influences Apoptosis-Related Proteins, including Apoptosis Protein Bax, Caspase-3, and Antiapoptosis Protein Bcl-2 (Figure 6). The result of western blot showed that icariin has a strong antiapoptosis effect on human NP cells, when cells were exposed to an inflammatory environment ($^{**}P < 0.01$ versus control group, $^{*}P < 0.05$ versus control group).

3.7. Icariin Had a Strong Stimulative Effect on the PI3K/AKT Pathway of Human Nucleus Pulposus (Figure 7(a)). Over time, the stimulative effects of icariin on the PI3K/AKT pathway became more evident. During the observation period, after the 4 h intervention, a remarkable stimulative effect of icariin on the PI3K/AKT pathway was evident.

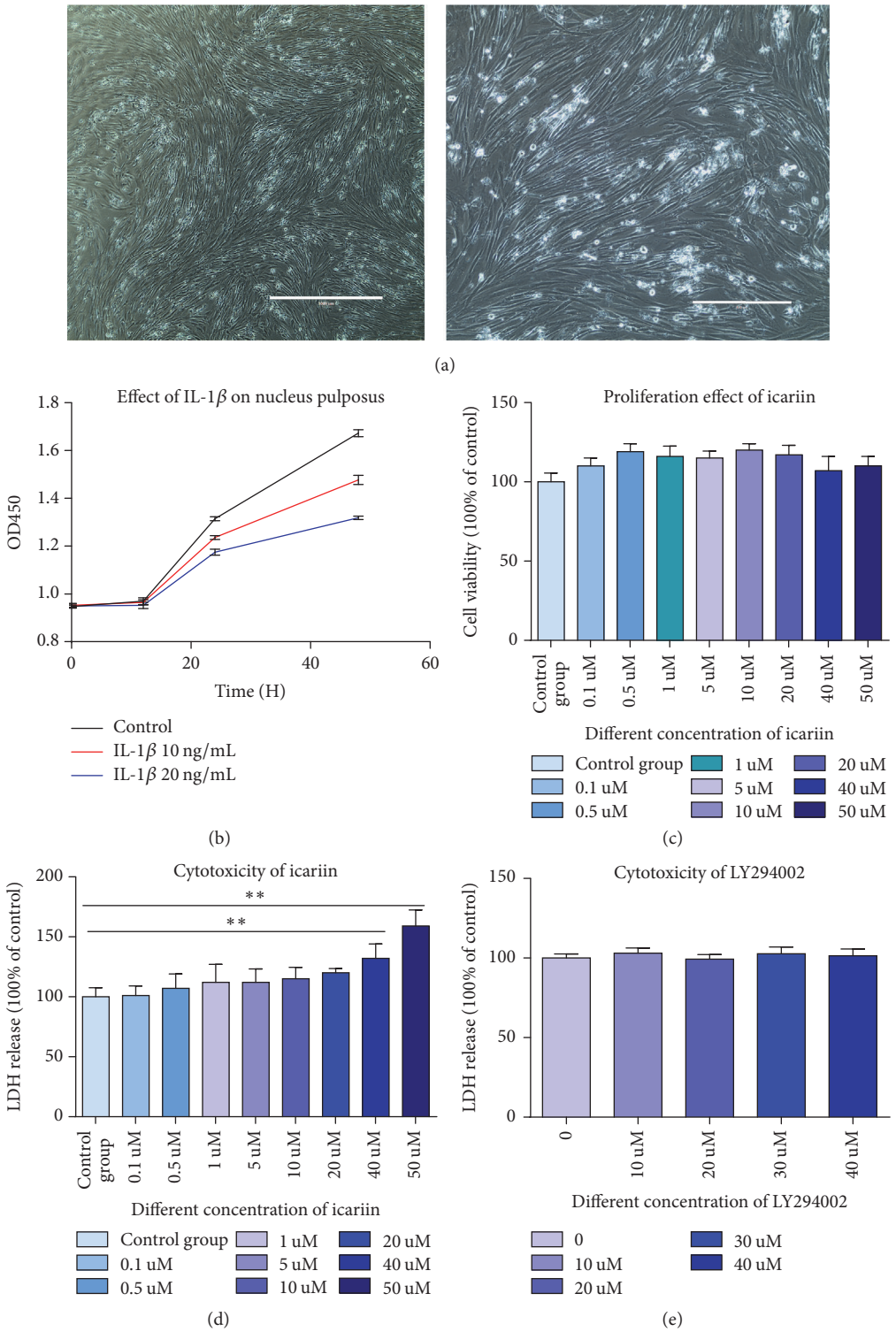


Figure 1: (a) Human NP cells showed a long spindle shape and exhibited good growth. (b) IL-1β retarded human nucleus pulposus growth over time and depending on its concentration. (c) Icariin has no promoting or inhibiting effects on cell proliferation at the concentration of 0.1 uM to 20 uM. (d) There was no cytotoxicity of icariin on cell at the concentration of 0.1 uM to 40 uM. When its concentration reached 40 uM, the cell membrane was observed to be unstable. There was significant difference between control group and 40 uM and 50 uM ($^{**}p < 0.01$).

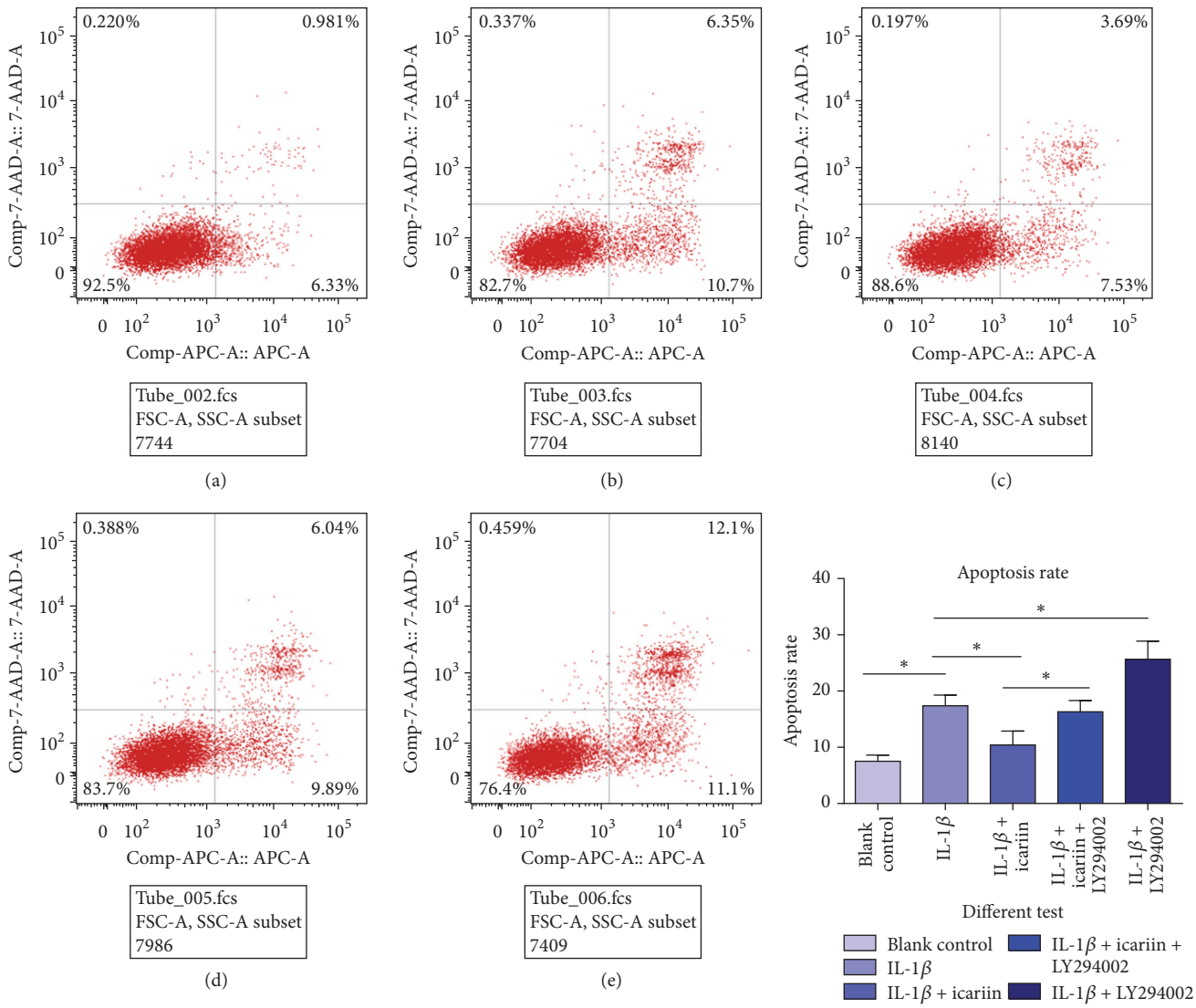


FIGURE 2: (a) Blank control; (b) 20 ng/ml IL-1β; (c) 20 ng/ml IL-1β + 20 μM icariin; (d) 20 μM icariin + 20 ng/ml IL-1β + 25 μM LY294002; (e) 20 ng/ml IL-1β + 25 μM LY294002. Compared with group A, IL-1β could induce apoptosis in human NP cells. When cells were pretreated with icariin, the apoptosis rate decreased. However, we noted that the PI3K/AKT pathway was involved in this protective effect, as the apoptosis rate increased when the PI3K/AKT pathway was blocked. All results were statistically significant ($^{*}P < 0.05$).

These results provided strong evidences that icariin had the ability to stimulate the PI3K/AKT pathway alone. What is more, the PI3K/AKT pathway was involved in the protective process (Figure 7(b)) ($^{**}P < 0.01$ versus control group, $^{*}P < 0.05$ versus control group). AKT is a vital molecule in the PI3K/AKT pathway and p-AKT is biologically active in the same pathway. P53 is a protein downstream and is inhibited by activated AKT (p-AKT). This variation indicated an important effect of the PI3K/AKT pathway in the process.

4. Discussion

Inflammation is an important risk factor of intervertebral disc degeneration (IDD). Some inflammatory cytokines, such as IL-1β, IL-6, IL-10, and TNF-α, have been explored to be related to IDD [20–27]. Excessive deposition of inflammatory cytokines in the microenvironment of intervertebral disc contributes to degeneration of the intervertebral disc [28]. As a traditional Chinese medicine, icariin has a strong antioxidative [29–32] and anti-inflammatory effect [33–36] in many vital tissues; however, there were no studies reporting its effect in the intervertebral disc.

We have simulated an inflammatory microenvironment in human NP cells using IL-1β, an important inflammatory factor. We found cytotoxicity of IL-1β at a concentration of 20 ng/mL, which could induce apoptosis for human NP cells; thus we used this concentration for our experiment.

Icariin had a strong protective effect on IL-1β pretreated human NP cells. This anti-inflammatory effect was detected by the stabilization of mitochondrial membrane potential,

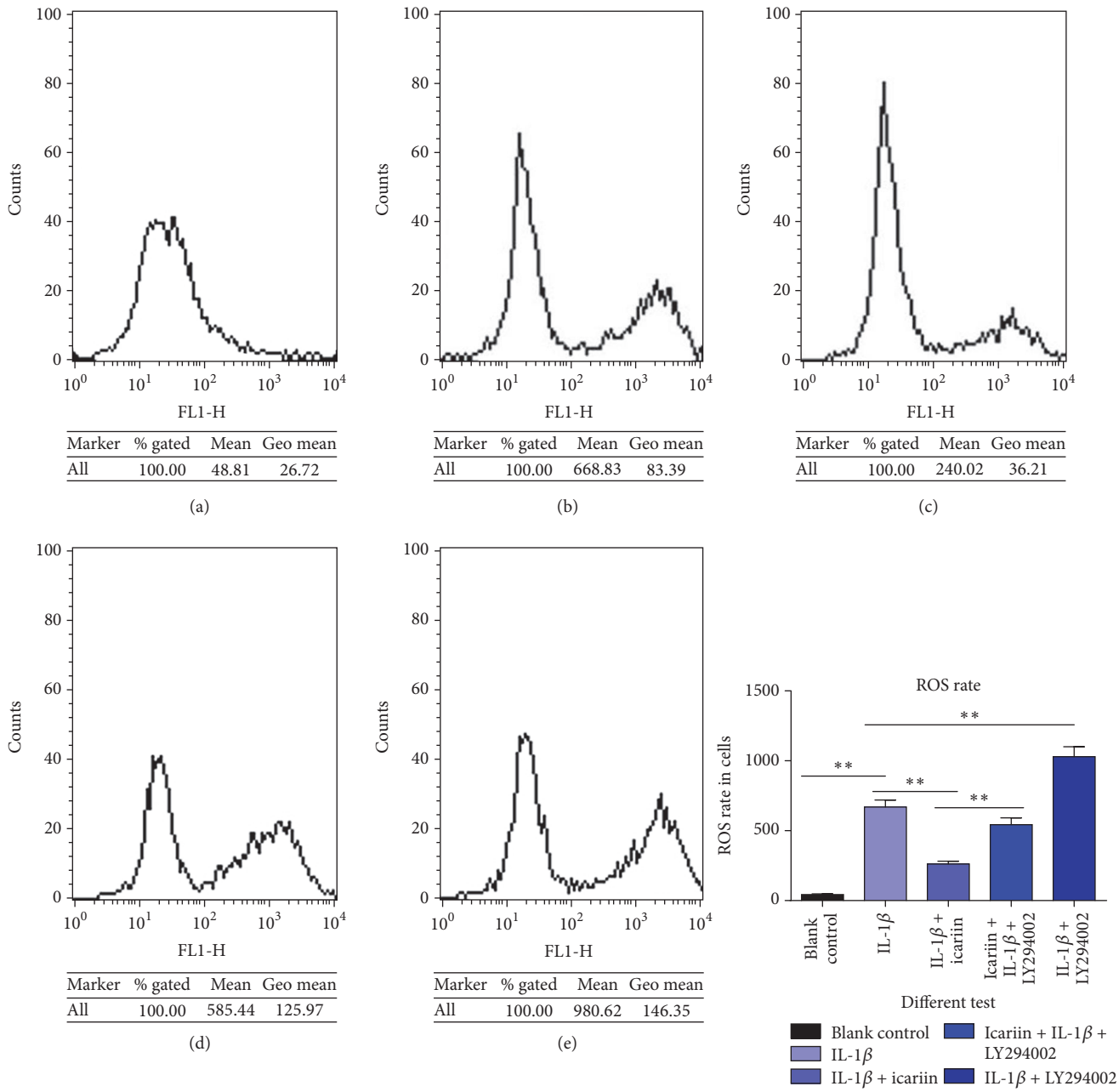


Figure 3: (a) Blank control; (b) 20 ng/ml IL-1β; (c) 20 ng/ml IL-1β + 20 μM icariin; (d) 20 μM icariin + 20 ng/ml IL-1β + 25 μM LY294002; (e) 20 ng/ml IL-1β + 25 μM LY294002. Intercellular ROS rates increased when human NP cells were exposed to 20 ng/mL IL-1β. Icariin attenuated the effect of IL-1β, to a certain extent. When PI3K/AKT pathway was blocked by LY294002, the protective effect of icariin was weakened. What is more, LY294002 could be an independent damage factor or had a synergistic effect with IL-1β to raise the intercellular ROS rate.

apoptosis rate, apoptosis relative proteins, intracellular ROS rate, and imaging cells in an electron microscope. All of these results supported each other and indicated that icariin could protect against IL-1β-induced cell apoptosis and cellular instability. In many other areas, the similar function of icariin was observed, such as cerebral ischemia-reperfusion injury [37, 38], osteolysis and inflammatory response in bone [39], and lipopolysaccharide-induced brain dysfunction [40]. We conclude that icariin has an anti-inflammatory effect and, to our knowledge, this paper is the first one to point out that it may have potential application in NP cells and intervertebral disc degeneration.

In addition to the protect effect of icariin in an inflammatory environment, we explored possible mechanism behind this phenomenon. The PI3K/AKT pathway is important to and responsible for cell life cycle, proliferation, aging, survival, and apoptosis [41]. There are many literatures proposing that PI3K/AKT may be a target molecular signaling

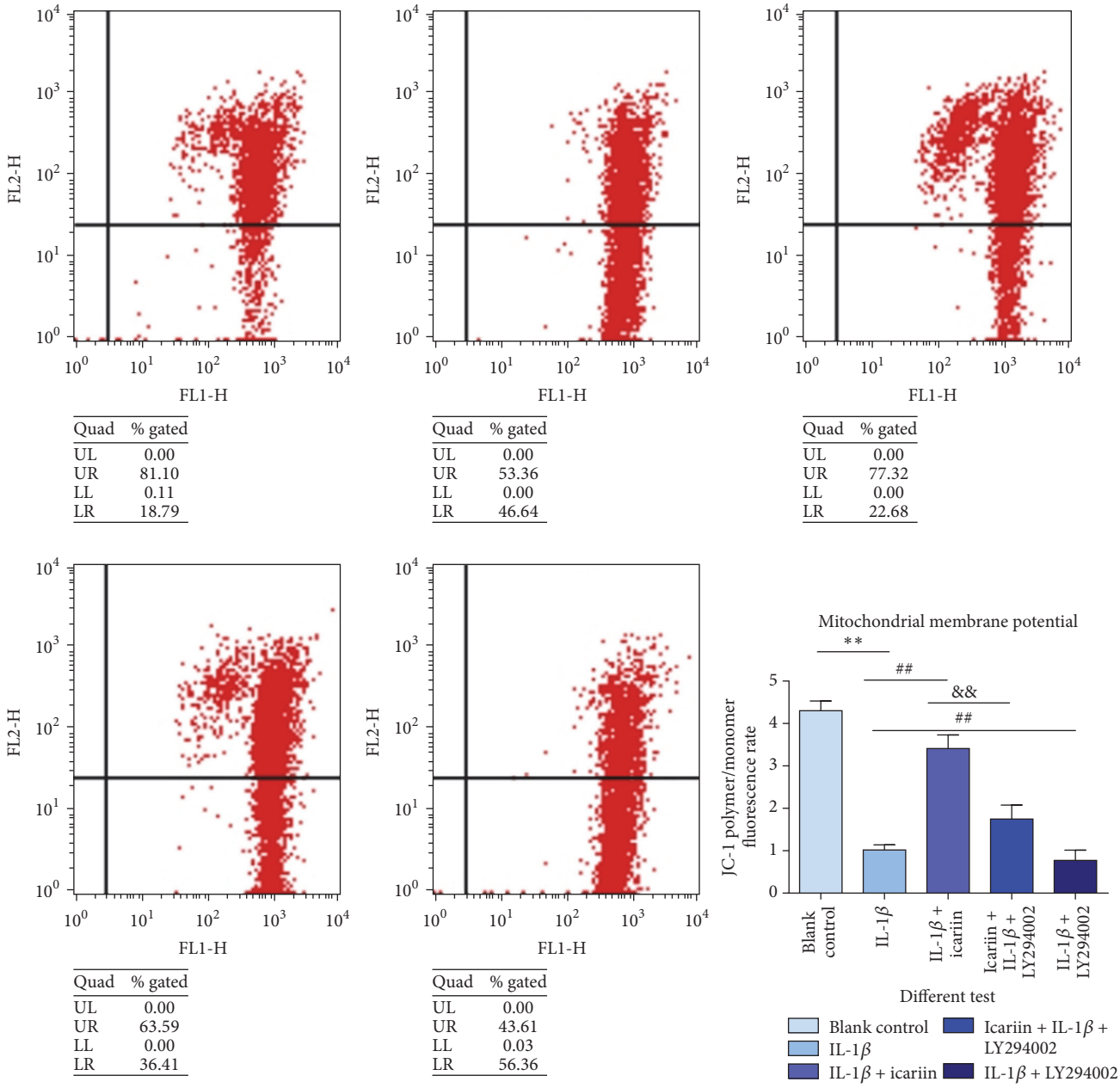
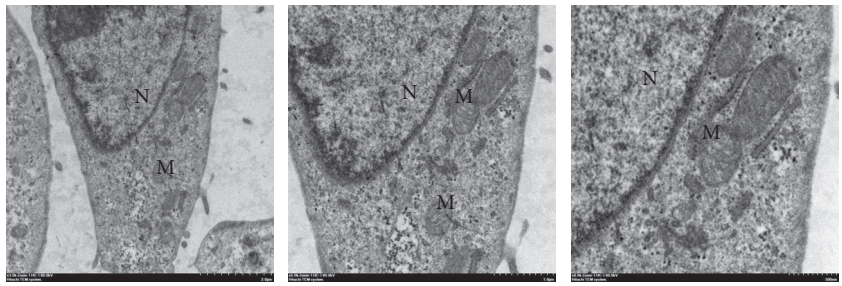
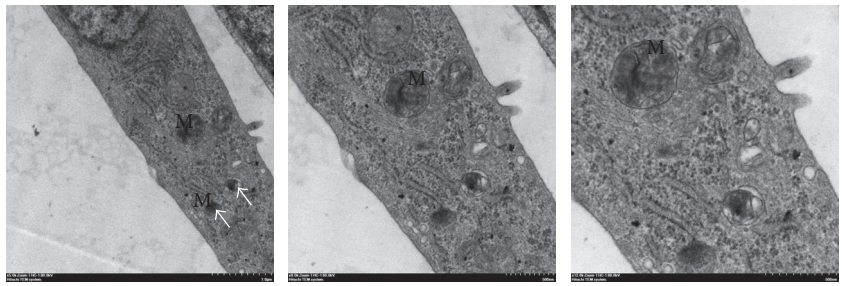


FIGURE 4: Given 20 μg/mL of IL-1β for 48 h, human NP cells exhibited a decline in MMP. The group pretreated with icariin exhibited a stable mitochondrial membrane potential. When the PI3K/AKT pathway was blocked, this protective effect was weakened. There were significant statistical differences between groups, as labeled in the figure ($^{**}p < 0.01$ versus control group, $^{\#\#}p < 0.05$ versus IL-1β group, and $^{\&\&}p < 0.05$ versus IL-1β + icariin group).

