Evidence-Based Complementary and Alternative Medicine

Chinese Herbal Medicine for Rheumatic Diseases

Lead Guest Editor: Jing Luo
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Research Article

Chondroprotective Effects of Gubitong Recipe via Inhibiting Excessive Mitophagy of Chondrocytes

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Objective. Osteoarthritis (OA) is the most common degenerative joint disorder and a leading cause of disability. A previous randomized controlled trial has shown that Gubitong (GBT) recipe can improve OA-related symptoms and articular function without noticeable side effects. However, the underlying mechanisms remain unclear. This study aims to explore the therapeutic mechanisms of the GBT recipe for OA through in vivo and in vitro experiments. *Methods*. Rats of the OA model were established by Hulth surgery and intervened with the GBT recipe and then were subjected to pathological assessment of the cartilage. Matrix metalloproteinase 13 (MMP-13) expression in cartilage tissues was assessed by immunohistochemical staining. Chondrocytes were isolated from sucking rats and stimulated with LPS to establish an in vitro model. After intervened by water extraction of the GBT recipe, the fluorescent signal of Mtphagy Dye and mitochondrial membrane potential ($\Delta\psi$m) were detected to determine the states of mitophagy and mitochondrial dynamics of chondrocytes in vitro, respectively. Western blot test was used to detect levels of proteins related to catabolism of the cartilage matrix, mitophagy, and PI3K/AKT pathway. *Results*. In in vivo experiments, the GBT recipe can effectively inhibit the cartilage degeneration of chondrocytes in OA rats, as well as markedly suppress the expression of MMP-13. In vitro experiments on LPS-induced chondrocytes exhibited increase in mitochondrial depolarization and excessive mitophagy, and the GBT recipe can alleviate these changes. LPS-stimulated chondrocytes showed increases in MMP-13, PINK1, and Parkin in cell lysates and LC3II/LC3I ratio in the mitochondrial fraction, and the GBT recipe can inhibit these increases in a dose-dependent manner. Moreover, the GBT recipe can attenuate the abnormal activation of PI3K/AKT pathway induced by LPS. *Conclusion*. The GBT recipe exhibits chondroprotective effects through inhibiting excessive mitophagy of chondrocytes, which may be associated with its inhibitory effect on the abnormal activation of PI3K/AKT pathway.

1. Introduction

Osteoarthritis (OA) is a common and disabling condition characterized by degeneration of articular cartilage [1]. With an aging population and increasing rates of obesity, OA is becoming more prevalent than in previous decades [2]. A study based on the National Health Interview Survey (NHIS) of the United States showed that 22.7% of the adult population had at least one joint affected by OA and the incidence increased to 49.6% for those beyond the age of 65 [3]. Patients with OA often have pain, morning stiffness, crepitus on joint motion, and even instability or physical disability of joint, which impair quality of life and lead to a considerable socioeconomic burden [4].

Articular cartilage consists of a rich extracellular matrix (ECM) with a sparse dispersion of chondrocytes [5]. Adult articular cartilage is devoid of both innervation and vascularization, and chondrocytes are responsible for the

anabolism and catabolism balance of ECM. Injured chondrocytes in osteoarthritic cartilage manufacture less ECM than usual, contributing to the irreversible degenerative process of OA [6, 7]. The anabolic and catabolic processes of chondrocytes are intimately linked to mitochondrial functions [8]. Mitophagy, a selective form of autophagy that removes damaged or excessive mitochondria, is essential in maintaining cellular energy homeostasis and function [9]. However, excessive mitophagy can result in excessive mitochondrial oxidative stress and mitochondrial functions decline, leading to chondrocytes degeneration and cartilage destruction, and finally to OA [10, 11].

Current management for OA focuses on relieving pain and inflammation, alleviating cartilage degeneration, and improving articular function [12]. Commonly, the treatment of OA includes pharmacological therapies, non-pharmacological therapies, and joint replacement surgery [13–15]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly recommended drugs for OA [15]. However, long-term applications of NSAIDs in patients with OA are controversial due to the gastrointestinal and cardiovascular side effects [16,17]. There are no specific and efficacious disease-modifying drugs for OA yet [14]. Thus, it is desirable to develop a more effective and safer drug.

Traditional Chinese medicine (TCM) has been used to treat a variety of medical conditions including OA in China for thousands of years. Recently, some randomized controlled trials (RCTs) have demonstrated the therapeutic effect and safety of herbal medicine in treating OA [16–19]. Gubitong (GBT) recipe, a TCM prescription consisting of eight herbs, has also shown its therapeutic potential for patients with OA in a previous RCT [19]. However, the underlying mechanisms remain unclear. This study aims to explore the therapeutic mechanisms of the GBT recipe for OA through in vivo and in vitro experiments.

2. Materials and Methods

2.1. Reagents and Antibodies. 0.25% Trypsin-EDTA, DMEM medium, phosphate-buffered saline (PBS), fetal bovine serum (FBS), and collagenase II were purchased from Gibco. Lipopolysaccharides (LPS), penicillin-streptomycin and poly-L-lysine, and Triton X-100 were purchased from Sigma. Electrochemiluminescence (ECL) luminous fluid and polyvinylidene difluoride (PVDF) membranes were purchased from Millipore. Animal-free blocking solution was purchased from Cell Signaling. Mitochondrial extraction kit, RIPA lysis buffer, phenylmethanesulfonyl fluoride (PMSF), protein phosphatase inhibitor, Tween-20, and EDTA-Na_2 were purchased from Solarbio Life Sciences. SDS-PAGE running buffer powder, Tris-buffered saline (TBS) powder, and SDS-PAGE transfer buffer powder were purchased from Servicebio. A fluorescent mounting medium with DAPI (4,6-diamidino-2-phenylindole) was purchased from Zhongshan Jingqiao Biotechnology. Mtphagy Dye was purchased from Dojindo Laboratories.

Anticollagen II antibody (ab34712) and anti-MMP-13 antibody (ab39012) were purchased from Abcam. Anti-AKT antibody (4691S) and anti-phospho-AKT (Ser473) antibody (4060S) were purchased from Cell Signaling Technology. Anti-Parkin antibody (sc-32282) was purchased from Santa Cruz. Anti-PINK1 antibody (A11435), anti-LC3B antibody (A19665), anti-COX IV antibody (A6564), and anti-PI3K p85 antibody (A4992) were purchased from ABclonal. Anti-phospho-PI3K antibody (YP0224) was purchased from Immunoway. β-Actin antibody (TA-09), horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (ZB-5305), HRP-conjugated goat anti-rabbit IgG (ZB-2301), and Alexa Fluor 488-conjugated goat anti-rabbit IgG (H + L) (ZF-0511) were purchased from Zhongshan Jingqiao Biotechnology.

2.2. Preparation of GBT Recipe. GBT recipe, which consists of Rhizoma Drynariae 20 g, Epimedii Folium 15 g, Fructus Psoraleae 15 g, Cortex Eucommiae 30 g, Rhizoma Cibotii 30 g, Rhizoma Bolbostemmae 20 g, Caulis Sinomenii 30 g, and Caulis Spatholobi 30 g, was purchased from TCM Pharmacy of China-Japan Friendship Hospital. Each drug of the GBT recipe was validated by an herbal medicinal botanist from the Beijing University of Chinese Medicine. Drugs were soaked in a 10-fold volume of distilled water for 4 hours. After 1 hour of decoction, the suspension of the GBT recipe was filtered three times. The filtered decoction was concentrated under reduced pressure on a rotary evaporator. The concentrated solution was refrigerated for 12 hours at 4°C and then centrifuged to obtain a suspension. After boiling the final supernatant, dehydrated alcohol was gently added with quick agitation until the concentration reached 75% alcohol (v/v). The filtrate was then centrifuged after cooling and decompressed into a paste having a relative density of 1.25 g/ml (w/v). The paste was then lyophilized under vacuum.

2.3. Liquid Chromatography-Mass Spectrometry Analysis. Reversed-phase chromatography was performed using a Nexera High-Performance Liquid Chromatograph (Japan Shimadzu Co., Ltd) coupled to the SCIEX 5600 Triple-TOF Mass Spectrometer (Sciex, Toronto, Canada). The samples were eluted on HSS T3 C18 analytical column (2.1×100 mm, 1.8 μm) with a 30 min gradient at a flow rate of 0.3 mL/min. The two mobile phases consisted of buffer A (0.1% formic acid/99.9% H_2O) and buffer B (99.9% acetonitrile/0.1% formic acid) and operated under the following program: 0–5 min, 15%–15% B; 5–13 min, 15%-16% B; 13–17 min, 16%–16% B; 17–20 min, 16%-17% B; 20–26 min, 11%–95% B; 26-27 min, 95%–15% B; and 27–30 min, 15%-15% B. The scan mode for high-resolution mass spectrometry acquisition was full-scan/dd-MS2 mode, and data were acquired in the m/z range 100–1,500.

2.4. Animals Feeding. Seven-week-old male Sprague–Dawley (SD) rats were purchased from SPF (Beijing) Biotechnology Co., Ltd. The rats were raised in a clean-grade animal room with a temperature of 23 ± 2°C and a humidity of 50 ± 10% in the Experimental Animal Center of China-Japan Friendship Hospital. All animal experiment procedures were approved by the Animal Care and Welfare Committee of China-Japan Friendship Hospital (No. zryhyy21-21-05-09).

After 7 days of adaptive feeding, the rats were randomly divided into four groups, including blank control group, model group, GBT recipe group, and glucosamine sulfate group, according to the random digital table method.

2.5. OA Model Preparation and Intervention. The rats were anesthetized by isoflurane inhalation. After fur was shaven off, the skins of the left keen joint were sterilized with iodophor. A surgical knife was used to transversely incise the skin of the left keen joint and patellar ligament. After the medial collateral ligament was severed and meniscus was drawn, anterior and posterior cruciate ligaments were severed. The incision was sutured layer by layer after a drawer test was positive. Penicillin (200,000 units) was injected into each rat that underwent surgery for successive three days to avoid infection. A typical daily dose of GBT recipe and glucosamine sulfate in the treatment of OA was 190 g and 1440 mg, and a human reference weight of an adult is 60 kg. Thus, the dose of GBT recipe and glucosamine to be administered is 3.2 g/kg and 1.7 mg/kg for an adult. According to the guide for dose conversion between animals and humans, the dose for rats is 6.3 times that of humans [20]. Based on this, the dose of GBT recipe in rats should roughly be 20.2 g/kg/day, and the dose of glucosamine sulfate should roughly be 150 mg/kg/d. Beginning on the 4th day following surgery, glucosamine sulfate and GBT recipe were given to rats once a day. Rats were sacrificed by inhalation of CO_2 (compressed CO_2 gas cylinder) at 4 weeks after administration. The left knee joint was separated for pathological assessment.

2.6. Pathological Assessment. The knee joints of rats were fixed with 4% paraformaldehyde for 72 hours and decalcified in 10% ethylenediaminetetraacetic acid-Na_2 (EDTA-Na_2) for 6 weeks. The tissues were dehydrated, embedded, and sliced into 4 μm sections. The sections were then stained with hematoxylin and eosin (HE) and safranin O-fast green (SCO) staining for histological examination. Mankin's scoring method was used to evaluate the articular cartilage degeneration [21]. Each section was assessed by a well-trained researcher without being informed of the grouping.

2.7. Immunohistochemistry. The paraffin-embedded tissues were used for the immunohistochemical analysis of MMP-13 expression in the articular cartilage of rats. In brief, after the slides were incubated with a blocking serum for 30 min, they were blotted and then overlaid with the primary antibody against MMP-13 for 2 h at room temperature. Subsequently, biotinylated secondary antibodies were added into the sections, followed by a peroxidase-labeled streptavidin-biotin staining technique. Finally, the samples were observed under a light microscope. The images from the immunohistochemistry samples were quantified using Image-Pro Plus software.

2.8. Isolation and Culture of Chondrocytes. Five-day sucking rats were sacrificed by inhalation of CO_2 (compressed CO_2 gas cylinder). The knee joint cartilage was isolated in a sterile environment and digested using 0.25% trypsin for 1 hour. The cartilage was neutralized with DMEM containing 10% FBS and washed three times with PBS. After digested with 2 mg/ml type II collagenase for 6 hours, the joint cartilage was filtered with a 100-mesh stainless steel screen to prepare for chondrocytes suspensions. The suspensions were centrifuged to collect chondrocytes. Chondrocytes were seeded in a DMEM medium with 10% FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin and cultured on polylysine-coated dishes. A second or third generation of chondrocytes was used for cell experiments.

2.9. MTS Assay. MTS assay was used to evaluate the effect of the GBT recipe on the vitality of chondrocytes. 1×10^4 chondrocytes were seeded into 96-well culture plates. After cells were completely adherent, the culture medium was discarded and the cells were treated with different concentrations of the GBT recipe for 12 hours. Then, the cells were incubated for 4 hours in a DMEM medium. Subsequently, 20 μL MTS reagent was added to the cells, followed by another 20 min of incubation. A microplate reader was used to detect absorbance at a wavelength of 490 nm.

2.10. Cell Culture and Treatment. Chondrocytes were cultured in a DMEM medium containing 10% FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin. Cells were subcultured every 2–3 days at 37°C in a humidified 5% CO_2 environment. Chondrocytes were pretreated with DEX (100 nmol/ml) or different GBT recipe concentrations (50, 100, and 200 ug/ml) for 1 hour, and then, LPS (100 ng/ml) was added for 12 hours.

2.11. Mitochondrial Isolation. Chondrocyte mitochondria were isolated using a mitochondrial extraction kit. Chondrocytes were homogenized using a Dounce-type glass homogenizer in precold lysis buffer and centrifuged at 1000 g for 10 minutes. The mitochondria were then pelleted by centrifuging the crude supernatant for 10 minutes at 12,000 g. Precipitates were resuspended in 0.5 mL wash buffer and centrifuged at 1000 g for 5 min. Mitochondria were ultimately separated from the supernatant by centrifugation at 12,000 g for 10 minutes at 4°C.

2.12. Reverse Transcription and Quantitative Real-Time PCR. RNA was extracted from chondrocytes and SW1353 cells using the column-based HiPure Total RNA Mini Kit. Then, RNA concentration and purity were measured. 1 ug RNA was reverse transcribed into cDNA using a reverse transcription system. The qRT-PCR was performed in a total volume of 20 μl, with 2 μl of cDNA, 10 μl of SYBR Green qPCR Mix, and 2 μM of the forward and reverse primers in each tube. The CT values of each sample were acquired after the end of the reaction.

2.13. Western Blot Analysis. The samples were lysed by RIPA buffer with 1% PMSF and 1% phosphatase inhibitors on ice

for 30 min. After centrifugation at 10,000 g for 10 minutes at 4°C, the supernatant was collected. The bicinchoninic acid (BCA) method was used to quantify the protein content. Protein samples were boiled after the addition of loading buffer, and 40 μg of each protein sample was loaded onto the SDS-PAGE gels. The protein was separated by SDS-PAG electrophoresis (SDS-PAGE) and transferred to PVDF membrane (70 v, 55 min). PVDF membrane was then blocked with 5% nonfat milk powder for 1 hour at room temperature. The membranes were then incubated with primary antibodies overnight at 4°C. After washing with TBS containing 0.05% Tween-20 (TBST), membranes were incubated with secondary antibodies for 2 hours at room temperature. The protein samples were visualized using a chemiluminescence machine (Bio-Rad, USA). The primary antibodies associated parameters are listed in Table 1.

2.14. Immunofluorescence Staining. Chondrocytes were inoculated on a 48-well plate and cultured for 24 hours. The culture medium was discarded after completion and the plate was washed with PBS at 37°C. The cells were fixed for 15 minutes at room temperature with 4% paraformaldehyde and then permeabilized for 5 minutes with 0.1% Triton X-100/PBS. After washing three times with PBS, cells were blocked for 1 hour with an animal-free blocking solution. Then, cells were incubated with antibodies against type II collagen (1 : 100 dilution) overnight at 4°C. Cells were washed three times with PBS and incubated with fluorescein (FITC)-conjugated goat anti-rabbit IgG (1 : 50 dilution) at room temperature for 1 hour. After the cells were washed three times with PBS, the nuclei were stained with DAPI and observed under an inverted fluorescence microscope.

2.15. Mitophagy Assay. A Mitophagy Detection Kit (Dojindo Molecular Technologies) was used to detect mitophagy [22]. The chondrocytes were washed twice with DMEM and incubated with 100 nM Mtphagy Dye diluted in DMEM for 30 min at 37 °C. After incubation, cells were washed twice with DMEM and continued to be incubated for another 1 h in the previous culture conditions. After mitochondrial staining, the dye was immobilized and fluorescence intensity varied according to pH value. In mitochondrial-lysosome fusion, Mtphagy Dye displayed higher fluorescence intensity, indicating mitophagy. The level of mitophagy was defined by the area of Mtphagy Dye per cell.

2.16. Flow Cytometry. In the measurement of type II collagen, chondrocytes were fixed and incubated with type II collagen primary antibody (1 : 50 dilution) for 1 hour after discarding the culture supernatant. Subsequently, the cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit antibody for 30 minutes and then detected by flow cytometry. Mitochondrial membrane potential was measured using a mitochondrial membrane potential assay kit with JC-1 according to the manufacturer's protocol.

2.17. Statistical Analysis. Statistical analysis was performed using SPSS 19 statistical package. Student's *t*-test was used to detect the statistically significant differences between experimental groups. Data were presented as mean ± standard deviation; a two-sided $p < 0.05$ was considered statistically significant.

3. Results

3.1. Quality Control of GBT Recipe Extraction. Representative components of each herb in the GBT recipe were selected according to the Chinese Pharmacopoeia (2020 edition) and relevant literature [23]. There were nine representative components in GBT recipe including naringin (from Rhizoma Drynariae), icariin (from Epimedii Folium), psoralen (from Fructus Psoraleae), pinoresinol diglucoside (from Cortex Eucommiae), protocatechuic acid (from Rhizoma Cibotii), tubeimoside I (from Rhizoma Bolbostemmae), sinomenine (from Caulis Sinomenii), and catechin and epicatechin (from Caulis Spatholobi). Liquid chromatography coupled with mass spectrometry (LC-MS) results showed that all the nine components were present in GBT recipe extraction (Figure 1), and the detailed information is shown in Table 2.

3.2. Protective Effects of GBT Recipe on Joints Cartilage of OA Rats. Pathological changes in knee joint cartilage in each group are shown in Figure 2. Compared with the blank control group, the cartilage in the model group exhibited a successful establishment of the OA model and a significantly higher Mankin's score: the articular cartilage surface layer was fiberized, chondrocytes were severely proliferated and hypertrophied, cell lamination was completely disordered, and cytoplasmic vacuolization resulting from apoptotic cells was increased (Figures 2(a), 2(b), 2(e), and 2(f)). After GBT recipe treatment, cartilage lesion and matrix degradation were well ameliorated (Figures 2(c) and 2(g)). Meanwhile, the improvement of cartilage degeneration in the glucosamine sulfate group was inferior to that in the GBT recipe group (Figures 2(d) and 2(h)).

3.3. Effect of GBT Recipe on MMP-13 in the Articular Cartilage. The results of immunohistochemistry showed that MMP-13 increased in cartilage of the OA model group (Figures 3(a) and 3(b)). The GBT recipe and glucosamine sulfate could reduce the abnormal increase caused by OA, and the GBT recipe was more effective than glucosamine sulfate ($p < 0.05$) (Figures 3(c) and 3(d)).

3.4. Identification of Chondrocytes. The identification of chondrocytes by type II collagen expression is shown in Figure 4. The immunofluorescence analysis showed that nearly all chondrocytes were positive for type II collagen (Figures 4(a), 4(b), and 4(c)). The proportion of type II collagen-positive chondrocytes was further assessed by flow cytometry. The results indicated that the proportion of chondrocytes expressing type II collagen was 98 ± 1.1%

Table 1: Information of primary antibodies.

Antibody	Manufacturer	Catalog no.	Molecular weights	Dilution	Electrophoretic gel concentration (%)
Anti-MMP-13	Abcam	ab39012	60 kD	WB 1 : 1000	10.0
Anti-AKT	Cell Signaling Technology	4691S	60 kD	WB 1 : 1000	10.0
Anti-phospho-AKT (Ser473)	Cell Signaling Technology	4060S	60 kD	WB 1 : 1000	10.0
Anti-Parkin	Santa Cruz	sc-32282	52 kD	WB 1 : 1000	10.0
Anti-PINK1	ABclonal	A11435	63 kD	WB 1 : 1000	10.0
Anti-LC3B	ABclonal	A19665	14 kD/16 kD	WB 1 : 1000	12.5
Anti-COX IV	ABclonal	A6564	17 kD	WB 1 : 1000	12.5
Anti-PI3K p85	ABclonal	A4992	85 kD	WB 1 : 1000	10.0
Anti-phospho-PI3K	Immunoway	YP0224	55 kD/85 kD	WB 1 : 1000	10.0
β-Actin	Zhongshan Jingqiao Biotechnology	TA-09	42 kD	WB 1 : 1000	10.0

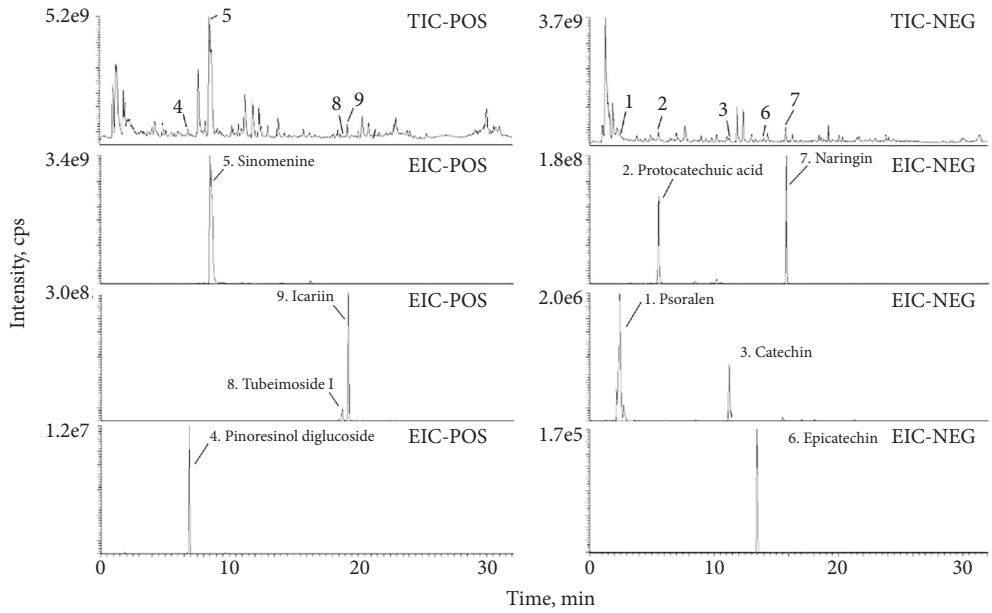

Figure 1: Total ion chromatography (TIC) on positive and negative and extraction ion chromatography of GBT recipe.

Table 2: Chemical identification of GBT recipe.

No.	RT (min)	Name	Formula	Ion	Cal. m/z	Mea. m/z	Error (ppm)	MS/MS
1	2.39	Psoralen	$C_{11}H_6O_3$	M−H	185.0244	185.0251	9.617	185.0251, 147.0325
2	5.52	Protocatechuic acid	$C_7H_6O_4$	M − H	153.0193	153.0194	7.743	153.0194, 109.0296
3	6.09	Catechin	$C_{15}H_{14}O_6$	M − H	289.0717	289.0717	3.582	289.0717
4	6.84	Pinoresinol diglucoside	$C_{32}H_{42}O_{16}$	M + H	683.2545	683.2535	−3.167	519.1923, 357.2159
5	8.49	Sinomenine	$C_{19}H_{23}NO_4$	M + H	330.1699	330.1709	2.772	330.1709
6	13.42	Epicatechin	$C_{22}H_{18}O_{10}$	M − H	441.0827	441.0829	2.895	441.0829
7	15.77	Naringin	$C_{27}H_{32}O_{14}$	M − H	579.1719	579.1722	2.328	579.1722, 271.0612, 151.0037
8	18.65	Tubeimoside I	$C_{63}H_{98}O_{29}$	M + H	1319.627	1319.628	4.783	1319.628, 677.2439
9	19.19	Icariin	$C_{33}H_{40}O_{15}$	M + H	677.2439	677.2439	3.822	677.2439, 369.1335, 313.0708

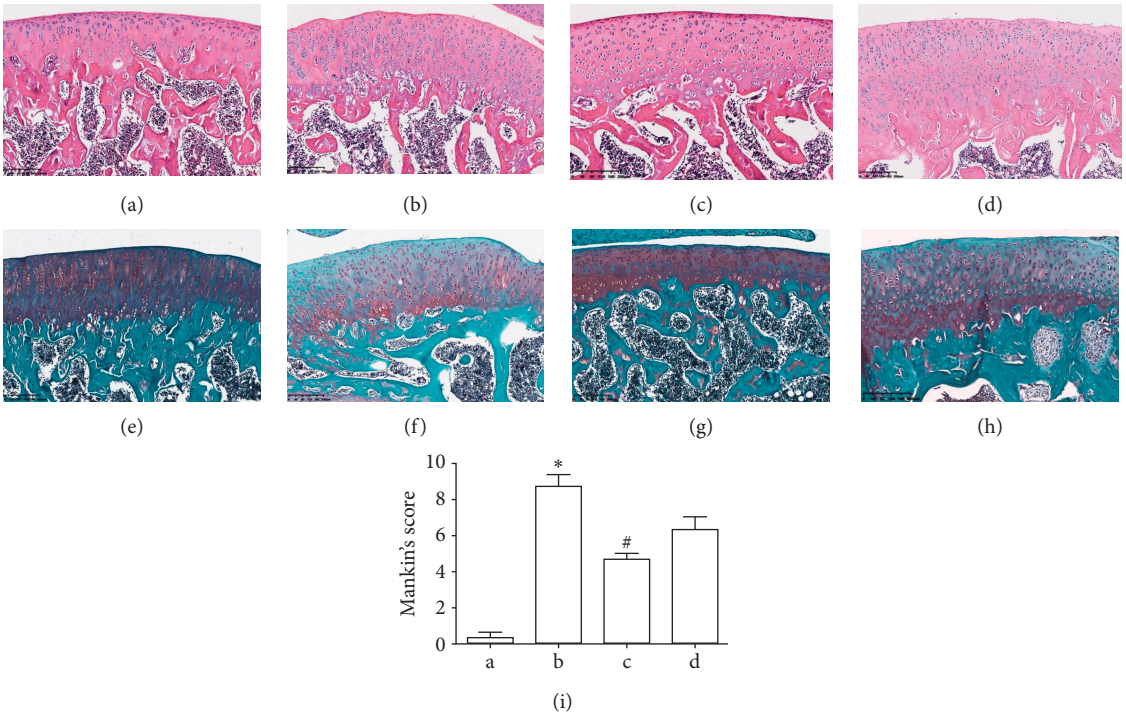

Figure 2: Pathological changes of cartilage. (a, e) Blank control group of hematoxylin-eosin (HE) staining and safranin O/fast green (S-O) staining. (b, f) Model group of HE and S-O staining. (c, g) GBT recipe group of HE and SCO staining. (d, h) Glucosamine sulfate group of HE and S-O staining. (I) Mankin's score of each group. Data were presented as mean ± standard deviation ($^{*}p < 0.05$ compared with the blank control group; $^{\#}p < 0.05$ compared with the OA model group). (a) Blank control group; (b) model group; (c) GBT recipe group; (d) glucosamine sulfate group.