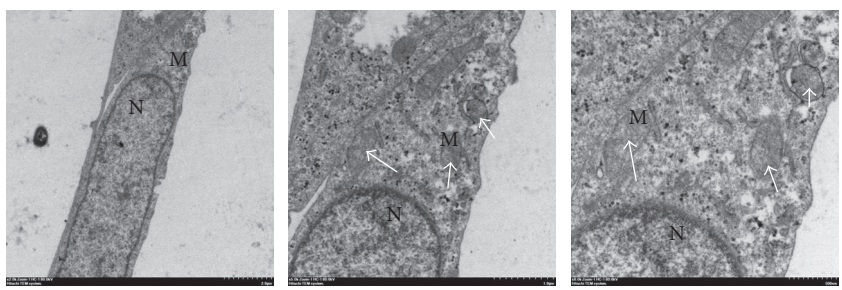
pathway in oncotherapy [42–44] which raises the importance of PI3K/AKT pathway. However, excessive activation of PI3K/AKT pathway may lead to neoplastic lesion [45]; appropriate activation is essential when this pathway is used in antiapoptosis. Our research exhibited that when cells were exposed to adverse environmental factors, the activation of PI3K/AKT pathway had positive meanings. Icariin's ability to stimulate the PI3K/AKT pathway has been pointed out many times and is related to antiapoptosis [9, 46], antioxidative stress [15], the promotion of differentiation [14] and sex [47], and the protective effect of ischemia reperfusion [48]. In our research, we observed significant stimulative effects on the PI3K/AKT pathway by icariin when the interaction time reached 4-5 h; therefore there are evidences to believe that the antiapoptosis effect of icariin is linked to this pathway. In subsequent experiments, we designed five groups to explore the protective effect of icariin and the role of the PI3K/AKT pathway in this process. All of the results supported that icariin had a strong protective effect on IL-1β pretreated human NP cells and that the PI3K/AKT pathway was, at least



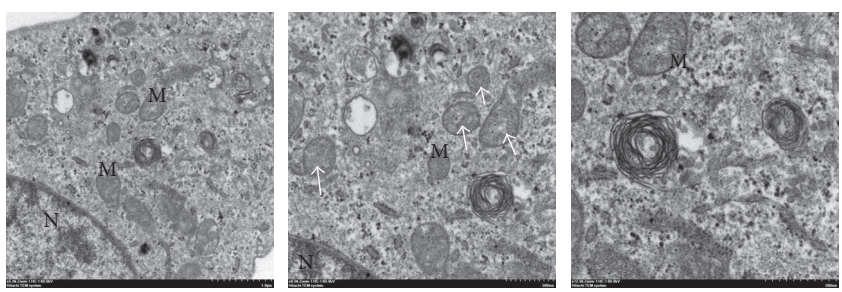
(a) Control group



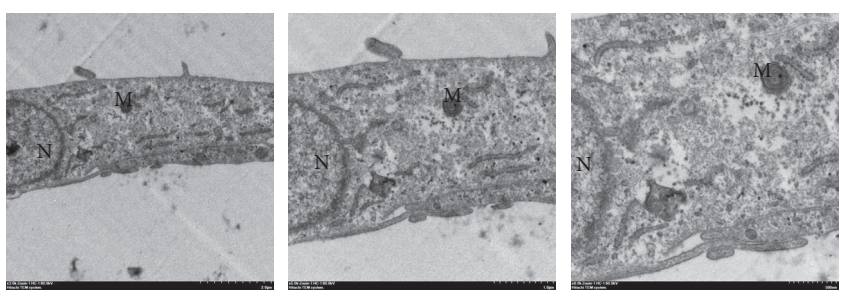
(b) IL-1β



(c) ICA + IL-1β



(d) ICA + IL-1β + LY294002



(e) IL-1β + LY294002

FIGURE 5: As shown above, IL-1β could induce mitochondrial swelling and membrane breakup and icariin could protect cells from this damage. LY294002 could attenuate the protecting effect and be an independent damage factor in the process of IL-1β-induced mitochondria damage.

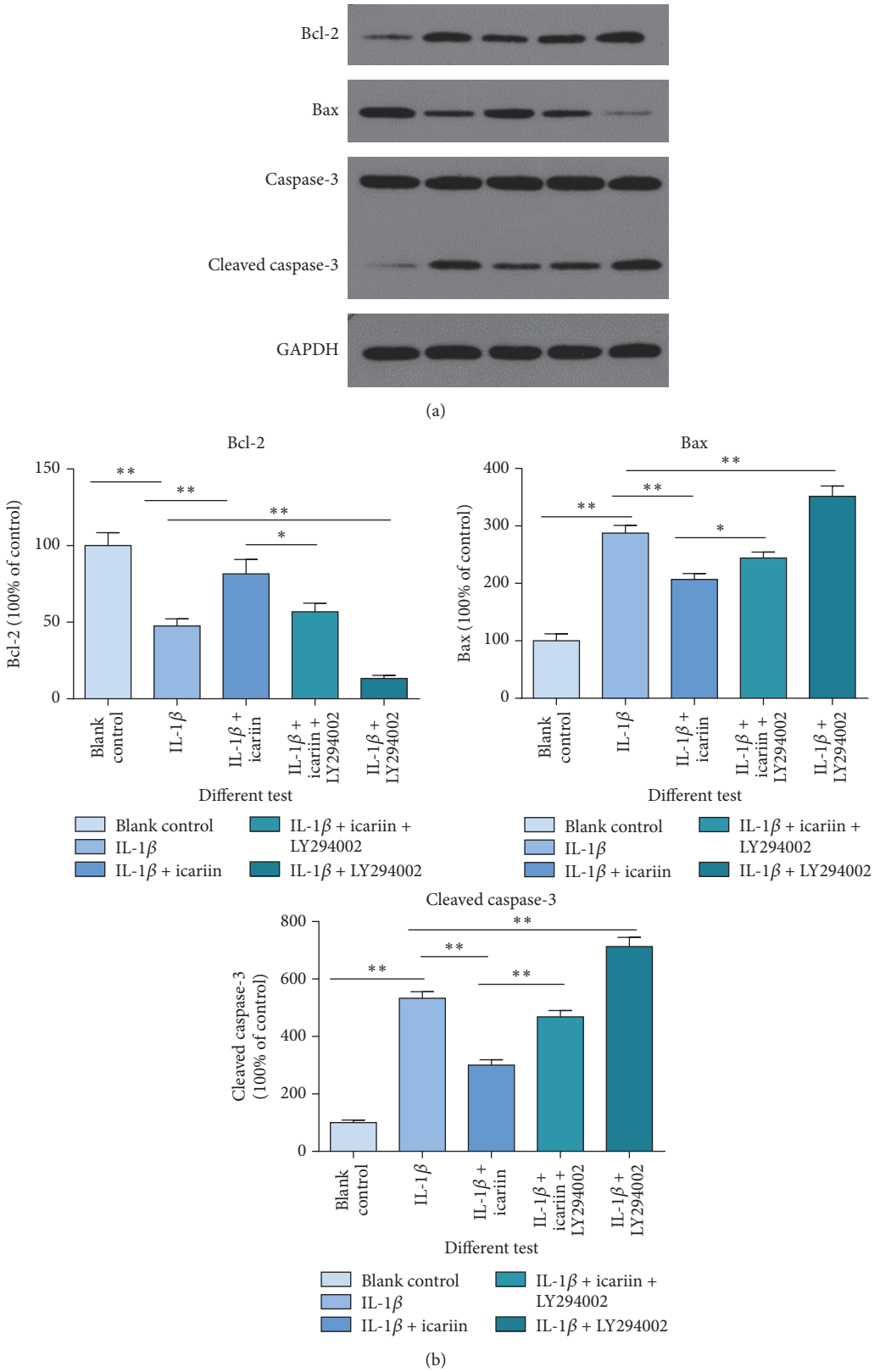


Figure 6: As shown above, IL-1β could induce apoptosis-related protein changes. (a) Bax and cleaved caspase-3 are two important apoptosis-promoter proteins and Bcl-2 is an antiapoptosis protein. An increase of Bcl-2 and a decline of bax and cleaved caspase-3, when pretreated with icariin, showed a protective effect of icariin. (b) The bar graph comes from (a) ($^{**}p < 0.01$ versus control group, $^{*}p < 0.05$ versus control group). When the PI3K/AKT pathway was blocked by LY294002, this protective effect was diminished.

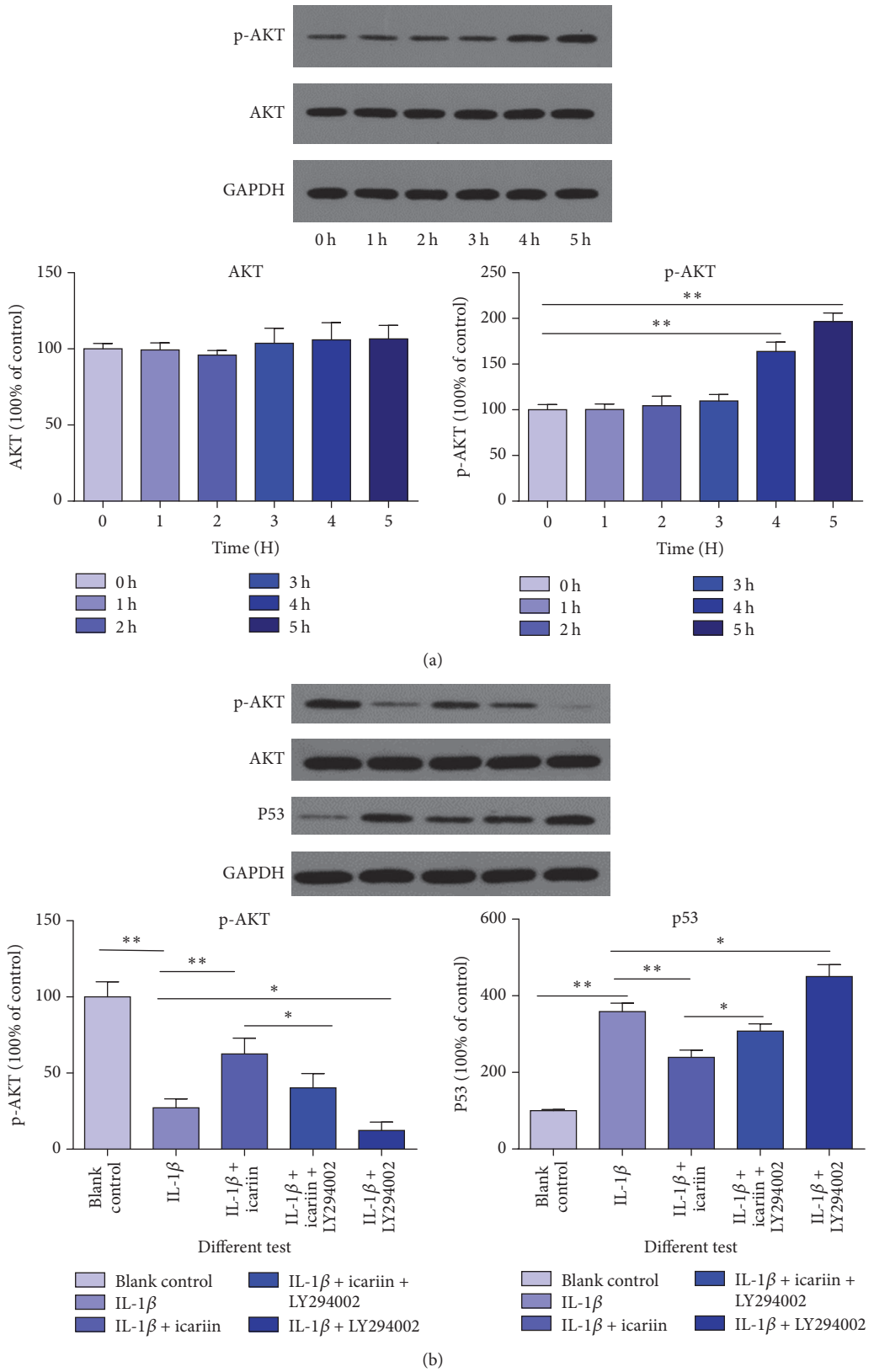


Figure 7: (a) Icariin had significant stimulative effects on the PI3K/AKT pathway when the reaction time reached 4 h and greater; results were detected by western blot of p-AKT ($^{**}p < 0.01$ versus control group). (b) IL-1β inhibited the PI3K/AKT pathway, but icariin attenuated this effect ($^{**}p < 0.01$ versus control group, $^{*}p < 0.05$ versus control group). We think the PI3K/AKT pathway is partly involved in the protective effect of icariin through an antiapoptosis process.

partly, involved in this protective effect. The apoptosis rate, ROS rate, JC-1 detection, and apoptosis-related proteins had similar results.

In summary, to our knowledge, this study is the first one to propose the anti-inflammatory effect of icariin on human NP cells. The present research provides a theoretical basis for icariin's application in the treatment of IDD.

Conflicts of Interest

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

Authors' Contributions

Xiangyu Deng and Wei Wu contributed equally to this study and share first authorship.

Acknowledgments

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References

[1] W. Liu, Y. Zhang, P. Xia et al., "MicroRNA-7 regulates IL-1β-induced extracellular matrix degeneration by targeting GDF5 in human nucleus pulposus cells," *Biomedicine & Pharmacotherapy*, vol. 83, pp. 1414–1421, 2016.

[2] A. T. H. Choy and B. P. Chan, "A structurally and functionally biomimetic biphasic scaffold for intervertebral disc tissue engineering," *PLoS ONE*, vol. 10, no. 6, Article ID e0131827, 2015.

[3] Z. Wang, Z. Qu, C. Fu et al., "Interleukin 1 polymorphisms contribute to intervertebral disc degeneration risk: A meta-analysis," *PLoS ONE*, vol. 11, no. 6, Article ID e0156412, 2016.

[4] S. Dagenais, J. Caro, and S. Haldeman, "A systematic review of low back pain cost of illness studies in the United States and internationally," *The Spine Journal*, vol. 8, no. 1, pp. 8–20, 2008.

[5] X. Yang, L. Jin, L. Yao, F. H. Shen, A. L. Shimer, and X. Li, "Antioxidative nanofullerol prevents intervertebral disk degeneration," *International Journal of Nanomedicine*, vol. 9, no. 1, pp. 2419–2430, 2014.

[6] A. L. Karunanayake, A. Pathmeswaran, and L. S. Wijayaratne, "Chronic low back pain and its association with lumbar vertebrae and intervertebral disc changes in adults. A case control study," *International Journal of Rheumatic Diseases*, 2017.

[7] A. Pantovic, A. Krstic, K. Janjetovic et al., "Coordinated time-dependent modulation of AMPK/Akt/mTOR signaling and autophagy controls osteogenic differentiation of human mesenchymal stem cells," *Bone*, vol. 52, no. 1, pp. 524–531, 2013.

[8] J. Hong, C. Reed, D. Novick, and M. Happich, "Costs associated with treatment of chronic low back pain: an analysis of the UK general practice research database," *The Spine Journal*, vol. 38, no. 1, pp. 75–82, 2013.

[9] D. Zhang, Z. Wang, C. Sheng et al., "Icariin prevents amyloid beta-induced apoptosis via the Pi3k/Akt pathway in PC-12 cells," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 235265, pp. 1–9, 2015.

[10] C.-Q. Xu, B.-J. Liu, J.-F. Wu et al., "Icariin attenuates LPS-induced acute inflammatory responses: involvement of PI3K/Akt and NFκB signaling pathway," *European Journal of Pharmacology*, vol. 642, no. 1–3, pp. 146–153, 2010.

[11] M.-C. Jiang, X.-H. Chen, X. Zhao, X.-J. Zhang, and W.-F. Chen, "Involvement of IGF-1 receptor signaling pathway in the neuroprotective effects of Icaritin against MPP+-induced toxicity in MES23.5 cells," *European Journal of Pharmacology*, vol. 786, pp. 53–59, 2016.

[12] X.-H. Duan, C.-Q. Xu, J.-H. Huang, W.-J. Zhou, and B. Sun, "Icariin delays homocysteine-induced endothelial cellular senescence involving activation of the PI3K/AKT-eNOS signaling pathway," *Pharmaceutical Biology*, vol. 51, no. 4, pp. 433–440, 2013.

[13] Z. Wang, L. Yang, Y. Xia, C. Guo, and L. Kong, "Icariin enhances cytotoxicity of doxorubicin in human multidrug-resistant osteosarcoma cells by inhibition of ABCB1 and down-regulation of the PI3K/Akt pathway," *Biological & Pharmaceutical Bulletin*, vol. 38, no. 2, pp. 277–284, 2015.

[14] Y. K. Zhai, X. Y. Guo, B. F. Ge et al., "Icariin stimulates the osteogenic differentiation of rat bone marrow stromal cells via activating the PI3K–AKT–eNOS–NO–cGMP–PKG," *Bone*, vol. 66, pp. 189–198, 2014.

[15] J. Wu, H. Xu, P. F. Wong, S. Xia, J. Xu, and J. Dong, "Icaritin attenuates cigarette smoke-mediated oxidative stress in human lung epithelial cells via activation of PI3K-AKT and Nrf2 signaling," *Food and Chemical Toxicology*, vol. 64, pp. 307–313, 2014.

[16] S. Li, P. Dong, J. Wang et al., "Icariin, a natural flavonol glycoside, induces apoptosis in human hepatoma SMMC-7721 cells via a ROS/JNK-dependent mitochondrial pathway," *Cancer Letters*, vol. 298, no. 2, pp. 222–230, 2010.

[17] J. Wang, Y. Tao, Z. Ping et al., "Icariin attenuates titanium-particle inhibition of bone formation by activating the Wnt/β-catenin signaling pathway in vivo and in vitro," *Scientific Reports*, vol. 6, Article ID 23827, 2016.

[18] F. Jin, Q.-H. Gong, Y.-S. Xu et al., "Icariin, a phoshphodiesterase-5 inhibitor, improves learning and memory in APP/PS1 transgenic mice by stimulation of NO/cGMP signalling," *The International Journal of Neuropsychopharmacology*, vol. 17, no. 6, pp. 871–881, 2014.