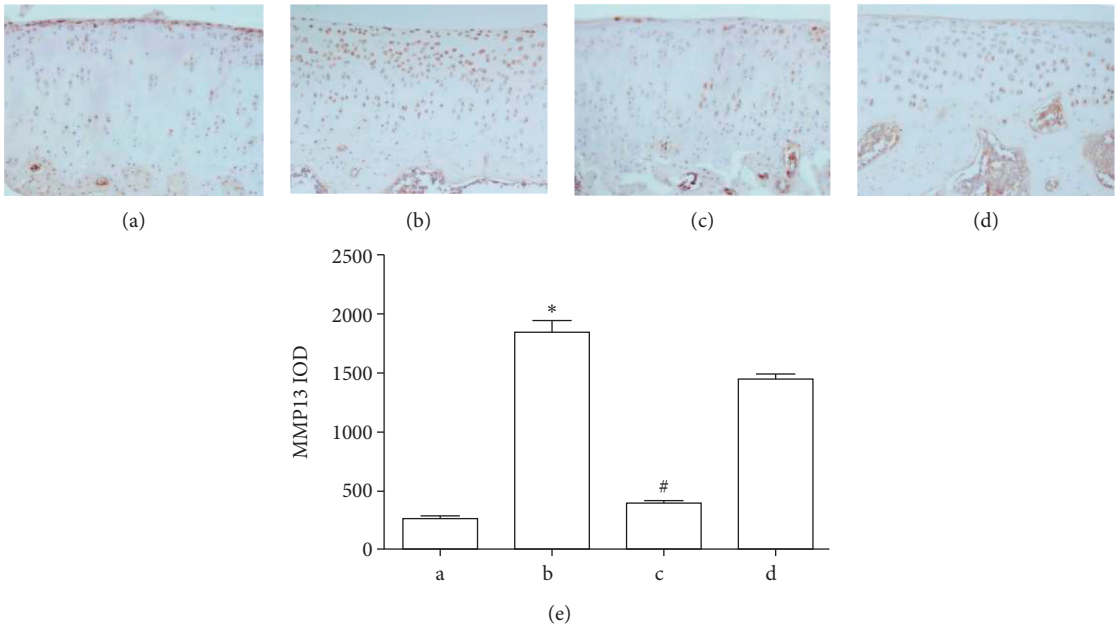

Figure 3: (a) Immunohistochemistry (IHC) for MMP-13 in cartilage from blank control group rat knee joints. (b) IHC for MMP-13 in the cartilage from the OA model group rat knee joints. (c) IHC for MMP-13 in the cartilage from the GBT recipe group rat knee joints. (d) IHC for MMP-13 in the cartilage from the glucosamine sulfate group rat knee joints, ×200. (e) IHC quantitative analysis was shown as IOD ($n = 3$). $^{*}p < 0.05$ compared with the blank control group, $^{\#}p < 0.05$ compared with the Hulth model group. (a) Blank control group, (b) OA model group, (c) GBT recipe group, and (d) glucosamine sulfate group.

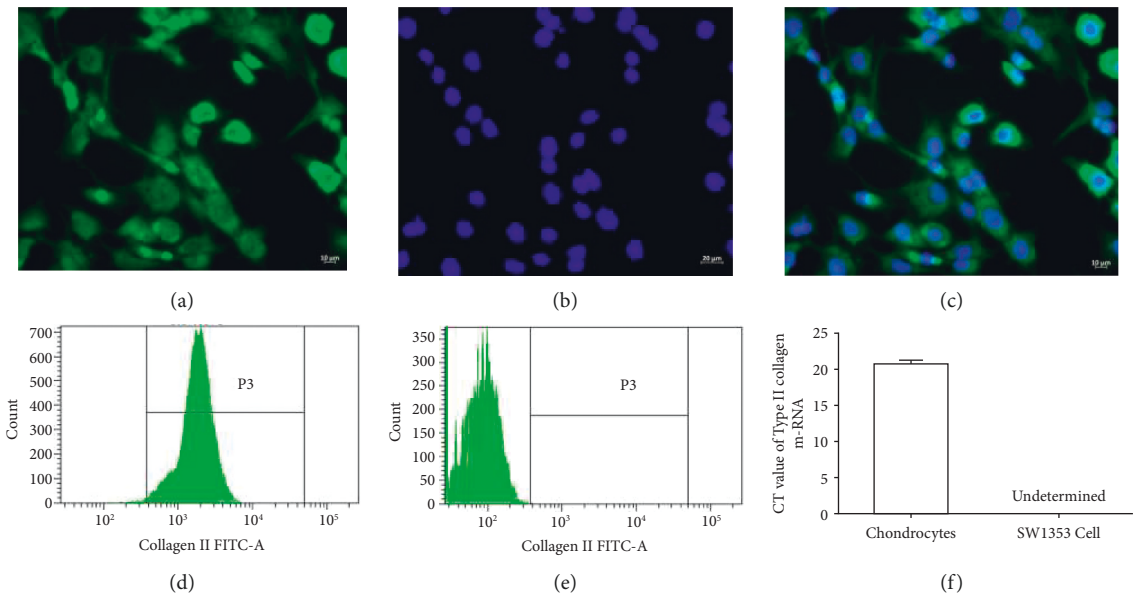

Figure 4: The localization of vimentin and DAPI was visualized under fluorescence microscopy after immunofluorescence staining. (a) Antivimentin antibody (green). (b) DAPI staining of nuclei (blue). (c) The merge of (a) and (b). Superficial markers of chondrocytes were detected using flow cytometry, with SW1353 cells as negative control. (d) 98% of chondrocytes show positive for type II collagen. (e) SW1353 cells show negative for type II collagen. (f) Type II collagen mRNA in chondrocytes and SW1353 cells was detected by qRT-PCR.

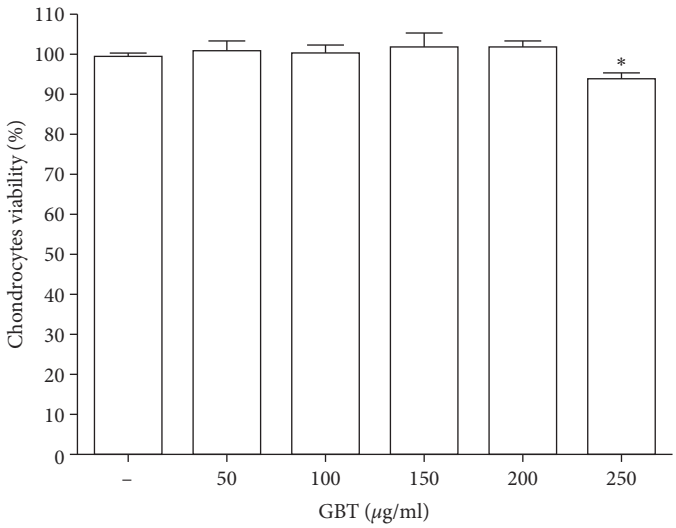

Figure 5: Effects of GBT recipe on chondrocytes viability. Chondrocytes were treated with different concentrations of GBT recipe (0, 50, 100, 150, 200, and 250 μg/ml) for 12 h, and the viability of chondrocytes was assessed by the MTS assay; data are presented as mean ± standard deviation. ($^*p < 0.05$ compared with the blank control group).

($n = 3$), and none for SW1353 cell-expressed type II collagen (Figures 4(d) and 4(e)). The qRT-PCR results demonstrated that chondrocytes expressed large amounts of type II collagen mRNA but the mRNA expression of type II collagen was not detectable in SW1353 cells (Figure 4(f)).

3.5. Effects of GBT Recipe on Chondrocytes Viability. To evaluate the effects of the GBT recipe on chondrocytes, the chondrocytes were incubated in a medium containing various concentrations of the GBT recipe (0, 50, 100, 150, 200, and 250 μg/ml) for 12 hours. MTS assay was performed to evaluate the viability of chondrocytes after treating with the GBT recipe. There was no significant effect on chondrocytes viability among concentrations of 0, 50, 100, 150, and 200 μg/ml. The chondrocytes viability was significantly decreased at the concentration of 250 μg/ml (Figure 5). These results demonstrated that 200 ug/ml was the maximum intervention concentration of the GBT recipe for chondrocytes. Therefore, 50 μg/ml, 100 μg/ml, and 200 μg/ml were chosen as low, intermediate, and high concentrations of the GBT recipe, respectively.

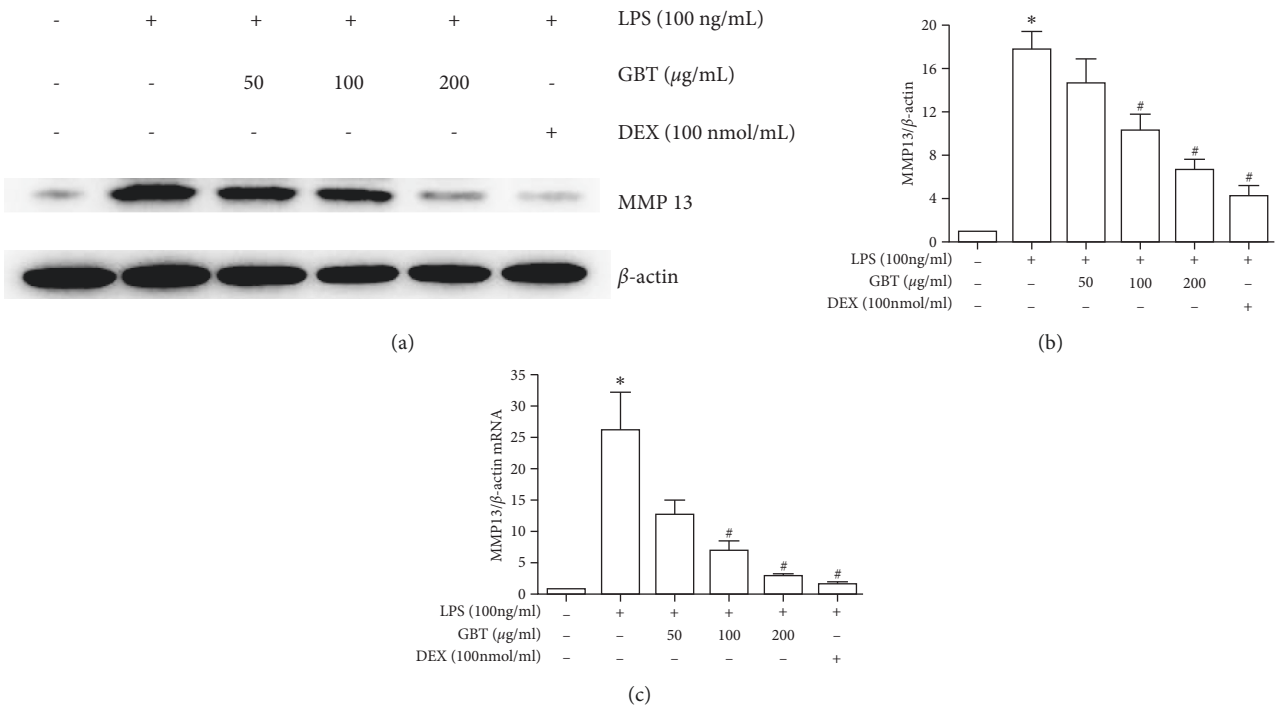

Figure 6: Expression of MMP-13 in chondrocytes. Chondrocytes were pretreated with 50, 100, or 200 μg/ml of GBT recipe for 1 hour and then stimulated with 100 ng/ml LPS for 12 h. (a) MMP-13 Western Blot band. (b) MMP-13 Western blot mean gray value. (c) MMP-13 mRNA expression. The data are derived from three independent experiments and expressed as the mean ± standard deviation. ($^{*}p < 0.05$ compared with the control group; $^{\#}p < 0.05$ compared with the LPS-treated group).

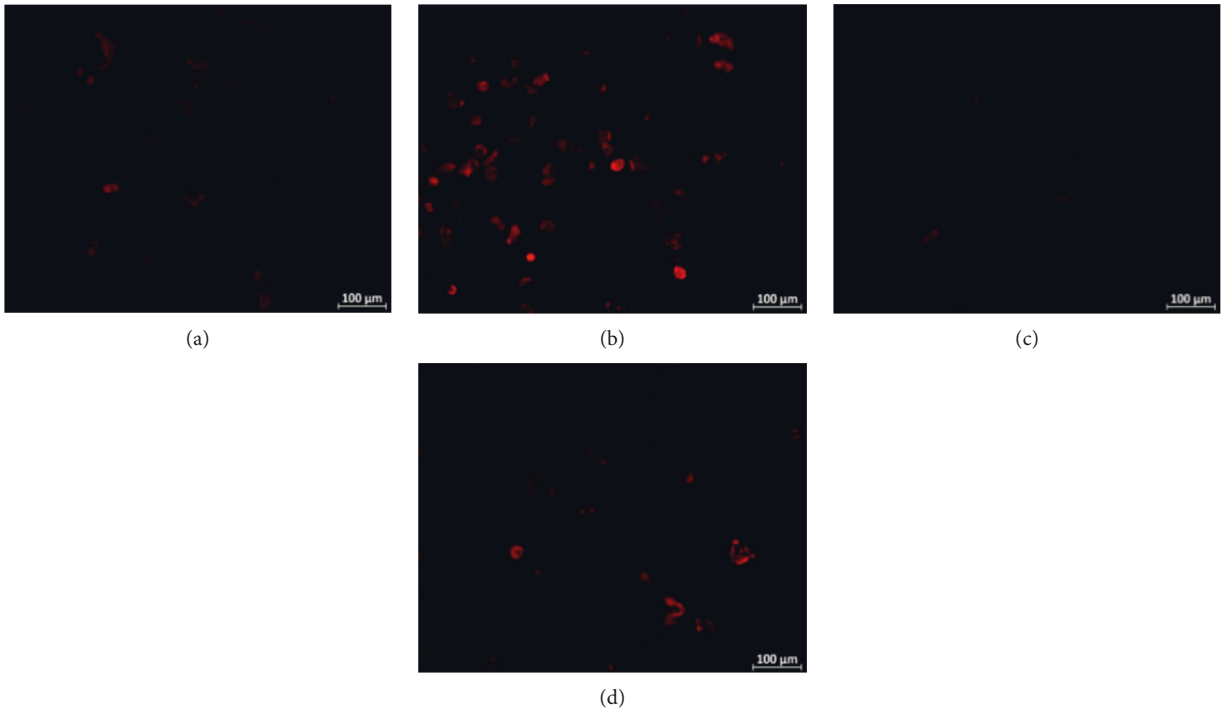

Figure 7: Detection of mitophagy in chondrocytes using Mtphagy Dye (red). (a) Chondrocytes unstimulated control. (b) Chondrocytes stimulated with 100 ng/ml LPS for 12 h. (c) Chondrocytes pretreatment with 200 μg/ml GBT recipe for 1 hour and then stimulated with 100 ng/ml LPS for 12 h. (d) Chondrocytes pretreatment with 100 nmol/ml DEX for 1 hour and then stimulated with 100 ng/ml LPS for 12 h.

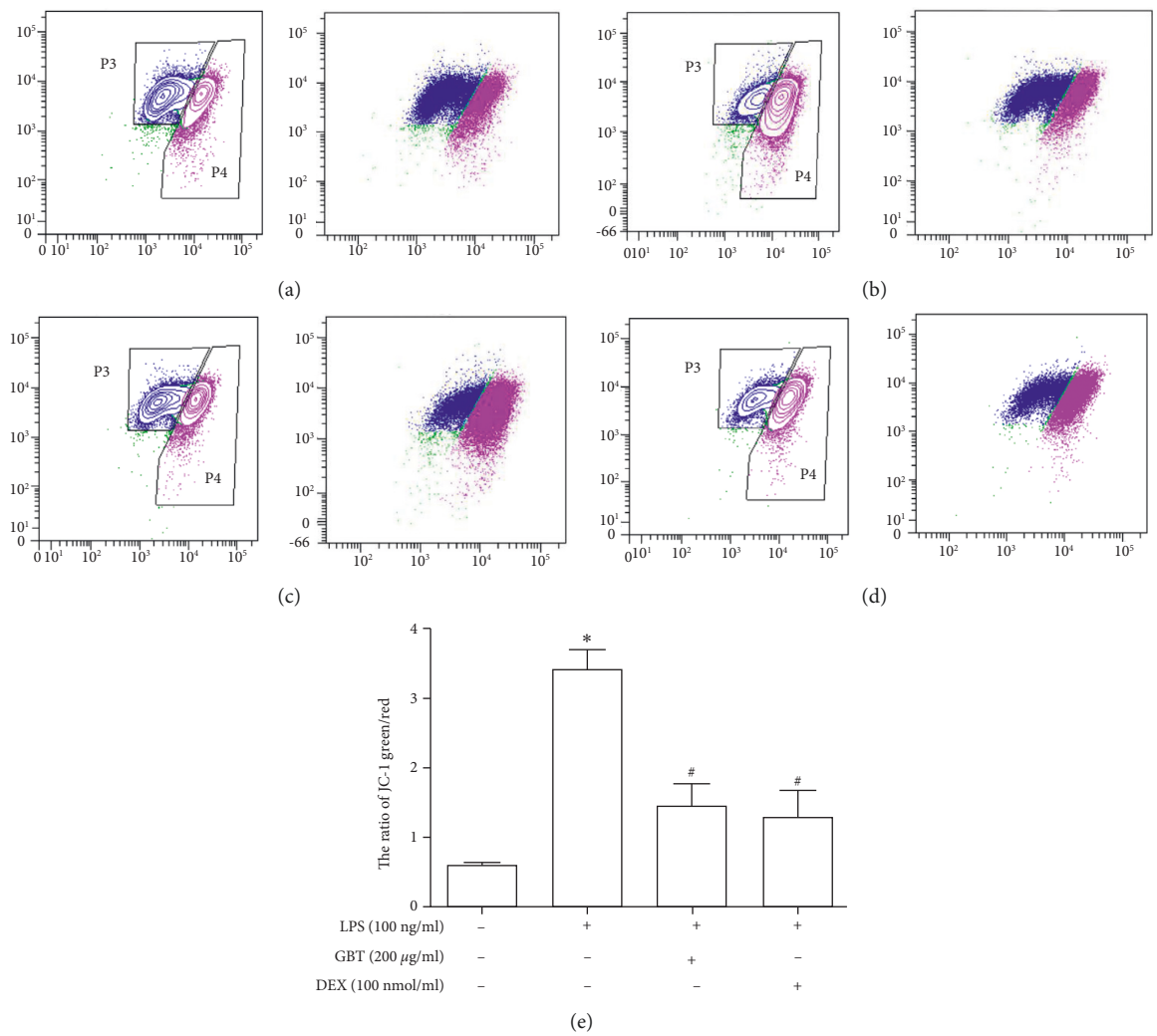

Figure 8: Measurement of mitochondrial membrane potential ($\Delta\psi$m) in chondrocytes using flow cytometric analysis after incubating with JC-1. P3 represents JC-1 red and P4 represents JC-1 green. (a) Chondrocyte unstimulated control. (b) 100 ng/ml LPS-stimulated chondrocyte for 12 h. (c) Chondrocyte pretreatment with 200 μg/ml GBT recipe for 1 hour and then stimulated with 100 ng/ml LPS for 12 h. (d) Chondrocyte pretreatment with 100 nmol/ml DEX for 1 hour and then stimulated with 100 ng/ml LPS for 12 h. (e) The ratio of JC-1 green/red in each group; data are presented as mean ± standard deviation ($^{*}p < 0.05$ compared with the blank control group; $^{\#}p < 0.05$ compared with the OA model group).

3.6. Effects of GBT Recipe on LPS-Induced MMP-13 Expression in Chondrocytes. Western blot and RT-PCR analyses showed that compared with LPS-free chondrocytes, LPS-induced chondrocytes had an increased expression of MMP-13 at the gene and protein levels. However, these changes in LPS-induced chondrocytes were reversed by the GBT recipe and DEX, and the GBT recipe showed a dose-dependent effect (Figure 6).

3.7. Effects of GBT Recipe on LPS-Induced Mitophagy in Chondrocytes. Mitophagy was detected using Mtphagy Dye. Compared with unstimulated chondrocytes, a significant increase in the fluorescent signal of Mtphagy Dye was detected in LPS-stimulated chondrocytes (Figures 7(a) and 7(b)). These changes could be suppressed by 200 μg/ml GBT recipe and 100 nmol/ml DEX (Figures 7(c) and 7(d)).

3.8. Effects of GBT Recipe on LPS-Induced Mitochondrial Dynamics in Chondrocytes. We used the JC-1 assay to measure mitochondrial membrane potential ($\Delta\psi$m) changes to see if LPS-induced mitophagy affects mitochondrial dynamics. LPS treatment significantly increased mitochondrial depolarization, as evidenced by a decrease in the red/green fluorescence intensity ratio (Figures 8(a) and 8(b)). 200 μg/ml GBT recipe and 100 nmol/ml DEX stabilize the mitochondrial membrane potential ($\Delta\psi$m) significantly (Figures 8(c) and 8(d)).

3.9. Effects of GBT Recipe on Protein Expression Associated with Chondrocyte Mitophagy in Chondrocytes. Western blot analysis showed that LPS elevated the ratio of LC3II and LC3I (LC3II/LC3I) in mitochondria of chondrocytes. Meanwhile, compared with LPS-free chondrocytes, LPS-

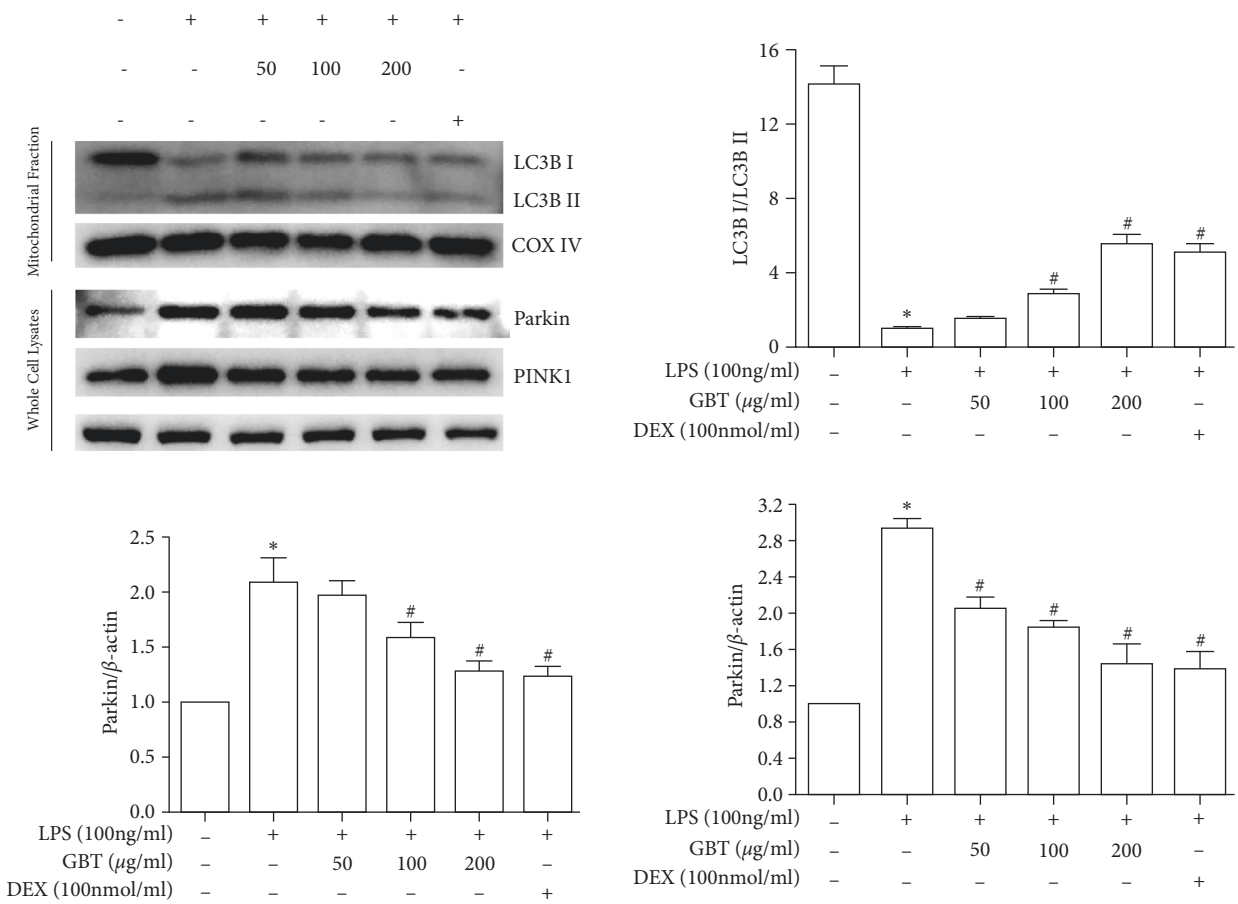

Figure 9: Expression of the ratio of LC3II and LC3I in mitochondria of chondrocytes, PINK1, and Parkin in chondrocytes. Chondrocytes were pretreated with 50, 100, or 200 μg/ml of GBT recipe or 100 nmol/ml DEX for 1 hour and then stimulated with 100 ng/ml LPS for 12 h The data are derived from three independent experiments and expressed as the mean ± standard deviation ($^{*}p < 0.05$ compared with the control group; $^{\#}p < 0.05$ compared with the LPS-treated group).

induced chondrocytes had an increased expression of PINK1 and Parkin. However, these changes in LPS-induced chondrocytes were reversed by the GBT recipe and DEX, and the GBT recipe showed a dose-dependent effect (Figure 9).

3.10. Effects of GBT Recipe on PI3K/AKT Signaling Pathway in Chondrocytes. Western blot revealed that the levels of PI3K and AKT proteins in LPS-induced chondrocytes were not changed compared with LPS-free chondrocytes, but the expression of p-PI3K and p-AKT was increased, suggesting that LPS abnormally activated the PI3K/AKT signaling pathway in chondrocyte (Figure 10). In addition, the phosphorylation levels of PI3K and AKT were reversed by the GBT recipe in a dose-dependent manner.