[19] X. Deng, S. Chen, D. Zheng, Z. Shao, H. Liang, and H. Hu, "Icariin Prevents H2O2-induced apoptosis via the PI3K/Akt pathway in rat nucleus pulposus intervertebral disc cells," *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, Article ID 2694261, pp. 1–10, 2017.

[20] C.-M. Zhao, Q. Chen, W.-J. Zhang et al., "17β-estradiol protects rat annulus fibrosus cells against apoptosis via α1 integrin-mediated adhesion to type I collagen: An in-vitro study," *Medical Science Monitor*, vol. 22, article 178, pp. 1375–1383, 2016.

[21] I. Altun, "Cytokine profile in degenerated painful intervertebral disc: variability with respect to duration of symptoms and type of disease," *The Spine Journal*, vol. 16, no. 7, pp. 857–861, 2016.

[22] B. Hu, C. Shi, C. Xu et al., "Heme oxygenase-1 attenuates IL-1β induced alteration of anabolic and catabolic activities in intervertebral disc degeneration," *Scientific Reports*, vol. 6, Article ID 21190, 2016.

[23] F. Xu, F. Gao, Y. Liu et al., "Bioinformatics analysis of molecular mechanisms involved in intervertebral disc degeneration induced by TNF-α and IL-1β," *Molecular Medicine Reports*, vol. 13, no. 3, pp. 2925–2931, 2016.

[24] S.-D. Yang, L. Ma, D.-L. Yang, and W.-Y. Ding, "Combined effect of 17β-estradiol and resveratrol against apoptosis

induced by interleukin-1b in rat nucleus pulposus cells via PI3K/Akt/caspase-3 pathway," *PeerJ*, vol. 2016, no. 1, Article ID e1640, 2016.

[25] J. Shen, J. Fang, J. Hao et al., "SIRT1 inhibits the catabolic effect of IL-1β through TLR2/SIRT1/NF-κB pathway in human degenerative nucleus pulposus cells," *Pain Physician*, vol. 19, no. 1, pp. E215–E226, 2016.

[26] Z. Sun, Z. Yin, C. Liu, H. Liang, M. Jiang, and J. Tian, "IL-1β promotes ADAMTS enzyme-mediated aggrecan degradation through NF-κB in human intervertebral disc," *Journal of Orthopaedic Surgery and Research*, vol. 10, article 159, 2015.

[27] K. Xu, W. Chen, X. Wang et al., "Autophagy attenuates the catabolic effect during inflammatory conditions in nucleus pulposus cells, as sustained by NF-κB and JNK inhibition," *International Journal of Molecular Medicine*, vol. 36, no. 3, pp. 661–668, 2015.

[28] K. Xu, X. Wang, Q. Zhang et al., "Sp1 downregulates proinflammatory cytokine-induced catabolic gene expression in nucleus pulposus cells," *Molecular Medicine Reports*, vol. 14, no. 4, pp. 3961–3968, 2016.

[29] H. Xiao, G. Sui, and X. Lu, "Icariin improves eNOS/NO pathway to prohibit the atherogenesis of apolipoprotein E-null mice," *Canadian Journal of Physiology and Pharmacology*, vol. 95, no. 6, pp. 625–633, 2017.

[30] Y. Chen, L. Zeng, Y. Lu et al., "Treatment effect of a flavonoid prescription on duck virus hepatitis by its hepatoprotective and antioxidative ability," *Pharmaceutical Biology*, vol. 55, no. 1, pp. 198–205, 2016.

[31] Y.-H. Song, H. Cai, Z.-M. Zhao et al., "Icariin attenuated oxidative stress induced-cardiac apoptosis by mitochondria protection and ERK activation," *Biomedicine & Pharmacotherapy*, vol. 83, pp. 1089–1094, 2016.

[32] Z.-S. Huang, H.-J. Xiao, T. Qi et al., "Antioxidative protective effect of icariin on the FeSO4/H2O2-damaged human sperm based on confocal raman micro-spectroscopy," *Journal of Huazhong University of Science and Technology (Medical Sciences)*, vol. 34, no. 5, pp. 755–760, 2014.

[33] B. Liu, C. Xu, X. Wu et al., "Icariin exerts an antidepressant effect in an unpredictable chronic mild stress model of depression in rats and is associated with the regulation of hippocampal neuroinflammation," *Neuroscience*, vol. 294, pp. 193–205, 2015.

[34] Z. Wang, L. Ding, S. Zhang, T. Jiang, Y. Yang, and R. Li, "Effects of icariin on the regulation of the OPG-RANKL-RANK system are mediated through the MAPK pathways in IL-1β-stimulated human SW1353 chondrosarcoma cells," *International Journal of Molecular Medicine*, vol. 34, no. 6, pp. 1720–1726, 2014.

[35] J. Cui, M. Zhu, S. Zhu, G. Wang, Y. Xu, and D. Geng, "Inhibitory effect of icariin on Ti-induced inflammatory osteoclastogenesis," *Journal of Surgical Research*, vol. 192, no. 2, pp. 447–453, 2014.

[36] L. Zeng, W. Wang, X.-F. Rong et al., "Chondroprotective effects and multi-target mechanisms of Icariin in IL-1 β-induced human SW 1353 chondrosarcoma cells and a rat osteoarthritis model," *International Immunopharmacology*, vol. 18, no. 1, pp. 175–181, 2014.

[37] Y. Deng, D. Xiong, C. Yin, B. Liu, J. Shi, and Q. Gong, "Icariside II protects against cerebral ischemia-reperfusion injury in rats via nuclear factor-κB inhibition and peroxisome proliferator-activated receptor up-regulation," *Neurochemistry International*, vol. 96, pp. 56–61, 2016.

[38] D. Xiong, Y. Deng, B. Huang et al., "Icariin attenuates cerebral ischemia-reperfusion injury through inhibition of inflammatory response mediated by NF-κB, PPARα and PPARγ in rats," *International Immunopharmacology*, vol. 30, pp. 157–162, 2016.

[39] H. Shao, J. Shen, M. Wang et al., "Icariin protects against titanium particle-induced osteolysis and inflammatory response in a mouse calvarial model," *Biomaterials*, vol. 60, pp. 92–99, 2015.

[40] J. Guo, F. Li, Q. Wu, Q. Gong, Y. Lu, and J. Shi, "Protective effects of icariin on brain dysfunction induced by lipopolysaccharide in rats," *Phytomedicine*, vol. 17, no. 12, pp. 950–955, 2010.

[41] P. Chen, H. Yao, and L. J. Huang, "Cytokine receptor endocytosis: new kinase activity-dependent and -independent roles of PI3K," *Frontiers in Endocrinology*, vol. 8, article 78, 2017.

[42] F. Zhao, J. Zhao, L. Song, Y. Zhang, Z. Guo, and K. Yang, "The induction of apoptosis and autophagy in human hepatoma SMMC-7721 cells by combined treatment with vitamin C and polysaccharides extracted from Grifola frondosa," *Apoptosis*, vol. 22, no. 11, pp. 1461–1472, 2017.

[43] X. Wang, Y. Xie, and J. Wang, "Overexpression of MicroRNA-34a-5p Inhibits Proliferation and Promotes Apoptosis of Human Cervical Cancer Cells by Downregulation of Bcl-2," *Oncology Research*, 2017.

[44] Z. Amini-Farsani, M. H. Sangtarash, M. Shamsara, and H. Teimori, "MiR-221/222 promote chemoresistance to cisplatin in ovarian cancer cells by targeting PTEN/PI3K/AKT signaling pathway," *Cytotechnology*, pp. 1–11, 2017.

[45] J. Kim, H. Lee, E. A. Shin, D. H. Kim, J. B. Choi, and S. Kim, "Implications of Bcl-2 and its interplay with other molecules and signaling pathways in prostate cancer progression," *Expert Opinion on Therapeutic Targets*, vol. 21, no. 9, pp. 911–920, 2017.

[46] K.-W. Zeng, H. Ko, H. O. Yang, and X.-M. Wang, "Icariin attenuates β-amyloid-induced neurotoxicity by inhibition of tau protein hyperphosphorylation in PC12 cells," *Neuropharmacology*, vol. 59, no. 6, pp. 542–550, 2010.

[47] J. Ding, Y. Tang, Z. Tang et al., "Icariin improves the sexual function of male mice through the PI3K/AKT/eNOS/NO signalling pathway," *Andrologia*, 2017.

[48] Z. Ke, J. Liu, P. Xu, A. Gao, L. Wang, and L. Ji, "The Cardioprotective Effect of Icariin on Ischemia-Reperfusion Injury in Isolated Rat Heart: Potential Involvement of the PI3K-Akt Signaling Pathway," *Cardiovascular Therapeutics*, vol. 33, no. 3, pp. 134–140, 2015.

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Research Article

Antiarthritic Activity of Qi-Wu Rheumatism Granule (a Chinese Herbal Compound) on Complete Freund's Adjuvant-Induced Arthritis in Rats

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Objective. The aim was to study the therapeutic effects and mechanisms of QWRG on adjuvant-induced RA in rats. *Methods*. The RA rat models were manipulated and subsequently divided into five experimental groups: AIA, DEX, and QWRG groups. The paw volume, body weight, arthritic score, and mechanical nociceptive threshold were assessed. The serum levels of the RF, MDA, ALP, AST, ALT, IL-1β, IL-2, IL-16, and TNF-α were measured. The proliferative capacity of lymphocytes was evaluated, and the synovial tissue was histopathologically examined. *Results*. The paw swelling and arthritic scores were relieved, and the variation of relative body weight and mechanical nociceptive threshold had improved in the AIA rats. The serum levels of RF, MDA, ALP, AST, and ALT were alleviated, and the inflammation and cartilage damage were effectively attenuated in the AIA rats. Simultaneously, the inflammation of the synovial cavity was alleviated, and the grading of synovitis reduced by inhibiting the expressions of IL-1β, TNF-α, and IL-16 in the serum and synovium tissue. *Conclusion*. Our results suggested that the antiarthritic properties of QWRG may be due to immunodepression and downregulation of inflammatory cytokines, which may be a potential candidate for the treatment of RA.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic, symmetric, inflammatory, and systemic manifestations and affects between 0.3 and 1% of the population worldwide [1]. The disease appears in most cases between 50 and 60 years of age and women are more affected than men [2, 3]. In patients with RA, a joint deformation is observed with an increase in the extent of loss of function and cartilage and bone destruction [4]. In terms of the characteristics of the disease, RA shows stages of pathological process, and early symptoms of heat, swelling, pain, and decreased joint function; the late stage shows different degrees of joint stiffness and deformity accompanying bone damage and disability risk [5]. With the development of RA, it characteristically affects the small joints of the hands and feet resulting in a gradual painful swelling, exaggerated, and abnormal development of the synovium, pannus formation, and changes in the morphology of the joint [6]. Although the pathogenesis and mechanisms of RA are not fully understood, it is stated that part of the RA pathogenesis is the retention of microbial products in the synovial tissue and persistent infection of the joint articular surface, which induces an immune reaction, ultimately altering the integrity of these joint components.

Currently, there is no effective cure for RA [7]. Although many current therapies including nonsteroidal anti-inflammatory drugs, glucocorticosteroids, and biological agents improve pain, fatigue, and disability, they mainly focus on controlling synovitis. Furthermore, long-term, large-dose administrations of these agents could lead to relative limited effectiveness and severe negative side effects [8, 9]. In addition, therapeutic effects in RA might be achieved by antagonizing these proinflammatory mediators. Newer therapies such as antitumor necrosis factor- (TNF-) α therapy,

anti-CD20 therapy, and CD80/86 blockade are required to inhibit the underlying immune process. However, all these antirheumatic drugs are associated with numerous side effects coupled with lengthy treatment duration and potential unknown threats [10–12]. Consequently, it has become an inevitable trend to identify an effective anti-RA drug with high therapeutic effects and fewer side effects. The search for traditional herbal drugs that are more effective, safer, and economical has attracted a great attention, since 80% of the world population mainly rely on herbal drugs [13]. With the long history of traditional Chinese medicine (TCM) in the treatment of RA, RA is included in the theory of TCM "arthralgia" category. The TCM treatments focus on reinforcing qi and nourishing the blood, dispelling cold and removing dampness, promoting blood circulation, dispelling wind and relieving pain, and addressing both the symptoms and root cause and strengthening the body resistance to eliminate pathogenic factors. TCM not only has the advantages of fewer side effects and lower costs but can also be used for individual treatment with multiway, multilink, multitarget effects and integral regulation [14]. It is confirmed that TCM treatment on RA could significantly improve the living quality of patients, which provides a new way to overcome many difficulties [15, 16].

Qi-Wu Rheumatism Granule (QWRG), a herbal formulation consisting of five crude drugs, namely, *milkvetch root, Radix Aconiti Preparata, scorpion, centipede,* and *geosaurus* (w : w), is considered an integral part of TCM and widely used in *Affiliated Traditional Chinese Medical Hospital of Xinjiang Medical University*. QWRG is effective for cold resistance with "antiarthromyodynia" [17], which has been long used as a folk medicine to treat RA. In RA, QWRG exerts an analgesic, anti-inflammatory, and antipyretic effect and improves the joint function. Our previous research also demonstrated that QWRG possessed a substantial anti-arthritic activity.

In this study, a rat model of adjuvant-induced arthritis (AIA) was established to investigate the potential therapeutic effects and mechanism of QWRG, which reflects a number of clinical characteristics of RA in humans [18, 19]. First, the safety of QWRG was evaluated in mice. Subsequently, in order to provide an effective experimental basis, and to lay a theoretical foundation for the development of new drugs for the treatment of RA with QWRG, the therapeutic effects of QWRG were evaluated in AIA rats. For this reason, the pathological change of serum biochemical indicators, immune indicators, spleen index, spleen lymphocyte proliferation, synovial membrane, and synovium expression of interleukin 1β (IL-1β), IL-2, IL-16, and tumor necrosis factor α (TNF-α) were analyzed.

2. Materials and Methods

2.1. Animals. Twenty SPF Kunming mice weighing 18–22 g (10 males and 10 females) were purchased from the Xinjiang Uygur Autonomous Region Animal Research Center (license number SCXK [Xin] 2011-0003). Sixty male Wistar rats (160 ± 20 g) were purchased from the Animal Center of Xinjiang Medical University (license number 65000700000087). All animals were provided food and water ad libitum and were maintained in a room at a controlled temperature (23–25°C) and humidity (40–50%) and under a 12/12 h light/dark cycle. The mice were allowed 7 days to adapt to the laboratory environment before the experiments. The experiments were approved by the Animal Ethical Council of Xinjiang Medical University.

2.2. Preparation of the QWRG Extract. The laboratory QWRG consisted of a mixture of five fruits, namely the *milkvetch root, Radix Aconiti Preparata, scorpion, centipede,* and *geosaurus* (Kangmei Pharmaceutical Co., Ltd.) in the ratio of 1 : 1 : 1 : 1 : 1 (w : w). The fruit pieces were broken and soaked in water for 12 h, decocted and boiled for 60 min, and finally gauze filtered and decocted for 30 min with another 8-fold water. Subsequently, the water filtrates were combined and heat-concentrated to a thick paste. The water filtrate was concentrated under reduced pressure at 55°C by a vacuum rotary evaporator (RE-52A, Shanghai Yarong, China) and further dried in a vacuum drying oven (DZF-6090, Shanghai Jinghong, China) to yield a solid QWRG extract at 8.6%. The dried QWRG extract was freshly prepared with normal saline before each experiment. The clinical dosage of QWRG was 124.5 g of crude extract. The water extraction content corresponded to 1 g, equivalent to 4.12 g crude drug. All other reagents used were standard laboratory reagents of analytical grade and were purchased locally.

2.3. Safety Evaluation of QWRG. After fasting for 16 h, 20 SPF Kunming mice were randomly divided into the control and QWRG group and were treated by intragastric administration with normal saline (control; 40 mL/kg) or QWRG (1.43 g/mL), respectively. Subsequently, the behavior, performance, characteristics, toxic reaction time, recovery time, and death rate were recorded before and after the treatment. Subsequently, the animals were weighed on the day of the treatment (day 0) and on days 4, 7, 10, and 14 after the treatment.

2.4. AIA Rats Model and Experimental Design. Male Wistar rats weighting 140–180 g were randomly divided into a normal group (10 rats) and an AIA group (50 rats). Complete Freund's Adjuvant (CFA) was obtained by mixing 7 mg/mL *mycobacterium* cheese (Lot. 0260570, Difco Int, USA), which has an efficacy 3-fold higher than that of the *Mycobacterium tuberculosis*, with the incomplete Freund's Adjuvant (Sigma, Inc., WA, USA). Rats in the AIA group were administered with a single intradermal injection of 0.1 mL CFA into the right hind paw to induce arthritis [20], while the equivalent volume of normal saline was injected to the rat in the normal group. After 7 days of inflammation, the volume of paw swelling was detected by using the Volumetric Meter (Chengdu Taimen Company, China). Subsequently, the rats were randomly divided into five experimental groups ($n = 10$ each) based on the paw swelling volume: control group, dexamethasone group (5.0 mg/kg, intraperitoneal), low QWRG group (1.0 g/kg, gavage), medium QWRG group (2.0 g/kg, gavage), and high QWRG group (4.0 g/kg, gavage). All treatments were administered orally 30 min before the

CFA induction (day 0) and daily thereafter up through 35 days. Periodically, the development of arthritis was monitored by measuring the paw thickness. On day 35, at the end of the experimental period, the animals were killed by euthanasia and the blood was collected for various biochemical estimations. The spleen was immediately dissected and homogenized in ice-cold Tris HCl buffer (0.01 mol/L, pH 7.4).

2.5. Evaluation of AIA Development. Measurements of the paw volume, arthritic score, mechanical nociceptive threshold, thermal hyperalgesia, and body weight were recorded on days 0, 7, 14, 21, 28, and 35. The paw swelling was calculated using the following equation: paw swelling degree = $(\text{paw swelling volume})_{\text{after}} - (\text{paw swelling volume})_{\text{before}}$. Randall Selitto analgesiometer (UGO Basile) was used to measure the mechanical pain threshold [20]. For each animal, the change of body weight and paw withdrawal latency responses (pain threshold) were expressed as % values relative to the preadministration value (100%) [21]. The severity of arthritis was assessed by three independent observers by visual observation. The rats were observed periodically for the severity of joint inflammation every 7 days. The severity of arthritis was graded on a five-point scale [19], with *4* indicating edema and erythema from the ankle to the entire leg, *3* indicating moderate edema and erythema from the ankle to the tarsal bone, *2* indicating slight edema and erythema from the ankle to the tarsal bone, *1* indicating slight edema and limited erythema, and *0* indicating no edema or swelling. The arthritis score for each mouse was the sum of the severity in all the right paw (maximum four points for individual rats).

2.6. Serum Biochemical Indicators and Immune Indicators. Blood was collected from the inferior vena cava of rats in all experimental groups without anticoagulant and was centrifuged at 3,000 rpm, 4°C for 10 min at day 35 after treatments. The serum was separated and divided into aliquots at 4°C. The serum levels of the arthritis factor (RF), malondialdehyde (MDA), alkaline phosphatase (ALP), amino transaminase (AST), and alanine amino transaminase (ALT) were investigated by commercially available colorimetric assay kits (Jian Cheng Bioengineering Institute, China) according to the manufacturer's instructions. Furthermore, the serum levels of inflammation-related cytokines (IL-1β, TNF-α, IL-16, and IL-2) were evaluated using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Bender MedSystem, Vienna, Austria). Briefly, a biotinylated antibody reagent was added to the 96-well plates, which were then filled with the homogenized serum and incubated at 37°C in CO_2 for 2 h. After washing with phosphate-buffered saline (PBS), streptavidin-horseradish peroxidase (HRP) solution was added and the plates were incubated for 30 min at room temperature. The absorbance was measured at 450 nm using a microplate reader (iMark; Bio-Rad, Hercules, CA, USA).

2.7. Lymphocyte Proliferation and Spleen Index. The lymphocyte proliferation was evaluated in all rats by using the methyl thiazolyl tetrazolium (MTT) assay. Briefly, the spleen cell suspension was cultured in RPMI1640 medium, and the cell concentration was subsequently adjusted to 5×10^5/mL. Subsequently, 100 μL of cells was added in each hole of the 96 well-plate with five repeats for every rat. Each plate was induced with bovine globulin (ConA) and lipopolysaccharide (LPS), incubated in 5% CO_2 at 37°C for 48 h, and added to 10 μL of MTT culture solution with further incubation for another 2 h. The proliferation of lymphocytes was detected with dual wavelength at 450 and 650 nm (reference wavelength) using the enzyme labeling instrument. The spleens of all rats were dissected and weighed. The spleen index was calculated as the ratio (mg/g) of spleen wet weight versus body weight.

2.8. Histology Assay of the Synovial Tissue. At the end of the experiment (day 35), the rats' right hind limbs were dissected and fixed with 10% formaldehyde solution for 48 h. The tissues were embedded in paraffin and sliced. The sections were stained with hematoxylin and eosin (HE) and evaluated by two trained observers who were blinded to the experimental groups. Histological assessment of joint damage was carried out on the basis of articular cartilage damage, underlying bone destruction, and inflammatory cells infiltrate. The cartilage damage was semiquantified with a Mankin scale [22], with *0-1* indicating invasion of the tidemark by blood vessels, *0–4* indicating loss of matrix staining, and *0–6* indicating cartilage structural compromise, with a maximum score of 12 points. The histological scores on bone destruction and inflammatory cells infiltration were scored on a four-scale: *3*, severe; *2*, moderate; *1*, mild; and *0*, normal [23]. The mean of three sections per rat was used as independent data for statistical analysis.

3. Results

3.1. Safety Evaluation of QWRG. Compared with the normal control group, the normal physiological function of the mice following intragastric administration of QWRG remained intact. In particular, there were no obvious abnormalities, toxic reactions, or death occurrence up to 14 days after the treatment. In contrast, in the early growth stage of the mice (before the age of 10 days), the growth in the QWRG group was significantly slower than that in the control group as evidenced by the body weight changes. Subsequently, the trend of the weight change was basically comparable between the two groups. This might indicate that the initial differences were due to the large QWRG dose that may have limited the mice feeding, thus resulting in slower growth at early administration. After 10 days of adaptation, the weight growth was comparable between the two groups (Figure 1), which implied that QWRG had no negative effects on growth. In addition, there were no obvious changes in the location, size, color, and adhesion of the organs and no abnormal changes such as fluid or tumor in the viscera surfaces and sections. Taken together, these results confirmed that a large QWRG dose (40 mL/kg) had no acute toxic effects and no influence on the growth of mice, thus implying its safety.

Table 1: Effects of QWRG in AIA model on paw swelling ($\overline{x} \pm s$, $n = 10$).

Groups	Paw swelling (mL)					
	0 d	7 d	14 d	21 d	28 d	35 d
Control	0.03 ± 0.02	0.03 ± 0.12	0.09 ± 0.07	0.03 ± 0.11	0.04 ± 0.12	0.04 ± 0.16
AIA group	1.58 ± 0.41**	2.15 ± 0.40**	2.71 ± 0.66**	2.93 ± 0.77**	2.94 ± 0.73**	3.32 ± 1.26**
DEX (5 mg/kg)	1.49 ± 0.20	0.53 ± 0.43$^{\#\#}$	0.64 ± 0.24$^{\#\#}$	0.86 ± 0.32$^{\#\#}$	0.78 ± 0.32$^{\#\#}$	0.90 ± 0.57$^{\#\#}$
QWRG						
1.0 g/kg	1.64 ± 0.52	1.68 ± 0.55$^{\#\&\&}$	2.25 ± 0.37$^{\#\&\&}$	2.31 ± 0.12$^{\#\&\&}$	2.37 ± 0.49$^{\#\&\&}$	2.44 ± 1.18$^{\#\&\&}$
2.0 g/kg	1.64 ± 0.42	1.76 ± 0.48$^{\#\&\&}$	2.17 ± 0.49$^{\#\&\&}$	2.13 ± 0.28$^{\#\&\&}$	2.14 ± 0.88$^{\#\&\&}$	2.06 ± 0.13$^{\#\&\&}$
4.0 g/kg	1.62 ± 0.71	1.12 ± 0.58$^{\#\#\&\&}$	1.53 ± 0.22$^{\#\#\&\&}$	1.29 ± 0.736$^{\#\#\&\&}$	1.20 ± 0.75$^{\#\#\&\&}$	1.26 ± 0.94$^{\#\#\&\&}$

The values from 10 different rats in each group. Data are mean ± SD. $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus model; $^{\&\&}P < 0.01$ versus DEX.

Table 2: Effects of QWRG in AIA model on relative body weight ($\overline{x} \pm s$, $n = 10$).

Groups	Relative body weight (%)					
	0 d	7 d	14 d	21 d	28 d	35 d
Control	100.0	101.4 ± 12.2	102.3 ± 12.6	104.6 ± 12.1	106.5 ± 11.9	109.1 ± 10.2
AIA group	100.0	96.5 ± 10.3	94.2 ± 9.6*	93.8 ± 9.6**	92.0 ± 10.2**	92.5 ± 11.6**
DEX (5 mg/kg)	100.0	99.2 ± 11.3	98.3 ± 9.4	99.2 ± 9.2$^{\#}$	98.8 ± 10.5$^{\#}$	100.5 ± 9.7$^{\#}$
QWRG						
1.0 g/kg	100.0	97.1 ± 10.2	96.1 ± 10.6	95.2 ± 10.8$^{\&}$	94.5 ± 10.9$^{\&}$	94.7 ± 9.4$^{\&}$
2.0 g/kg	100.0	98.3 ± 9.9	97.1 ± 10.1	97.3 ± 9.8$^{\#}$	96.8 ± 9.8$^{\#}$	96.1 ± 10.2$^{\#\&}$
4.0 g/kg	100.0	99.2 ± 11.3	99.1 ± 9.4$^{\#}$	98.1 ± 9.7$^{\#}$	97.3 ± 8.9$^{\#\#}$	98.5 ± 9.7$^{\#\#}$

The values from 10 different rats in each group. Data are mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus model; $^{\&}P < 0.05$ versus DEX.

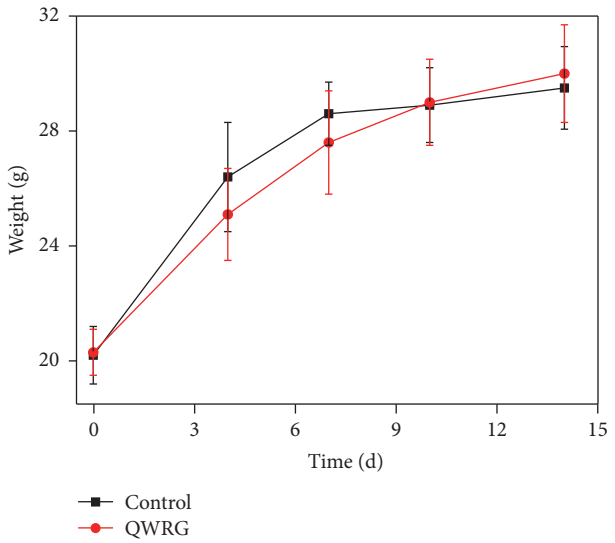

Figure 1: Effects of QWRG on normal mice weight (animals received intragastric administrating with QWRG during 14 d; the values of weight are means, with their standard errors represented by vertical bars. Herein, circle and squares represented the QWRG treatment group and control, resp.).

3.2. The Role of QWRG in the Treatment of AIA Rats

3.2.1. Effects of QWRG on the Paw Swelling. The paw swelling of the AIA group significantly increased compared with the control group ($P < 0.01$) and gradually increased after the model manipulation, indicating that the AIA model was established successfully. After administration of QWRG (1.0, 2.0, and 4.0 g/kg), the paw swelling volume was significantly reduced in the treated rats compared with the AIA group ($P < 0.05$ or $P < 0.01$); this reduction was less pronounced than that of the DEX group ($P < 0.01$; Table 1). The above results show that the QWRG has obviously relieved RA paw swelling, even though still significantly weaker than DEX.

3.2.2. Effect of QWRG on the Body Weights. In the AIA group, the rats' body weights gradually decreased and became significantly different compared to the control group starting from treatment day 7 ($P < 0.05$ or $P < 0.01$). In the rats treated with QWRG (2.0 and 4.0 g/kg), the body weights were significantly different throughout the treatment compared with the AIA group ($P < 0.05$ or $P < 0.01$, resp.), but not with the DEX group (Table 2).

3.2.3. Effect of QWRG on the Arthritic Score. The morphological variation materialized by the arthritic score was significant in all animals of the AIA group ($P < 0.01$). DEX and QWRG (2.0 and 4.0 g/kg) effectively protected the animals against the exaggeration of morphological variation observed in the untreated animals; this was reflected by a significant variation of the arthritic scores between the treated and untreated rats (Table 3).

3.2.4. Effect of QWRG on the Mechanical Nociceptive Threshold. After the administration of QWRG (2.0 and 4.0 g/kg),

Table 3: Effects of QWRG in AIA model on arthritic score ($\overline{x} \pm s$, $n = 10$).

Groups	Arthritic score					
	0 d	7 d	14 d	21 d	28 d	35 d
Control	0	0	0	0	0	0
AIA group	$4.3 \pm 0.8^{**}$	$5.3 \pm 0.3^{**}$	$8.2 \pm 0.6^{**}$	$7.4 \pm 0.6^{**}$	$7.0 \pm 0.9^{**}$	$7.1 \pm 11.6^{**}$
DEX (5 mg/kg)	4.1 ± 0.9	$4.2 \pm 0.8^{\#}$	$2.3 \pm 0.4^{\#\#}$	$2.2 \pm 0.2^{\#\#}$	$1.8 \pm 0.5^{\#\#}$	$1.5 \pm 0.7^{\#\#}$
QWRG						
1.0 g/kg	4.0 ± 0.6	5.1 ± 0.7	7.8 ± 0.6	7.4 ± 0.8	7.1 ± 0.4	6.9 ± 0.6
2.0 g/kg	4.2 ± 0.5	$4.8 \pm 0.4^{\&}$	$6.8 \pm 0.9^{\#\&\&}$	$6.3 \pm 0.5^{\#\&\&}$	$6.0 \pm 0.8^{\#\&\&}$	$6.1 \pm 0.5^{\#\&\&}$
4.0 g/kg	4.3 ± 0.8	$4.3 \pm 0.1^{\#}$	$3.4 \pm 0.7^{\#\#}$	$3.1 \pm 0.5^{\#\#}$	$2.9 \pm 0.8^{\#\#}$	$3.0 \pm 0.4^{\#\#}$

The values from 10 different rats in each group. Data are mean ± SD. $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus model; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ versus DEX.

Table 4: Effects of QWRG in AIA model on mechanical nociceptive threshold ($\overline{x} \pm s$, $n = 10$).

Groups	Paw withdrawal latency (%)					
	0 d	7 d	14 d	21 d	28 d	35 d
Control	100.0	99.2 ± 3.5	99.4 ± 5.5	98.7 ± 4.2	97.6 ± 3.4	99.1 ± 6.2
AIA group	100.0	$35.4 \pm 5.3^{**}$	$33.6 \pm 7.6^{**}$	$32.4 \pm 5.5^{**}$	$32.0 \pm 6.2^{**}$	$30.1 \pm 4.8^{**}$
DEX (5 mg/kg)	100.0	$44.4 \pm 6.9^{\#}$	$52.7 \pm 6.8^{\#\#}$	$63.5 \pm 9.1^{\#\#}$	$65.8 \pm 8.5^{\#\#}$	$72.3 \pm 11.7^{\#\#}$
QWRG						
1.0 g/kg	100.0	35.4 ± 5.7	36.3 ± 9.6	37.4 ± 7.6	36.8 ± 6.8	37.1 ± 8.2
2.0 g/kg	100.0	37.7 ± 6.8	$42.8 \pm 7.8^{\#\#\&}$	$52.9 \pm 9.2^{\#\#\&}$	$51.5 \pm 6.9^{\#\#\&}$	$53.6 \pm 8.1^{\#\#\&}$
4.0 g/kg	100.0	$41.4 \pm 6.4^{\#}$	$45.5 \pm 6.4^{\#\#}$	$58.8 \pm 7.3^{\#\#}$	$60.2 \pm 8.15^{\#\#}$	$60.1 \pm 5.9^{\#\#}$

The values from 10 different rats in each group. Data are mean ± SD. $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus model; $^{\&}P < 0.05$ versus DEX.

a significant protective effect against the mechanical pain was observed compared with the AIA group ($P < 0.05$ or $P < 0.01$). However, there was little improvement observed in the mechanical withdrawal threshold in the group treated with low-dose QWRG (1.0 g/kg). DEX showed significant improvement in the mechanical withdrawal threshold between day 1 and day 35 ($P < 0.05$ or $P < 0.01$), which was more pronounced than that induced by QWRG (Table 4). These results showed that QWRG has significantly relieved the mechanical nociceptive threshold.

3.3. The Impact of QWRG on Serum Biochemical Indicators and Immune Indicators

3.3.1. Effects of QWRG on Serum RF, MDA, ALP, AST, and ALT. Based on Table 5, the serum levels of RF, MDA, ALP, AST, and ALT significantly increased in the AIA group compared with the control group ($P < 0.01$). In animals treated with higher QWRG doses (2.0 and 4.0 g/kg) or DEX, all biochemical parameters evaluated tended to return to normal values ($P < 0.05$ or $P < 0.01$, resp.). Nevertheless, the low-dose QWRG (1.0 g/kg) had nearly no effect on the biochemical indexes ($P > 0.05$), and the effects of high-dose QWRG (4.0 g/kg) basically equaled those of DEX ($P < 0.05$; Table 5).

3.3.2. Effects of QWRG on Serum IL-1β, TNF-α, IL-16, and IL-2. The inflammatory cytokines IL-1β, TNF-α, IL-16, and IL-2 of the AIA group were significantly higher than those of the controls ($P < 0.01$). The positive control groups (DEX) and QWRG (2.0 and 4.0 g/kg) displayed all significantly reduced IL-1β, TNF-α, IL-16, and IL-2 levels compared with the AIA group ($P < 0.05$ or $P < 0.01$), and both QWRG doses (2.0 g/kg and 4.0 g/kg) did not differ significantly. However, the 2.0 g/kg QWRG dose induced significantly weaker effects than the DEX treatment ($P < 0.05$). All these results indicated that QWRG enhanced the immune function in rats, regulated the secretion of inflammatory cytokines, and improved the inflammatory symptoms of RA (Table 6).

3.3.3. Effect of QWRG on the Spleen Index and Spleen Lymphocyte Proliferation. Due to the inflammatory stimulation by adjuvant, the spleen index and T lymphocyte proliferation rate were increased in the AIA group ($P < 0.01$); DEX and QWRG (2.0 and 4.0 g/kg) obviously reduced the spleen index and the proliferation rate of T lymphocytes compared with the AIA group ($P < 0.05$ or $P < 0.01$), while treatment with 2.0 g/kg QWRG resulted in significantly weaker effects than the DEX treatment ($P < 0.05$). These results showed that QWRG can inhibit the ConA- and LPS-induced proliferation of lymphocytes in the spleen (Table 7).

3.4. Pathological Changes of the Synovial Membrane following QWRG Treatment. Typical microphotographs of knee joints sections stained with HE illustrated the severity of the joint damage, and histological analyses were performed to investigate whether QWRG relieved the histological changes in knee joint of the AIA rats (Figure 2). In the normal group, the articular cavity was clearly visible, and there was

Table 5: Effects of QWRG in AIA model on serum biochemical indicators ($\overline{x} \pm s$, $n = 10$).

Groups	RF (IU/mL)	MDA (nmol/mL)	ALP (U/L)	AST (U/L)	ALT (U/L)
Control	—	3.8 ± 0.6	80.4 ± 4.2	42.4 ± 2.0	45.7 ± 5.3
AIA group	$87.4 \pm 2.4^{**}$	$7.1 \pm 1.4^{**}$	$483.2 \pm 29.0^{**}$	$142.3 \pm 9.5^{**}$	$172.6 \pm 9.2^{**}$
DEX (5 mg/kg)	$35.5 \pm 1.9^{\#\#}$	$4.6 \pm 0.6^{\#\#}$	$178.4 \pm 16.9^{\#\#}$	$78.9 \pm 5.9^{\#\#}$	$99.3 \pm 8.1^{\#\#}$
QWRG					
1.0 g/kg	81.8 ± 3.0	7.0 ± 2.1	455.3 ± 23.2	142.0 ± 11.2	168.6 ± 8.4
2.0 g/kg	$46.9 \pm 3.2^{\#\#\&}$	$6.2 \pm 1.5^{\#\&}$	$345.5 \pm 12.3^{\#\#\&\&}$	$112.3 \pm 8.4^{\&}$	$143.3 \pm 9.5^{\#\&}$
4.0 g/kg	$37.9 \pm 4.1^{\#\#}$	$4.9 \pm 2.2^{\#\#}$	$248.0 \pm 22.6^{\#\#}$	$89.3 \pm 7.8^{\#\#}$	$111.4 \pm 10.4^{\#\#}$

The values from 10 different rats in each group. Data are mean ± SD. $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus AIA group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ versus DEX.

Table 6: Effects of QWRG in AIA model on serum immune indicators ($\overline{x} \pm s$, $n = 10$).

Groups	IL-1β (pg/mL)	TNF-α (pg/mL)	IL-16 (pg/mL)	IL-2 (pg/mL)
Control	215.3 ± 58.5	402.4 ± 73.19	10.2 ± 0.6	85.3 ± 8.3
AIA group	$451.9 \pm 37.5^{**}$	$825.9 \pm 61.15^{**}$	$28.4 \pm 1.6^{**}$	$163.4 \pm 9.0^{**}$
DEX (5 mg/kg)	$328.4 \pm 33.9^{\#\#}$	$591.6 \pm 81.42^{\#\#}$	$13.4 \pm 1.6^{\#\#}$	$96.7 \pm 6.8^{\#\#}$
QWRG				
1.0 g/kg	429.6 ± 47.5	818.8 ± 114.42	25.9 ± 2.2	158.5 ± 5.9
2.0 g/kg	$373.4 \pm 49.3^{\#\#\&}$	$702.5 \pm 94.11^{\#\#\&}$	$17.2 \pm 1.9^{\#\#\&}$	$108.6 \pm 6.0^{\#\#\&}$
4.0 g/kg	$342.5 \pm 61.1^{\#\#}$	$618.6 \pm 91.06^{\#\#}$	$12.4 \pm 2.3^{\#\#}$	$90.5 \pm 4.9^{\#\#}$

The values from 10 different rats in each group. Data are mean ± SD. $^{**}P < 0.01$ versus control; $^{\#\#}P < 0.01$ versus AIA group; $^{\&}P < 0.05$ versus DEX.

Table 7: Effects of QWRG in AIA model on spleen index and spleen lymphocyte proliferation ($\overline{x} \pm s$, $n = 10$).