4. Discussion

Chondrocytes are the only cell type in cartilage and numerous studies have shown that OA often occurs with chondrocyte senescence and apoptosis [6, 24, 25]. During the pathologic process of OA, the capacity of chondrocytes to synthesize cartilage matrix was greatly reduced and large amounts of proteolytic enzymes were released, such as MMP-13, causing damage to the cartilage matrix [26]. Thus, improving the function of OA chondrocytes may be a target in treating OA [27].

Our study showed that the GBT recipe can effectively suppress the cartilage degeneration and apoptosis of the chondrocytes in the OA rat model. This was consistent with a previous study [28]. We found an increased expression of MMP-13 in the OA rat model by immunohistochemistry. MMP-13 was a hallmark of chondrocyte senescence and apoptosis and thus considered an important cause of the cartilage degeneration [29–31]. The intragastric administration of the GBT recipe can effectively reverse the high expression of MMP-13 in cartilage tissue of the OA rat model, which may indicate the therapeutic potential and mechanism of the GBT recipe for OA.

To further explore the therapeutic mechanisms of the GBT recipe for OA, we established an OA cell model by LPS-induced chondrocytes in vitro. The results showed that the mRNA and protein expressions of MMP-13 were significantly increased in LPS-induced chondrocytes and the GBT recipe can reverse this phenomenon in a dose-dependent manner. This suggests that the GBT recipe can promote chondroprotection by inhibiting the expression of MMP-13 in inflammatory chondrocytes.

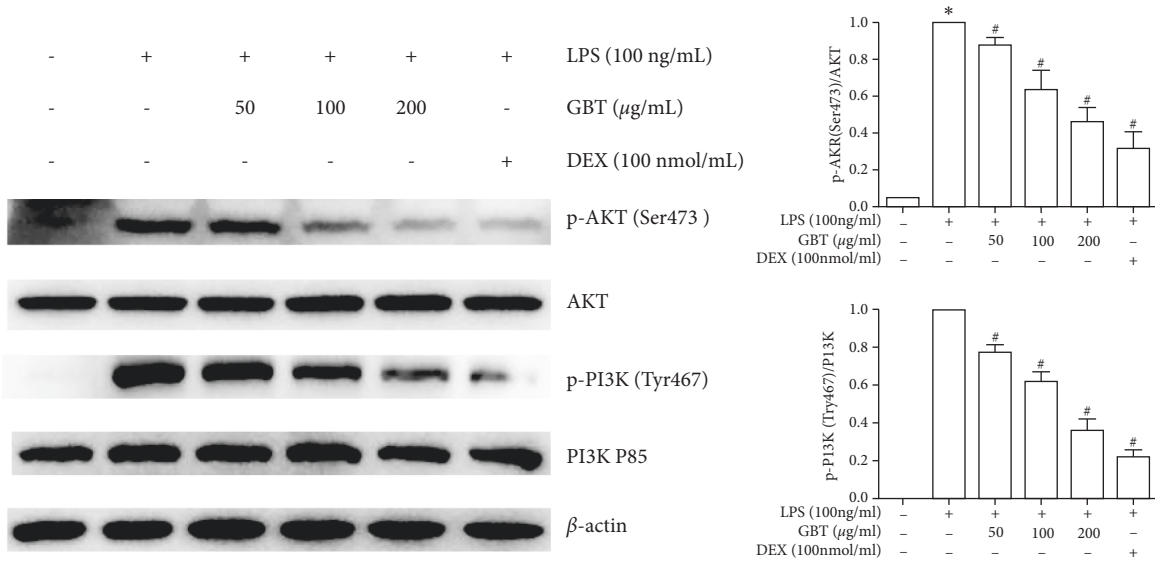

Figure 10: Expressions of PI3K, p-PI3K, AKT, and p-AKT in chondrocytes. Chondrocytes were pretreated with 50, 100, or 200 μg/ml of GBT recipe or 100 nmol/ml DEX for 1 hour and then stimulated with 100 ng/ml LPS for 12 h. The data were derived from three independent experiments and expressed as mean ± standard deviation ($^{*}p < 0.05$ compared with the control group; $^{\#}p < 0.05$ compared with the LPS-treated group).

JC-1 has been widely used to detect mitochondrial membrane potential as a fluorescent probe [32, 33]. The JC-1 dye accumulates in mitochondria in a potential-dependent manner, which is usually indicated by a shift in fluorescence emission from green (535 nm) to red (595 nm). JC-1 forms aggregates with intense red fluorescence in healthy mitochondria with high $\Delta\Psi m$, and monomers with green fluorescence when $\Delta\Psi m$ diminishes (depolarization) [34]. When the mitochondria are damaged, the mitochondrial membrane potential decrease in cells, the J-aggregate (red fluorescence) declines and the JC-1 monomer (green fluorescence) increases. The mitophagy detection kit is a common detection method for mitophagy after mitochondrial staining; the dye is immobilized and fluorescence intensity varies according to pH value [35, 36]. In mitochondrial-lysosome fusion, Mtphagy Dye displays higher fluorescence intensity, indicating mitophagy.

Our study showed that there was a decrease in the red/green fluorescence intensity ratio in LPS-induced chondrocytes, which indicated that the mitochondria membrane potential was depolarized and the mitochondrial function was impaired. Concurrently, the level of mitophagy in inflammatory chondrocytes was significantly elevated by analyzing the fluorescence intensity of Mtphagy Dye. These results revealed that excessive mitophagy was induced by LPS stimulation causing mitochondrial dysfunction. In further chondrocyte experiments, we found that the expression of the following two key molecules in mitophagy regulation was much higher in the LPS group than in the control group: Parkin and PINK1, and the LC3II/LC3I ratio. These results corroborate the excessive mitophagy in LPS-induced chondrocytes.

ROS are primarily generated in mitochondria, and mitochondrial abnormalities can induce ROS overproduction, leading to abnormal activation of the NFκB signaling pathway, eventually promoting MMPs expression [37–40]. The GBT recipe and the positive control drug (Dex) can inhibit the mitochondrial depolarization and the expression of mitophagy-related proteins and thus alleviate excessive mitophagy. Overall, inhibiting the excessive mitophagy of inflammatory chondrocytes may be the key mechanism by which the GBT recipe protects chondrocytes.

Previous studies have demonstrated that the PI3K/AKT pathway is associated with the inflammatory response, apoptosis, and autophagy of chondrocytes and thus is closely related to the development of OA [41–43]. The inhibition of the PI3K/AKT pathway can reduce cartilage degeneration by regulating the expression of multiple downstream targets. Hence, the PI3K/AKT pathway is considered a potential therapeutic target in treating OA [44]. In our in vitro experiments, chondrocytes showed elevated levels of phosphorylated PI3K and phosphorylated AKT after LPS stimulation. Meanwhile, the GBT recipe reduced these changes in LPS-induced chondrocytes, suggesting that the GBT recipe can inhibit the abnormal activation of the PI3K/AKT pathway, which may be associated with its chondroprotective effects and need further research in the future.

In conclusion, the GBT recipe showed chondroprotective effects on OA rats and rat chondrocytes stimulated by LPS. The chondroprotective effects may be associated with the inhibition of excessive mitophagy and abnormal activation of the PI3K/AKT pathway. Taken together, we suggest that the GBT recipe can be an effective alternative therapy in preventing and treating OA. However, we did not explore the effect of the GBT recipe in synovitis and human OA chondrocytes in this experiment. More studies are still needed to further investigate and validate the protective effects of the GBT recipe for OA.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon request.

Disclosure

Xin-bo Yu and Guang-yao Chen share the first authorship.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Conceptualization was done by Jing Luo and Qing-wen Tao; Xin-bo Yu, Guang-yao Chen, and Jing Luo contributed to the methodology; Li Zhou, Li-li Deng, Wei-jiang Song, Jia-qi Chen, Qian He, and Cai-qin Xu investigated the study; Xin-bo Yu and Guang-yao Chen wrote the manuscript; Jing Luo revised the manuscript.

Acknowledgments

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Review Article

A Systematic Review and Network Meta-Analysis about the Efficacy and Safety of *Tripterygium wilfordii* Hook F in Rheumatoid Arthritis

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Objective. This study aims to evaluate the efficacy of various conventional synthetic DMARDs, including *Tripterygium wilfordii* Hook F (TwHF) for treating rheumatoid arthritis (RA) by network meta-analysis. *Methods*. We retrieved the related literature from online databases and supplemented it by using a manual retrieval method. Data was extracted from the literature and analyzed with STATA software. *Results*. A total of 21 trials (5,039 participants) were identified. Assessment of ACR20 response found that TwHF combined with methotrexate (MTX) had the greatest probability for being the best treatment option among the treatments involved, while TwHF used singly was second only to TwHF combined with MTX. Assessment of ACR50 response found that TwHF combined with MTX ranked second in all treatment options after cyclosporine A (CsA) combined with leflunomide (LEF) and TwHF alone, followed by TwHF combined with MTX. Assessment of ACR70 response found that CsA combined with LEF ranked first, TwHF combined with LEF ranked second, TwHF combined with MTX ranked third, and TwHF used singly ranked fourth. In the safety analysis, TwHF had the least probability of adverse event occurrence, followed by TwHF combined with MTX, which ranked first and second, respectively. *Conclusion*. Compared with the current csDMARDs for treating RA, the efficacy of TwHF was clear, and TwHF combined with MTX performed well under various endpoints. In the future, large, rigorous, and high-quality RCTs are still needed to confirm the benefits of TwHF therapy on RA.

1. Introduction

Rheumatoid arthritis (RA) is a common systemic immune disease which is characterized by joint inflammation, destruction, and deformity associated with chronicity and a high rate of disability. Improvement in treatment, stopping progression, and optimizing quality of life are priorities in the field of rheumatology in China. *Tripterygium wilfordii* Hook F (TwHF) refers to the dry root or root xylem of the celastraceae plant *Tripterygium wilfordii*, a widely used herb in traditional Chinese medicine (TCM). In accordance with TCM theory, TwHF is considered a key herb for treating persistent rheumatoid arthritis, due to its strong efficacy in eliminating wind-damp and promoting blood circulation to dredge collaterals. In recent years, TwHF prepared by extracting the essence of the active components of

Tripterygium wilfordii has been used in clinical practice to treat a variety of rheumatic immune diseases, including RA [1–4]. It is noted that TwHF has been found to possess toxicity and is associated with having adverse events, such as hepatorenal toxicity, reproductive toxicity, and hematologic toxicity. Network meta-analysis (NMA) is a technique for comparing three or more interventions simultaneously in a single analysis by combining direct and indirect evidence and ranking the efficacy. Compared with traditional meta-analysis, NMA may assist in comparing the efficacy of multiple interventions for a disease more comprehensively, to provide more rigorous evidence through greater synthesis of information. There are many meta-analyses on TwHF in treating RA [5,6], but most are pairwise comparisons, which have limited ability to illustrate the individual differences among multiple disease-modifying antirheumatic drugs (DMARDs). This study is distinguished as a NMA which includes new and recent studies to evaluate the efficacy and safety of commonly used conventional synthetic DMARDs as both monotherapy and combination therapy for treatment of RA, including TwHF, methotrexate (MTX), leflunomide (LEF), sulfasalazine (SSZ), cyclosporine A (CsA), tacrolimus (FK506), minocycline (MINO).

2. Methods

2.1. Data Sources and Searches. This review was performed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement. We systematically searched the electronic databases PubMed, Embase, CNKI, Cochrane Library, SinoMed, and Wanfang Data from inception to February 28, 2020. We adopted a search method of subject words combined with free words, while manual retrieval was also performed to avoid omission. Searches included a combination of free text and Medline Subject Headings (MeSH) terms for "disease terms" with "drug names," and were limited to published RCTs. For the English databases, we used free text terms, such as "*Tripterygium wilfordii* Hook F″, methotrexate, leflunomide, sulfasalazine, hydroxychloroquine, cyclosporine A, azathioprine, cyclophosphamide, mycophenolate mofetil, tacrolimus (FK506), intramuscular gold, auranofin, minocycline, D-penicillamine, chlorambucil, "rheumatoid arthritis", and "randomized controlled trials". For the Chinese databases, free texts were used, such as "Lei gong teng", "Lei Gong Teng Zhiji", "Lei Gong Teng Duo Gan", "Jia An Die Ling (MTX)", "Lai Fu Mi Te (LEF)", "Liu Dan Huang Bi Ding (SSZ)", "Huan Bao Su A (CsA)", "Ta Ke Mo Si (FK506)", "Mi Nuo Huan Su (MINO)", "Lin Chuang Yan Jiu (clinical research)","Lei feng shi guan jie yan (rheumatoid arthritis)", "Sui Ji Dui Zhao Shi Yan (RCT)".

2.2. Study Selection

2.2.1. Inclusion Criteria. Literature that met all the following requirements were included:

Types of studies:

(i) Randomized controlled trials of conventional synthetic DMARDs for treatment of RA, published in either English or Chinese language.

Types of participants:

(i) The subjects were diagnosed with RA in accordance with the 1987 Guidelines of the American Rheumatology Association [7] or the 2010 ACR/European League against Rheumatism (EULAR) Criteria [8]; without diagnosis of other autoimmune diseases or serious cardiovascular and cerebrovascular diseases; no restrictions on age, sex, race, or nationality.

Types of intervention:

(i) TwHF, MTX, LEF, SSZ, CsA, FK506, and MINO used singly or as a two-drug combination in the treatment of RA. TwHF includes both tripterygium glycoside tablet and tripterygium tablet, the two root preparations of TwHF that have shown therapeutic promise [9,10]. The time limit for intervention was ≥12 weeks. Use of nonsteroidal anti-inflammatory drugs, folic acid, vitamins, calcium tablets, and low-dose hormones as adjuvant therapy during the treatment was not limited.

Types of outcome measures:

(i) Primary outcome: the American College of Rheumatology (ACR) response criteria ACR20 [11].
(ii) Secondary outcomes: ACR50, ACR70, and incidence of adverse events. All literature studies on adverse events were included, inclusive of all types of adverse events;
(iii) The analyses of outcomes were conducted on an intent-to-treat (ITT) basis, or modified ITT (number actually receiving treatment at baseline) if the number randomized to treatment was not reported.

2.3. Exclusion Criteria

(i) Publications where full text literature cannot be obtained;
(ii) Studies where research data are incomplete or cannot be extracted for analysis;
(iii) Interventions as herbs containing TwHF.

2.4. Data Extraction and Quality Assessment. The literature screening and extraction were carried out by two researchers, respectively, according to the inclusion criteria for literature retrieval. After the preliminary screening of titles and abstracts, the full text was screened, and the literature inclusion and data extraction were carried out based on intentionality analysis. Finally, the data extracted was compared and sorted. Two authors independently evaluated the methodological quality of eligible publications by using the Cochrane Collaboration's tool for assessing the risk of bias [12] (random sequence generation, allocation

concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias). If there were differences, a third-party researcher was invited to assist the ruling.

2.5. Data Synthesis and Analysis. The primary outcome of this analysis was the American College of Rheumatology (ACR) response criteria: ACR20. The ACR20 is defined as a reduction by 20% or more, in the number of tender and swollen joints plus 20% improvement in at least three of the following five measures: pain, patient global assessment, physician global assessment, a score of physical disability, and blood acute-phase reactants. The secondary outcomes were ACR50, ACR70, and adverse events. The ACR50 is defined as an improvement of 50% or more in the number of tender and swollen joints, plus 50% improvement in at least three of the aforementioned five measures. The ACR70 is defined as an improvement of 70% or more in the number of tender and swollen joints, plus 70% improvement in at least three of the aforementioned five measures.

2.6. Network Meta-Analysis. Results are reported as odds ratios (ORs) with 95% confidence intervals (CI) for all comparisons of interventions. Initially, traditional pairwise meta-analysis was performed by using a random-effects model. Then network meta-analysis was performed to compare different therapies by using a frequentist approach. We included multi-arm trials in the analysis by breaking multi-arm trials into separate two-arm trials. We employed a multi-variate random-effects meta-analysis model for each outcome separately, combining direct evidence for each comparison [13,14].

For each "loop" of treatment comparisons from three or more independent sources and for each outcome, we computed the difference between estimates from direct and indirect evidence on the log OR scale. Inconsistency was defined as disagreement between direct and indirect evidence with a 95% CI excluding 0. For each outcome, we estimated the probability of which intervention was the best for each outcome, the second best, the third best, and so on, from the ranked order of the treatments at each interaction. These ranking probabilities were used to calculate the surface under the cumulative ranking curve (SUCRA), which is expressed as percentage (100% for the best intervention, 0% for the worst intervention, and approximately 50% for equivalent interventions) [15].

2.7. Funnel Plot and Publication Bias. The difference between the observed effect size and comparison-specific summary effect for each study was calculated. This variable was then regressed on the standard error (SE), thus adding a simple linear regression line in the funnel plot. This method could help to visually determine if there is a publication bias in the results between small and large studies. We performed traditional and network meta-analysis by using Stata software (version 12.0, the StataCorp, College Station, Texas, USA).

3. Results

The flow chart of studies considered for inclusion is shown in Figure 1. On the basis of the title and abstract, 113 publications were selected and analyzed in full text versions. Eventually, 21 publications were included in the systematic review, and the characteristics of the literature were extracted as Table 1. Figure 2 shows the network of all treatment comparisons analyzed according to ACR 20, 50, 70, and adverse events. All reviews followed the methods in the Cochrane Handbook, including standardized searches, inclusion criteria, and outcomes.

3.1. Characteristics of Included Studies. Table 1 summarizes the clinical and methodological characteristics as well as the main outcomes of each trial. A total of 21 trials (5,039 participants) were identified, and the characteristics of the literature were extracted as shown in Table 1. The risk of bias assessments for the included trials is illustrated in Figure S2 and Figure S3. Most of the evidence was of moderate-to-good quality. All 21 RCTs mentioned the word "randomization". Over half of the studies did not report adequate information about allocation sequence generation and allocation sequence concealment. Unblinded designs were used in over half of the trials included.

3.2. NMA Results

3.2.1. ACR20. In the evaluation of the ACR20 response, 21 studies were included, involving a total of 5039 patients, including a total of 12 kinds of interventions. The interventions were MTX, TwHF, TwHF combined with MTX, LEF, TwHF combined with LEF, SSZ, SSZ combined with MTX, CsA, CsA combined with LEF, FK506, MINO, and placebo (Figure S4A). Efficacy was evaluated by drawing cumulative probability diagram, probability efficacy ranking table (Table 2), and inverted triangle table (Table 3). According to the analysis results, TwHF combined with MTX had the greatest probability of the best efficacy among the treatments involved, and TwHF used singly ranked second (Figure S5A).

3.2.2. ACR50. In the evaluation of ACR50 response, 15 literature studies were included, involving 2,968 patients, including 11 interventions: MTX, TwHF, TwHF combined with MTX, LEF, TwHF combined with LEF, SSZ combined with MTX, CsA, CsA combined with LEF, FK506, and placebo (Figure S4B). The efficacy was evaluated by drawing a cumulative probability diagram, a probability efficacy ranking table (Table 2), and an inverted triangle table (Table S1). According to the analysis results, the efficacy of TwHF combined with MTX ranked second only to CsA combined with LEF in all treatment schemes, while TwHF alone ranked third in all treatment schemes, second only to TwHF combined with MTX (Figure S5B).

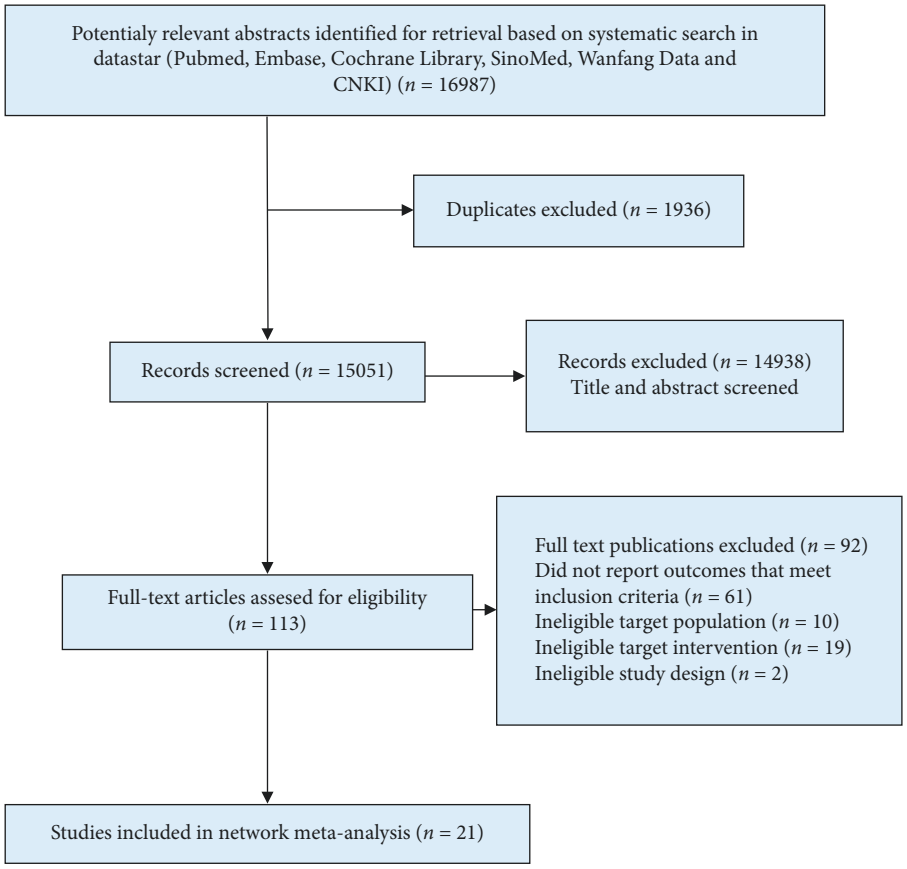

Figure 1: The flowchart.

3.2.3. ACR70. In the evaluation of ACR70 response, 10 literature studies were included, involving 2,374 patients, including 11 interventions: MTX, TwHF, TwHF combined with MTX, LEF, TwHF combined with LEF, SSZ, SSZ combined with MTX, CsA, CsA combined with LEF, FK506 and placebo (Figure S4C). The efficacy was evaluated by drawing a cumulative probability diagram, a probability efficacy ranking table (Table 2) and an inverted triangle table (Table S2). According to the analysis results, CsA combined with LEF ranked first, TwHF combined with LEF ranked second, TwHF combined with MTX ranked third, and TwHF used singly ranked fourth (Figure S5C).

3.2.4. Adverse Events. In the analysis of incidence of adverse events, 13 literature studies were included, involving a total of 3,415 patients, including 11 interventions: MTX, TwHF, TwHF combined with MTX, LEF, TwHF combined with LEF, SSZ combined with MTX, CsA, CsA combined with LEF, FK506 and placebo (Figure S4D). Incidence of adverse events was evaluated by drawing a cumulative probability diagram, a probability efficacy ranking table (Table 2), and an inverted triangle table (Table S3). According to the analysis results, TwHF and TwHF combined with MTX, ranked first and second, respectively (Figure S5D).

3.2.5. Forest Plots. In this study, a forest plot was drawn to assess for inconsistency, as shown in Figure S6A through to S6D. With exception of the M-S-T closed loop with ACR20 as the endpoint, there was no obvious inconsistency in all other closed loops. After analyzing the literature included in the M-S-T closed loop with ACR20 as the endpoint, it is considered that the sources of inconsistency may include different treatment time, different drug doses, heterogeneity caused by allowable adjuvant drugs.

3.2.6. Publication Bias. In addition, this study also evaluated publication bias with funnel plots (Figure S7A through to S7D). The scatters in the 4 funnel plots were almost symmetrical visually, and occasionally a small number of scatters were slightly less symmetrical, indicating that the publication bias in the included studies was overall satisfactory.

4. Discussion

TwHF is considered one of the most effective traditional Chinese herbal medicines against rheumatoid arthritis. Extracts of TwHF have been used for hundreds of years in China to treat various symptoms and, over the past 30 years, extracts of TwHF have become a standard therapy for rheumatoid arthritis in China. An earlier meta-analysis on treating RA bone destruction with TwHF was conducted by the team, and the results showed that the TwHF group was superior to the positive drugs MTX and SSZ used in the control group in Van der Heijde modified total sharp score (mTSS), joint erosion (JE), and joint space narrowing (JSN)

Table 1: Literature characteristics.