Groups	Spleen index	OD_{450}	
		ConA	LPS
Control	3.8 ± 0.5	402.43 ± 73.2	10.2 ± 0.6
AIA group	$5.9 \pm 0.7^{**}$	$825.88 \pm 61.2^{**}$	$28.4 \pm 1.6^{**}$
DEX (5 mg/kg)	$4.4 \pm 0.9^{\#\#}$	$591.6 \pm 81.4^{\#\#}$	$13.4 \pm 1.6^{\#\#}$
QWRG			
1.0 g/kg	5.6 ± 0.5	818.82 ± 114.4	25.9 ± 2.2
2.0 g/kg	$5.0 \pm 0.6^{\&}$	$702.53 \pm 94.1^{\#\&}$	$17.2 \pm 1.9^{\#\#\&}$
4.0 g/kg	$4.5 \pm 1.1^{\#\#}$	$618.58 \pm 91.6^{\#\#}$	$12.4 \pm 2.3^{\#\#}$

The values from 10 different rats in each group. Data are mean ± SD. $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus AIA group; $^{\&}P < 0.05$ versus DEX.

no pathological change in the synovium. The synovial layer was composed of synovial cells and orderly arranged with no inflammatory cell infiltration and a smooth articular cartilage surface. The pathological slides of the AIA group showed matrix thickening, subsynovial collagen fiber structural changes, inflammatory cell infiltration, and obvious capillary hyperplasia. Compared with the AIA rats, QWRG (2.0 and 4.0 g/kg) and DEX ameliorated the above-mentioned pathological changes to varying degrees. Mankin semiquantified analysis of the knee joint sections further indicated that QWRG effectively reduced the cartilage damage (Figure 3(a)), with statistical significance at the higher doses (2.0 and 4.0 g/kg; $P < 0.01$). In addition, accompanied with the relief of cartilage damage, the severity of the underlying bone destruction and inflammatory cells infiltration was also attenuated by QWRG in a dose-dependent manner ($P < 0.05$ or $P < 0.01$; Figures 3(b) and 3(c)).

3.5. Immunohistochemical Changes of IL-1β, TNF-α, and IL-16 in Synovium. The IL-1β, TNF-α, and IL-16 positive cells were found distributed throughout the synovium, especially in the synovial sublining regions (Figures 4(a), 5(a), and 6(a)). Compared with the control group, the expression of IL-1β, TNF-α, and IL-16 in the AIA group was significantly enhanced ($P < 0.01$). In addition, compared with the AIA group, there was a significant reduction in the expression of IL-1β, TNF-α, and IL-16 in rats treated with 2.0 g/kg and 4.0 g/kg QWRG and DEX-treated rats ($P < 0.05$ or $P < 0.01$). Furthermore, the efficacy of QWRG treatment at of 4.0 g/kg was similar to that of DEX treatment ($P > 0.05$), while the 2.0 g/kg QWRG dose exerted weaker effects than the DEX treatment ($P < 0.05$; Figures 4(b), 5(b), and 6(b)). These results suggested that QWRG could significantly reduce the expression of IL-1β, TNF-α, and IL-16 in the synovium of AIA rats.

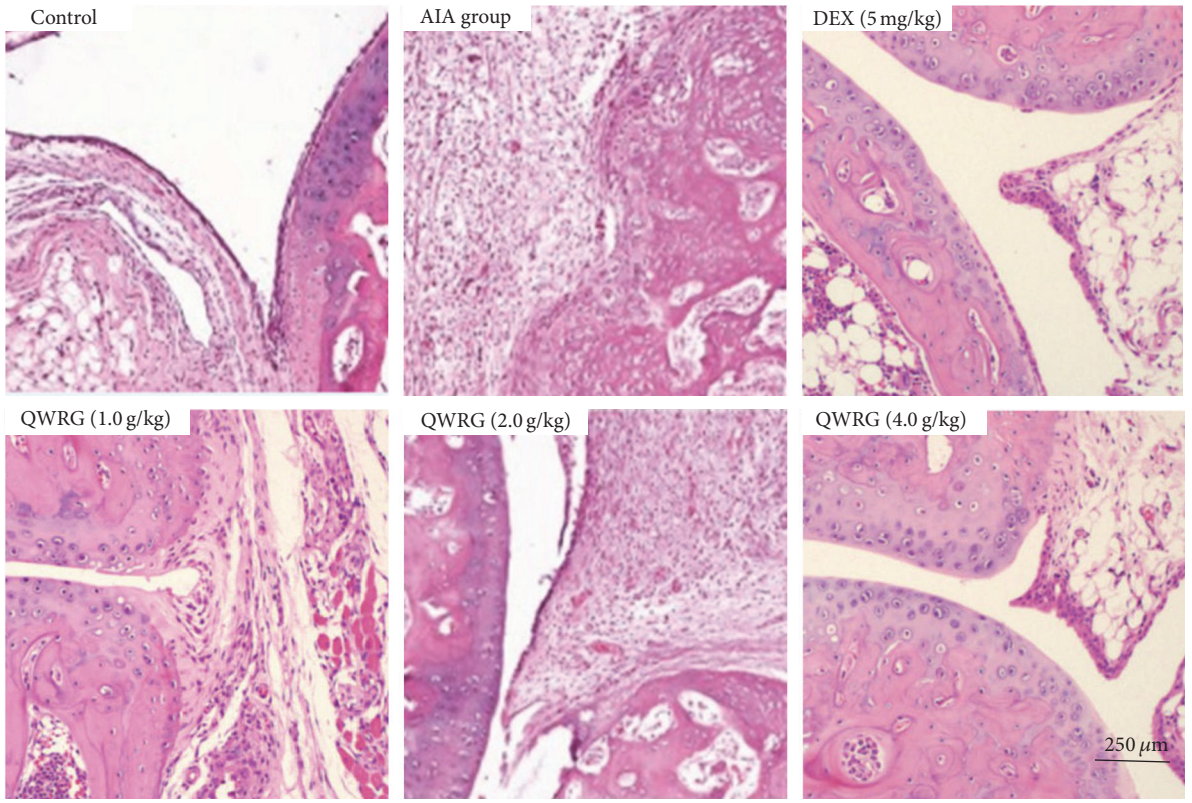

Figure 2: Effect of QWRG on synovium damage of rats with AIA (representative histopathologic photos of knee joint sections from different groups with H&E staining, taken from control rats, AIA model rats, QWRG-treated rats of 1.0 g/kg, 2.0 g/kg, and 4.0 g/kg, and DEX-treated group).

4. Discussion

Rheumatoid, with symptoms such as swelling, release of RF (autoantibody), deformity, and systemic change, is an autoimmune disease characterized by chronic inflammation of the synovial joint. In RA, swelling of the synovium due to the proliferation of synovial cells is the main actor in the cartilage deterioration [24]. Bone erosion, associated with increased and prolonged inflammation, affects 80% of patients and occurs rapidly [25]. In addition, the imbalance of the immune function mainly reflects the imbalance between cellular immunity and humoral immunity. Cellular immunity relatively increases and Th1 cells become activated, thus leading to the secretion of proinflammatory cytokines, while the humoral immunity decreases, and the Th2 inflammatory cytokines secretion decreases [26]. Studies showed that the cytokines produced from mononuclear macrophages and lymphocytes in the synovium play an important role in the pathogenesis of RA [27, 28]. Furthermore, the complex role of the cytokine networks is a key factor in the persistence of RA lesions and the progression of the disease [29]. In the TCM theory, the RA-related symptoms belong to the category of Bi Zheng, which can be manifested as arthralgia and dyskinesia of the joints and dampness and heat of the limbs. QWRG is a TCM compound, and it can dispel cold and relieve pain. In this study, from the evaluation of the safety experiment, the oral administration of 40 mL/kg QWRG did not induce any toxicity in normal mice and did not affect growth, suggesting that the administration of QWRG at this clinical dose would be safe and would not ensue any adverse effects in mice in the context of RA treatment.

The AIA rats are an experimental model of RA that shares many pathological features with RA including extremities swelling, synovial hyperplasia, proliferation of synovial tissue, destruction of cartilage, and excessive inflammation. After the AIA rat model was successfully established, we explored the antiarthritic effect of different QWRG doses in the AIA rats. Our results indicated that QWRG (2.0 and 4.0 g/kg) had obviously relieved the AIA paw swelling (Table 1), while the change in body weight was significant throughout the treatment (Table 2), although to a lesser extent it was compared with the DEX treatment. In addition, the QWRG treatment could significantly alleviate the variation of arthritic scores (Table 3) and improve the mechanical nociceptive threshold in the AIA rats (Table 4). These results suggested that QWRG possesses obvious anti-inflammation effects, though with a lesser efficacy than DEX. Previous studies have indicated that TCMs are effective for RA, including *Nux vomica*, *Tripterygium wilfordii*, and orientvine in treating RA [30–33]. In terms of the mechanisms, several TCMs primarily inhibit the expression of cytokines associated with RA to exert their anti-RA effects [34, 35].

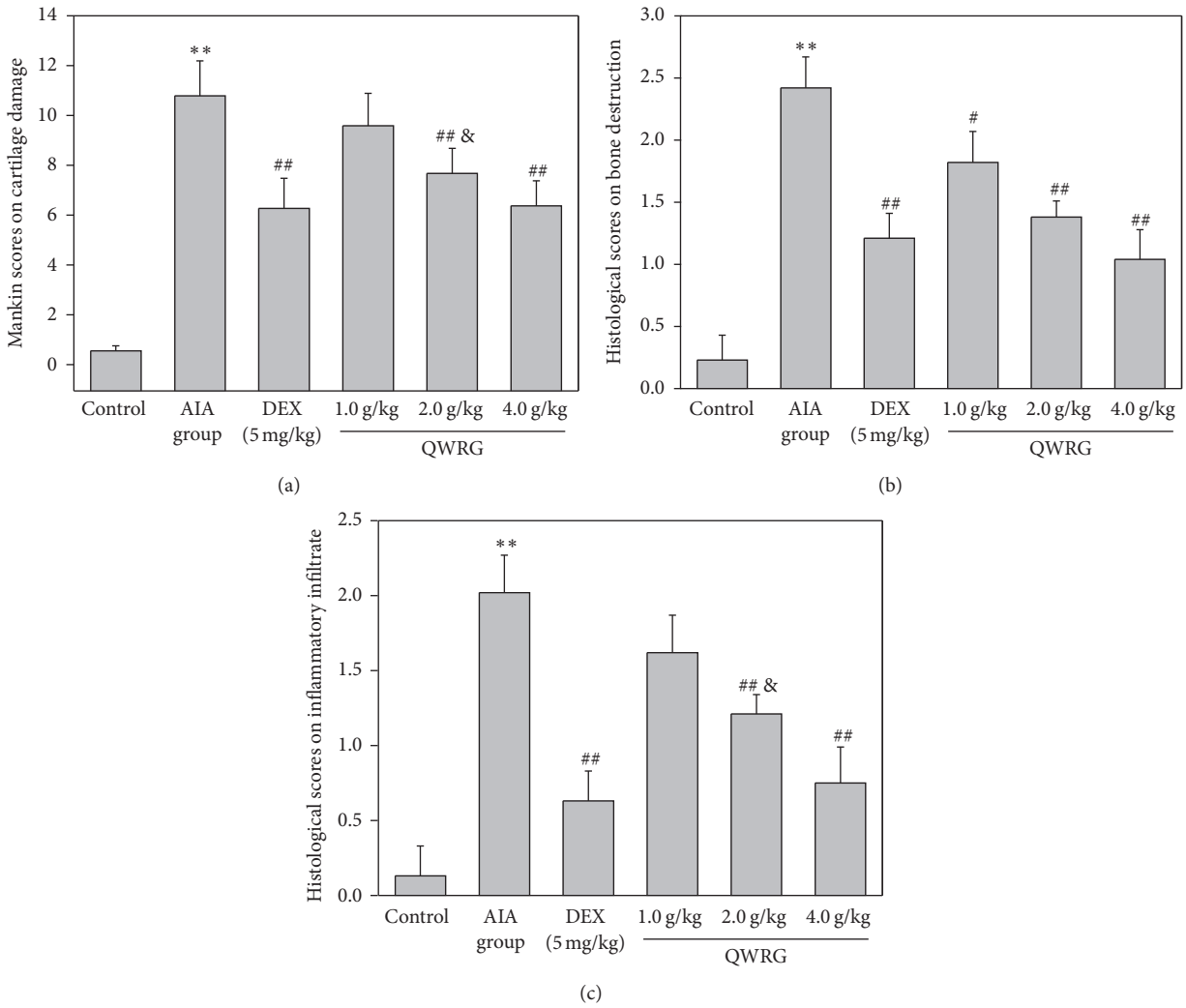

Figure 3: Semiquantified analysis of the protective effect of QWRG on synovium damage. (a) Mankin scores on cartilage damage; (b) histological scores on underlying bone destruction; (c) histological scores on inflammatory cells infiltration. Tissues from three different rats in each group and 10 randomly selected areas from each slide were analyzed. Data are mean ± SD ($n = 10$). $^{**}P < 0.01$ versus control; compared with AIA group, when P is less than 0.01 or 0.05, it means very significant difference, designated by ## or #; $^{\&}P < 0.05$ versus DEX.

To evaluate the antiarthritic property of a drug, the levels of RF, MDA, ALP, AST, and ALT provide an excellent and simple tool. In the present study, the activities of these biomarkers significantly increased in the AIA rats [36]. These enzymes, when released into the circulation during the bone formation and resorption, will be involved in localized bone loss such as bone erosion and periarticular osteopenia [37]. In this study, the levels of AST and ALT were decreased in rats treated with QWRG. This result implies that QWRG can relieve the liver toxicity induced by AIA. In addition, with the QWRG treatments, the increased levels of serum RF, MDA, and ALP were also significantly attenuated (Table 5), indicating that QWRG is effective on AIA.

The histological examination and analysis of the knee joint damage were measured by HE staining (Figure 2). Mankin scores were calculated to assess the severity of the cartilage damage. The results suggested that QWRG effectively reduced the severity of cartilage damage in the AIA rats, with statistically significant effects at 2.0 and 4.0 g/kg QWRG (Figure 3(a)). In addition, the severity of the underlying bone destruction and inflammatory cells infiltration was also attenuated by QWRG in a dose-dependent manner (Figures 3(b) and 3(c)), suggesting that QWRG significantly relieved the inflammation of the synovium and improved the cartilage in the AIA rats, even though still significantly weaker than DEX.

In RA, the cytokines can be divided into two categories according to their different sources. One is produced by T lymphocytes, and the other is mainly produced by monocytes/macrophages, including IL-1, TNF, IL-5, IL-18, IL-15, IL-6, IL-12, and IL-17. A variety of factors are interdependent and their interaction results in a large network of cytokines, which restrict or promote the occurrence and development of various diseases. For example, IL-1β is an important factor in the development of RA pathology, which can be

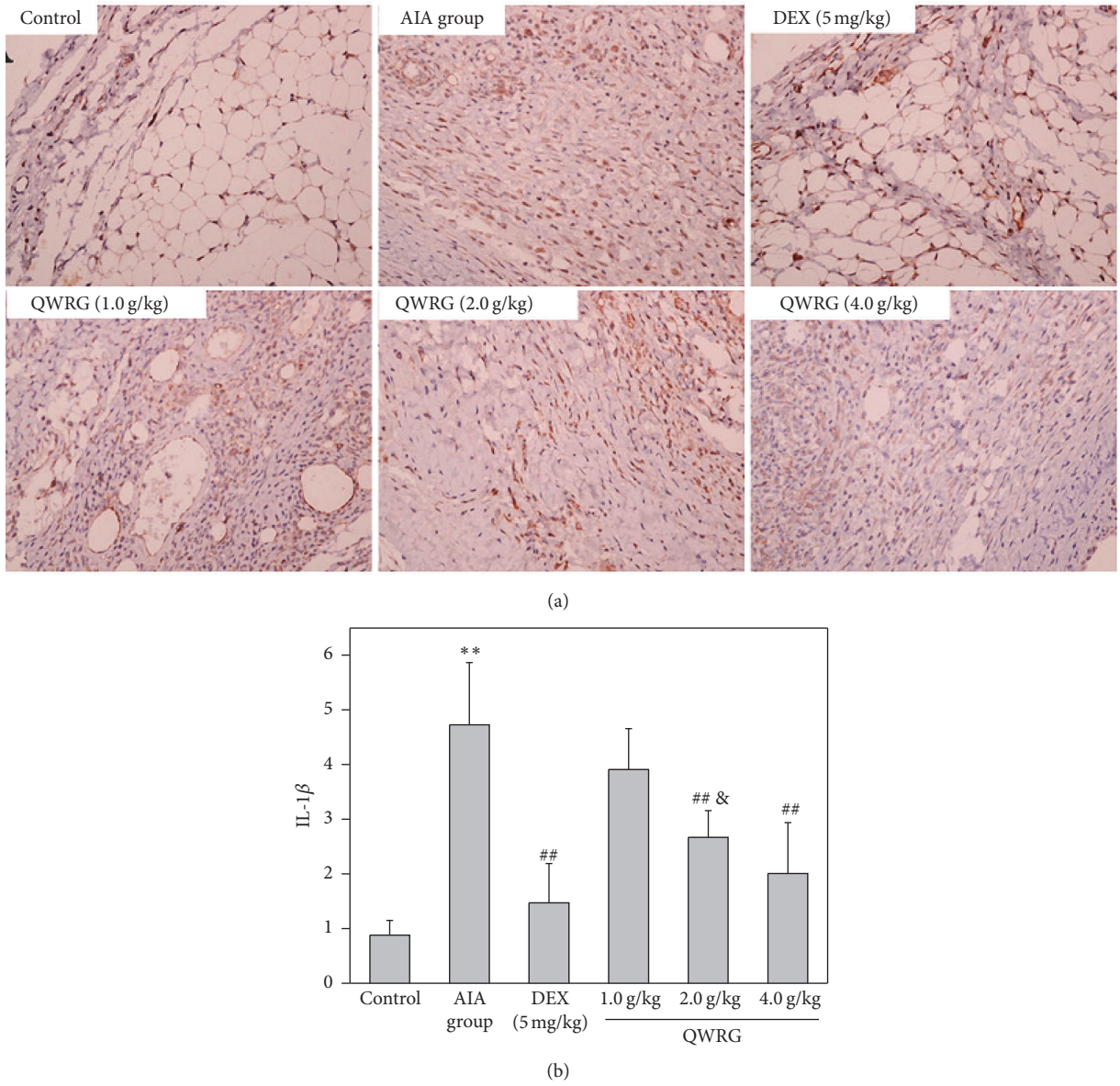

FIGURE 4: Immunohistochemical results of IL-1β in synovium (immunohistochemistry of IL-1β in the knee joints taken from control rats, AIA model rats, QWRG-treated rats of 1.0 g/kg, 2.0 g/kg, and 4.0 g/kg, and DEX-treated group. Magnification ×200. Tissues from three different rats in each group and 10 randomly selected areas from each slide were analyzed. Quantitative data (mean ± SD) are presented using the average density values of the IL-1β positive regions. $^{**}P < 0.01$ versus control; $^{\#\#}P < 0.01$ versus AIA group; $^{\&}P < 0.05$ versus DEX).

detected in the joint cavity, and it can assist cell migration and stimulate endothelial cells. Therefore, inhibiting the production of IL-1β is one approach for treating RA. As "sister cell cytokines" of IL-1, the effective TNF-α target cells and their functions are also very similar. The high expression of TNF-α in RA can cause local joint tissue destruction and clinical symptoms [38]. The overexpression of TNF-α could result in severe arthritis in mice, while the pharmacological inhibition of the TNF-α activity can significantly improve the clinical symptoms of RA [39]. Furthermore, IL-16 is another proinflammatory cytokine secreted by T lymphocytes. In RA, IL-16 can not only destroy the cartilage collagen but also stimulate the differentiation of osteoclast and inhibit the bone synthesis [40]. In addition, IL-16 can also play a synergistic role with TNF-α and IL-1β to amplify the inflammatory response [41]. This present study focused on investigating the lymphocytes and inflammatory cytokines in AIA rats in addition to exploring the pharmacodynamics of QWRG for a preliminarily inquiry of the cellular and molecular mechanisms of QWRG on relieving AIA. In accordance with the histologic and immunochemical results, QWRG (2.0 and 4.0 g/kg) can significantly alleviate the inflammation of the synovial cavity in RA rats and reduce the grading of synovitis through inhibiting the expressions of IL-1β, TNF-α, and IL-16 in the serum and synovium of RA rats. It can also antagonize the proliferation of lymphocytes

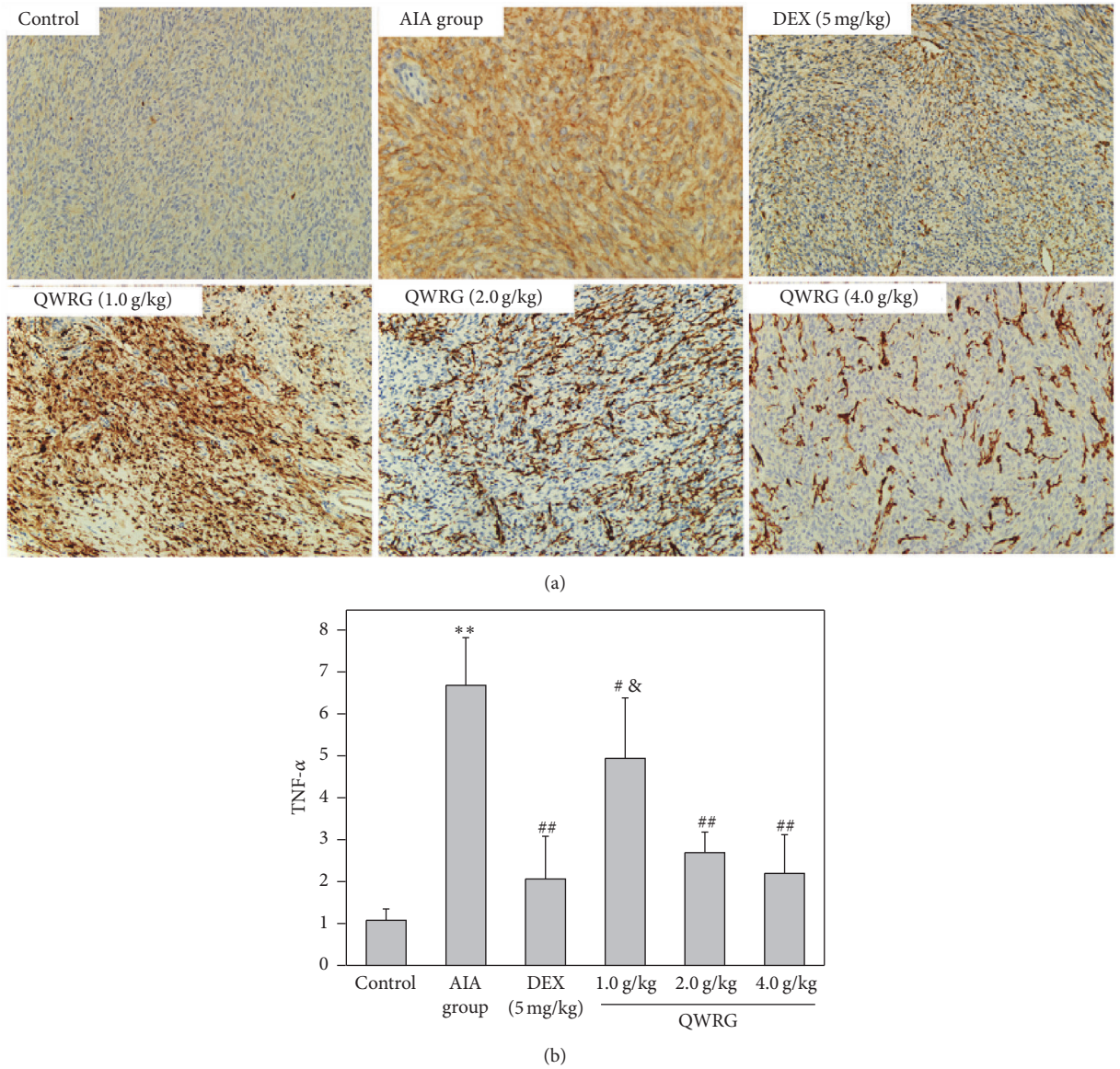

Figure 5: Immunohistochemical results of TNF-α in synovium (immunohistochemistry of TNF-α in the knee joints taken from control rats, AIA model rats, QWRG-treated rats of 1.0 g/kg, 2.0 g/kg, and 4.0 g/kg, and DEX-treated group. Magnification ×200. Tissues from three different rats in each group and 10 randomly selected areas from each slide were analyzed. Quantitative data (mean ± SD) are presented using the average density values of the TNF-α positive regions. $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus AIA group; $^{\&}P < 0.05$ versus DEX).

induced by ConA and LPS and the spleen index in rats. The present results indicated that the anti-RA effect of QWRG is profound and that its underlying mechanism might be associated with decreasing the release of cytokines, regulating the function of the spleen, and elevating the immunologic function.

In conclusion, our study revealed that QWRG effectively inhibited inflammation and cartilage damage in AIA rats. Taken together with the preventive effects on cartilage damage and relatively lower adverse effects, it is reasonable to regard QWRG as a potential antiarthritic drug. However, further work is still needed to identify the detailed mechanisms underlying this effect. Our findings present some experimental evidence that the reduction of IL-1β, TNF-α, and IL-16 in the serum and synovial tissue and the reduction of lymphocyte proliferation might be of potential clinical interest in RA treatment. Thus, in the light of the above-mentioned findings, it could be asserted that QWRG could serve as a promising herbal drug that will open a new window for the treatment of RA.

Disclosure

Qi Xu and Yong Zhou are not co-first author.

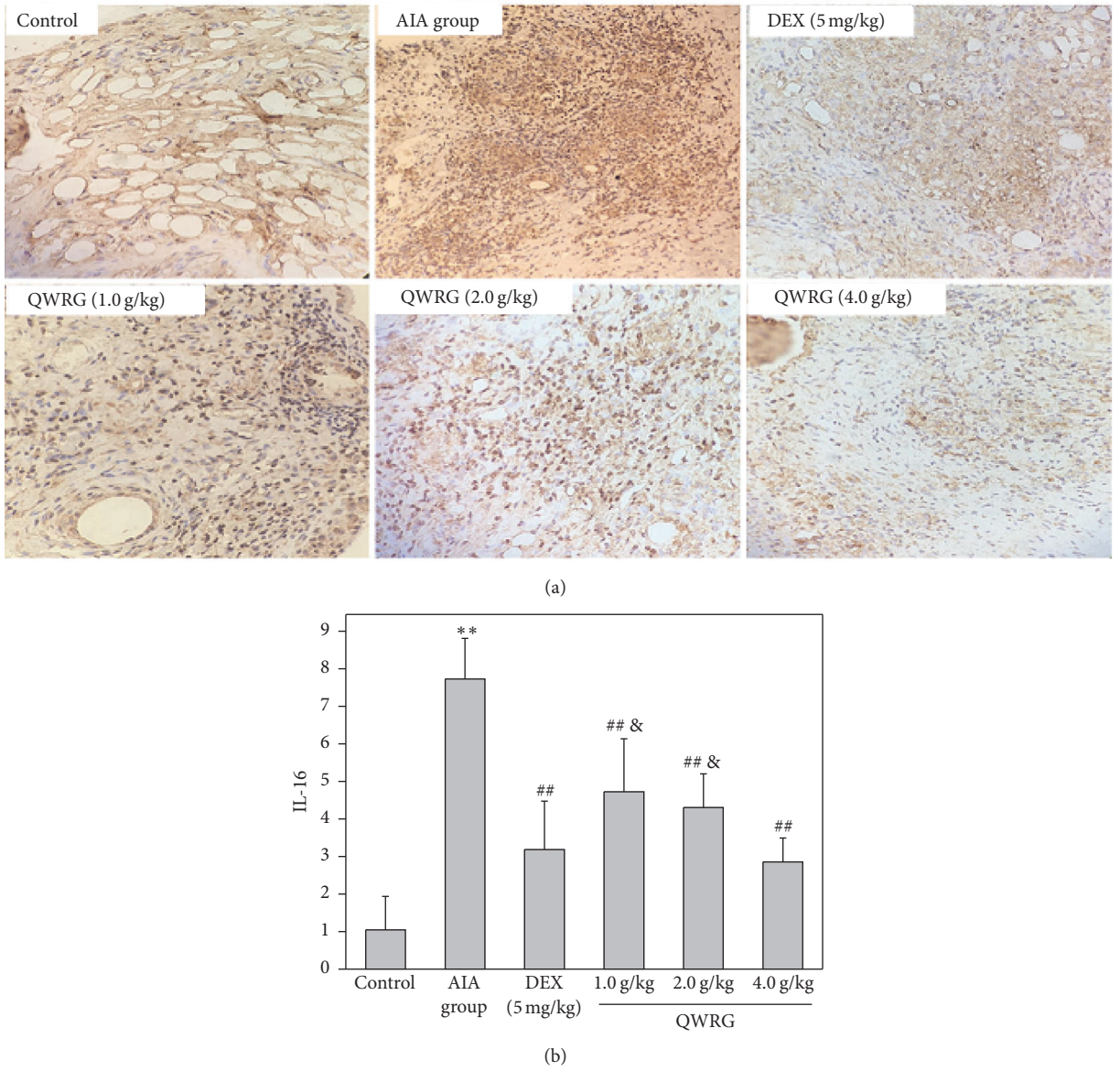

FIGURE 6: Immunohistochemical results of IL-16 in synovium (immunohistochemistry of IL-16 in the knee joints taken from control rats, AIA model rats, QWRG-treated rats of 1.0 g/kg, 2.0 g/kg, and 4.0 g/kg, and DEX-treated group. Magnification ×200. Tissues from three different rats in each group and 10 randomly selected areas from each slide were analyzed. Quantitative data (mean ± SD) ($n = 10$) are presented using the average density values of the IL-16 positive regions. $^{**}P < 0.01$ versus control; $^{\#\#}P < 0.01$ versus AIA group; $^{\&}P < 0.05$ versus DEX).

Conflicts of Interest

The authors declare that they have no conflicts of interest to disclose.

Acknowledgments

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References

[1] G. Coiffier, B. Bouvard, F. Chopin et al., "Common bone turnover markers in rheumatoid arthritis and ankylosing spondylitis: a literature review," *Joint Bone Spine*, vol. 80, no. 3, pp. 250–257, 2013.

[2] S. E. Gabriel, "The epidemiology of rheumatoid arthritis," *Rheumatic Disease Clinics of North America*, vol. 27, no. 2, pp. 269–281, 2001.

[3] A. Gibofsky, "Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis," *The American Journal of Managed Care*, vol. 18, no. 13, pp. S295–S302, 2012.

[4] Q. Wang, H. Kuang, Y. Su et al., "Naturally derived anti-inflammatory compounds from Chinese medicinal plants," *Journal of Ethnopharmacology*, vol. 146, no. 1, pp. 9–39, 2013.

[5] K. M. Huffman, R. Jessee, B. Andonian et al., "Molecular alterations in skeletal muscle in rheumatoid arthritis are related

to disease activity, physical inactivity, and disability," *Arthritis research and therapy*, vol. 19, no. 1, p. 12, 2017.

[6] M. J. H. Hair, M. G. H. Sande, and T. H. Ramwadhdoebe, "Features of the synovium of individuals at risk of developing rheumatoid arthritis : implications for understanding preclinical rheumatoid arthritis," *Arthritis and Rheumatology*, vol. 66, no. 3, pp. 513–522, 2014.

[7] D. A. Walsh and D. F. McWilliams, "Mechanisms, impact and management of pain in rheumatoid arthritis," *Nature Reviews Rheumatology*, vol. 10, no. 10, pp. 581–592, 2014.

[8] D. Deane, "Can rheumatoid arthritis be prevented," *Best Practice and Research Clinical Rheumatology*, vol. 27, no. 4, pp. 87–92, 2013.

[9] S. Ramiro, C. Gaujoux-Viala, J. L. Nam et al., "Safety of synthetic and biological DMARDs: a systematic literature review informing the 2013 update of the EULAR recommendations for management of rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 73, no. 3, pp. 529–535, 2014.

[10] M. Schiff, M. Keiserman, C. Codding et al., "Clinical response and tolerability to abatacept in patients with rheumatoid arthritis previously treated with infliximab or abatacept: Open-label extension of the ATTEST Study," *Annals of the Rheumatic Diseases*, vol. 70, no. 11, pp. 2003–2007, 2011.

[11] L. Harmse and H. Reuter, "An overview of the biological disease modifying drugs available for arthritic conditions in South Africa," *South African Family Practice*, vol. 58, no. 6, pp. 6–10, 2016.

[12] P. Roll, A. Palanichamy, C. Kneitz, T. Dorner, and H.-P. Tony, "Regeneration of B cell subsets after transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis," *Arthritis & Rheumatology*, vol. 54, no. 8, pp. 2377–2386, 2006.

[13] V. Murunikkara and M. K. Rasool, "Trikatu, a herbal compound mitigates the biochemical and immunological complications in adjuvant-induced arthritic rats," *International Journal of Rheumatic Diseases*, vol. 20, no. 3, pp. 298–308, 2017.

[14] M. C. Huang, F. T. Pai, C. C. Lin et al., "Characteristics of traditional Chinese medicine use in patients with rheumatoid arthritis in Taiwan: a nationwide population-based study," *Journal of Ethnopharmacology*, vol. 176, pp. 9–16, 2015.

[15] C. Lu, Q.-I. Zha, A.-I. Chang, Y. T. He, and A.-P. Lu, "Pattern differentiation in traditional chinese medicine can help define specific indications for biomedical therapy in the treatment of rheumatoid arthritis," *The Journal of Alternative and Complementary Medicine*, vol. 15, no. 9, pp. 1021–1025, 2009.

[16] J. Qiu, "When the East meets the West: The future of traditional Chinese medicine in the 21st century," *National Science Review*, vol. 2, no. 3, pp. 377–380, 2015.

[17] Y. He, A. Ou, X. Yang et al., "Traditional Chinese medicine versus western medicine as used in China in the management of rheumatoid arthritis: a randomized, single-blind, 24-week study," *Rheumatology international*, vol. 34, no. 12, pp. 1647–1655, 2014.

[18] H. M. Tag, O. E. Kelany, and H. M. Tantawy, "Potential anti-inflammatory effect of lemon and hot pepper extracts on adjuvant-induced arthritis in mice," *The Journal of Basic and Applied Zoology*, vol. 67, no. 5, pp. 149–157, 2014.

[19] M. Hegen, J. C. Keith Jr., M. Collins, and C. L. Nickerson-Nutter, "Utility of animal models for identification of potential therapeutics for rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 67, no. 11, pp. 1505–1515, 2008.

[20] J. Zhao, Q. Zha, M. Jiang, H. Cao, and A. Lu, "Expert consensus on the treatment of rheumatoid arthritis with chinese patent medicines," *The Journal of Alternative and Complementary Medicine*, vol. 19, no. 2, pp. 111–118, 2013.

[21] G. Coruzzi, M. Adami, E. Guaita, I. J. P. de Esch, and R. Leurs, "Antiinflammatory and antinociceptive effects of the selective histamine H4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation," *European Journal of Pharmacology*, vol. 563, no. 1-3, pp. 240–244, 2007.

[22] Y. Feng-Lai, C. Fei-Hu, L. Wei-Guo et al., "Inhibition of acid-sensing ion channels in articular chondrocytes by amiloride attenuates articular cartilage destruction in rats with adjuvant arthritis," *Inflammation Research*, vol. 59, no. 11, pp. 939–947, 2010.

[23] O. Kehoe, A. Cartwright, A. Askari, A. J. El Haj, and J. Middleton, "Intra-articular injection of mesenchymal stem cells leads to reduced inflammation and cartilage damage in murine antigen-induced arthritis," *Journal of Translational Medicine*, vol. 12, article 157, 2014.

[24] D. K. Rhee, J. Marcelino, M. Baker et al., "The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth," *The Journal of Clinical Investigation*, vol. 115, no. 3, pp. 622–631, 2005.

[25] H. Visser, S. Le Cessie, K. Vos, F. C. Breedveld, and J. M. W. Hazes, "How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis," *Arthritis & Rheumatology*, vol. 46, no. 2, pp. 357–365, 2002.

[26] M.-C. Boissier, E. Assier, G. Falgarone, and N. Bessis, "Shifting the imbalance from Th1/Th2 to Th17/treg: The changing rheumatoid arthritis paradigm," *Joint Bone Spine*, vol. 75, no. 4, pp. 373–375, 2008.

[27] G. R. Burmester, E. Feist, and T. Dörner, "Emerging cell and cytokine targets in rheumatoid arthritis," *Nature Reviews Rheumatology*, vol. 10, no. 2, pp. 77–88, 2014.

[28] F. Hu, R. Mu, J. Zhu et al., "Hypoxia and hypoxia-inducible factor-1α provoke toll-like receptor signalling-induced inflammation in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 73, no. 5, pp. 928–936, 2014.

[29] J. Sokolove, D. S. Johnson, L. J. Lahey et al., "Rheumatoid factor as a potentiator of anti-citrullinated protein antibody-mediated inflammation in rheumatoid arthritis," *Arthritis & Rheumatology*, vol. 66, no. 4, pp. 813–821, 2014.

[30] E. Choy, K. Ganeshalingam, A. G. Semb, Z. Szekanecz, and M. Nurmohamed, "Cardiovascular risk in rheumatoid arthritis: recent advances in the understanding of the pivotal role of inflammation, risk predictors and the impact of treatment," *Rheumatology*, vol. 53, no. 12, pp. 2143–2154, 2014.

[31] Y. Hu, W. Cheng, W. Cai, Y. Yue, J. Li, and P. Zhang, "Advances in research on animal models of rheumatoid arthritis," *Clinical Rheumatology*, vol. 32, no. 2, pp. 161–165, 2013.

[32] J. R. O'Dell, T. R. Mikuls, T. H. Taylor et al., "Therapies for active rheumatoid arthritis after methotrexate failure," *The New England Journal of Medicine*, vol. 369, no. 4, pp. 307–318, 2013.

[33] F. Calmon-Hamaty, R. Audo, B. Combe, J. Morel, and M. Hahne, "Targeting the Fas/FasL system in Rheumatoid Arthritis therapy: Promising or risky?" *Cytokine*, vol. 75, no. 2, pp. 228–233, 2015.

[34] H.-M. Liu and K.-J. Wang, "Therapeutic effect of Captopril on rheumatoid arthritis in rats," *Asian Pacific Journal of Tropical Medicine*, vol. 7, no. 12, pp. 996–999, 2014.

[35] R. Heo, J.-S. Park, H. J. Jang et al., "Hyaluronan nanoparticles bearing γ-secretase inhibitor: In vivo therapeutic effects on

rheumatoid arthritis," *Journal of Controlled Release*, vol. 192, pp. 295–300, 2014.

[36] J. Zhao, T. Liu, F. Xu, S. You, C. Li, and Z. Gu, "Anti-arthritic effects of total flavonoids from Juniperus sabina on complete freund's adjuvant induced arthritis in rats," *Pharmacognosy Magazine*, vol. 12, no. 47, pp. 178–183, 2016.

[37] T. Pan, T. Cheng, Y. Jia, P. Li, and F. Li, "Anti-rheumatoid arthritis effects of traditional Chinese herb couple in adjuvant-induced arthritis in rats," *Journal of Ethnopharmacology*, vol. 205, pp. 1–7, 2017.

[38] G. Murdaca, F. Spano, and F. Puppo, "Use of leflunomide plus TNF-α inhibitors in rheumatoid arthritis," *Expert Opinion on Drug Safety*, vol. 12, no. 6, pp. 801–804, 2013.

[39] D. H. Solomon, J. R. Curtis, K. G. Saag et al., "Cardiovascular risk in rheumatoid arthritis: comparing tnf-α blockade with nonbiologic DMARDs," *American Journal of Medicine*, vol. 126, no. 8, pp. 730.e9–730.e17, 2013.

[40] W. Ouyang, J. K. Kolls, and Y. Zheng, "The biological functions of T helper 17 cell effector cytokines in inflammation," *Immunity*, vol. 28, no. 4, pp. 454–467, 2008.

[41] L. A. Fouser, J. F. Wright, K. Dunussi-Joannopoulos, and M. Collins, "Th17 cytokines and their emerging roles in inflammation and autoimmunity," *Immunological Reviews*, vol. 226, no. 1, pp. 87–102, 2008.