	Intervention			Endpoint	Average age (Years old)	Gender (%F)	Duration oftreatment	Sample size
	Treatment group	Control group	Other group					
Reece, 2002 [16]	L	M		ACR20	L:60 M:61	total:54	16 weeks	39
Cohen, 2001 [17]	L	M		ACR20, 50, 70	L:54 M:53	total:73	48 weeks	380
Lv, 2015 [1]	T	M	*M* + *T*	ACR20, 50, 70	T:51.3 M:51.0 *M* + *T*:50.6	T:81.2 M:85.5 *M* + *T*:79.7	24 weeks	207
Goldbach-mansky R, 2009 [18]	S	T		ACR20, 50, 70	T:54 S:52	T:73 S:87	24 weeks	121
Strand , 1994 [19]	L	M	P	ACR20, 50, 70	L:54.1 M:53.3 P:54.6	L:72.5 M:75.3 P:70.3	52 weeks	482
Emery , 2000 [20]	L	M		ACR20	L:58.3 M:57.8	L:70.7 M:71.3	52 weeks	999
Kraan , 2000a [21]	L	M		ACR20, 50	L:60 M:59	L:43.8 M:52.6	16 weeks	35
Kraan , 2000b [22]	L	M		ACR20, 50	L:63 M:66	L:57.1 M:37.5	16 weeks	15
Bao, 2003 [23]	L	M		ACR20	L:46.59 M:45.81	L:81.1 M:79.8	12 weeks	504
Capell, 2007 [24]	S	M	*M* + *S*	ACR20, 50, 70	S:55 M:53 *M* + *S*:56	S:75 M:79 *M* + *S*:75	48 weeks	165
Haagsm, 1997 [25]	S	M	*M* + *S*	ACR20	S:56.8 M:54.9 *M* + *S*:57.0	S:61.8 M:65.7 *M* + *S*:66.7	52 weeks	105
Dougads, 1999 [26]	S	M	*M* + *S*	ACR20	S:52 M:50 *M* + *S*:52	S:71 M:74 *M* + *S*:77	52 weeks	205
Smolen, 1999 [27]	L	S	P	ACR 20, 50	S:58.9 L:58.3 P:58.8	S:69 L:76 P:75	24 weeks	358
Karanikolas, 2006 [28]	C	L	L + *C*	ACR20, 50, 70	—	—	48 weeks	102
Scott, 2001 [29]	L	S		ACR20, 50, 70	S:59 L:58	S:69 L:76	24 weeks	262
Yocum,2003 [30]	F	P		ACR20, 50	F:55.9 P:55.8	F:77.2 P:75.8	24 weeks	464
Kawai,2011 [31]	F	P		ACR20, 50, 70	F:47.1 P:50.0	F:90.2 P:80.6	28 weeks	123
Pillemer, 1997 [32]	Mi	P		ACR20	Mi:55.0 P:53.5	Mi:76 P:80	48 weeks	219
Chao-yang Long, 2019 [33]	T	M		ACR20, 50	T:65.03 M:64.79	T:73.3 M:80.0	12 weeks	60
Yong-qiang Wang, 2013 [34]	M	*M* + *T*		ACR20, 50	total:43.4	total:55.6	12 weeks	126
Ming-li Zhao, 2017 [35]	L + *T*	L		ACR20, 50, 70	L:62.24 L + *T*:64.32	L:72.4 L + *T*:83.4	12 weeks	68

TwHF, *Tripterygium wilfordii* Hook F; MTX, methotrexate; LEF, leflunomide; SSZ, sulfasalazine; CsA, cyclosporine; FK506, tacrolimus; and MINO, minocycline; M, MTX; T, TwHF; M + T, TwHF combined with MTX; L, LEF; L + T, TwHF combined with LEF; S, SSZ; M + S, SSZ combined with MTX; C, CsA; L + C, CsA combined with LEF; F, FK506; Mi, MINO; P, placebo.

on X-ray films, with statistical differences ($P < 0.01$). In the aspects of mTSS, joint erosion, and joint space narrowing, TwHF is better than MTX and SSZ. The analysis results showed that TwHF can effectively delay the bone destruction process of RA [5]. Network meta-analysis is a further development and extension of traditional meta-analysis. The biggest advantage of NMA is that it can evaluate different interventions for the treatment of similar diseases for quantitative statistical analysis and comparison. In recent years, the number of NMAs published in various journals and magazines has increased to provide guidance for clinicians in choosing effective interventions. In a previous NMA analysis that was conducted on the efficacy and safety of using DMARDs singly represented by TwHF in the treatment of RA, we found that TwHF is safe and effective [6]. This study provides an updated evaluation based on the

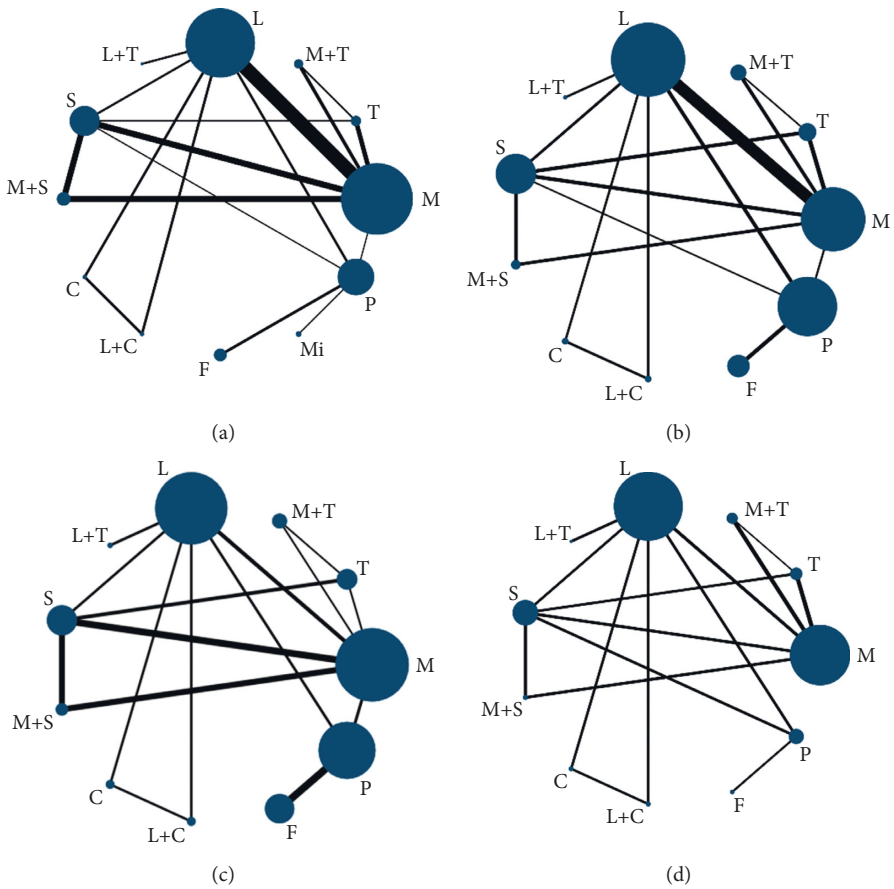

Figure 2: The network of all treatment comparisons analyzed according to ACR 20, 50, 70 response, and adverse events. (a) Network evidence plot based on ACR20. (b) Network evidence plot based on ACR50. (c) Network evidence plot based on ACR70 (d) and adverse events.

Table 2: Ranking probability of different conventional synthetic DMARDs.

Treatment	ACR20		ACR50		ACR70		Adverse events	
	SUCRA	Rank	SUCRA	Rank	SUCRA	Rank	SUCRA	Rank
T	0.749	2	0.726	3	0.606	3	0.107	11
M + *T*	0.867	1	0.87	2	0.646	2	0.146	10
M	0.371	9	0.457	6	0.261	9	0.441	6
M + *S*	0.603	6	0.603	5	0.457	7	0.616	4
L	0.263	10	0.397	7	0.508	6	0.524	5
L + *T*	0.397	8	0.607	4	0.852	4	0.723	3
L + *C*	0.661	4	0.95	1	0.915	1	0.352	9
C	0.664	3	0.374	8	0.542	5	0.415	7
S	0.245	11	0.246	10	0.156	10	0.403	8
F	0.639	5	0.254	9	0.428	8	0.836	2
Mi	0.505	7	—	—	—	—	—	—
P	0.035	12	0.016	11	0.131	11	0.936	1

TwHF, *Tripterygium wilfordii* Hook F; MTX, methotrexate, LEF; leflunomide; SSZ, sulfasalazine; CsA, cyclosporine; FK506, tacrolimus; and MINO, minocycline; M, MTX; T, TwHF; *M* + *T*, TwHF combined with MTX; L, LEF; L + *T*, TwHF combined with LEF; S, SSZ; *M* + *S*, SSZ combined with MTX; C, CsA; L + *C*; CsA combined with LEF; F, FK506; Mi, MINO; P, placebo.

results of previous research and with additional interventions, including combined medications. Based on our results on ACR20 response, we found that TwHF combined with MTX had the greatest probability of having the best efficacy among the treatment schemes involved, and TwHF used singly was the second best in the scheme of rankings. The efficacy ranking from best performing to the least are listed as the following: 1st rank TwHF combined with MTX, 2nd rank TwHF, 3rd rank CsA, 4th rank CsA combined with LEF, 5th rank FK506, 6th rank SSZ combined with MTX, 7th rank

Table 3: Inverted triangle table based on ACR20

OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
M	1.78 (1.01, 3.14)	2.28 (1.23, 4.21)	0.90 (0.68, 1.17)	1.01 (0.34, 3.02)	0.87 (0.60, 1.26)	1.38 (0.82, 2.35)	1.67 (0.47, 5.91)	1.67 (0.47, 5.91)	1.49 (0.71, 3.13)	1.22 (0.49, 3.01)	0.56 (0.34, 0.92)
0.56 (0.32, 0.99)	*T*	1.28 (0.62, 2.63)	0.50 (0.27, 0.92)	0.57 (0.17, 1.93)	0.49 (0.27, 0.89)	0.78 (0.37, 1.63)	0.94 (0.24, 3.72)	0.94 (0.24, 3.72)	0.84 (0.33, 2.09)	0.69 (0.24, 1.96)	0.31 (0.15, 0.65)
0.44 (0.24, 0.81)	0.78 (0.38, 1.60)	*M + T*	0.39 (0.20, 0.76)	0.44 (0.13, 1.55)	0.38 (0.19, 0.77)	0.61 (0.27, 1.35)	0.73 (0.18, 2.98)	0.73 (0.18, 2.98)	0.65 (0.25, 1.70)	0.53 (0.18, 1.59)	0.24 (0.11, 0.54)
1.12 (0.85, 1.46)	**1.99 (1.08, 3.64)**	**2.55 (1.31, 4.95)**	*L*	1.13 (0.39, 3.26)	0.97 (0.67, 1.40)	1.54 (0.88, 2.70)	1.86 (0.54, 6.41)	1.86 (0.54, 6.41)	1.66 (0.80, 3.45)	1.36 (0.56, 3.32)	0.62 (0.39, 1.00)
0.99 (0.33, 2.98)	1.77 (0.52, 6.01)	2.26 (0.64, 7.94)	0.89 (0.31, 2.58)	*L + T*	0.86 (0.28, 2.66)	1.37 (0.41, 4.57)	1.65 (0.32, 8.46)	1.65 (0.32, 8.46)	1.48 (0.41, 5.37)	1.21 (0.30, 4.85)	0.55 (0.17, 1.78)
1.15 (0.79, 1.67)	**2.05 (1.12, 3.74)**	**2.63 (1.30, 5.28)**	1.03 (0.71, 1.49)	1.16 (0.38, 3.58)	*S*	1.59 (0.94, 2.69)	1.92 (0.53, 6.98)	1.92 (0.53, 6.98)	1.71 (0.80, 3.69)	1.40 (0.56, 3.53)	0.64 (0.38, 1.09)
0.72 (0.43, 1.23)	1.29 (0.61, 2.70)	1.65 (0.74, 3.67)	0.65 (0.37, 1.13)	0.73 (0.22, 2.43)	0.63 (0.37, 1.06)	*M + S*	1.20 (0.31, 4.69)	1.20 (0.31, 4.69)	1.08 (0.45, 2.60)	0.88 (0.32, 2.45)	0.40 (0.20, 0.80)
0.60 (0.17, 2.13)	1.07 (0.27, 4.24)	1.37 (0.34, 5.58)	0.54 (0.16, 1.86)	0.61 (0.12, 3.10)	0.52 (0.14, 1.90)	0.83 (0.21, 3.23)	*C*	1.00 (0.26, 3.79)	0.89 (0.21, 3.76)	0.73 (0.16, 3.37)	0.33 (0.09, 1.26)
0.60 (0.17, 2.13)	1.07 (0.27, 4.24)	1.37 (0.34, 5.58)	0.54 (0.16, 1.86)	0.61 (0.12, 3.10)	0.52 (0.14, 1.90)	0.83 (0.21, 3.23)	1.00 (0.26, 3.79)	*L + C*	0.89 (0.21, 3.76)	0.73 (0.16, 3.37)	0.33 (0.09, 1.26)
0.67 (0.32, 1.42)	1.20 (0.48, 2.99)	1.53 (0.59, 4.00)	0.60 (0.29, 1.25)	0.68 (0.19, 2.46)	0.58 (0.27, 1.26)	0.93 (0.38, 2.25)	1.12 (0.27, 4.71)	1.12 (0.27, 4.71)	*F*	0.82 (0.32, 2.09)	0.37 (0.22, 0.65)
0.82 (0.33, 2.02)	1.46 (0.51, 4.17)	1.87 (0.63, 5.55)	0.73 (0.30, 1.79)	0.83 (0.21, 3.32)	0.71 (0.28, 1.79)	1.13 (0.41, 3.15)	1.37 (0.30, 6.28)	1.37 (0.30, 6.28)	1.22 (0.48, 3.11)	Mi	0.46 (0.22, 0.97)
1.79 (1.09, 2.95)	**3.19 (1.54, 6.63)**	**4.09 (1.87, 8.96)**	1.61 (1.00, 2.59)	1.81 (0.56, 5.81)	1.56 (0.92, 2.65)	**2.48 (1.25, 4.94)**	2.99 (0.79, 11.26)	2.99 (0.79, 11.26)	**2.67 (1.54, 4.64)**	**2.19 (1.03, 4.65)**	*P*

Weighted mean difference with 95% CIs of network meta-analysis. Treatments are reported in alphabetical order. Results of direct comparisons are listed in the lower-left triangle, and the estimation is calculated as the row-defining treatment compared with the column-defining treatment. Results of network meta-analysis are listed in the upper-right triangle, and the estimation is calculated as the column-defining treatment compared with the row-defining treatment. Bolding indicates that the difference has a statistical significance.

MINO, 8th rank TwHF combined with LEF, 9th rank MTX, 10th rank LEF, 11th rank SSZ, and 12th rank placebo. Based on our results on ACR50 response, the analysis showed that TwHF combined with MTX ranked second only to CsA combined with LEF, while TwHF ranked third. The detailed ranking list is as follows: 1st rank CsA combined with LEF, 2nd rank TwHF combined with MTX, 3rd rank TwHF, 4th rank TwHF combined with LEF, 5th rank SSZ combined with MTX, 6th rank MTX, 7th rank LEF, 8th rank CsA, 9th rank FK506, 10th rank SSZ, and 11th rank placebo. Based on our results on ACR70 response, the analysis showed that CsA combined with LEF ranked first, TwHF combined with LEF ranked second, TwHF combined with MTX ranked third, and TwHF used singly ranked 4th. The detailed rankings are as follows: 1st rank CsA combined with LEF, 2nd rank TwHF combined with LEF, 3rd rank TwHF combined with MTX, 4th rank TwHF, 5th rank CsA, 6th rank LEF, 7th rank SSZ combined with MTX, 8th rank FK506, 9th rank MTX, 10th rank SSZ, and 11th rank placebo. In the analysis of incidence of adverse events, we found the least possibility of incidence with TwHF used singly, followed by TwHF combined with MTX, ranking first and second, respectively. The details of the interventions are as follows: 1st rank TwHF, 2nd rank TwHF combined with MTX, 3rd rank CsA combined with LEF, 4th rank SSZ, 5th rank CsA, 6th rank MTX, 7th rank LEF, 8th rank SSZ combined with MTX, 9th rank TwHF combined with LEF, 10th rank FK506, and 11th rank placebo. In conclusion, from the results of the current analysis it can be considered that compared with the DMARDs currently used to treat RA, TwHF has shown a clear efficacy in treatment of RA, and TwHF combined with MTX performed well under various endpoints. In the ACR20, ACR50, and ACR70 responses, the analysis showed that the efficacy of combination therapy of TwHF was better than its monotherapy. In the ACR20 and ACR50 responses, both monotherapy and combination therapy of TwHF were found to have good efficacy. In analysis of the incidence of adverse events, we found the least possibility of incidence with TwHF used singly. This study also has some limitations. For example, due to the insufficient number of studies, it was not feasible to assess for different dosages of the same drug across different treatment schemes, which may impact the study's results. In addition, some of the included literature studies do not explicitly mention details of randomization method or method of blinding; thus, there is a risk of publication bias. In clinical practice, TwHF is often considered to possess liver and kidney toxicity and to easily cause adverse events. While our study found that TwHF has little possibility of incidence in adverse events, we cannot completely exclude the possibility of publication bias or selective reporting. Thus further review after the publication of more rigorous, high-quality RCTs is warranted.

5. Conclusions

This NMA found that in assessment of ACR20 response, TwHF combined with MTX had the greatest probability of achieving the best efficacy among the treatment schemes involved, while TwHF used singly was ranked as second best. In assessment of ACR50 response, the efficacy of TwHF combined with MTX ranked second only to CsA combined with LEF, while TwHF used singly ranked third. In assessment of ACR70 response, CsA combined with LEF ranked first, TwHF combined with LEF ranked second, TwHF combined with MTX ranked third, while TwHF alone ranked fourth. In analysis of incidence of adverse events, the possibility of incidence ranked the lowest with TwHF used singly and the second lowest with TwHF combined with MTX. In conclusion, it can be considered that compared with the DMARDs currently used to treat RA, TwHF has a clear efficacy on RA. Among all treatments, the monotherapy of TwHF and the combination therapy of TwHF and MTX performed well at various endpoints.

In a previous study [6] we conducted an NMA analysis on the efficacy and safety of TwHF and traditional synthetic DMARDs monotherapy in RA. The results indicated that in the direct comparison, TwHF was better than sulphasalazine in ACR 20, ACR 50, and ACR 70 responses; TwHF was superior to placebo in ACR 20 and ACR 50 responses. In indirect comparison, TwHF was superior to MTX, LEF, FK506, MINO, and placebo in ACR 20 response. In the efficacy ranking, TwHF ranked first in ACR 20 and ACR 50 response, and was the preferred treatment. Also, in ACR 70 response, TwHF ranked second (57.8%), second only to LEF (69.6%), which confirmed its efficacy and safety in RA. In clinical practice, combination therapy is also our conventional treatment for RA. Therefore, in this study, based on the previous research, we performed an updated NMA on monotherapy and combination therapy of TwHF and conventional synthetic DMARDs in RA. The research results showed that the clinical protocol of TwHF combination therapy for RA is more in line with clinical practice and has more advantages than other clinical protocols of conventional synthetic DMARD drugs in RA. TwHF can be considered as a potential first-line DMARD for the treatment of RA, but high-quality randomized trial data are still needed to guide the use of TwHF in clinical RA treatment.

Ethical Approval

All analyses were based on previously published studies. As a result, ethical approval and patient consent are not relevant.

Disclosure

Chun-ping Liu and Yu-zheng Yang are co-correspondence authors. Hai-long Wang and Qi Zhao are co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Chun-ping Liu and Yu-zheng Yang conceived and designed the study, and the two of them contributed equally to this work. Hai-long Wang and Qi Zhao wrote the paper, and the two of them contributed equally to this work. Yu-zheng Yang analyzed the data and performed the statistical

analysis. Wei Li, Hua-chao Zhu, Liu Lv, Zhen-hong Zhu, Xi-xi Wang, Zheng-zheng Yang, Yu-cao Ma, Ming-xuan Liu, and Yi-wen Wang collected the data. Hezheng Lai provided revision of both the content and language expression of the study. All authors read and approved the final manuscript.

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Supplementary Materials

Figure S1: PRISMA-2009-Flow-Diagram-MS-Word: PRISMA flowchart. Figure S2: Risk of bias graph. Figure S3: Risk of bias summary. Figure S4: The cumulative probability diagram. A. With ACR20 as the endpoint. B. With ACR50 as the endpoint. C. With ACR70 as the endpoint. D. The analysis of adverse events. Figure S5: Forest plots. A. With ACR20 as the endpoint. B. With ACR50 as the endpoint. C. With ACR70 as the endpoint. D. The analysis of adverse events. Figure S6: Inconsistent assessment. A. With ACR20 as the endpoint. B. With ACR50 as the endpoint. C. With ACR70 as the endpoint. D. The analysis of adverse events. Figure S7: The publication bias. A. With ACR20 as the endpoint. B. With ACR50 as the endpoint. C. With ACR70 as the endpoint. D. The analysis of adverse events. Table S1: Inverted triangle table based on ACR50. Table S2: Inverted triangle table based on ACR70. Table S3: Inverted triangle table based on adverse events. Table S4: Search strategy. (*Supplementary Materials*)

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Research Article

Tripterygium wilfordii Hook. f. Preparations for Rheumatoid Arthritis: An Overview of Systematic Reviews

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Objectives. To summarize the quantity and quality of evidence for using *Tripterygium wilfordii Hook. f.* (TwHF) preparations in patients with rheumatoid arthritis (RA) and to find the reasons of the disparity by comprehensively appraising the related systematic reviews (SRs). *Methods*. We performed an overview of evidence for the effectiveness and safety of TwHF preparations for patients with RA. We searched seven literature databases from inception to July 15, 2021. We included SRs of TwHF preparations in the treatment of RA. Four tools were used to evaluate the reporting quality, methodological quality, risk of bias, and the certainty of evidence for the included SRs, which are the PRISMA, the AMSTAR-2, the ROBIS, and the GRADE approach. *Results*. We included 27 SRs (with 385 studies and 33,888 participants) for this overview. The AMSTAR-2 showed that 19 SRs had critically low methodological quality and the remaining 8 had low methodological quality. The rate of overlaps was 68.31% (263/385), and the CCA (corrected covered area) was 0.53, which indicated the degree of overlap is slight. Based on the assessment of ROBIS, all 27 SRs were rated as low risk in phase 1; one SR was rated as low risk in domain 1, 9 SRs were in low risk in domain 2, 16 SRs were in low risk in domain 3, and 16 SRs were in low risk in domain 4 in phase 2; 7 SRs were rated as low risk in phase 3. Among 27 items of PRISMA, 15 items were reported over 70% of compliance, the reporting quality of 16 SRs was rated as "fair," and 11 were "good." Using GRADE assessment, moderate quality of evidence was found in 5 outcomes, and 5 outcomes were low quality. *Conclusion*. The use of TwHF preparations for the treatment of RA may be clinically effective according to the moderate-quality evidence. There are methodological issues, risk of bias, and reporting deficiencies still needed to be improved. SRs with good quality and further randomized clinical trials that focus on clinical important outcomes are needed.

1. Introduction

Rheumatoid arthritis (RA) is the most common autoimmune inflammatory arthritis in adults with a prevalence of 0.5–1.0% of the general population [1, 2]. A recent meta-analysis found the global prevalence of RA was 460 per 100,000 population in the period 1980 to 2019 [3]. RA is characterized by progressive symmetric arthritis with chronic joint inflammation, synovial hyperplasia, and systemic manifestations [4]. The most common symptoms reported by people with RA are arthralgia, swelling, redness, and limited motion range [5, 6]. Without adequate treatment, RA can lead to severe joint deformity and disability, impacting upon patients' quality of life and work ability [7]. Complications associated with RA lead to high morbidity and rising mortality [7, 8]. Significant progress in studying the mechanisms of RA has been made in the field of genetic predisposition and environmental research area, which were involved in its onset and progression, emphasizing the heterogeneity of RA [9].

Treatment algorithms for patients with RA involve measuring disease activity with composite indices, and its treatment target is the maintenance of remission/low disease activity or prevention of joint destruction and deformity and

improvement of joint function [10]. The 2021 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis addressed the treatment for patients with RA is the disease-modifying antirheumatic drugs (DMARDs) [11]. As the first line of therapy for RA, (cs) DMARDs (e.g., methotrexate (MTX), leflunomide, and sulfasalazine) and several recommendations against the use of glucocorticoid therapy are made in the newest guideline [12]. Although the prospects for most patients are now favorable, many still do not respond to current therapies. Adverse effects (e.g., immunosuppression, bone marrow dysfunction, interstitial lung disease, liver damage, hyperglycemia, and hypertension) occurred in RA patients with longtime medication given for treatment [13, 14], and the cost of treating RA has also risen strikingly, largely as a consequence of the biologic therapies [15]. Accordingly, there are still some unmet needs for patients who do not achieve remission and who continue to worsen despite treatment. Hence, patients often seek more complementary therapies.

The popularity of complementary and alternative medicine (CAM) in the management of RA has grown considerably, which covered both the interest of patients and the research community over the past decade [16, 17]. Botanical extract, among the CAM approaches, is an effective option against RA symptoms owing to several anti-inflammatory, palliative, and antiarthritic properties. *Tripterygium wilfordii Hook. f.* (TwHF) is a traditional Chinese herb, which is widely used in the treatment of RA in China [18, 19] due to its anti-inflammatory and immunosuppressive effects. Several TwHF preparations and patented preparations derived from TwHF extracts are clinically available, including Tripterygium wilfordii tablets (TWTs) and Tripterygium wilfordii glycosides tablets (TWGTs) and Tripterygium hypoglaucum hutch tablets. Both TWTs and TWGTs exhibited efficacy similar to MTX as well as enhanced efficacy when a combined remedy of the tablets and MTX was administered to patients with RA in randomized controlled clinical trials [20, 21]. Biochemical and pharmacokinetic studies found that triptolide (TP) and celastrol are two of the most bioactive, yet toxic, constituents identified in TwHF preparations [22]. Triptolide is regarded as the most potent systematic anti-inflammatory and immunoregulating natural products [23]. Previous reviews summarized that the mechanisms associated with the significant therapeutic effects of TP and celastrol against T helper cell-mediated immunity, including RA, have been extensively studied [24, 25]. Emerging evidence suggests that TP suppresses inflammatory responses by attenuating MAPK/NF-κB activation and inhibiting downstream responses [24, 25]. Several studies have demonstrated that TwHF preparations' therapeutic effect may be dependent on the immune balance of Th17 cells and Tregs, the regulation of the proportion between CD4+ and CD8+ T cells, and the differentiation of dendritic cells [26, 27]. A large number of individual trials and systematic reviews (SRs)/meta-analyses (MAs) of TwHF preparations in the treatment of RA have been published. However, the results and quality of the SRs have been mixed. As an increasingly popular form of evidence synthesis, an overview of SRs/MAs uses explicit and systematic methods to extract and analyze their results across important outcomes from multiple SRs/MAs on related research questions [28, 29]. Thus, we conducted an overview of SRs/MAs about TwHF preparations in the treatment of RA to inform healthcare decision-makers and address new questions that were not reported in the included SRs/MAs.

2. Methods

We adhered to two guidelines for conducting an overview, one is the Cochrane Handbook [30], and the other is the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [31]. Literature search and selection, data extraction, and quality evaluation were completed by two authors (Huimin Li and Simin Xu) independently. All discrepancies were resolved by consulting an experienced third reviewer (Xing Liao) firstly and then reached consensus in the team of all authors. We referred to two published overviews with good quality in this step [32, 33].

2.1. Protocol and Registration. We registered our protocol in the International Platform of Registered Systematic Review and Meta-Analysis Protocols (DOI number is 10.37766/inplasy2021.8.0081). There was no need for the ethical approval.

2.2. Search Strategy. We searched the following literature databases using the words of TwHF, RA, and systematic review/meta-analysis from inception to July 13, 2021: The Cochrane Library, PubMed, Embase, VIP database, China National Knowledge Infrastructure, CBM, and WanFang. The details of the literature search strategy are presented in Appendix A.

2.3. Inclusion and Exclusion Criteria. We selected related SRs meeting inclusion criteria: (a) SRs of randomized controlled trials (RCTs) or other research designs; (b) the participants were diagnosed as RA by common criteria, or it was clearly stated that the population of the SR was RA patients; (c) the comparisons were any type of TWHs extract with or without standardization treatment used as the treatment for RA versus standardization treatments, such as drug therapy, routine activities, no therapy, placebo, and other treatment; (d) outcomes including clinical, physiological, or caregiver-reported outcomes; patient-reported outcomes; and adverse effects. Only SRs published in English and Chinese were included. SRs with unavailable full text were excluded.

2.4. Data Management and Data Collection. We used the literature manager NoteExpress (V3.5.0.9054) to perform literature selection. We firstly screened title and abstract to eliminate duplication for potentially relevant SRs. Full texts of possible eligible SRs were downloaded and assessed based on inclusion and exclusion criteria. We applied a predesigned form to extract related information from each eligible SR: general information (e.g., the publication year, title, first

author, country, and language); review characteristics (e.g., literature database, number of included studies and participants, diagnosis criteria, interventions and comparisons, meta-analysis, quality assessment tool, and outcomes); and the main conclusion.

2.5. Assessment of Methodological Quality. We used the tool Assessing the Methodological Quality of Systematic Reviews 2 (AMSTAR-2) [34] to estimate the methodological quality for all included SRs, which provides guidance to rate the overall confidence in the results of a review. The AMSTAR-2 includes four critical domains, which are preparation for review, search for and selection of primary studies, data coding and reporting, and data synthesis. It contains 16 items, of which seven were critical domains (items: 2, 4, 7, 9, 11, 13, and 15) that can critically influence the validity of an SR and its conclusion. For each item, three options could be chosen to answer the question: "yes" indicating high quality, "partial yes" being partially compliant, or "no" being poor quality. The overall rating depends on weaknesses in the critical domains (items: 2, 4, 7, 9, 11, 13, and 15). The rating is divided into four categories depending on the number of critical flaws and/or noncritical weaknesses: "high" means no or one noncritical weakness; "moderate" means more than one noncritical weakness but no critical flaws; "low" means one critical flaw with or without noncritical weaknesses; and "critically low" means more than one critical flaw with or without noncritical weaknesses.

2.6. Assessment of Risk of Bias. We also evaluated the risk of bias of each included SR/MA using ROBIS statement [35], which assesses whether an SR is at risk of bias based on its methods and conduct. ROBIS is comprised of three phases: (a) assess relevance (optional), (b) identify concerns with the SR process, and (c) judge risk of bias of the SR. Phase one is optional, which assesses the relevance. Phase two includes four domains formed by 21 signaling questions, which aims to identify concerns with the review process. Phase three, with three signaling questions, concentrates to judge the risk of bias of the SR. All signaling questions were answered as "yes," "probably yes," "probably no," "no," and "no information." Based on the answers to the signaling questions in each domain, each domain is assigned a risk of bias grade. If all of signaling questions of phase 3 were answered as "yes," the SR was judged as "low risk." Any of signaling question of phase 3 was answered as "probably no" or "no," the SR was assessed as "high risk." If the information provided was insufficient to judge, the SR was rated as "unclear risk." After completing phase three, a summary judgment (e.g., high, low, or unclear) regarding the risk of bias for the SR will be rendered.

2.7. Assessment of Reporting Quality. We applied the checklist Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [36] to appraise the report quality for each SR/MA. PRISMA consists of seven main domains: title, abstract, introduction, methods, results, discussion, and funding. It comprises 27 items and a four-phase flow diagram, which focus on the reporting of methods and results in SRs/MAs. Each item was answered as "yes," "no," and "partially reported." With the purposes of statistical analysis, we judged whether an SR fully reported what was required by PRISMA and scored each item with a 1 point (fully reported), 0.5 point (partially reported), or 0 point (not reported) for each item. The sum of all items scored for each question was divided by its maximum possible score as a percentage to assess the report quality for each SR. The report quality of SRs related to its PRISMA score percentage was rated as very poor (<30%), poor (30–50%), fair (50–70%), good (70–90%), and excellent (>90%).

2.8. Assessment of Quality of Evidence. The Grades of Recommendations, Assessment, Development, and Evaluation (GRADE) [37] approach was used to assess and report the certainty of evidence for the clinically important outcome of interest in the current overview. In the GRADE system, five factors for rating down the quality of evidence were considered for the current overview: risk of bias (also called "study limitations"), inconsistencies, indirectness, inaccuracy, and publication bias. Quality of evidence of each outcome was judged as "high," "moderate," "low," and "very low."

2.9. Data Synthesis and Presentation. We narratively described the characteristics of included SRs and the efficacy and safety of TwHF preparations for RA in this overview. We made use of tabulation and figures to summarize the results of all SRs/MAs as well as the appraisal results from AMSTAR-2, PRISMA, and ROBIS. We generated the evidence profile and summary of findings table with the aid of the GRADEpro GDT online software (https://www.gradeworkinggroup.org/).

3. Results

3.1. Results on SRs/MAs Search and Selection. The initial search strategy yielded 280 records from the selected databases. After removal of 42 duplicates, 238 records were screened based on title and abstract. Afterward, fifty-six articles were read in full text, of which 27 SRs [20, 21, 38–62] were included in the current overview. The excluded review list has been recorded in Appendix A. The PRISMA diagram for the process of screening and selecting SRs is displayed in Figure 1.

3.2. Characteristics of Included Reviews. Of the 27 included SRs, 19 [38, 40–44, 46, 47, 50–54, 57–62] were published in Chinese and 8 [20, 21, 39, 45, 48, 49, 55, 56] in English. They were published from 2013 to 2021, including 26 SRs from China and 1 from the United Kingdom. All 27 SRs included RCTs, of which 26 conducted meta-analysis, while only one SR [39] did qualitative analysis; of which 25 SRs evaluated efficacy and safety of TwHF preparations, while the remaining 2 [58, 60] only explored the safety profile of TwHF

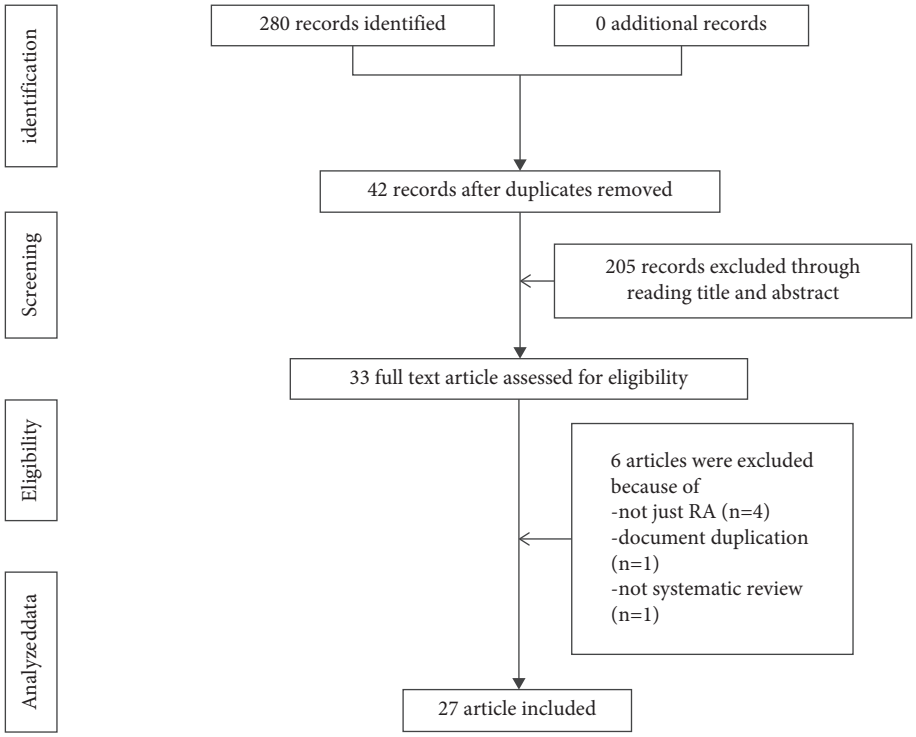

Figure 1: Flow chart showing the selection of SRs from search to inclusion.

preparations; and of which 24 SRs only included RCTs, while the remaining 3 included mixed studies. The number of studies included in each SR varied from 2 to 79, and sample sizes of individual study ranged from 105 to 5255. Among the 27 SRs, 20 SRs [20, 21, 40–47, 49, 51, 53–57, 59, 61, 62] specified the diagnostic criteria of the included studies, while the remaining seven [38, 39, 48, 50, 52, 58, 60] were unclear. As for intervention, 13 SRs [20, 38–46, 54, 60, 62] were TwHF preparations plus other treatment (e.g., routine drug therapy or placebo) versus other treatment alone, and 14 SRs [21, 47–53, 55–59, 61] were TwHF preparations versus other treatments (e.g., routine drug therapy). The outcomes reported by the 27 SRs covered tender joint count (TJC), swollen joint count (SJC), morning stiffness (MS), grip strength (GS), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), American College of Rheumatology (ACR), adverse events (AEs), interleukin 1 (IL-1), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor-alpha (TNF-α), 15-m walking time (15Mwt), 15/20-m walking time (15/20Mwt), tenderness score, physician-rated and patient-rated overall assessments, X-ray score, radiological changes of joints, withdrawal rate related to adverse reactions, joint symptoms, disease activity score, cyclic citrullinated peptide (CCP), mean grip strength, analgesic onset time (AOT), short form 36 health questionnaire (SF-36), health assessment questionnaire, traditional Chinese medicine symptom score of the joint swelling, and painful joint count. Among the 25 SRs that aimed to evaluate both efficacy and safety of TwHF preparations, only 12 SRs [20, 40–45, 49, 50, 53, 55, 56] reported AEs. The quality assessment tools of the original studies varied among the 27 SRs, out of which 17 employed Cochrane risk of bias tool, 9 adopted the Jadad score, and the remaining 1 used an unknown tool. Out of the 27 SRs, 26 SRs [20, 21, 38, 40–62] completed subgroup analysis, and 6 [47, 51, 54, 55, 57, 61] conducted sensitivity analysis. Of the 27 SRs, 12 SRs [41, 44, 46–48, 50, 52, 54, 55, 57, 59, 61] concluded TwHFPs were probably beneficial, 11 SRs [20, 40, 42, 43, 45, 48, 49, 51, 53, 56, 62] were beneficial, 3 [38, 58, 60] were no effect and 1 [39]) was harmful. The detailed characteristics the SRs are presented in Table 1.

3.3. Results on Review Quality Assessment

3.3.1. Methodological Quality. Table 2 presents the results of methodological quality of the 27 included SRs/MAs assessed by the AMSTAR-2. Out of the 27 included SRs, the quality of 20 SRs was rated critically low since they had more than one critical weakness (items 2, 4, 7, 9, 11, 13, and 15). Severe limitation existed in item 2, item 3, item 7, item 10, and item 16 (percentage of items with "yes" < 50%). The methodological quality appraised by the AMSTAR-2 for the 27 SRs can be reflected as follows: 92.6% of the 27 SRs did not explicitly report the review methods, which should be established before conducting the review and significant deviations from the protocol was found (item 2); 91.49% did not provide a list of excluded studies and justified the exclusions (item 7); 96.3% did not explain the selection of the study designs for inclusion in the review (item 3); 81.49% did not use a comprehensive literature search strategy (item 4), 66.67% did not report any potential sources of conflicts of

Table 1: Characteristics of 27 included systematic reviews.

Author, year	Design and number of included studies	Participants (*n*)	Literature databases	Population diagnostic criteria	Intervention/comparison	Methodological quality assessment tool	Meta analysis (yes/no)	Outcomes	Conclusion
Liu, 2013 [20]	RCT: 10	733	(1)(2)(3)(4)(5)(6)(7)(9)	ACR 1987	TwHFPs vs NM	JS	Yes	TJC; SJC; MS; GS. RF; ESR; CRP; AEs	Beneficial
Wang, 2017 [21]	RCT: 6	643	(6)(7)(8)	ACR	TwHFPs + MTX vs NM	JS	Yes	ACR (20/50); SJC; TJC; MS; ESR; CRP; RF	Probably beneficial
Xu, 2001 [38]	RCT: 3; CCT: 4	784	(4)	NM	TwHFPs vs CWM + COP	CROB	Yes	CTE	No effect
Canter, 2006 [39]	RCT: 2	105	(9)(10)(11)(12)	NM	TwHFPs vs CWM + COP	JS	No	TS; SJC; MS; GS; 15Mwt; ESR; CRP; IgG; IgM; IgA, PPOA	Harmful
Jiang, 2009a [40]	RCT: 8	470	(1)(2)(4)(5)(7)(9)(10)(12)	ACR 1987	TwHFPs vs NT + CWM + COP	CROB	Yes	ACR (20); RF; AEs	Beneficial
Jiang, 2009b [41]	RCT: 7	393	(1)(2)(4)(5)(7)(9)(10)(12)	ACR 1987	TwHFPs vs CWM + COP	CROB	Yes	CTE; X-RS; SJC; ESR; CRP; RF; AEs	Probably beneficial
Tang, 2010 [42]	RCT: 11	2327	(1)(2)(3)(4)	ACR 1987	TwHFPs vs NT + CWM + COP	NA	Yes	ESR; CRP; TJC; SJC; MS; MS; RF; CTE; AEs	Beneficial
Wang, 2011 [43]	RCT: 10	632	(1)(4)(5)(14)	ACR	TwHFPs vs NT + CWM + COP	JS	Yes	ACR (20/50/70) PAOS; VAS; HAQ; CRP; TJC; SJC; ESR; CRP; MS; GS; RCJ; AEs	Beneficial
Wang, 2014 [44]	RCT: 15	1031	(1)(4)(5)(6)(10)(14)	ACR 1987	TwHFPs vs CWM + COP	JS	Yes	TJC; SJC; MS; GS,ESR; CRP; RF; ACR (20/50/70); AEs	Probably beneficial
Wang, 2016 [45]	RCT: 22	5255	(1)(2)(3)(4)(5)(6)(9)	ACR 1987	TwHFPs vs CWM + COP	CROB	Yes	ACR (20/50/70), PPOA; AEs	Beneficial
Yang, 2016 [46]	RCT: 10	889	(1)(3)(4)(5)(6)(9)(10)	ACR 1987	TwHFPs vs CWM + COP	CROB	Yes	TJC; SJC; MS,GS; RF; ESR; CRP	Probably beneficial
Zeng, 2017 [47]	RCT: 6	362	(1)(2)(3)(6)(9)	ACR 1987; ACR/EULAR 2009	TwHFPs + MTX vs NT + CWM + COP	JS	Yes	WDAR; TWR ACR (20/50/70); CRP;CTE; RF; DAS28; SJC; TJC; MS; PPOA; VAS; HAQ; ESR; CRP; EPOTNF-a; IL-10; DAS28	Probably beneficial

Table 1: Continued.

Author, year	Design and number of included studies	Participants (*n*)	Literature databases	Population diagnostic criteria	Intervention/comparison	Methodological quality assessment tool	Meta analysis (yes/no)	Outcomes	Conclusion
Wang, 2018 [48]	RCT: 11	1055	(1)(2)(3)(6)(7)(9)(15)(16)	NM	TwHFPs vs CWM + COP	CROB	Yes	ACR (20/50/70); DAS; ESR; RF; CRP; CCP; hsCRP; TJC; SJC; MGS; 15/20Mwt; AOT; SOT; SF-36; HAQ	Beneficial
Zhou, 2018 [49]	RCT: 14	1254	(1)(3)(4)(5)(6)(9)(10)	ACR 1987	TwHFPs or TwHFPs + DMARDs vs NM	CROB	Yes	TJC; SJC; GS; MS; ESR; CRP; AEs	Beneficial
He, 2018 [50]	RCT: 4	230	(1)(2)(3)(5)(6)(8)	NA	TwHFPs + LEF vs CWM + COP	CROB	Yes	JS; ESR; CRP; CTE; MS; TJC; SJC; AEs	Probably beneficial
Wang, 2019 [51]	RCT: 18	1764	(1)(2)(3)(5)(6)(9)	ACR/EULAR 2010; ACR 1987	TGT + MTX vs MTX	CROB	Yes	TJC; SJC; MS	Beneficial
Li, 2019 [52]	RCT: 25	2507	(1)(2)(3)(5)(6)(9)	NM	TwHFPs or TwHFPs + MTX vs MTX	CROB	Yes	ESP; CRP; RF	Probably beneficial
Yin, 2019 [53]	RCT: 10	792	(1)(2)(3)(4)(6)(9)	ACR/EULAR 2009	TwHFPs + MTX vs MTX	CROB	Yes	CTE; MS; TJC; SJC; TCMSJS; ESP; CRP; RF; AEs	Beneficial
Zhu, 2019 [54]	RCT: 3	233	(1)(2)(3)(4)(5)(6)(7)(8)(9)	ACR1987; ACR/EULAR 2010	TwHFPs vs MTX + SASP	JS	Yes	Mtss; JE; JSN	Probably beneficial
Wen, 2020 [55]	RCT: 40	3092	(1)(2)(3)(4)(5)(6)(8)(9)	ACR 1987; ACR/EULAR 2010	TwHFPs + DMARDs vs DMARDs	CROB	Yes	MS; TJC; SJC; VAS; CRP; ESR; RF; CTE; AEs	Probably beneficial
Yang, 2020 [56]	RCT: 12	830	(1)(2)(3)(6)(9)(10)	ACR/EULAR 2010	TwHFPs + LEF vs LEF + CWM + COP	CROB	Yes	CTE; MS; TJC; SJC; ESR; CRP; RF; IL-1; IL-6; TNF-α; AEs	Beneficial

Table 1: Continued.

Author, year	Design and number of included studies	Participants (n)	Literature databases	Population diagnostic criteria	Intervention/comparison	Methodological quality assessment tool	Meta analysis (yes/no)	Outcomes	Conclusion
Chen, 2020 [57]	RCT: 10	1184	(1)(2)(3)(4)(6)(9)	ACR 1987; ACR/EULAR 2010	TwHFPs or TwHFPs + MTX vs MTX	CROB	Yes	ACR (20/50/70)	Probably beneficial
Li, 2020 [58]	RCT: 54; CCT: 11; case series: 7; case report: 7	3358	(1)(2)(3)(4)(5)(6)(9)	NM	TwHFPs or TwHFPs + CWM + COP vs NM	CROB; IHE; MIORS; JBI; standard for case report	Yes	AEs	No effect
Wang, 2020 [59]	RCT: 10	876	(1)(2)(3)(4)(5)(6)(7)(8)(9)	ACR 1987; ACR/EULAR 2010	TwHFPs + MTX vs CWM + COP	CROB	Yes	IL-17; IL-23; TNF-α; IL-1; IL-6; IL-4; IL-10	Probably beneficial
Gao, 2020 [60]	RCT: 22	2085	(1)(2)(3)(4)(5)(6)(9)(8)	NM	TwHFPs vs CWM + COP	JS	Yes	AEs	No effect
Ying, 2021 [61]	RCT: 13	1004	(1)(2)(3)(4)(6)(8)(9)	ACR 1987; ACR/EULAR 2009	TwHFPs + LEF vs LEF	CROB	Yes	CTE; MS; TJC; SJCAEs; CRP; ESR; RF; IgA; IgG; IgM; IL-1; IL-6; IL-10; TNF-α; sICAM-1	Probably beneficial
Wang, 2021 [62]	Mixed RCT and CCT: 10	696	(1)(2)(3)(6)(8)	ACR 1987; ACR/EULAR 2009	TwHFPs vs LEF	JS	Yes	TJC; SJC; PJC; ESR; CRP; CTE	Beneficial

(1) CNKI, (2) VIP, (3) WanFang, (4) CBM-disk, (5) EMBASE, (6) PubMed, (7) CCTR, (8) WOS, (9) CL, (10) MEDLINE, (11) AMED, (12) CINAH, (13) CMFD, (14) CENTRAL, (15) ScienceDirect, (16) FMRS, (17) Elsevier; RCT: randomized controlled trial, CCT: controlled clinical trial, CTE: clinical treatment efficacy, AEs: adverse events, vs: versus, LEF: leflunomide, MTX: methotrexate, SASP: sulfasalazine, 3M: 3 months, 6M: 6 months, 1M: 1 month, MS: morning stiffness, SJC: swollen joint count, TJC: tender joint count, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, RF: rheumatoid factor, IL-1: interleukin 1, IL-4: interleukin 4, IL-6: interleukin 6, IL-10: interleukin 10, TNF-α: tumor necrosis factor-alpha, NM: not mentioned, GS: grip strength, 15 Mwt: 15 m walking time, 15/20 Mwt: 15/20 m walking time, TS: tenderness score, PPOA: physician-rated and patient-rated overall assessments, X-RS: X-ray score, RCJ: radiological changes of joints, WDAR: withdrawal rate related to adverse reactions, JS: joint symptoms, DAS: disease activity score, CCP: cyclic citrullinated peptide, MGS: mean grip strength, AOT: analgesic onset time, SF-36: short form 36 health questionnaire, HAQ: health assessment questionnaire, TCMSJS: TCM symptom score of the number of joint swelling, PJC: painful joint count, DMARDs: disease-modifying antirheumatic drugs, TwHFPs: Tripterygium wilfordii Hook. f. preparations, NT: no therapy, CM: conventional medicine, COP: Chinese patent medicine or placebo, CROB: Cochrane Risk of Bias tool, JS: Jadad Scale, CL: Cochrane Library, Mtss: Van der Heijde modified. Note. The conclusions reported by the included SRs were classified into five categories by referring to another evidence mapping study [63]. Inconclusive: reported the results differed across or within reviews due to conflicting results or limitations of individual studies. No effect: reported that there is no difference between intervention and comparator. Harmful: reported clearly a harmful effect. Probably beneficial: did not report firm benefits despite the reported positive treatment effect. Beneficial: reported a clear beneficial effect without major concerns regarding the supporting evidence.

Table 2: The results of AMSTAR-2.

Study ID	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12	Item 13	Item 14	Item 15	Item 16	Ranking of quality
Xu 2001 [38]	Y	N	N	N	N	N	N	PY	Y	N	N	N	Y	N	N	N	−−
Canter 2006 [39]	Y	N	N	Y	N	Y	Y	Y	Y	N	N	N	Y	Y	N	N	−
Jiang 2009a [40]	Y	N	N	PY	Y	Y	Y	PY	Y	N	Y	Y	Y	Y	N	N	−
Jiang 2009b [41]	Y	N	N	PY	Y	Y	Y	PY	Y	N	Y	Y	Y	Y	N	N	−
Tang 2010 [42]	Y	N	N	PY	Y	Y	N	N	N	N	Y	N	N	N	N	N	−−
Wang 2011 [43]	Y	N	N	PY	Y	Y	Y	Y	Y	N	Y	N	N	N	N	N	−
Liu 2013 [20]	Y	N	Y	PY	Y	Y	N	PY	Y	N	Y	Y	Y	Y	Y	N	−−
Wang 2014 [44]	Y	N	N	PY	Y	Y	N	Y	Y	Y	N	N	Y	N	N	Y	−−
Yang 2016 [46]	Y	N	N	N	Y	Y	Y	PY	Y	N	Y	Y	N	N	Y	Y	−
Wang 2016 [45]	Y	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	−
Zeng 2017 [47]	Y	N	N	PY	Y	Y	N	PY	Y	N	Y	N	N	N	N	N	−−
Wang 2017 [21]	Y	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	−
He 2018 [50]	Y	N	N	PY	Y	Y	N	PY	Y	Y	N	N	N	N	N	N	−−
Wang 2018 [48]	Y	N	N	PY	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	Y	−−
Zhou 2018 [49]	Y	N	N	PY	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	N	−−
Wang 2019 [51]	Y	N	N	PY	Y	Y	N	PY	Y	Y	Y	N	N	Y	Y	N	−−
Li 2019 [52]	Y	N	N	PY	Y	Y	N	PY	Y	Y	Y	Y	Y	Y	Y	N	−−
Ying 2019 [53]	Y	N	N	PY	Y	Y	N	PY	Y	Y	N	N	N	N	Y	Y	−−
Zhu 2019 [54]	Y	N	N	PY	Y	Y	N	PY	Y	Y	N	N	N	N	N	N	−−
Chen 2020 [57]	Y	N	N	PY	Y	Y	N	PY	PY	Y	N	N	N	N	Y	N	−−
Li 2020 [58]	Y	N	N	Y	Y	Y	N	PY	PY	Y	Y	Y	Y	Y	Y	N	−−

Table 2: Continued.

Study ID	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11	Item 12	Item 13	Item 14	Item 15	Item 16	Ranking of quality
Wang 2020 [59]	Y	N	N	PY	Y	Y	N	PY	Y	Y	Y	Y	Y	Y	Y	N	−−
Gao 2020 [60]	Y	N	N	PY	Y	Y	N	PY	Y	Y	Y	N	N	N	N	N	−−
Wen 2020 [55]	Y	N	N	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	−−
Yang 2020 [56]	Y	N	N	PY	Y	Y	N	Y	PY	N	Y	Y	N	Y	Y	Y	−−
Ying 2021 [61]	Y	N	N	PY	Y	Y	N	Y	PY	Y	Y	Y	Y	Y	Y	N	−−
Wang 2021 [62]	Y	N	N	PY	Y	Y	N	PY	PY	Y	N	N	Y	Y	Y	Y	−−

Note. Y: yes; PY: partial yes; N: no; ++: high; +: moderate, −: low; −−: critically low.

interest (item 16); and 66.67% described the included studies insufficiently (item 8).

3.3.2. Risk of Bias. The ROBIS was used to assess the risk of bias for each SR, the results of which are presented in Appendix B. All 27 SRs were judged with low risk of bias in phase 1 (assessing relevance). Regarding phase 2, across all 27 SRs, the individual bias domains at the highest risk of bias were domains 1 (protocol and eligibility criteria, 26/27, 96.30%) and 2 (methods to identify and select studies, 18/27, 66.67%). Specific areas of concern in these two domains were the lack of information about publication of an SR protocol, language restrictions, choice of literature databases, and searches for gray literature. Eleven (40.74%) SRs were at high risk of bias for both domain 3 (collection and study appraisal) and domain 4 (synthesis and findings). Seven (25.92%) SRs were rated as low risk of bias in phase 3(risk of bias in the review). Finally, 20 of the 27 SRs were rated as "high risk," and the remaining 7 SRs were rated as "low risk." In general, 20 of the 27 SRs were rated as "high risk," and the remaining 7 SRs were rated as "low risk." Reviews with high risk of bias mainly have problems with the completeness of the search for relevant studies, inadequate report of the protocol, and lack of explicit method to select studies.

3.3.3. Reporting Quality. The results of PRISMA assessment are presented in Appendix B. Of the 27 items, 12 items had adherence greater than 70% in most of the included SRs; however, five items had only one SR, and four items had no adherence. The section of rationale, objectives, eligibility criteria, title, introduction, study characteristics, and results of individual studies were all well reported by all included SRs, but there were still inadequate reports in other sections. Five items with adherence lower than 5% were the main reporting deficiency, which are if a protocol exists or is registered (item 5, percentage of items with "yes," 3.7%); certainty assessment (item 15, percentage of items with "yes," 3.7%); search strategy (item 7, yes = 3.7%); structured summary (item 2, yes = 3.7%); and certainty assessment (item 22, yes = 0%). Additionally, only one SR [45] mentioned the study protocol and the protocol registration number. Finally, the reporting quality of 16 SRs was rated as "fair," and 11 "good."