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Research Article

Effect of Sheng-Jiang Powder on Obesity-Induced Multiple Organ Injuries in Rats

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Background and Aims. Obesity has become the main public health issue nowadays with poor control and has been associated with increased risk of multiorgan disease, but the specific mechanism and effective medication are still to be addressed. Sheng-jiang powder (SJP) showed great potential in preventing obesity in Chinese researches but has no trace in English reports. This study was designed to investigate the effect of SJP on obesity and obesity-mediated multiorgan injuries. *Methods*. Rats were randomized into normal group (NG), obese group (OG), and SJP treatment group (SG). Obesity was induced by high-fat diet feeding. Rats were gavaged with SJP/normal saline daily from the third week and all rats were sacrificed after 12 weeks' feeding. Tissues were obtained for cytokines tests. *Results*. Firstly, high-fat diet feeding led to significant obesity. Compared to NG, the level of SOD in the liver, spleen, lung, and kidney was much lower in OG ($p < 0.05$), while the pathological scores of pancreas, liver, spleen, lung, and kidney were much higher. SJP significantly increased SOD level in the liver, spleen, and lung and reduced the pathological scores of pancreas, liver, spleen, lung, and kidney correspondingly ($p < 0.05$). *Conclusions*. SJP ameliorates inflammatory response and mitigates obesity-induced multiple organ injuries.

1. Introduction

The rising prevalence of obesity has achieved an unprecedented pandemic around the world. According to the results of Global Burden of Disease 2013 Study (GBD), the prevalence of overweight and obesity combined has risen by 27.5% for adults and 47.1% for children globally during the past three decades. And the number of overweight and obese individuals has increased to 2.1 billion in 2013, which is 2.28 times that of 1980 [1]. Obesity has been related to an increased risk of a series of diseases that involve multiple organ-systems of the body and was estimated to cause 3.4 million deaths, 3.9% of years of life loss, and 3.8% of disability adjusted life years (DALYs) globally in 2010 [2]. The specific mechanisms of obesity leading to diseases have not yet been fully elucidated, but increasing evidences have linked obesity to inflammation according to the work over the past decades.

Obesity-associated inflammation is a chronic, persistent, low-grade inflammation but with insidious effect against multiple organs. Lipid overaccumulation and energy metabolism disorder lead to inflammatory changes of microenvironment of adipose tissue with abundant macrophage infiltration, unbalanced secretion of adipokines, and overexpression of proinflammatory cytokines [3]. Subsequent activation of several inflammation signal pathways by those cytokines helps amplify the inflammatory response, which in turn promotes the expression and secretion of proinflammatory cytokines and eventually leads to inflammatory injuries of all organs without a single one having narrow escape [4]. Studies in recent years have demonstrated the independent role of obesity in the development of a series of diseases, such as nonalcoholic fatty liver disease [5], cardiovascular disease [6, 7], skeletal and muscular disorders [8], intestinal microbiota imbalance [9], metabolic disorders [10], respiratory disease [11], kidney disease [12], and neurological disease [13, 14], while the liver, heart, pancreas, bone, muscle, brain, and many other organs are relevant [15]. Therefore, increasing trials are carried out which try to find proper methods or

drugs to control the ongoing trend of obesity and relevant organ injuries, such as sea buckthorn leaves extract [16], shao fu zhu yu decoction [17], anthocyanin-rich foods [18], and probiotics supplement [9].

According to the traditional Chinese medicine (TCM) theory, obesity belongs to the category of "Turbidity," a syndrome caused by "ascending and descending disfunction" of spleen [19]. Spleen disfunction leads to abnormal motion of qi, further cause qi stagnation, phlegm retention, and blood stasis, and finally induces the occurrence of obesity. To treat turbidity is to regulate the generation, transportation, and distribution of lipid. And the permanent cure is to resume the "ascending and descending" function of spleen. SJP is derived from "wan bing hui chun," which was compiled by ting-xian gong during the Ming dynasty of China, and consists of Jiangchan *(Bombyx Batryticatus)*, Chantui *(periostracum cicada)*, Jianghuang *(Curcuma longa)*, and Dahuang *(Rheum palmatum)* [20]. As a classic representative formula to treat "ascending and descending disfunction," SJP was demonstrated to be effective in lowering body weight and anti-inflammation, antiviral, antiallergic, antipyretic, and immune regulation [21]. Early in the 1990s, there had been a study focused on the effect of SJP combined with auricular point sticking in lowering body weight [22]. SJP combined with acupuncture treatment can significantly increase serum adiponectin level, decrease serum leptin and intracellular ROS expression, and mitigate obesity-related inflammation in obese patients [23, 24]. However, almost all studies about SJP were reported in Chinese, and the effects of SJP on obesity-related multiple organ injuries have not been fully elucidated so far. Therefore, we designed this study to explore the effect of SJP on obesity-related inflammatory damage of multiple organs to give the world a comprehensive impression about SJP in ameliorating obesity-associated multiple organ injuries.

2. Materials and Methods

2.1. Design. This study is a prospective, randomized controlled trial.

2.2. Settings. The study was set at Ethnopharmacology Laboratory at West China Hospital.

2.3. Ethics Statement. The protocol was approved by the Ethics Committee for Animal Experiments of Sichuan University. All rats were handled according to the University Guidelines and the Animal Care Committee Guidelines of West China Hospital. All surgeries were performed under chloral hydrate anesthesia, and all efforts were made to minimize suffering of rats.

2.4. Preparation of Sheng-Jiang Powder. Sheng-jiang powder (SJP) was derived from the famous Chinese medical book "wan bing hui chun," and was composed of Jiangchan (*Bombyx Batryticatus*, 6 g), Chantui (*periostracum cicada*, 3 g), Jianghuang (*Curcuma longa*, 9 g), and Dahuang (raw rhubarb, 12 g). Jiangchan (1701117), Chantui (1608027), Jianghuang (1506067), and Dahuang (1610039) were purchased from Chengdu New Green Herbal Pharmaceutical Co., Ltd. (Chengdu, China). The crude drugs were identified and the prescription for this study was an aliquot from the same batch. SJP was boiled twice in distilled water (1 : 12, w/v) for 30 min each time. The blended supernatants were then lyophilized (yield = 23% w/w, dried extract/crude drug). The dried extract was dissolved in distilled water before use. According to the original prescription recorded, the dose of an adult was 0.5 g/Kg·BW. Therefore, we adopt a 10-fold dose (5 g/Kg·BW) to treat the experimental animals.

2.5. Animals and Treatment. Male Sprague-Dawley rats weighed 60–80 g were purchased from Chengdu Dashuo Experimental Animal Co., Ltd (Chengdu, China). All animals were kept under controlled temperature (22-23°C) and on a 12-h light/12-h dark cycle and had free access to a high-fat diet (60% of calories derived from fat; TP23300; Trophic Animal Feed High-tech Co., Ltd, China) to induce obesity or control diet (16.7% of calories derived from fat; TP23302; Trophic Animal Feed High-tech Co., Ltd, China) (http://trophic.biomart.cn). Animals were randomly allocated to normal group (NG, control diet, $n = 6$), obese group (OG, high-fat diet, $n = 8$), and Sheng-jiang powder group (SG, high-fat diet plus Sheng-jiang powder, $n = 8$) by random number table. The whole study lasted for 12 weeks with 10 weeks' administration of SJP (5 g/Kg) one time a day and body weight was recorded every week. Rats in SG were gavaged with SJP from the third week, while rats in the other two groups were gavaged with equal volume of normal saline instead. All rats were sacrificed after 12 weeks' feeding (Figure 1(a)). Tissue samples were obtained for cytokines tests and histopathological analysis. This study adhered to the ARRIVE Guidelines for reporting animal research (S1 ARRIVE Checklist).

2.6. Tissue Sampling and Cytokines Analysis. All rats were sacrificed after 12 weeks' feeding and blood samples were obtained from heart. Liver, heart, spleen, lung, kidney, intestine, and pancreas tissues were dissected immediately and collected for cytokines and histopathological analysis. Tissue samples were homogenized using a tissue homogenizer (Biospec Products, Bartlesville, OK). Homogenates were incubated at 4°C for 30 min and then centrifuged at 1000 ×g for 10 minutes. Supernatants were collected for cytokine analysis. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-px), reactive oxygen species (ROS), and myeloperoxidase (MPO) were measured by means of enzyme-linked immunosorbent assay (ELISA) (eBio, Wuhan, China) with commercially available materials. According to the manufacturer's protocol, absorbance was measured at 450 nm with High Throughput Universal Microplate Assay. The sample values were then read off the standard curve and the relative concentrations were calculated.

2.7. Histopathological Analysis. Fresh tissue samples were fixed in 10% neutral formalin and embedded in paraffin and then sectioned into 5 μm slices and followed with hematoxylin and eosin (H&E) staining. All the histopathology specimens were reviewed and scored in a blinded fashion

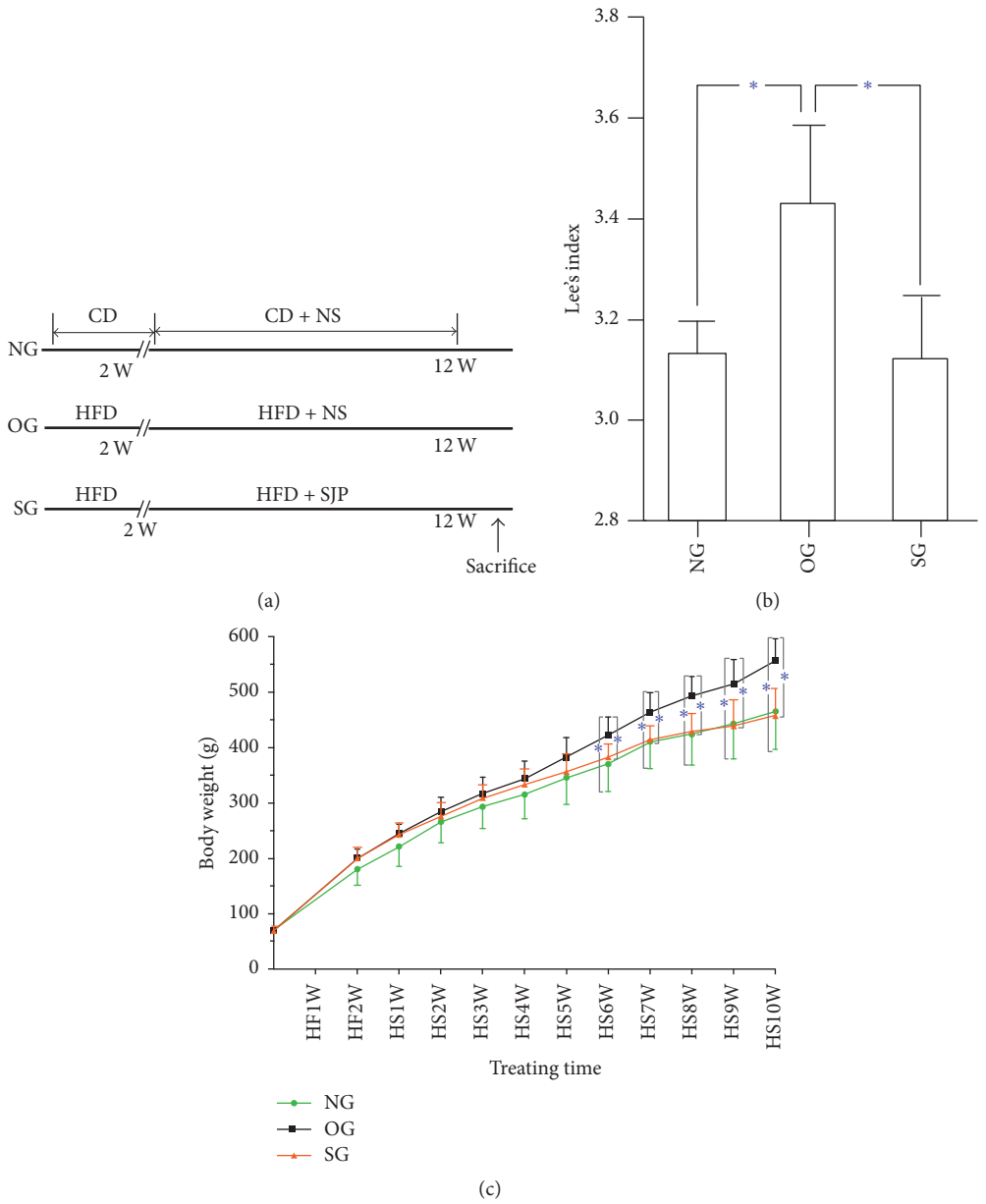

Figure 1: *Study design of feeding methods and the body weight and Lee's index of rats with high-fat diet feeding with/without Sheng-jiang powder (SJP) administration*. Normal group (NG), obese group (OG), and SJP treatment group (SG). CD: chew diet; HFD: high-fat diet; NS: normal saline; SJP: Sheng-jiang powder. HF: high-fat diet feeding; HS: high-fat diet feeding and SJP administration. (a) Feeding and intervention methods of the study; (b) Lee's index of rats before sacrifice; (c) body weight of rats in the three experimental groups during the whole process of feeding. The whole study lasted 12 weeks with 10 weeks' administration of SJP (5 g/Kg) one time a day. All rats were sacrificed after 12 weeks' feeding. $*$ indicates that $p < 0.05$.

by two independent pathologists using a scoring system for the extent and severity of tissue injury (points 0–4, edema, neutrophil infiltration, necrosis, and hemorrhage) as previously described [25]. The total histopathology score is the mean of the combined scores for each parameter from both investigators.

3. Statistical Analysis

All data were expressed as mean ± SD. Statistical analysis was performed with PEMS3.1 statistical program for Windows. One-way ANOVA was used to analyze group differences in the study. Differences with a $p < 0.05$ were considered to be statistically significant.

4. Results

4.1. SJP Protect against High-Fat Diet Induced Obesity in Experimental Rats. Obesity were successfully induced after 8 weeks' high-fat diet feeding with the body weights of rats in OG significantly increased by 20% more than that of the NG. However, rats in SG showed a much slower weight gain

Table 1: Expression of inflammatory indicators in tissues of rats in the three experimental groups.

Organ	NG ($n = 6$)	OG ($n = 8$)	SG ($n = 8$)
Liver			
MDA (pmol/ml)	1730 ± 258	1755 ± 204	1788 ± 327
ROS (IU/ml)	672 ± 97	707 ± 45	767 ± 56
SOD (U/ml)	321 ± 26	275 ± 9*	336 ± 51#
GSH-px (mIU/ml)	83 ± 5	68 ± 4*	93 ± 4#
Heart			
MDA (pmol/ml)	1287 ± 229	1826 ± 76*	1512 ± 148#
SOD (U/ml)	341 ± 38	363 ± 13	370 ± 12
Spleen			
MDA (pmol/ml)	1403 ± 184	1535 ± 303	1455 ± 254
SOD (U/ml)	271 ± 42	151 ± 24*	365 ± 20#
Lung			
MDA (pmol/ml)	1549 ± 158	1591 ± 227	1395 ± 166
MPO (ng/ml)	65 ± 9	69 ± 8	68 ± 6
SOD (U/ml)	315 ± 68	208 ± 26*	301 ± 46#
Kidney			
MDA (pmol/ml)	1437 ± 45	1558 ± 135	1409 ± 215
ROS (IU/ml)	740 ± 164	362 ± 72*	552 ± 60#
SOD (U/ml)	283 ± 34	229 ± 15*	218 ± 35
GSH-px (mIU/ml)	75 ± 19	98 ± 5*	72 ± 7#
Intestine			
MDA (pmol/ml)	1452 ± 378	1475 ± 262	1591 ± 305
MPO (ng/ml)	68 ± 16	84 ± 4	46 ± 6#
SOD (U/ml)	316 ± 38	319 ± 18	237 ± 27#
Pancreas			
MDA (pmol/ml)	346 ± 65	321 ± 83	235 ± 48#
SOD (U/ml)	167 ± 19	153 ± 29	135 ± 38

MDA: malondialdehyde; SOD: superoxide dismutase; GSH-px: glutathione peroxidase; ROS: reactive oxygen species; MPO: myeloperoxidase. ∗ indicates that, compared with NG, $p < 0.05$; # means that, compared with OG, $p < 0.05$.

with SJP gavage and the body weights of rats in SG were significantly lower than that of OG after 6 weeks' gavage. At the end of the experimental period, rats in NG and SG showed an almost similar body weight gain and Lee's index; both were significantly lower than that of OG (Figures 1(b) and 1(c)).

4.2. SJP Ameliorate Tissue Inflammation of Obese Rats. Obesity led to distinct changes of cytokines in tissue samples of rats. In our study, according to all the indicators we selected in different tissues, obesity contributed to the significant elevated level of MDA (a product of lipid peroxide degradation which reflects the degree of oxidative stress response) in heart and GSH-px (a peroxide decomposition enzyme) in liver, while it decreased level of SOD (a free radical scavenger) in liver, spleen, lung, and kidney. However, SJP inversely decreased the level of MDA in heart and GSH-px in liver and elevated the level of SOD in liver, spleen, lung, and kidney (Table 1).

4.3. SJP Mitigate Multiple Organ Injuries in Obese Rats. Inflammation led to obvious tissue damage of multiple organs in obese rats. The histopathological evaluation results uncovered significant higher pathological scores of pancreas, liver, spleen, lung, and kidney of obese rats with more inflammatory cell infiltration and/or much severe tissue edema or cell vacuolation or cell necrosis. Reversely, SJP distinctly lowered the pathological score of pancreas, liver, spleen, lung, and kidney with less inflammatory cell infiltration and/or mild tissue edema or less cell vacuolation and necrosis (Figures 2 and 3).

5. Discussion

In the present study, we investigated the effect of SJP on systemic inflammatory injuries of multiple organs in obese rats. Our results uncovered significantly lower expression of SOD in the liver, spleen, lung, and kidney of obese rats and more severe injuries of pancreas, liver, spleen, lung, and kidney, while SJP was effective in increasing tissue levels of SOD in the liver, spleen, and lung and ameliorating inflammatory injuries of pancreas, liver, spleen, lung, and kidney correspondingly.

According to traditional Chinese Medicine theory, SJP was applied in "febrile symptoms" for evacuating wind and clearing heat, ascending lucidity, and descending turbidity. With the deepening of the studies to the mechanism of

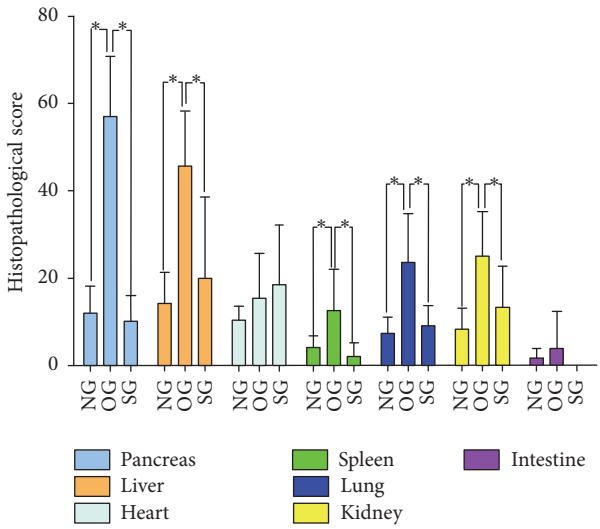

Figure 2: *The pathologic scores of rats' organs in all of the three experimental groups.* $*$ indicates that $p < 0.05$.

obesity and the characters of the formula, SJP was found quite appropriate to treat obesity and first reported in the treatment of Norplant subcutaneous preparations induced obesity for its prominent effect of lowering body weight [22, 24]. Studies afterwards further demonstrated that SJP was effective in anti-inflammation and immune regulation and was widely used in inflammatory diseases, such as flu, asthma, glomerulonephritis, acute pancreatitis, sepsis, and obesity.