3.3.4. Evidence Quality of Outcomes. The information about the efficacy and safety of TwHF preparations for RA from included SRs is summarized and displayed in Table 3. Ten of the 27 SRs that selected rheumatoid factor as the primary outcome suggested that patients with RA who received TwHF preparations had better effects than their counterparts who were treated with DMARDs. Eighteen of the 27 SRs (66.66%) reported that both tender joint count and swollen joint count were significantly reduced in the TwHF preparations group. As for the ACR (20/50/70), 7 of the 27 SRs (25.92%) reported that ACR (20/50/70) was significantly improved in the TwHF preparations group. As for the levels of ESR and CRP, 18 of the 27 SRs (66.66%) reported that both of them were significantly reduced following the TwHF preparations treatment, while one SR reported there was no statistical significance for ESR. Among the 15 included SRs that reported morning stiffness (MS), 8 SRs reported that MS was significantly reduced in the TwHF preparations group. The combination therapy with TwHF preparations and other treatment significantly decreased the duration of morning stiffness; alleviated tender joint count; relieved swollen joint count, ACR (20/50/70), ESR, CRP, and RF; and lowered the level of TNF-α. The most common AEs with TwHF preparations were gastrointestinal discomfort, menstruation disorders, amenorrhea, decreased sperm motility, liver function damage, and skin diseases.

Table 3: The results of GRADE.

Outcomes	Study ID	Synthesis of results	Total patient number in the treatment or control group	No. of participants (studies)	Quality of the evidence (GRADE)
SJC	Liu 2013 [20]	MD −4.13, 95% CI (−5.69, −2.58), $I^2 = 0\%$, $P < 0.00001$	45/47	2	☐☐⊕⊕LOW[a,b]
	Yang 2020 [56]	MD −1.24, 95% CI (−1.59, −0.88), $I^2 = 97\%$, $P < 0.00001$	417/417	12	☐☐☐⊕MODERATE[a]
	Zhou Y 2018 [49]	MD −1.92, 95% CI (−3.85, 0.03), $I^2 = 0\%$, $P < 0.00001$	219/218	6	☐☐⊕⊕LOW[a,b]
	He 2018 [50]	MD 0, 95% CI (−0.19, 0.2), $I^2 = 41\%$, $P = 1.00$	30/30	3	☐☐⊕⊕LOW[a,b]
	Wang 2019 [51]	MD 3.01, 95% CI (2.09, 3.93), $I^2 = 88\%$, $P < 0.00001$	635/633	14	☐☐☐⊕MODERATE[a]
	Wang 2021 [62]	SMD −0.64, 95% CI (−1.32, 0.05), $I^2 = 93\%$, $P = 0.07$	287/287	8	☐☐☐⊕MODERATE[a]
	Yang 2016 [46]	MD −1.96, 95 % CI (−3.56, 0.35), $I^2 = 87\%$, $P = 0.14$	69/68	2	☐⊕⊕⊕VERY LOW[a,b]
	Yin 2019 [53]	SMD −1.46, 95% CI (−2.4, −0.44), $I^2 = 97\%$, $P = 0.005$	342/330	8	☐☐☐⊕MODERATE[a]
	Yin 2021 [61]	SMD −0.78, 95% CI (−1.52, −0.04) ,$I^2 = 95\%$, $P = 0.04$	362/362	10	☐☐☐⊕MODERATE[a]
	Wen 2020 [55]	SMD −1.72, 95% CI (−2.04, −1.41), $I^2 = 89\%$, $P = 0.0001$	1196/1188	30	☐☐⊕⊕LOW[a]
MS	Yang, 2020 [56]	MD −0.29, 95% CI (−0.42, −0.12) , $I^2 = 99\%$, $P = 0.0005$	296/296	8	☐☐☐⊕MODERATE[a]
	Zhou 2018 [49]	MD −30.94, 95% CI (−37.85, −24.04), $I^2 = 86\%$, $P = 0.21$	144/142	3	☐☐⊕⊕LOW[a]
	He 2018 [50]	MD −0.32, 95% CI (−0.4, −0.24), $I^2 = 38\%$, $P = 0.0001$	66/66	2	☐☐⊕⊕LOW[a]
	Wang 2019 [51]	MD −18.24, 95% CI (−12.64, 23.84), $I^2 = 36.9\%$, $P < 0.00001$	383/383	9	☐☐☐⊕MODERATE[a]
	Yin 2019 [53]	SMD −1.51, 95% CI (−2.31, −0.71), $I^2 = 94\%$, $P = 0.00002$	267/267	6	☐☐⊕⊕LOW[a]
	Yin 2021 [61]	SMD −2.29, 95% CI (−3.36, −1.12), $I^2 = 0\%$, $P < 0.00001$	100/100	3	☐☐⊕⊕LOW[a]

Table 3: Continued.

Outcomes	Study ID	Synthesis of results	Total patient number in the treatment or control group	No. of participants (studies)	Quality of the evidence (GRADE)
RF	Liu 2013 [20]	MD −32.4, 95% CI (−89.76, −24.96), $I^2 = 24\%$, $P = 2.7$	45/47	2	☐☐⊛⊛LOW[a]
	Wang 2018 [48]	MD −5.41, 95% CI (−7.46, −3.37), $I^2 = 13\%$, $P < 0.00001$	197/202	3	☐☐⊛⊛LOW[a]
	Yang 2020 [56]	MD −50.88, 95% CI (−72.3, 29.45), $I^2 = 99\%$, $P < 0.00001$	257/257	7	☐⊛⊛⊛VERY LOW[a,b,c]
	Jiang 2009b [41]	MD −0.5, 95% CI (−0.81, −0.18), $I^2 = 85.1\%$, $P = 0.002$	85/85	3	☐☐⊛⊛LOW[a,b]
	Li 2019 [52]	SMD 1.05, 95% CI (0.51, 1.6), $I^2 = 94\%$, $P = 0.00001$	521/521	12	☐☐☐⊛MODERATE[a]
	Wang 2011 [43]	MD 0.38, 95% CI (−0.42, 1.18), $I^2 = 64\%$, $P = 0.36$	55/55	2	☐☐⊛⊛LOW[a,b]
	Wang 2021 [62]	SMD −2.23, 95% CI (−3.27, −1.19), $I^2 = 95\%$, $P < 0.00001$	234/234	7	☐☐☐⊛MODERATE[a]
	Yin 2019 [53]	SMD −1.11, 95% CI (−1.96, −0.26), $I^2 = 94\%$, $P = 0.01$	215/215	5	☐☐⊛⊛LOW[a,b]
	Yin 2021 [61]	SMD −2.97, 95% CI (−4.22, −1.72), $I^2 = 97\%$, $P < 0.00001$	327/327	8	☐☐☐⊛MODERATE[a]
TJC	Yang 2020 [56]	SMD −50.88, 95% CI(−72.30, 29.45.48), $I^2 = 99\%$, $P < 0.00001$	894/893	23	☐⊛⊛⊛VERY LOW[a,b,c]
	Zhou 2018 [49]	MD −1.51, 95% CI (−2.2, −0.83), $I^2 = 0\%$, $P < 0.00001$	417/417	12	☐☐☐⊛MODERATE[a]
	Wang 2019 [51]	MD 2.15, 95% CI (−3.54, −−0.75), $I^2 = 78\%$, $P < 0.00001$	219/218	6	☐☐⊛⊛LOW[a,b]
	Wang 2021 [62]	SMD −0.92, 95% CI (−1.74, −0.09), $I^2 = 93\%$, $P = 0.03$	190/190	6	☐☐☐⊛MODERATE[a]
	Yang 2016 [46]	MD −2.73, 95% CI (−4.68, −0.78), $I^2 = 0\%$, $P = 0.06$	69/68	2	☐⊛⊛⊛VERY LOW[a,b,c]
	Yin 2019 [53]	SMD −1.28, 95% CI (−1.98, −0.57), $I^2 = 95\%$, $P = 0.0004$	382/370	9	☐☐☐⊛MODERATE[a]
	Yin 2021 [61]	SMD −0.92, 95% CI (−1.74, −0.09), $I^2 = 93\%$, $P = 0.003$	362/36	10	☐☐☐⊛MODERATE[a]
	Wen 2020 [55]	SMD −1.69, 95% CI (−2.01, −1.37), $I^2 = 89\%$, $P \leq 0.001$	1233/1225	31	☐☐☐⊛MODERATE[a]

Table 3: Continued.

Outcomes	Study ID	Synthesis of results	Total patient number in the treatment or control group	No. of participants (studies)	Quality of the evidence (GRADE)
Total effective rate	Wang 2018 [48]	RR 1.20, 95% CI (1.13, 1.27), $I^2 = 31\%$, $P < 0.00001$	950/516	7	□□□x̂MODERATE[a]
	Zeng 2017 [47]	OR 1.02, 95% CI (0.46, 2.28), $I^2 = 0\%$, $P = 0.95$	103/101	4	□□x̂x̂LOW[a,b]
	He 2018 [50]	RR 1.19, 95% CI (1.02, 1.38), $I^2 = 0\%$, $P = 0.02$	73/71	3	□□x̂x̂LOW[a,b]
	Wang 2021 [62]	OR 3.80, 95% CI (2.34, 6.16), $I^2 = 0\%$, $P < 0.00001$	253/253	7	□□□x̂MODERATE[a]
	Yin 2019 [53]	RR 1.23, 95% CI (1.13, 1.35), $I^2 = 20\%$, $P < 0.00001$	296/296	7	□□x̂x̂LOW[a,b]
	Yin 2021 [61]	OR 4.27, 95% CI (2.51, 7.27), $I^2 = 0\%$, $P < 0.00001$	3668/36	9	□□□x̂MODERATE[a]
	Wen 2020 [55]	RR1.23, 95% CI (1.133, 1.335), $I^2 = 0\%$, $P = 0.951$	452/452	12	□à□x̂MODERATE[a]

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI: confidence interval; RR: risk ratio; OR: odds ratio; MD: mean difference. GRADE Working Group grades of evidence—HIGH quality: further research is very unlikely to change our confidence in the estimate of effect; MODERATE quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; LOW quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; VERY LOW quality: we are very uncertain about the estimate. a: downgraded due to risk of bias; b: downgraded due to publication bias; c: downgraded due to inconsistency and imprecision.

(1) Swollen Joint Count. Nine MAs [42, 44, 47–49, 53, 54, 59, 60] reported the swollen joint count. Two interventions (TwHFPs with LEF and TwHFPs with MTX) reduced swollen joint count. The result of different comparisons were shown as follows: TwHFPs vs NM (MD: −4.13, 95% CI: −5.69, −2.58; low quality); TwHFPs + LEF vs LEF + CWM + COP (MD: −1.24, 95% CI: −1.59,−0.88; moderate quality); TwHFPs or TwHFPs + DMARDs vs NM (MD: −1.92, 95% CI: −3.85, 0.03; low quality); TwHFPs + LEF vs CWM + COP(MD: 0, 95% CI: −0.19, 0.2; low quality); TGT + MTX vs MTX(MD: 3.01, 95% CI: 2.09, 3.93; moderate quality); TwHFPs vs LEF (SMD: −0.64, 95% CI: −1.32, 0.05; moderate quality); TwHFPs vs CWM + COP (MD: −1.96, 95% CI: −3.56, 0.35; moderate quality); TwHFPs + MTX vs MTX (SMD: −1.46, 95% CI: −2.4, −0.44; moderate quality); TwHFPs + LEF vs LEF (SMD: −0.78, 95% CI: −1.52, −0.04; moderate quality); TwHFPs + DMARDs vs DMARDs (SMD: −1.72, 95% CI: −2.04, −1.41; low quality).

(2) Morning Stiffness. Six MAs [47–49, 51, 54, 59] reported the morning stiffness. Two interventions (TwHFPs with LEF and TwHFPs with MTX) reduced morning stiffness. The result of different comparisons were shown as follows: TwHFPs + LEF vs LEF + CWM + COP (MD: −0.29, 95% CI: −0.42,−0.12; moderate quality); TwHFPs or TwHFPs + DMARDs vs NM (MD: −30.94, 95% CI: −37.85, −24.04; low quality); CWM + COP (MD: −0.32, 95% CI: −0.4, −0.24; low quality); TGT + MTX vs MTX (MD: −18.24, 95% CI: −12.64, 23.84; moderate quality); TwHFPs + MTX vs MTX (SMD: −1.51, 95% CI: −2.31, −0.71; low quality); TwHFPs + LEF vs LEF (SMD: −2.29, 95% CI: −3.36, −1.12; moderate quality).

(3) Rheumatoid Factor. Nine MAs [21, 39, 41, 42, 50, 51, 54, 59, 60] reported the rheumatoid factor. Two interventions (TwHFPs and TwHFPs with LEF) reduced the rheumatoid factor. The result of different comparisons were shown as follows: TwHFPs + LEF vs LEF + CWM + COP (MD: −0.29, 95% CI: −0.42, −0.12; moderate quality); TwHFPs or TwHFPs DMARDs vs NM (MD: −30.94, 95% CI: −37.85, −24.04; low quality); CWM + COP (MD: −0.32, 95% CI: −0.4, −0.24; low quality); TGT + MTX vs MTX (MD: −18.24, 95% CI: −12.64, 23.84; moderate quality); TwHFPs + MTX vs MTX (SMD: −1.51, 95% CI: −2.31, −0.71; low quality); TwHFPs + LEF vs LEF (SMD: −2.29, 95% CI: −3.36, −1.12; moderate quality).

(4) Tender Joint Count. Eight MAs [44, 47, 49, 51, 53, 54, 59, 60] reported the tender joint count. Three interventions (TwHFPs, TwHFPs with DMARDs, and TwHFPs with LEF) reduced TJC. The result of different comparisons were shown as follow: TwHFPs vs NM (MD: −32.4, 95% CI: −89.76, −24.96; low quality); TwHFPs vs CWM + COP (MD: −5.41, 95% CI: −7.46, −3.37; low quality); TwHFPs + LEF vs LEF + CWM + COP (MD: −50.88, 95% CI: −72.3, 29.45; very low quality); TwHFPs vs CWM + COP (MD: −0.5, 95% CI: −0.81, −0.18; low quality); TwHFPs or TwHFPs + MTX vs MTX (MD: −0.5, 95% CI: −0.81, −0.18; moderate quality); TwHFPs vs NT + CWM + COP (MD: 0.38, 95% CI: −0.42, 1.18; low quality); TwHFPs vs LEF (SMD: −2.23, 95% CI: −3.27, −1.19; moderate quality); TwHFPs + MTX vs MTX (SMD: −1.11, 95% CI: −1.96, −0.26; low quality); TwHFPs + LEF vs LEF (SMD: −2.97, 95% CI: −4.22, −1.72; moderate quality).

(5) Total Effective Rate. Eight MAs [21, 46, 48, 49, 51, 53, 59, 60] reported the total effective rate. Three interventions (TwHFPs, TwHFPs with DMARDs, and TwHFPs with LEF) increased the total effective rate. The result of different comparisons were shown as follows: TwHFPs vs CWM + COP (RR: 1.20, 95% CI: 1.13, 1.27; moderate quality); TwHFPs + MTX vs NT + CWM + COP (OR: 1.02, 95% CI: 0.46, 2.28; low quality); TwHFPs + LEF vs CWM + COP (RR: 1.19, 95% CI: 1.02, 1.38; low quality); TwHFPs vs LEF (OR: 3.80, 95% CI: 2.34, 6.16; moderate quality); TwHFPs + MTX vs MTX (RR: 1.23, 95% CI: 1.13, 1.35; low quality); TwHFPs + MTX vs MTX (RR: 1.23, 95% CI: 1.13, 1.35; low quality); TwHFPs + DMARDs vs DMARDs (RR: 1.23, 95% CI: 1.133, 1.335; moderate quality).

3.3.5. Overall Quality of the Evidence. The details of GRADE summary of findings are described in Table 3. We only rated the body of evidence for main outcomes that were pooled based on RCTs using the GRADE system. Nineteen SRs involving 5 main outcomes related to the effects of TwHF preparations for RA were analyzed. Based on the analysis of the GRADE approach, moderate quality of evidence was found in 5 outcomes of the included SRs, whereas 5 outcomes were rated as low quality, and 2 outcomes were very low quality. There was no outcome with high-quality evidence found in the current overview. Risk of bias ($n = 13$) was the most common downgrading factors, followed by inconsistency ($n = 2$), imprecision ($n = 6$), publication bias ($n = 5$), and indirectness ($n = 9$). The reasons to downgrade the level of evidence are the poor methodological quality, imprecision of the results, and small sample size among relevant trials. The downgraded reason the small number of participants was for the majority outcomes. The number of participants included in the SR did not reach the optimal information size. Then, the quality of evidence was downgraded due to its imprecision. The effect estimates could not provide a convincing explanation for differences in results across studies for nearly half of the outcomes, owing to the statistically significant heterogeneity. Some of the outcomes had publication bias because of the incomprehensive literature search, which was already found by AMSTAR-2 and ROBIS.

4. Discussion

Overviews are most frequently employed where multiple systematic reviews already exist on similar or related topics and aim to systematically bring together, appraise, and synthesize the results of related systematic reviews [67]. Although there are an increasing number of SRs/MAs published on TwHF preparations for RA, the quality of those

SRs/MAs taken together has not been assessed until now. Thus, there is a need to systematically bring together, appraise, and synthesize the results of related systematic reviews in an overview of this issue.

4.1. Summary of Main Findings. Tripterygium wilfordii Hook f. (TwHF, also known as Thunder God Vine or Lei Gong Teng) is one of the most representative traditional Chinese herbs with therapeutic potential that has been broadly studied by scientists. In spite of some occasional, but severe, adverse effects (which may be harmful to the liver, kidneys, reproductive tissues, and immune tissues [64]) found in clinical practice, the use of TwHF preparations is still not reduced due to their significant efficacy against diseases. In the current overview, 27 included SRs on TwHF preparations were published from 2013 to 2021. Out of the included 27 SRs, 26 of which drew positive conclusions of TwHF preparations for RA; however, none of the review authors drew a firm conclusion owing to the small sample size of the included RCTs or their low methodological quality. Though it showed that adverse events caused by TwHF preparations were not significantly different from those caused by immunosuppressive agents, there is an urgent need for improving prevention and management of patients' tolerance and monitoring the administration of TwHF preparations in the clinical practice [65]. And TwHF preparations should not be used for RA patients with liver and kidney insufficiency and fertility planning, in view of the liver and kidney and reproductive toxicity. We reclassified and examined the 385 primary studies included in the 27 included SRs. We calculated the percentage of primary studies included in more than one SR and the rate of CCA (corrected covered area), which is a measure about the degree of overlap [68]. The rate of overlaps was 68.31% (263/385) and the CCA was 0.53, which indicated the degree of overlap is slight. There are two possible reasons for the overlap, one is SRs in TCM research area often having a broader research question, for instance, the majority of SRs investigating TwHFPs versus conventional medicine on different outcomes, leading to more primary studies included in an SR; the other is some authors of SRs reported that the quality of the published SRs was poor and there was necessity to perform a new one rather than an updated one. The quality of the SRs and the evidence quality of the outcomes in this overview are generally discouraging, on the basis of the evaluation from AMSTAR-2, ROBIS, PRISMA, and GRADE, implying that there is huge disparity between the included SRs/MAs and the real world. Thus, in view of these limitations, the trustworthy of evidence for TwHF preparations for RA was weakened. Consequently, recommending TwHF preparations as a complementary or even alternative treatment for patients with RA should be cautious.

The current overview found four main findings. First of all, the methodological quality of all the SRs was rated as critically low or low by the AMSTAR-2 tool, and the following deficiencies existed: 1) selective reporting bias arose due to the lack of SR protocol or the absent registration of the protocol of the included SRs, which affected their thoroughness; 2) the confidence of results was influenced by the decreased transparency, due to the omission of the lists of excluded studies with explanations; and 3) the reliability of the conclusions and its impact on different users of reviews were affected by missing disclosure of potential financial conflicts of interest or the authors' conflicts of interest. Secondly, high risk of bias evaluated by the ROBIS tool was found in the literature search, study selection, data synthesis method, and the explanation in the discussion among these included SRs, which made the current evidence unreliable. Thirdly, the assessment on included SRs' adherence to the PRISMA statement found that incomplete reporting occurred in the literature search strategies, the literature screening processes, the additional analyses, and the sources of funding, which decreased the trustworthiness of the findings. When information is absent or ambiguous in the reporting, SR users cannot implement the findings of SRs into clinical practice. Lastly, the results from the GRADE assessment in this overview revealed that moderate-quality evidence on some outcomes for TwHF preparations having potential effects for patients with RA. Low-quality evidence affected the confidence in the evidence, which made the uncertainty about the trade-offs when recommending the TwHF preparations as an intervention for RA. In regard to the safety of TwHF preparations for RA, 11 SRs reported that the combined therapy increased clinical efficacy significantly when compared with the Western medicine alone, whereas four SRs found no difference between the two groups. In the current overview, there is no high-quality evidence, and most of the outcomes were rated as low or very low quality. Evidence quality was downgraded due to the study limitations, inconsistency, and the publication biases. The publication bias in most of the included SRs was mostly caused by the small number of included RCTs with small sample size as well as positive results, which may lead to overestimating the effect size.

More than that, most of the original studies of TwHF preparations in treating RA have major limitations, including lack of allocation concealment; subjective outcomes without blinding; loss to follow-up; and no intention to treat analysis, which biased the estimates of the treatment effect and affected the confidence in the estimate of effect in SRs. Study heterogeneity prevented meaningful meta-analysis due to the various evaluation criteria for the assessment of clinical effectiveness and different treatment courses across studies. Only one SR [61] conducted subgroup analysis based on the different treatment courses.

4.2. Implications for Future Clinical Practice and Research. According to our results, TwHF preparations may be effective for RA patients, which is consistent with a related previous overview [68]. However, the administration should be monitored due to its adverse effects. TwHF preparations are likely to improve the physical function and quality of life in patients with RA, not just laboratory outcomes. More than half of the included SRs (66%) showed the significant

decrease for swollen joint count and tender joint count in the TwHF preparations group, 48.14% for morning stiffness, and only 26% for ACR (20/50/70). But as aforementioned, we should consider the inadequacy of the available evidence and be cautious when recommending TwHF preparations as a treatment for RA patients.

As we all know, the quality of a systematic review depends on the quality of the original research. Therefore, well-designed primary studies should be carried out in the future. The composite outcome total effective rate was used as a primary outcome with a simple rate calculation formula in most studies, whereas relieving joint pain was the internationally considered outcomes. For the sake of producing accepted efficacy evidence of TwHF preparations in the treatment of RA, future studies should select well-recognized outcomes and related measurements that are recommended by expert consensus or by international guidelines [66]. When evaluating the effects of TwHF preparations, we should not only consider the laboratory outcomes and physician-reported outcomes but also take into account patient-reported outcomes (such as quality of life), which can comprehensively evaluate the efficacy of TwHF preparations in the treatment of RA. Additionally, none of the included SRs mentioned follow-up. Considering that RA is a progressive disease with a long disease course, future studies should attach importance to the follow-up period to further assess the long-term efficacy of TwHF preparations for treating RA as well as fully monitoring its toxicity.

Last but not least, we strongly recommend authors of future SRs conduct and report SRs adhering to the AMSTAR-2 tool, ROBIS tool, and PRISMA statement.

4.3. Strengths and Limitations. To the best of our knowledge, this is the first systematic overview to explore the evidence of TwHF preparations for RA by using the AMSTAR-2, ROBIS, PRISMA, and GRADE. From the current overview, the quality of the SRs/MAs and body of evidence across outcomes are presented, which may be helpful for the research and clinical practice of TwHF preparations in treating RA. However, there are several limitations in this overview that should be taken into account. We only searched SRs in English and Chinese, which might produce publication bias. Although there are overlapping studies across the included SRs, we did not remove duplicate data and duplicate studies. As we are not aimed to resynthesize the data to evaluate the efficacy of the intervention, the overlap is unlikely to have an impact on the conclusion. The author team members may have their own subjective views during the evaluation, which could result in bias and influenced the research findings. Finally, out of the 27 included SRs, 26 from Chinese researchers supported the use of TwHF preparations for RA, whereas one SR from the British researchers disapproved the use of TwHF preparations, which may be judged as certain ethical bias.

5. Conclusion

TwHF preparations may be a complementary and alternative treatment for RA; however, it must be used carefully and monitored for its potentially severe toxicity. The quality of published SRs/MAs is unsatisfactory; hence, further standardized and rigorous SRs/MAs and RCTs are warranted to provide strong evidence for definitive conclusions.

Abbreviations

CNKI:	China National Knowledge Infrastructure
VIP:	VIP database
RCT:	Randomized controlled trial
CCT:	Controlled clinical trial
CTE:	Clinical treatment efficacy
AEs:	Adverse events
VS:	Versus
LEF:	Leflunomide
MTX:	Methotrexate
SASP:	Sulfasalazine
3M:	3 months
6M:	6 months
1M:	1 month
MS:	Morning stiffness
SJC:	Swollen joint count
TJC:	Tender joint count
ESR:	Erythrocyte sedimentation rate
CRP:	C-reactive protein
RF:	Rheumatoid factor
IL-1:	Interleukin 1
IL-4:	Interleukin 4
IL-6:	Interleukin 6
IL-10:	Interleukin 10
TNF-α:	Tumor necrosis factor-alpha
NM:	Not mentioned
GS:	Grip strength
15Mwt:	15 m walking time
15/20Mwt:	15/20 m walking time
TS:	Tenderness score
PAOS:	Physician and patient-rated overall assessments
X-RS:	X-ray score
RCJ:	Radiological changes of joints
WDAR:	Withdrawal rate related to adverse reactions
JS:	Joint symptoms
DAS:	Disease activity score
CCP:	Cyclic citrullinated peptide
MGS:	Mean grip strength
AOT:	Analgesic onset time
SF-36:	Short form 36 health questionnaire
HAQ:	Health assessment questionnaire
TCMSJS:	TCM symptom score of the number of joint swelling
PJC:	Painful joint count

DMARDs:	Disease-modifying antirheumatic drugs
TwHF preparations:	*Tripterygium wilfordii Hook. f.* preparations
NT:	No therapy
CM:	Conventional medicine
COP:	Chinese patent medicine or placebo
CROB:	Cochrane Risk of Bias tool
JS:	Jadad Scale
CL:	Cochrane Library.

Data Availability

The data supporting this overview of systematic reviews are from previous studies and data sets, which have been cited. The processed data are available from the corresponding author upon reasonable request.

Ethical Approval

Ethical assessment and informed consent were not required since our research did not involve individual data.

Disclosure

This study *Tripterygium wilfordii Hook. f.* has been registered in the International Platform of Registered Systematic Review and Meta-analysis Protocols (DOI number is 10.37766/inplasy2021.8.0081).

Conflicts of Interest

The authors declare that there are no conflicts of interest for this work.

Authors' Contributions

All the authors contributed substantially to the design, interpretation of the data, statistical analysis, drafting of the manuscript, and approval of the submission. Huimin Li and Ruixue Hu contributed equally to this work.