Obesity-induced inflammation is a chronic, low-grade inflammation first started in adipose tissue with abundant macrophage infiltration and then persistent production of dozens of proinflammatory molecules. Cytokines, reactive oxygen species (ROS), and many other inflammatory agents produced by adipocytes and immune cells are released and then they activated inflammatory pathways [26]. Hypoxia and oxidative stress are main mechanisms in obesity-induced chronic inflammation [27]. Studies have demonstrated that high-fat diet is a potent inducer of oxidative stress via altering oxygen metabolism. The accumulated intracellular lipid with insufficient oxygen supply stimulates substantial production of ROS and subsequent lipid peroxidation process with toxic metabolites production such as malondialdehyde (MDA) [28]. Antioxidant system was activated at the same time of oxygen stress injury and superoxide dismutase (SOD) is the best known antioxidant enzyme capable of scavenging superoxide radicals, inhabiting cell membrane lipid peroxidation, and neutrophil-mediated inflammation [29]. Another important antioxidant is glutathione peroxidase (GSH-px); it protects the structure and function of cell membrane from peroxide damage by catalyzing glutathione into oxidized glutathione, which makes a poisonous peroxide reduction into nontoxic hydroxyl compounds [30]. In the present study, high-fat diet feeding contributed to the growing body weight and SJP showed prominent effect in protecting against high-fat diet induced obesity. Lipid peroxidation led to increased production of MDA in the heart of obese rats. Although significant increase of MDA was not found in other organs, significant decrease of SOD in the liver, spleen, lung, and kidney of obese rats shed light on the oxidative stress response. From the present point of view, as lipid accumulation continues, macrophages in adipose tissue shift from M2 subtype to a proinflammatory M1 polarization [31], and the macrophages infiltration in adipose tissue was observed at the onset of weight gain [32]. The timely infiltration of macrophages directly contributed to and maintained the inflammatory state of fat, which in turn led to the development of obesity and chronic inflammation [33, 34]. Therefore, the antioxidant system might be initiated at the same time of weight gain and perpetuate throughout the whole process of oxidative response. So, in the process of pro- and anti-inflammation response, the increased metabolites of lipid peroxide in obese rats led to substantial SOD consumption rather than the inflammatory status that inhibited the production of endogenous SOD which might interpret our results. Fortunately, besides losing weight, SJP was efficient in elevating tissue levels of SOD in liver, spleen, and lung. Lowering body weight while improving antioxidant capacity to ameliorate inflammation or attenuating inflammation and then lowering body weight are two aspects that might be in reciprocal causation, just as reported in the papers of Pirola and Ferraz [35] and Hao et al. [24]. However, we did not get expected results in the kidney of obese rats, as there were higher level of GSH-px and lower level of ROS, although the SOD level was still lower than that of rats with normal body weight. The inconsistent results we get in the kidney of obese rats might be due to several factors such as the selection of limited indicator without adequate specificity, or at a certain phase of pro- and anti-inflammation response, or others. However it could not represent the oxidative injuries finally as visualized histologic images displayed clearly more severe damage of kidney with substantial inflammatory cells infiltration and intravascular congestion in obese rats. We think further studies with more specific indicators or longer feeding times will help address this problem.

Obesity-induced inflammatory injuries to organs were unshadowed in the histologic images. We observed distinct changes in different organs of obese rats, such as enlarged hepatocytes, extensive vacuolization, inflammatory cells infiltration, and fatty degeneration in the liver; myocardial edema, early infarction, myocardial cell vacuolization, and granular degeneration in the heart; follicular degeneration and edema in the spleen; edema and bleeding in the lung; substantial inflammatory cells infiltration and fibrogenesis in the kidney and edema; and necrosis and cyst formation in the pancreas. The above changes were attenuated with SJP treatment. In accordance with the present study, numerous studies have demonstrated similar changes in high-fat diet induced obesity and Traditional Chinese herb showed a great potential to improve obesity-induced inflammatory injuries via ameliorating inflammation response [36, 37], modulating microbiota hemostasis [38, 39] and lipid metabolism [40–43], and attenuating insulin resistance [44]. In a part of the above-mentioned Chinese herb formula, we found similar ingredients in SJP, such as rhein [38] and *Curcuma longa* [40], which might be the main effective constituent of the

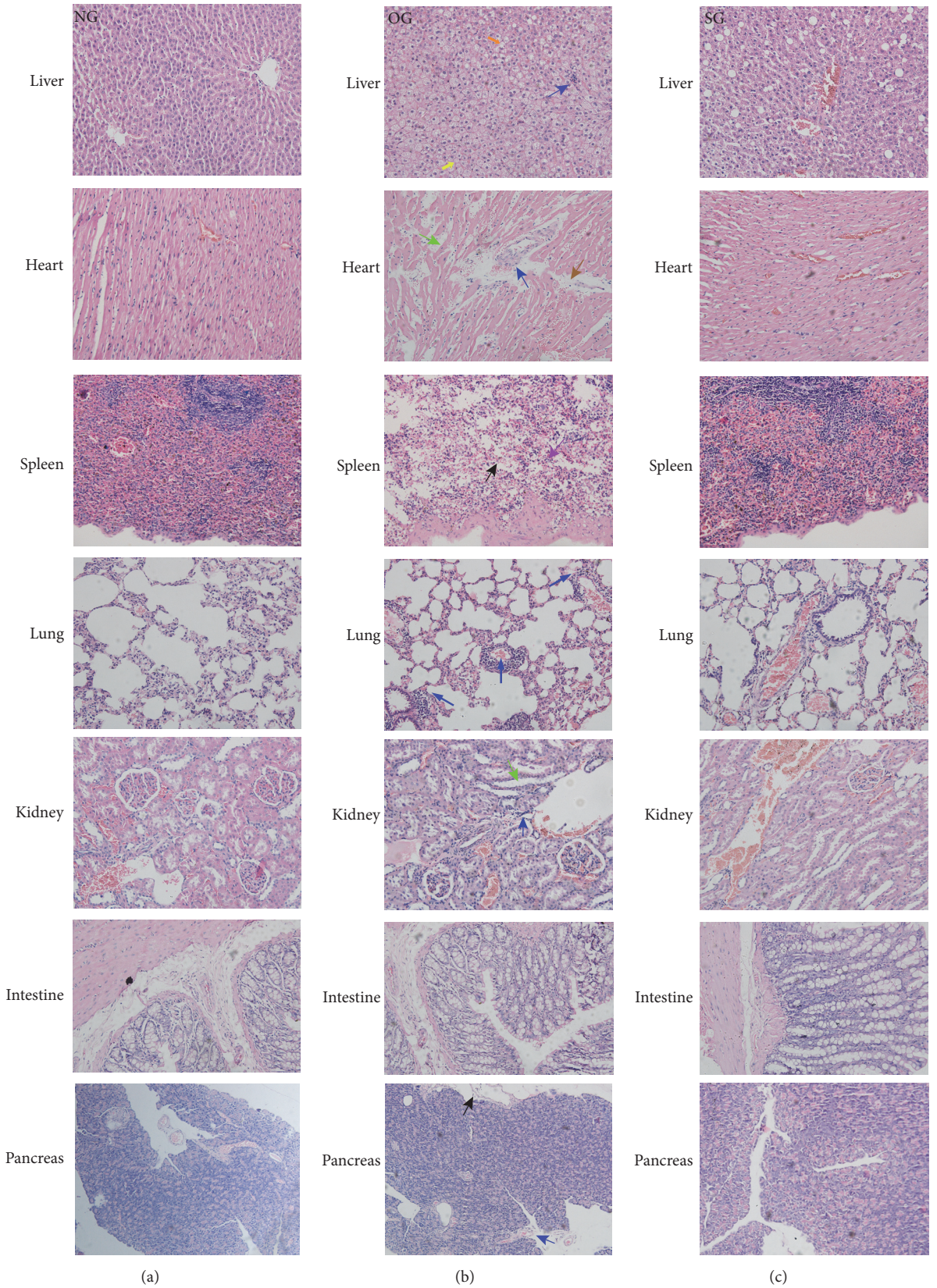

Figure 3: *The histological images of rats' organs in all of the three experimental groups*. Hematoxylin-eosin counterstain. Histological images are presented with original magnification 200x. The histological images of rats' organs from (a)–(c) exhibited tissue damage of rats in NG, OG, and SG, separately. Distinct changes were observed in different organs of obese rats (b), including enlarged hepatocytes (yellow arrow), extensive vacuolization (orange arrow), inflammatory cells infiltration (blue arrow), tissue edema (green arrow), myocardial early infarction (brown arrow), follicular degeneration (purple arrow), and necrosis (black arrow). These histological changes were partly reversed by SJP administration for 10 weeks (5 g/Kg·bw/day) (c).

classic formula. However, the specific effective ingredients and mechanisms of SJP in ameliorating obesity-associated inflammatory injuries still need further investigation.

The present study selected MDA, SOD, ROS, MPO, and GSH-px as indicators to reflect the extent of oxidative stress in organs and some changes were detected in selected indicators indeed. More specific indicators of organ damage and serum inflammatory cytokines detection might provide more comprehensive information about obesity-induced systemic inflammation and multiple organ injuries. Although SJP showed an obvious effect in preventing obesity and related multiple organ injuries in the present study, deeper investigation focused on the specific mechanism and effective ingredients basis might enable wide clinical usage.

In conclusion, high-fat diet induced obesity caused extensive inflammatory damage to rats, and SJP was effective in preventing high-fat diet induced obesity and related multiorgan injuries in rats.

Disclosure

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

Conflicts of Interest

There are no conflicts of interest.

Authors' Contributions

Juan Li and Yu-mei Zhang contributed equally to this work.

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References

[1] M. Ng, T. Fleming, M. Robinson, and et al, "Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013," *The Lancet*, vol. 384, no. 9945, pp. 766–781, 2014.

[2] S. S. Lim, T. Vos, and A. D. Flaxman, "A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010," *The Lancet*, vol. 380, no. 9859, pp. 2224–2260, 2013.

[3] A. R. Johnson, J. Justin Milner, and L. Makowski, "The inflammation highway: metabolism accelerates inflammatory traffic in obesity," *Immunological Reviews*, vol. 249, no. 1, pp. 218–238, 2012.

[4] L. Marseglia, S. Manti, G. D'Angelo et al., "Oxidative stress in obesity: a critical component in human diseases," *International Journal of Molecular Sciences*, vol. 16, no. 1, pp. 378–400, 2014.

[5] J. Fan, S. Kim, and V. W. Wong, "New trends on obesity and NAFLD in Asia," *Journal of Hepatology*, vol. 67, no. 4, pp. 862–873, 2017.

[6] T. DeMartini, M. Nowell, J. James et al., "High fat diet-induced obesity increases myocardial injury and alters cardiac STAT3 signaling in mice after polymicrobial sepsis," *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 2017.

[7] DC. Sulistyoningrum, R. Susilowati, E. Huriyati, W. NPD, HF. Luglio, and M. Julia, "Tumour necrosis factor-α and risk of cardiovascular disease among overfat Indonesian adolescents," *Asia Pacific Journal of Clinical Nutrition*, vol. 2, Supplement 1, pp. S50–S56, 2017.

[8] D. Azamar-Llamas, G. Hernández-Molina, B. Ramos-Ávalos, and J. Furuzawa-Carballeda, "Adipokine Contribution to the Pathogenesis of Osteoarthritis," *Mediators of Inflammation*, vol. 2017, pp. 1–26, 2017.

[9] C. Kang, B. Wang, K. Kaliannan et al., "Erratum for Kang et al., "Gut Microbiota Mediates the Protective Effects of Dietary Capsaicin against Chronic Low-Grade Inflammation and Associated Obesity Induced by High-Fat Diet"," *mBio*, vol. 8, no. 4, p. e00900-17, 2017.

[10] A. R. Johnson and L. Makowski, "Nutrition and metabolic correlates of obesity and inflammation: Clinical considerations," *Journal of Nutrition*, vol. 145, no. 5, pp. 1131–1136, 2015.

[11] K. Hidayat, X. Du, G. Chen, M. Shi, and B. Shi, "Abdominal obesity and lung cancer risk: Systematic review and meta-analysis of prospective studies," *Nutrients*, vol. 8, no. 12, article no. 810, 2016.

[12] A. Whaley-Connell and J. R. Sowers, "Obesity and kidney disease: From population to basic science and the search for new therapeutic targets," *Kidney International*, 2016.

[13] P. D. O'Brien, L. M. Hinder, B. C. Callaghan, and E. L. Feldman, "Neurological consequences of obesity," *The Lancet Neurology*, vol. 16, no. 6, pp. 465–477, 2017.

[14] O. Guillemot-Legris and G. G. Muccioli, "Obesity-Induced Neuroinflammation: Beyond the Hypothalamus," *Trends in Neurosciences*, vol. 40, no. 4, pp. 237–253, 2017.

[15] E. Lucas, M. Cruces-Sande, A. M. Briones et al., "Molecular physiopathology of obesity-related diseases: multi-organ integration by GRK2," *Archives of Physiology and Biochemistry*, vol. 121, no. 5, pp. 163–177, 2015.

[16] E. Kwon, J. Lee, Y. Kim et al., "Seabuckthorn Leaves Extract and Flavonoid Glycosides Extract from Seabuckthorn Leaves Ameliorates Adiposity, Hepatic Steatosis, Insulin Resistance, and Inflammation in Diet-Induced Obesity," *Nutrients*, vol. 9, no. 6, p. 569, 2017.

[17] M. Hong, J. Jung, H. Park et al., "Shaofu Zhuyu decoction ameliorates obesity-mediated hepatic steatosis and systemic inflammation by regulating metabolic pathways," *PLoS ONE*, vol. 12, no. 6, p. e0178514, 2017.

[18] G. Jamar, D. Estadella, and L. P. Pisani, "Contribution of anthocyanin-rich foods in obesity control through gut microbiota interactions," *BioFactors*, vol. 43, no. 4, pp. 507–516, 2017.

[19] x. x. Yang and Y. M. Zhang, "Syndrome analysis of regulating ascending and descending functions in spleen and stomach disease," *Chinese medicine science*, vol. 3, no. 9, pp. 101-102, 2013.

[20] L. Shao and L. M. Zhang, "Origin correction of Sheng jiang powder," *Journal of Chinese Medicine*, vol. 26, no. 1, pp. 50–52, 2011.

[21] W. J. Liu, Y. X. Xue, and D. P. Hu, "Research progress on the modern pharmacological mechanism of Sheng jiang powder," *Beijing Chinese medicine*, vol. 31, no. 12, pp. 939–943, 2012.

[22] H. L. Zhang, J. M. Zhao, and J. X. Yang, "Clinical observation of Jia wei sheng jiang powder combined with auricular point

sticking in the treatment of Norplant subcutaneous preparetions induced obesity," *Chinese Journal of Traditional Chinese*, vol. 17, pp. 12–749, 1997.

[23] Z. C. Ning and S. H. Zhang, "Effect of Jia wei sheng jiang powder combined with acupuncture on the microinflammatory state of patients with obesity-related nephropathy," *Sichuan Journal of Traditional Chinese Medicine*, vol. 35, no. 1, pp. 83–85, 2017.

[24] Y. J. Hao, Y. F. Dong, G. Y. Wang et al., "Effect of Sheng jiang powder combined with acupuncture on blood leptin and adiponectin in patients with obesity-related nephropathy," *Chinese Journal of preclinical Chinese Medicine*, vol. 21, no. 11, pp. 1438–1440, 2015.

[25] S. Wirtz, C. Neufert, B. Weigmann, and M. F. Neurath, "Chemically induced mouse models of intestinal inflammation," *Nature Protocols*, vol. 2, no. 3, pp. 541–546, 2007.

[26] A. W. Ferrante Jr., "Obesity-induced inflammation: a metabolic dialogue in the language of inflammation," *Journal of Internal Medicine*, vol. 262, no. 4, pp. 408–414, 2007.

[27] B. K. Beh, N. E. Mohamad, S. K. Yeap et al., "Anti-obesity and anti-inflammatory effects of synthetic acetic acid vinegar and Nipa vinegar on high-fat-diet-induced obese mice," *Scientific Reports*, vol. 7, no. 1, 2017.

[28] G. Frühbeck, V. Catalán, A. Rodríguez et al., "Involvement of the leptin-adiponectin axis in inflammation and oxidative stress in the metabolic syndrome," *Scientific Reports*, vol. 7, no. 1, 2017.

[29] N. Wu, F. Liao, H. Cheng, S. Sung, Y. Yang, and J. Wang, "Intravenous superoxide dismutase as a protective agent to prevent impairment of lung function induced by high tidal volume ventilation," *BMC Pulmonary Medicine*, vol. 17, no. 1, 2017.

[30] H. Wang, Y. Zhang, R. Bai, M. Wang, and S. Du, "Baicalin Attenuates Alcoholic Liver Injury through Modulation of Hepatic Oxidative Stress, Inflammation and Sonic Hedgehog Pathway in Rats," *Cellular Physiology and Biochemistry*, vol. 39, no. 3, pp. 1129–1140, 2016.

[31] H. Sell, C. Habich, and J. Eckel, "Adaptive immunity in obesity and insulin resistance," *Nature Reviews Endocrinology*, vol. 8, no. 12, pp. 709–716, 2012.

[32] M. S. Han, D. Y. Jung, C. Morel et al., "JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation," *Science*, vol. 339, no. 6116, pp. 218–222, 2013.

[33] H. Xu, G. T. Barnes, Q. Yang et al., "Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance," *The Journal of Clinical Investigation*, vol. 112, no. 12, pp. 1821–1830, 2003.

[34] S. H. Roerink, M. A. Wagenmakers, J. F. Langenhuijsen et al., "Increased Adipocyte Size, Macrophage Infiltration, and Adverse Local Adipokine Profile in Perirenal Fat in Cushing's Syndrome," *Obesity*, vol. 25, no. 8, pp. 1369–1374, 2017.

[35] L. Pirola and J. C. Ferraz, "Role of pro- and anti-inflammatory phenomena in the physiopathology of type 2 diabetes and obesity," *World Journal of Biological Chemistry*, vol. 8, no. 2, p. 120, 2017.

[36] H. Lee, E. H. Shim, M. S. Lee, and C.-S. Myung, "Traditional medicine, Sobokchukeo-Tang, modulates the inflammatory response in adipocytes and macrophages," *Molecular Medicine Reports*, vol. 15, no. 1, pp. 117–124, 2017.

[37] S.-H. Kim, H.-S. Park, M. J. Hong et al., "Tongqiaohuoxue decoction ameliorates obesity-induced inflammation and the prothrombotic state by regulating adiponectin and plasminogen activator inhibitor-1," *Journal of Ethnopharmacology*, vol. 192, pp. 201–209, 2016.

[38] S. Wang, X.-F. Huang, P. Zhang et al., "Chronic rhein treatment improves recognition memory in high-fat diet-induced obese male mice," *The Journal of Nutritional Biochemistry*, vol. 36, pp. 42–50, 2016.

[39] F. Chen, J. Jiang, D. Tian et al., "Targeting Obesity for the Prevention of Chronic Cardiovascular Disease Through Gut Microbiota-Herb Interactions: An Opportunity for Traditional Herbs," *Current Pharmaceutical Design*, vol. 23, no. 8, pp. 1142–1152, 2017.

[40] J.-H. Lee, J.-J. Lee, W.-K. Cho et al., "KBH-1, an herbal composition, improves hepatic steatosis and leptin resistance in high-fat diet-induced obese rats," *BMC Complementary and Alternative Medicine*, vol. 16, no. 1, article no. 355, 2016.

[41] X. LU, Z.-Y. Yuan, X.-J. Yan et al., "Effects of Angelica dahurica on obesity and fatty liver in mice," *Chinese Journal of Natural Medicines*, vol. 14, no. 9, pp. 641–652, 2016.

[42] Y. Li, X. Liu, Y. Fan, B. Yang, and C. Huang, "Radix Stellariae extract prevents high-fat-diet-induced obesity in C57BL/6 mice by accelerating energy metabolism," *PeerJ*, vol. 5, p. e3305, 2017.

[43] Q. Yang, M. Qi, R. Tong et al., "Plantago asiatica L. Seed Extract Improves Lipid Accumulation and Hyperglycemia in High-Fat Diet-Induced Obese Mice," *International Journal of Molecular Sciences*, vol. 18, no. 7, p. 1393, 2017.

[44] Y. H. Lee, B. Jin, S. H. Lee et al., "Herbal formula HT048 attenuates diet-induced obesity by improving hepatic lipid metabolism and insulin resistance in obese rats," *Molecules*, vol. 21, no. 11, article no. 1424, 2016.