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Supplementary Materials

Appendix A: search strategies and excluded systematic reviews. Appendix B: the results of ROBIS and PRISIMA. (*Supplementary Materials*)

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Research Article

The Impact of Traditional Chinese Medicine QingreHuoxue Treatment and the Combination of Methotrexate and Hydroxychloroquine on the Radiological Progression of Active Rheumatoid Arthritis: A 52-Week Follow-Up of a Randomized Controlled Clinical Study

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Traditional Chinese medicine (TCM) has been used successfully to treat rheumatoid arthritis (RA). QingreHuoxue treatment (QingreHuoxue decoction [QRHXD]/QingreHuoxue external preparation [QRHXEP]) is a Chinese medicine treatment for RA. To date, very few studies have compared the long-term effects of QRHXD with those of conventional disease-modifying antirheumatic drugs on RA disease activity and radiological progression. QRHXD delayed the radiological progression and showed long-term clinical efficacy of RA. In clinical experiments, the clinical evidence of delaying the radiological progression of RA patients was obtained. A portion of the patients who participated in the "Traditional Chinese Medicine QingreHuoxue Treatment vs. the Combination of Methotrexate and Hydroxychloroquine for Active Rheumatoid Arthritis" study were followed up for 52 weeks, and intention-to-treat (ITT) and compliance protocol (PP) analyses were used to collect and compare the clinical

indicators and imaging data between baseline and week 52. Two radiologists who were blind to treatment scored the images independently. Of the 468 subjects, 141 completed the 52-week follow-up. There were no significant differences among the three groups: the traditional Chinese medicine comprehensive treatment group, the Western medicine treatment group, and the integrated traditional Chinese and Western medicine treatment group. There were no differences in the total Sharp score, joint space stenosis score, and joint erosion score at baseline or 52 weeks. In the comparison of the estimated annual radiographic progression (EARP) and the actual annual Sharp total score changes among the three groups, the actual changes were much lower than the EARP at baseline. The radiological progress in all three groups was well controlled. Results of the ITT and PP data sets showed that the disease activity score 28 level of the three groups at 52 weeks was significantly lower than that at baseline. During the 52-week treatment period, the clearance of heat and promotion of blood circulation controlled disease activity and delayed the radiological progress of active RA.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease with erosive arthritis as the main clinical manifestation [1]. Data have shown that approximately 90% of RA patients experience bone erosion within 2 years of the onset of disease, which eventually leads to joint deformity and disability [2, 3]. A long-term clinical study by Scott et al. [4] indicated that the X-ray damage of RA is closely related to the level of disability, which increases with longer disease duration. The continuous progression of RA directly causes the loss of function and even disability in patients.

RA with moderate to severe activity results in bone destruction, disease activity, and bone erosion are the main clinical focus. How to effectively control RA synovial inflammation, reduce disease activity, and delay bone destruction are the primary problems addressed in clinical research. In recent years, a considerable number of RA patients have benefited under the guidance of advanced treatment concepts, such as early-stage standardized treatments, standard treatments based on the single or combined use of disease-modifying antirheumatic drugs (DMARDs), and the use of new biological agents. However, DMARDs and biological agents still exhibit problems such as serious adverse reactions and the significantly increased risk of lung infection and tuberculosis infection [5–8]. Moreover, some patients who achieve disease remission still exhibit continuous progression based on imaging [9]. The treatment of RA bone destruction is a primary, yet challenging, focus of clinical work. Traditional Chinese medicines (TCMs) may be particularly suitable for the treatment of RA. Clinical trials have demonstrated that TCMs can effectively reduce the disease activity of RA, increase the rate of RA disease remission, and effectively delay the progression of RA.

To further evaluate the role of TCMs in the treatment of RA by comparing its effects with a combination treatment of methotrexate (MTX) and hydroxychloroquine (HCQ), we recently conducted a clinical study named "Traditional Chinese Medicine QingreHuoxue Treatment vs. the Combination of MTX and Hydroxychloroquine for Active RA" [10]. In this multicenter, double-blind, randomized controlled trial (RCT), 468 Chinese patients with active RA (disease activity score [DAS] 28 > 3.2) were treated with QingreHuoxue decoction (QRHXD/QRHXEP) (the TCM group), MTX plus HCQ (the Western medicine [WM] group), or both (integrative medicine [IM] group). QRHXD/QRHXEP was effective in alleviating symptoms of active RA, albeit to a lesser degree than conventional synthetic DMARDs (csDMARDs), with fewer side effects. These results provide evidence that QRHXD can be used as a kind of adjuvant of csDMARDs in the treatment of RA.

Our current disease management goal for RA patients is to not only control disease activity for a long term but also reduce long-term-related joint damage. In clinical research, in addition to the evaluation of disease activity, such as by the DAS28 and American College of Rheumatology (ACR) 20% response (ACR20), 50% response (ACR50), and 70% response (ACR70) compliance rates, the prevention of progression on imaging and the reduction of joint damage are also important outcomes for determining the long-term therapeutic effect and are recommended as RA evaluation indices of patients' overall functional status [11]. Our study directly addressed the main difficulty in the treatment of RA: bone destruction. The Sharp score was used as the main efficacy indicator. A 24-week clinical study that focused on the treatment of RA with QingreHuoxue treatment was conducted. After the trial was completed, we continued to follow up the patients and conducted an observational efficacy comparison study. After its termination, the patients continued to be followed up for 52 weeks and monitored disease activity. Patients' radiological images and disease activity were collected at week 52 to determine whether the same level of efficacy observed during the previous 24 weeks was sustained. This observational curative effect real-world comparison study of RA patients allowed the evaluation of the long-term effects of QingreHuoxue treatment, the integrated traditional Chinese and Western medicine, and Western medicine on the radiological progression and disease activity of RA. The study aimed to provide effective interventions for the treatment of RA and obtain high-level clinical evidence demonstrating that the comprehensive program of clearing heat and promoting blood circulation delays the process of RA bone destruction.

2. Materials and Methods

The clinical study "Traditional Chinese Medicine QingreHuoxue Treatment vs. the Combination of MTX and Hydroxychloroquine for Active RA" was designed as a 24-week, multicenter, double-blind, RCT. Following the trial, researchers followed up subjects for 52 weeks and subsequently adopted an observational efficacy comparison study design to review the radiographs of patients' hands and wrists to assess the progression of joint bone destruction.

The primary endpoint of this study is the Sharp scoring system revised by van der Heijde at 52 weeks.

2.1. Patients. At the time of enrollment, patients who met the conditions of the trial also met the following criteria: (1) met RA classification criteria revised by the American Academy of Rheumatology (ARA) in 1987; (2) aged 18–65 years; (3) met TCM syndrome of damp-heat blockage and blood stasis blocking the collaterals; (4) had a DAS28 score >3.2; (5) patients were taking csDMARDs for at least 3 months at a stable dose and continued the same treatment for the duration of the present study; and (6) were willing to be followed up long term and provide 52-week hand radiology imaging data following the 24-week follow-up. Patients who completed the 24 weeks of the original study continued to be followed up for 52 weeks. All patients provided written informed consent at enrollment.

2.2. Study Treatment. The program was approved by the Ethical Review Committee of Guang'anmen Hospital, China Academy of Chinese Medical Sciences (no. 2013EC122).

Initially, 468 research cases from 17 research centers across the country were randomized and divided into three groups: the TCM group, the WM group, and the IM group. The specific treatments administered were as follows—the TCM group: QingreHuoxue recipe granules + QingreHuoxue external application + MTX tablet simulator + hydroxychloroquine sulfate tablet simulator; the WM group: MTX tablets + hydroxychloroquine sulfate tablets + TCM oral placebo particles + external placebo; the IM group: QingreHuoxue recipe granules + QingreHuoxue external application + MTX tablets + hydroxychloroquine sulfate tablets. MTX was taken 12.5 mg once weekly, and HCQ was taken 200 mg twice daily. QRHXD was taken twice daily for 24 weeks (1 bag boiled in water for each dose).

MTX and HCQ tablets were fabricated from Shanghai Xinyi Pharmaceutical Co. (Shanghai, China) and Shanghai Zhongxi Pharmaceutical Co. (Shanghai, China), respectively, and were taken orally. TCM QingreHuoxue treatment for RA "damp-heat-stasis syndrome" with QRHXD and QRHXEP supplied by the Guang'anmen Hospital China Academy of Chinese Medical Sciences was used in this study. QRHXD was gotten into granules that were packaged in a tin foil bag by Sichuan New Green Pharmaceutical Technology Development Co. (Chengdu, China). QRHXEP was processed into a gel formulation and packaged in a plastic tube at Guang'anmen Hospital China Academy of Chinese Medical Sciences (batch no. 15011303).

QRHXD has 12 components including animal drug wugong (centipede [4 g]) and the botanical drugs species or TCM plant preparations tufuling (*Smilax glabra* Roxb [30 g]), yinhua (*Lonicera japonica* Thunb [30 g]), huangqi (*Astragalus mongholicus* [30 g]), chaocangzhu (bran-fried *Atractylodes chinensis* [15 g]), huangbo (*Phellodendron amurense* [9 g]), chishao (*Paeonia lactiflora* [15 g]), bixie (Dioscoreae hypoglaucae rhizoma [15 g]), danshen (*Salvia miltiorrhiza* [15 g]), ezhu (*Curcuma zedoaria* [9 g]), qingfengteng (*Sinomenium acutum* [15 g]), and fengfang (Nidus vespae [5 g]). The granules were packaged as 10 g bags.

After the end of the study medication period (24 weeks), the follow-up period was up to 52 weeks, and the study was designed as an observational efficacy comparison study to achieve an open-label, case-control, and long-term follow-up study. During the 24–52 weeks, the treatment plan was adjusted and recorded according to the patient's condition. Most of the patients continued to use QingreHuoxue decoction, and some patients were changed to other treatment plans. Please refer to the study flowchart for details (Figure 1).

2.3. Outcomes and Measurements. The main indicator of this study was the evaluation of bone destruction. The primary endpoint of this study was the Sharp scoring system revised by van der Heijde (including joint erosion (JE) score, joint space reduction (JSN), revised total Sharpe score (TSS)) [12]. Subjects underwent radiological progression analysis at baseline and week 52, which involved frontal X-rays of both hands and wrists. Two radiologists read and analyzed the radiographic images according to the Sharp scoring system revised by van der Heijde. The radiologists had no knowledge of the treatment allocation, the chronology of radiographs, or patients' clinical responses. The joint erosion (JE) score and joint space narrowing (JSN) were added to calculate the revised total Sharp score (TSS) [13]. Differences between readers were assessed using the intraclass correlation coefficient, based on the status score, which ranged from 0.794 to 0.907. Secondary indicators were disease activity evaluation (DAS28), erythrocyte sedimentation rate, number of swollen joints, and number of tender joints. Safety evaluation indicators included the subjects' vital signs, such as blood pressure, respiration, and heart rate, as well as blood, urine, liver function, kidney function, and electrocardiograms of the subjects before and after the study at the baseline and 52 weeks.

2.4. Statistical Analysis. Intention-to-treat (ITT) and compliance protocol (PP) analyses were used to collect and compare the baseline and 52-week clinical indicators and radiographic data. Continuous data were presented as mean (SD), and categorical data were presented as numbers and/or percentages. A one-way ANOVA or Kruskal–Wallis rank test combined with the t-test or Wilcoxon rank test for post hoc testing was used for analyzing the continuous data. The chi-square or Fisher exact test was used for analyzing the categorical data. The Kruskal–Wallis test combined with the Wilcoxon rank test for post hoc testing was used for analyzing the ordinal data. For intragroup comparisons of continuous data, we used paired t-tests or Wilcoxon signed-rank tests.

For all statistical analyses, two-sided hypothesis tests were used, and the threshold of statistical significance of the two-tailed P value was 0.05. For the pairwise comparison between the three groups, the test level was adjusted to 0.0167 (0.05/3) according to the Bonferroni correction. The

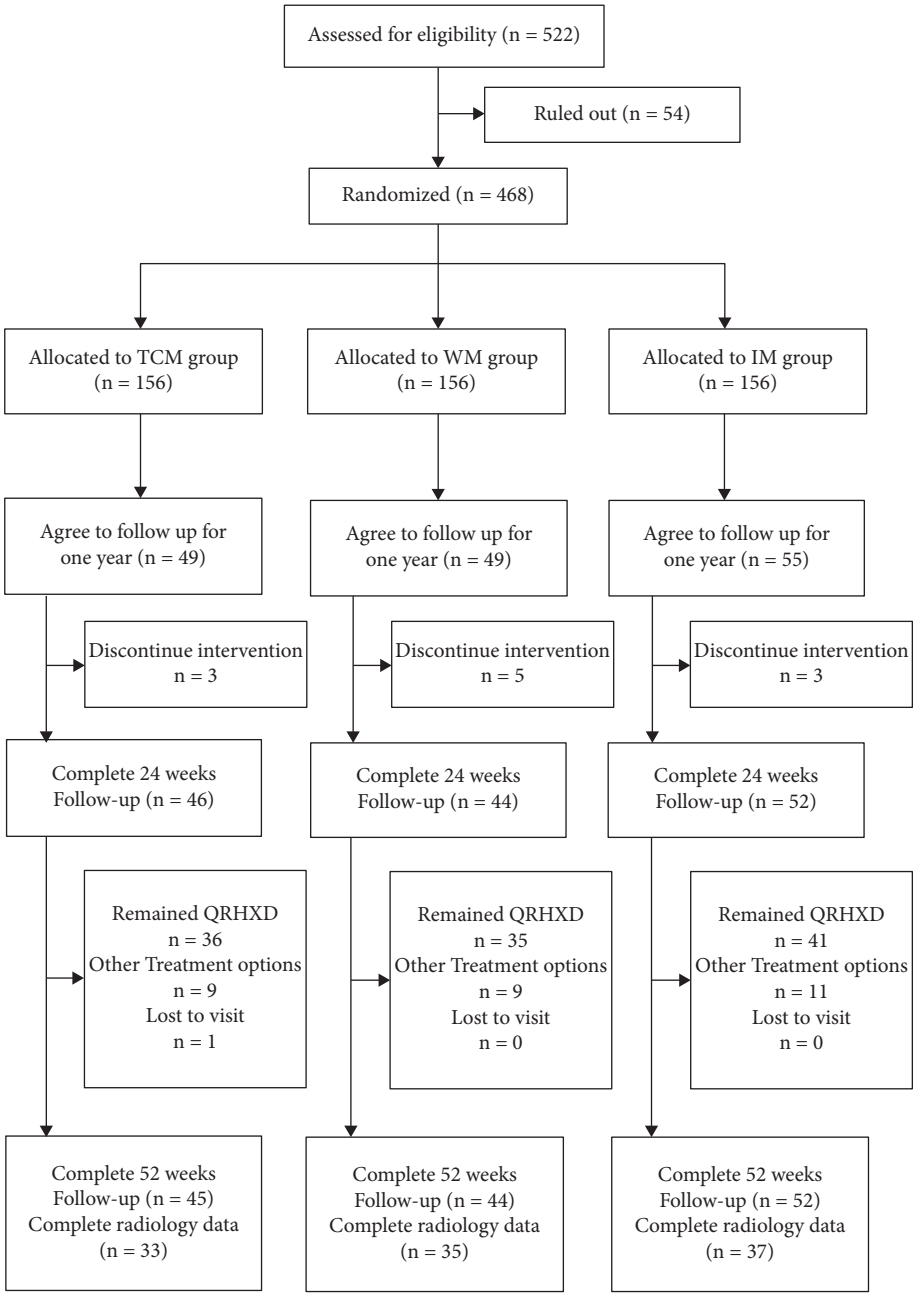

Figure 1: Study flowchart.

statistical analysis was performed using SAS V9.4 software (SAS Institute, Cary, NC, United States).

3. Results

There were 153 long-term follow-up patients, which comprised 141 patients who completed the 52-week follow-up of disease activity and 105 patients who completed the 52-week follow-up of radiological progression.

3.1. Patient Characteristics. Of the 153 patients followed up, 49 were in the TCM group, 49 were in the WM group, and 55 were in the IM group. In terms of sex, age, course of the disease, and other general population data, there were no significant differences between the three groups ($p > 0.05$; Table 1). In the baseline data, there was a higher proportion of women.

Patients were mainly middle-aged and older adults, which is consistent with the typical characteristics of RA patients. In terms of disease course, the data of the three groups were similar.

3.2. Clinical Efficacy

3.2.1. Radiographic Outcome. Results showed that baseline TSS, JSN, and JE scores did not differ among the three

Table 1: Comparison of the baseline population data among the three groups.

Items		TCM group ($n = 49$)	WM group ($n = 49$)	IM group ($n = 55$)	P-value
Gender	Male, N (%)	4 (8.16)	10 (20.41)	9 (16.36)	0.2237
	Female, N (%)	45 (91.84)	39 (79.59)	46 (83.64)	
Age	Mean (SD)	47.59 (11.11)	46.31 (10.71)	49.40 (10.07)	0.2998
Course	Mean (SD)	28.27 (37.61)	32.42 (37.08)	21.05 (16.62)	0.1123

groups. At 52 weeks, the TSS, JSN, and JE scores did not differ significantly between the three groups (Table 2).

TSS, JSN, and JE of the three groups at baseline and 52 weeks were calculated separately. It was found that the radiology of the WM group has progressed relatively quickly, and that of the TCM group came next, and the change value of IM group was the smallest. But the difference among them was not statistically significant (Table 2 and Figure 2).

Based on baseline TSS and disease course data, the estimated annual radiographic progression (EARP) was calculated, which is the TSS/disease course at baseline. There was no significant difference among the three groups or pairwise comparisons between groups (Table 2). Comparisons of the EARP with actual annual TSS changes of each group showed that the actual changes in each group were significantly lower than the baseline EARP, which suggested that the intervention measures were highly effective. Moreover, the radiological progression in three groups was well controlled (Figure 3).

A horizontal comparison was performed between the TCM, WM, and IM groups, and the change rates of TSS, JSN, and JE scores were calculated for each group. The average rate of progression at each evaluation point was calculated by (TSS − baseline score)/treatment time. Data showed that the three groups had similar rates of change of all three scores, as shown in Table 3. A horizontal comparison of the rates of change among the three groups indicated that they were comparable.

TSS, JSN, and JE scores after treatment were compared longitudinally with the baseline change rate. The baseline change rate was calculated by baseline TSS/disease course. The change rate of the three groups after treatment was significantly slower than the baseline change rate before treatment. The three groups of treatments could decrease the radiographic progression of RA patients (Figures 4–6).

3.2.2. Disease Activity Evaluation. After treatment, CRP, ESR, TJC, SJC, VAS, PhGA, PGA, and DAS28 in all three treatment groups decreased significantly from baseline to 52 weeks. There were no significant differences in the degree of improvement among the three groups, as shown in Tables 4 and 5.

3.3. Correlation Analysis between Radiology Score and DAS28. The correlation analysis showed that there was a significant correlation between the change values of the DAS28 and TSS. The change in DAS28 was smaller than that in TSS, as shown in Table 6. For those whose DAS28 change value was less than 1.41, the average TSS progression was 3.43. For those whose DAS28 change value was between 1.41 and 2.90 as well as above 2.90, the TSS change values were both 2.74.This association was observed between the DAS28 change value and both the JSN and baseline JE scores. This is consistent with previous findings.

3.4. Adverse Events. During the 24-week treatment and 52-week follow-up, there were no serious adverse events in any of the three groups, and none of the patients withdrew from the study because of the adverse reactions. The main adverse events were skin erythema, skin edema, skin itching, gastrointestinal reactions, blood system damage, and irregular menstruation. The incidence of adverse events in the TCM groups was the lowest at only 12 person-times, which included five cases of skin erythema, three cases of skin edema, two cases of skin pruritus, one case of gastrointestinal reactions, and one case of blood system damage. However, the incidence of adverse events in the WM and IM groups was relatively higher at 26 and 23 person-times, respectively (Table 7).

4. Discussion

RA is a chronic disabling autoimmune disease. With a prolonged disease course, the disability rate of patients increases significantly. Many experts now consider long-term management of disease activity and alleviation of related joint damage as the ultimate goals of RA management. This study focused on the clinical endpoint of RA bone destruction, which affects the long-term prognosis and life quality of RA patients. A follow-up clinical study was conducted to evaluate the long-term impact of TCM on the radiological progression of RA to promote the use of TCM.

Because RA is a chronic disease, we followed up a portion of patients in the study of "Rheumatoid arthritis TCM syndrome and comprehensive treatment plan for its 24-week RCT" at week 52 using similar evaluations. The current research was a long-term extension of the "24-week RCT" and is an innovative application based on real-world research concepts.

After the "24-week RCT" study, we adopted an observational comparative efficacy (CER) study design. As early as 2009, the American Agency for Healthcare Research and Quality (AHRQ) proposed the CER research method based on the concept of furthering detailed effectiveness research. CER is a highly valuable research method [14–16]. We noticed that a treatment plan that provided ideal outcomes in a well-controlled experimental environment, such as an

Table 2: Comparison of TSS among the three groups.

	TCM group, $N = 33$	WM group, $N = 35$	IM group, $N = 37$	P-value
TSS baseline				
Mean (SD)	7.48 (8.54)	19.23 (24.07)	17.62 (29.53)	0.3078
Median (Q1, Q3)	6 (1, 9)	10 (2, 33)	6 (0, 17)	
JSN baseline				
Mean (SD)	5.42 (4.66)	14.40 (18.03)	11.22 (16.44)	0.2763
Median (Q1, Q3)	5 (0, 8)	7 (1, 21)	5 (0, 16)	
JE baseline				
Mean (SD)	2.06 (5.23)	4.80 (6.57)	6.41 (13.94)	0.135
Median (Q1, Q3)	0 (0, 2)	1 (0, 9)	1 (0, 3)	
EARP				
Mean (SD)	9.61 (27.45)	19.45 (52.29)	9.56 (13.84)	0.3379
Median (Q1, Q3)	4 (0.35, 9.60)	6 (1, 24)	5.54 (0, 11.33)	
TSS (52 weeks)				
Mean (SD)	10.12 (11.77)	22.57 (26.99)	20.43 (34.86)	0.3274
Median (Q1, Q3)	7 (2, 14)	12 (3, 34)	6 (1, 17)	
JSN (52 weeks)				
Mean (SD)	7.24 (6.30)	16.43 (19.02)	12.97 (19.03)	0.3567
Median (Q1, Q3)	6 (2, 11)	8 (2, 23)	6 (1, 16)	
JE (52 weeks)				
Mean (SD)	2.88 (6.96)	6.17 (8.53)	7.46 (16.83)	0.2924
Median (Q1, Q3)	0 (0, 2)	1 (0, 12)	1 (0, 6)	
△TSS				
Mean (SD)	2.64 (5.02)	3.34 (5.31)	2.81 (6.18)	0.4997
Median (Q1, Q3)	1 (0, 3)	1 (0, 4)	1 (0, 4)	
△JSN				
Mean (SD)	1.82 (2.88)	2.03 (3.04)	1.76 (2.99)	0.8145
Median (Q1, Q3)	1 (0, 2)	1 (0, 3)	1 (0, 2)	
△JE				
Mean (SD)	0.82 (2.54)	1.37 (2.64)	1.05 (3.86)	0.1684
Median (Q1, Q3)	0 (0, 0)	0 (0, 2)	0 (0, 0)	

Note: TSS is the total Sharp score; JSN is the joint gap narrow score; JE is the joint erosion score; the EARP is baseline △TSS/disease duration; △TSS is 52-week TSS − baseline TSS; and △JE is 52-week JE − baseline JE.

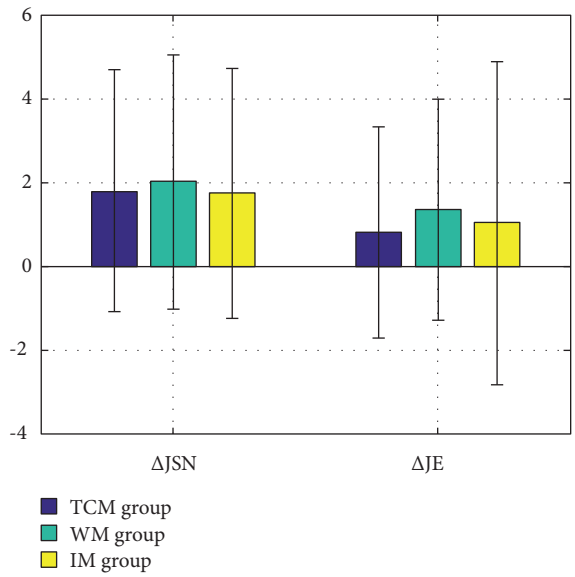

Figure 2: Comparison of JSN and JE among the three groups.

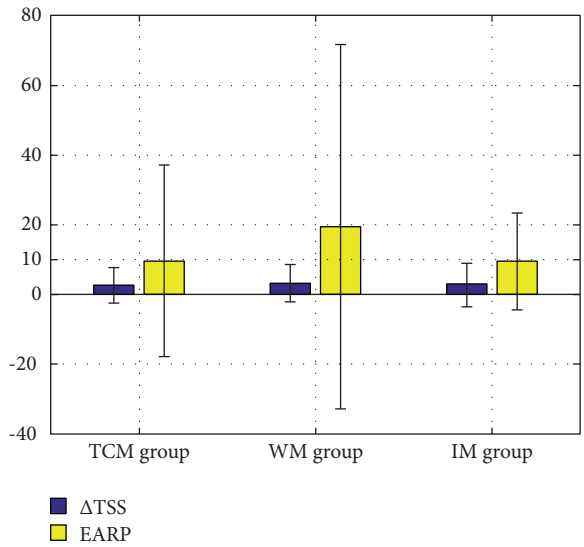

Figure 3: Comparison of TSS and EARP among the three groups.

Table 3: Change of TSS, JSN, and JE scores in the three groups.

	TCM group, $N = 33$	WM group, $N = 35$	IM group, $N = 37$	P-value
The rate of change in TSS				
Mean (SD)	0.31 (0.53)	0.34 (0.78)	0.21 (0.66)	0.2997
Median (Q1, Q3)	0.08 (0.00, 0.33)	0.08 (0.00, 0.30)	0.00 (0.00, 0.20)	
The rate of change in JSN				
Mean (SD)	0.25 (0.52)	0.35 (0.80)	0.10 (0.20)	0.447
Median (Q1, Q3)	0.00 (0.00, 0.27)	0.04 (0.00, 0.25)	0.00 (0.00, 0.13)	
The rate of change in JE				
Mean (SD)	0.07 (0.21)	0.13 (0.23)	0.17 (0.44)	0.1472
Median (Q1, Q3)	0.00 (0.00, 0.00)	0.00 (0.00, 0.15)	0.00 (0.00, 0.00)	

Note. Baseline rate = baseline TSS/course. Average rate of progression at each evaluation point of time = (TSS − baseline score)/treatment time.

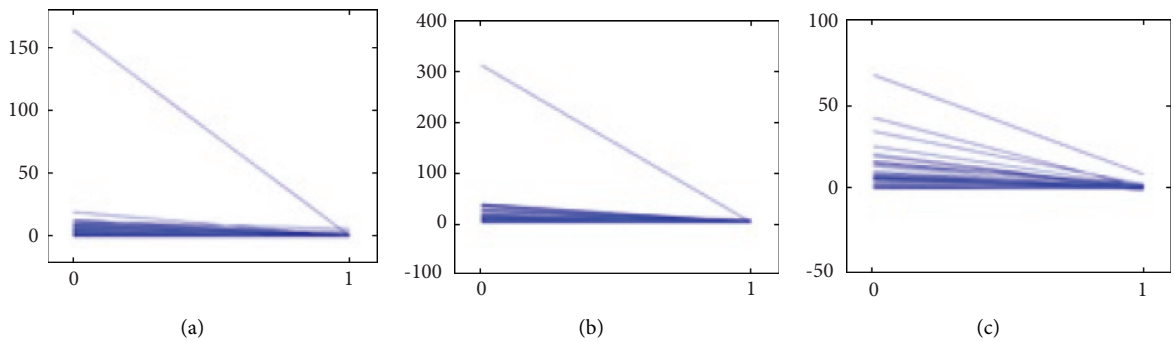

Figure 4: Comparison of TSS change among the three groups: (a) TCM groups, (b) WM group, and (c) IM group.

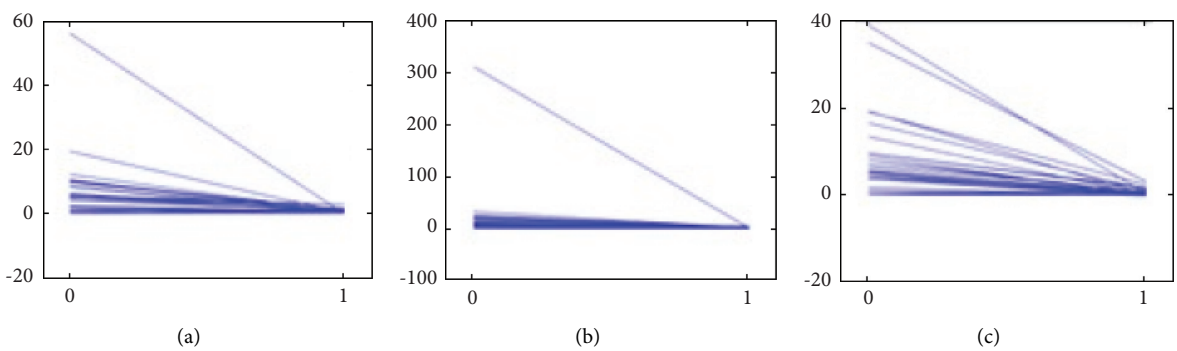

Figure 5: Comparison of JSN change among the three groups: (a) TCM groups, (b) WM group, and (c) IM group.

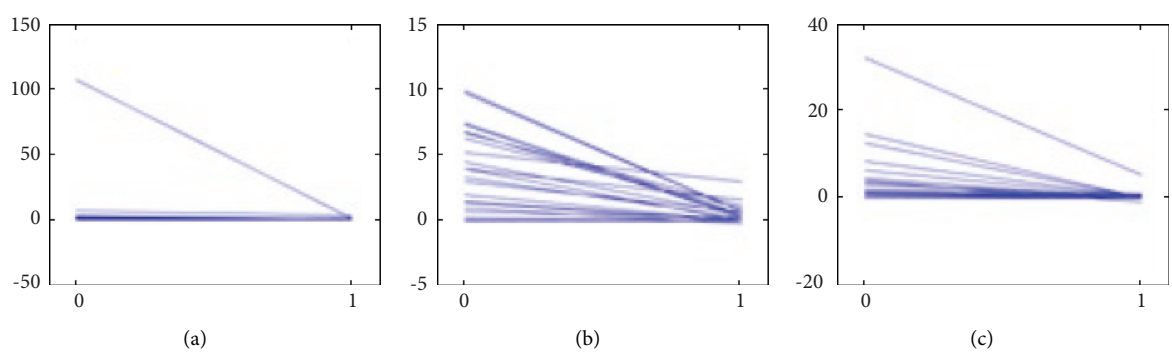

Figure 6: Comparison of JE change among the three groups: (a) TCM groups, (b) WM group, and (c) IM group.

Table 4: Disease activity comparison (ITT) between baseline and week 52.

Items	Baseline			52 weeks			P-value
	TCM group, $N = 36$	WM group, $N = 35$	IM group, $N = 41$	TCM group, $N = 36$	WM group, $N = 35$	IM group, $N = 41$	
ESR (mm/h)							
Mean (SD)	47.65 (29.36)	35.9 (21.72)	41.62 (27.42)	33.74 (28.19)	21.45 (18.67)	23.04 (16.08)	0.0843
Median (Q1, Q3)	34 (23, 68)	31 (23, 46)	33 (21, 65)	23.5 (13, 51)	14 (9, 29)	20 (10, 30)	
VAS (mm)							
Mean (SD)	54.49 (16.65)	54.24 (16.13)	54.85 (17.74)	19.73 (19.16)	21.3 (18.18)	18.38 (16.26)	0.7379
Median (Q1, Q3)	52 (40, 60)	50 (45, 60)	53 (40, 70)	20 (10, 30)	20 (10, 30)	11 (5.50, 30)	
PGA (mm)							
Mean (SD)	58.33 (17.55)	55.49 (19.14)	60.15 (19.45)	23.78 (17.74)	24.02 (20.26)	20.87 (15.24)	0.7631
Median (Q1, Q3)	60 (50, 70)	50 (45, 70)	60 (50, 80)	20 (10, 30)	15 (10, 40)	20 (10, 30)	
PhGA (mm)							
Mean (SD)	57 (14.67)	53.86 (15.06)	54.62 (18.87)	26.42 (17.89)	26.45 (18.9)	21.5 (14.71)	0.3392
Median (Q1, Q3)	60 (50, 70)	50 (45, 65)	60 (40, 70)	30 (10, 30)	20 (10, 40)	20 (10, 30)	
TJC							
Mean (SD)	10.78 (6.73)	9.96 (5.57)	10.36 (7.42)	3.53 (4.57)	3.91 (4.49)	2.63 (2.64)	0.3329
Median (Q1, Q3)	9 (6, 15)	10 (5, 13)	8 (5, 14)	2 (1, 5)	3 (1.50, 4)	2 (0, 4)	
SJC							
Mean (SD)	7.92 (5.23)	7.71 (5.32)	7.62 (5.31)	1.89 (2.41)	1.66 (2.89)	1.12 (1.62)	0.4277
Median (Q1, Q3)	7 (4, 11)	6 (3, 11)	6 (4, 10)	1 (0, 3)	0 (0, 2.50)	1 (0, 1)	
DAS28							
Mean (SD)	5.84 (0.98)	5.57 (0.99)	5.62 (1.26)	3.61 (1.45)	3.34 (1.33)	3.2 (1.05)	0.4752
Median (Q1, Q3)	5.9 (5.17, 6.36)	5.44 (4.93, 6.32)	5.47 (4.68, 6.69)	3.14 (2.75, 4.30)	3.17 (2.61, 4.01)	2.97 (2.44, 3.98)	

RCT, performed poorly in clinical practice. In this study, at the end of the 24-week RCT, we used the observational CER research method from 24 to 52 weeks. The observational CER method provided a real-world portrayal and systematic evaluation of true long-term curative effects of a comprehensive QingreHuoxue treatment in RA patients. In the real world, the clinical value and significance of long-term follow-up of diseases are considerable and vital for determining the actual clinical efficacy of QRHXD/QRHXEP for RA. Long-term follow-up of RA patients is challenging; thus, the information obtained from this study is invaluable. Observational CER methods have offered new opportunities for clinical research and are an innovative application of research design methods based on real-world research concepts, which can provide valuable guidance for practical clinical work.

Moderate to severe inflammation is a significant contributor to RA bone destruction. In RA patients, local synovitis promotes the differentiation and proliferation of osteoclasts, destroys bone structure, erodes local bone, and affects the normal function of joints and bones. Only when local and systemic inflammations have been effectively controlled, can the occurrence of RA bone destruction be effectively reduced. MTX is currently the first-line basic anchoring drug in the treatment of RA. Based on the concept of early and standard treatments, if DMARD treatment is ineffective, a combined treatment plan can be used [17]. In this study, the classic combination of MTX and HCQ was selected for the EM treatment, which was compared head to head with the comprehensive TCM and IM treatment plans.

QRHXD/QRHXEP can reduce RA disease activity and is extremely safe. After completion of the multicenter RCT of the comprehensive TCM program for clearing heat and promoting blood circulation, we continued to follow up the patients and monitor disease activity under realistic conditions. The 52-week follow-up results showed that all three groups had similar levels of disease activity. Although there

Table 5: Disease activity comparison (PP) between baseline and week 52.

Items	Baseline			52 weeks			P-value
	TCM group $N=36$	WM group $N=35$	IM group $N=41$	TCM group $N=36$	WM group $N=35$	IM group $N=41$	
ESR (mm/h)							
Mean (SD)	46.94 (28.71)	32.74 (19.51)	43.90 (28.67)	33.67 (28.38)	19.09 (15.92)	24.45 (15.65)	0.0563
Median (Q1, Q3)	34.00 (22.5, 66.5)	28.00 (20.0, 44.0)	38.00 (25.0, 69.0)	23.00 (13.0, 51.0)	14.00 (9.0, 25.0)	22.50 (10.0, 31.0)	
VAS (mm)							
Mean (SD)	54.75 (16.55)	52.37 (16.26)	53.90 (16.68)	18.56 (17.64)	19.34 (15.96)	19.54 (16.98)	0.9146
Median (Q1, Q3)	53.50 (40.0, 62.5)	50.00 (40.0, 60.0)	50.00 (40.0, 65.0)	15.00 (7.5, 30.0)	15.00 (10.0, 30.0)	20.00 (10.0, 30.0)	
PGA (mm)							
Mean (SD)	59.11 (17.06)	54.97 (19.80)	61.41 (18.97)	22.92 (16.61)	24.77 (20.77)	23.05 (15.29)	0.985
Median (Q1, Q3)	60.00 (50.0, 70.0)	50.00 (40.0, 70.0)	60.00 (50.0, 80.0)	20.00 (10.0, 30.0)	20.00 (10.0, 40.0)	20.00 (10.0, 30.0)	
PhGA (mm)							
Mean (SD)	57.03 (14.60)	53.40 (15.87)	53.61 (17.48)	25.25 (17.32)	24.40 (18.01)	22.27 (15.14)	0.7998
Median (Q1, Q3)	60.00 (50.0, 70.0)	50.00 (40.0, 70.0)	50.00 (40.0, 70.0)	22.50 (10.0, 30.0)	20.00 (10.0, 40.0)	20.00 (10.0, 30.0)	
TJC							
Mean (SD)	11.03 (7.30)	11.00 (5.78)	9.51 (7.66)	1.69 (2.12)	1.51 (2.20)	0.95 (1.53)	0.3336
Median (Q1, Q3)	9.00 (5.0, 15.5)	11.00 (6.0, 14.0)	7.00 (4.0, 13.0)	1.00 (0.0, 2.5)	0.00 (0.0, 3.0)	0.00 (0.0, 1.0)	
SJC							
Mean (SD)	8.44 (5.66)	7.69 (5.35)	7.20 (5.19)	3.89 (4.89)	3.54 (3.53)	2.44 (2.41)	0.3183
Median (Q1, Q3)	8.00 (3.5, 11.5)	6.00 (3.0, 11.0)	5.00 (4.0, 8.0)	2.00 (0.5, 5.0)	3.00 (2.0, 4.0)	2.00 (0.0, 4.0)	
DAS28							
Mean (SD)	5.87 (1.00)	5.56 (1.06)	5.52 (1.22)	3.62 (1.51)	3.21 (1.19)	3.22 (0.97)	0.2902
Median (Q1, Q3)	5.90 (5.16, 6.49)	5.50 (4.80, 6.31)	5.38 (4.59, 6.60)	3.15 (2.75, 4.79)	3.20 (2.31, 4.01)	3.15 (2.65, 3.98)	

Table 6: Correlation between DAS28 change values and TSS changes.

		△DAS28		
		<1.41 ($n=30$)	1.41–2.90 ($n=50$)	>2.90 ($n=23$)
△TSS	Mean (SD)	3.43 (6.49)	2.74 (4.65)	2.74 (5.88)
	Median (Q1, Q3)	1.5 (0, 4.75)	1 (0, 4)	0 (0, 2.5)
△JSN	Mean (SD)	2.07 (2.76)	1.86 (2.86)	1.74 (3.38)
	Median (Q1, Q3)	1 (0, 3.75)	0.5 (0, 2.75)	0 (0, 1)
△JE	Mean (SD)	1.43 (4.22)	0.88 (2.31)	1.09 (2.75)
	Median (Q1, Q3)	0 (0, 0.75)	0 (0, 1)	0 (0, 0.5)

Note. △TSS was 52-week TSS − baseline TSS; △JSN was 52-week JSN − baseline JSN; △JE was 52-week JE − baseline JE. Comparison between the three groups at $p<0.05$.

was no significant difference, there was a higher rate of compliance with DAS28 in the IM group.

The QRHXD/QRHXEP was effective in delaying the progression of RA bone destruction. The radiological progression and joint function of RA patients are key factors that affect their long-term prognosis and quality of life. This study indicated that TCM had a positive effect on inhibiting the bone destruction in RA patients and was no less inferior to the classic Western medicine treatment. In addition, we observed that the IM group had relatively lower scores of TSS, JSN, and JE than those of TCM group and WM group. However, there was no significant difference among the

Table 7: Adverse events in three groups.

	TCM group	WM group	IM group
Total	12	26	23
Erythema on the skin	5	5	0
Skin edema	3	3	1
Itchy skin	2	5	5
Gastrointestinal reaction	1	6	8
Damage to the blood system	1	0	0
Menstruation is not normal	0	1	0
Stomachache	0	1	0
Headache, dizziness	0	2	2
Hair loss	0	2	2
Proteinuria increases	0	1	1
Upper respiratory tract infection	0	0	1
Urinary tract infection	0	0	1
Arrhythmia	0	0	1
Insomnia	0	0	1

three groups, which may be due to the relatively small number of cases. We plan to conduct large-scale clinical studies in the future to further optimize the treatment plans for RA.

A recent meta-analysis [18] showed that TCM significantly improved the bone density, reduced the level of serum matrix metalloproteinase 3, and protected the bone condition of RA patients. At present, there are an increasing number of clinical treatments that intervene in RA bone destruction. Yet, there have been relatively few long-term efficacy studies. Additional randomized controlled trials with objective study designs are needed to obtain rich high-level clinical evidence to support TCM intervention for RA bone destruction and ultimately benefit more RA patients.

We found that there were no serious adverse events during the entire study. Furthermore, we found that the TCM group had the lowest incidence of adverse events and the highest safety among the three groups.

This study has several limitations. Firstly, the inherent limitations of study includes potential bias of open-label design and populations possibly representing only responders to therapy. However, properly designed and conducted open-label extension studies can provide rigorous information on long term efficacy of new therapy. Secondly, because of funding constraints, the radiographic images included in this study were only radiographic images of the hands and wrists and did not include those of the feet. Studies have shown that the joints of the feet are usually affected more easily and earlier than the joints of the hands. Therefore, including data on feet may help improve the sensitivity of assessment in the joint damage of early RA [19–22]. We plan to conduct further clinical trials in the future to evaluate the effect of TCM on RA bone destruction.

5. Conclusion

We used a comprehensive QingreHuoxue treatment to treat active RA and conducted a long-term follow-up of patients. This was a real-world observational comparative study that evaluated the long-term effects of TCM treatment on RA. We found that a comprehensive QingreHuoxue treatment delayed the radiological progression of RA and continued to reduce disease activity. The following conclusions are drawn:

(1) The program of clearing heat and promoting blood circulation had a therapeutic effect on RA bone destruction. The TCM group significantly slowed radiological progression and was not inferior to the combined MTX and HCQ treatment group.

(2) TCM treatment helped RA patients achieve rapid relief of symptoms (such as joint swelling and pain), reduce inflammatory indicators (such as ESR), and reduce disease activity.

(3) The incidence of adverse events was the lowest, and safety was the highest in RA patients who were offered the TCM treatment.

Data Availability

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

Conflicts of Interest

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

Authors' Contributions

QJ and XG designed the study. QJ and XG contributed equally to this work. RZ, X-PT, JW, JL, Q-CH, WL, Y-FF, D-YH, YL, M-LG, Q-JW, SC, Z-BL, YW, Y-MX, J-LZ, C-YZ, LM, W-XL, and X-CW performed the experiments. RZ and XG wrote the manuscript. All the authors read and approved the final version of the manuscript for publication.

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Supplementary Materials

File 1: supplementary materials_data_baseline. The supplementary materials are the original data of this study, mainly including the basic information of patients, grouping, disease activity, and X-ray Sharp score. The basic information of the subjects includes the name, gender, year of birth, and course of disease. Disease activity was recorded by

the researchers at baseline, including DAS28 (28-joint count Disease Activity Score), CRP (C-reactive protein), ESR (erythrocyte sedimentation rate), TJC (tender joint count), SJC (swollen joint count), VAS (visual analogue scale), PhGA (physician's global assessment of disease activity), PGA (patient's global assessment of disease activity), and HAQ (Health Assessment Questionnaire). Subjects underwent radiological progression analysis at baseline, which involved frontal X-rays of both hands and wrists. Two radiologists read and analyzed the radiographic images according to the Sharp scoring system revised by van der Heijde. The radiologists had no knowledge of the treatment allocation, the chronology of radiographs, or patients' clinical responses. The Sharp scoring system is revised by van der Heijde, including TSS (total Sharp score), JSN (joint gap narrow score), and JE (joint erosion score). The sum of joint erosion (JE) score and joint space narrowing (JSN) was the value of total Sharp score (TSS). File 2: supplementary materials_data_52w. The supplementary materials are the original data of this study, mainly including the basic information of patients, grouping, disease activity and X-ray sharp score. The basic information of the subjects includes the name, gender, and year of birth. Disease activity was recorded by the researchers at 52 weeks of follow-up, including DAS28 (28-joint count Disease Activity Score), CRP (C-reactive protein), ESR (erythrocyte sedimentation rate), TJC (tender joint count), SJC (swollen joint count), VAS (visual analogue scale), PhGA (physician's global assessment of disease activity), PGA (patient's global assessment of disease activity), and HAQ (Health Assessment Questionnaire). Subjects underwent radiological progression analysis at week 52, which involved frontal X-rays of both hands and wrists. Two radiologists read and analyzed the radiographic images according to the Sharp scoring system revised by van der Heijde. The radiologists had no knowledge of the treatment allocation, the chronology of radiographs, or patients' clinical responses. The Sharp scoring system is revised by van der Heijde, including TSS (total Sharp score), JSN (joint gap narrow score), and JE (joint erosion score). The sum of joint erosion (JE) score and joint space narrowing (JSN) was the value of total Sharp score (TSS) . (*Supplementary Materials*)

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Research Article

Effects and Safety of the Tripterygium Glycoside Adjuvant Methotrexate Therapy in Rheumatoid Arthritis: A Systematic Review and Meta-Analysis

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Objective. This study aimed to systematically review the efficacy and clinical safety of different courses and doses of tripterygium glycoside (TG) adjuvant methotrexate (MTX) therapy in the treatment of rheumatoid arthritis (RA). *Methods*. Randomized controlled trials (RCTs) of TG adjuvant MTX therapy in patients with RA were retrieved from SinoMed, China Network Knowledge Infrastructure, WanFang Data, PubMed, Cochrane Library, and Embase from inception to September 30, 2021. The effects and clinical safety evaluations were conducted using RevMan 5.3 software. *Results*. A total of 9 RCTs and 892 patients with RA were included in this study. In the meta-analysis, a total of 463 and 429 patients were enrolled into the TG adjuvant MTX therapy group and MTX monotherapy group, respectively. In comparison with MTX monotherapy, the results of the analyzed effects showed that the TG adjuvant MTX therapy can achieve 20%, 50%, and 70% improvements in American College of Rheumatology (ACR) criteria ACR20, ACR50, and ACR70 at $P = 0.005$, $P = 0.0001$, and $P = 0.004$, respectively. Simultaneously, the efficacy of the TG adjuvant MTX therapy was improved at either 30 or 60 mg/day over a six-month course compared to MTX monotherapy ($P < 0.0001$). There was no statistical difference in the effects between the doses of 30 and 60 mg/day after three months ($P = 0.82$). TG adjuvant MTX also reduced the expression rate of the swollen joint count, tender joint count, erythrocyte sedimentation rate, rheumatoid factor, and C-reactive protein in subgroup analyses with different courses and doses. In terms of hepatic adverse effects ($P = 0.28$), leukopenia ($P = 0.78$), gastrointestinal adverse effects ($P = 0.17$), cutaneous adverse effects ($P = 0.94$), and irregular menstruation adverse effects ($P = 0.29$), there was no statistically significant difference with TG adjuvant MTX therapy and MTX monotherapy with different courses and doses. *Conclusions*. TG adjuvant MTX therapy is more effective than MTX monotherapy and is a safe strategy for RA treatment in doses of 30 or 60 mg/day over a treatment course of six months. However, high-quality multicenter RCT studies with large sample sizes are still needed to confirm the effects and clinical safety of different courses and doses of TG adjuvant MTX therapy.

1. Introduction

Rheumatoid arthritis (RA) is a complex, inflammatory, and systemic autoimmune disease. It is associated with progressive disability, and it primarily affects the lining of the synovial joints [1, 2]. Patients with RA typically present with symmetrical polyarthritis of the small joints of the hands and feet with early morning stiffness and occasional constitutional symptoms [3]. Methotrexate (MTX) is the first-line treatment given to patients with RA. MTX can reduce the level of inflammation and prevent joint erosion and functional damage. The clinical effect of using MTX monotherapy is only 60% to 70%, whereas adjuvant therapy has positive significance in improving clinical effects [4]. Therefore, the clinical treatment of RA often uses MTX and adjuvant drugs, such as sinomenine and iguratimod, to enhance effects [5, 6].

Traditional Chinese herbal medicine has achieved considerable progress in treating RA [7, 8]. Tripterygium glycosides (TGs), which are the extracts of *Tripterygium wilfordii* Hook F, have been widely used as anti-inflammatory drugs and immunosuppressants for treating RA. The effective parts of TGs mainly include diterpenoids, alkaloids, triterpenoids, and glycosides. Most of these active constituents of TGs are effective in anti-inflammation and immunosuppression [9]. In the United States, several clinical trials have shown that TG has good effects in patients with RA because of its anti-inflammatory and immunosuppressive activities [10–12].

During RA treatment, adjuvant therapeutics, which involve several drugs that interact with multiple targets in the molecular networks of RA, may achieve better effects compared with monotherapy [13, 14]. TG has been empirically applied in adjuvant therapy with MTX for RA treatment [15, 16]. Previous studies have shown that TG adjuvant MTX therapy is a more effective strategy than MTX monotherapy for RA treatment and adverse reactions were not aggravated [17–19]. However, the effects and safety of different courses and doses of TG adjuvant MTX therapy in RA need to be further explored.

2. Methods

This study was investigated according to the preferred reporting items for systematic review and meta-analysis (PRISMA) 2020 checklist (Supplementary Information 1).

2.1. Protocol Registration. This meta-analysis study and its protocol were registered in PROSPERO of the Centre for Reviews and Dissemination (NO. CRD42021224095).

2.2. Eligibility Criteria. The eligibility criteria for the enrollment of studies into meta-analysis are as follows: (1) randomized controlled trials (RCTs) comparing TG adjuvant MTX therapy and MTX monotherapy, (2) patients with a diagnosis of RA based on the criteria revised by the American College of Rheumatology (ACR) in 1987 or the ACR/European Association of Anti-Rheumatology Annual in 2010 [20, 21], (3) primary outcomes with 20% improvement in ACR criteria (ACR20), 50% improvement in ACR criteria (ACR50), and 70% improvement in ACR criteria (ACR70), (4) secondary outcomes of swollen joint count (SJC), tender joint count (TJC), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), C-reactive protein (CRP), hepatic adverse effects, leukopenia, gastrointestinal adverse effects, cutaneous adverse effects, irregular menstruation adverse effects before and after treatment, and (5) studies published in English or Chinese.

The exclusion criteria are as follows: (1) studies that focused only secondary outcomes or safety outcomes without primary outcomes, (2) patients in studies with additional treatment factors in TG adjuvant MTX therapy group and/or the MTX monotherapy group, and (3) incomplete or duplicate data.

2.3. Search Strategy. An electronic search of databases, including SinoMed, China Network Knowledge Infrastructure (CNKI), WanFang Data, PubMed, Cochrane Library and Embase, was performed and completed on September 30, 2021. The references of all retrieved articles were also reviewed for potentially relevant studies. The search strategy involved the use of the following keywords: "Tripterygium*," "Tripterygium hypoglaucum," "Tripterygium hypoglaucums," "Tripterygiums," "hypoglaucums, Tripterygium," "Tripterygium wilfordii," "Tripterygium wilfordius," "wilfordius, Tripterygium," "Leigong Teng," "Leigong Tengs," "Teng, Leigong," "Tengs, Leigong," "Thundergod Vine," "Thundergod Vines," "Vine, Thundergod," "Vines, Thundergod," "Arthritis, Rheumatoid*," "Caplan Syndrome*," "Felty Syndrome*," "Rheumatoid Nodule*," "Rheumatoid Vasculitis*," "Sjogren's Syndrome*," "Still's Disease, Adult-Onset*," "Rheumatoid Arthritis," "Caplan Syndromes," "Caplan's Syndrome," "Caplans Syndrome," "Syndrome, Felty," "Felty's Syndrome," "Feltys Syndrome," "Syndrome, Felty's," "Familial Felty's Syndrome," "Familial Feltys Syndrome," "Felty's Syndrome, Familial," "Syndrome, Familial Felty's," "Rheumatoid Arthritis," "Splenomegaly and Neutropenia," "Familial Felty Syndrome," "Felty Syndrome, Familial," "Syndrome, Familial Felty," "Nodule, Rheumatoid," "Nodules, Rheumatoid," "Rheumatoid Nodules," "Rheumatoid Nodulosis," "Rheumatoid Noduloses," "Rheumatoid Vasculitides," "Vasculitides, Rheumatoid," "Vasculitis, Rheumatoid," "Sjogrens Syndrome," "Syndrome, Sjogren's," "Sjogren Syndrome," "Sicca Syndrome," "Syndrome, Sicca," "Still's Disease, Adult-Onset," "Stills Disease, Adult-Onset," "Adult-Onset Still's Disease," "Adult-Onset Still's Disease," "Adult-Onset Stills Disease," "Still Disease, Adult-Onset," "Still Disease, Adult-Onset," "Adult-Onset Still Disease," "Adult-Onset Still Disease," "Methotrexate*," "Amethopterin," "Methotrexate, (D)-Isomer," "Methotrexate, (DL)-Isomer," "Mexate," "Methotrexate Sodium," "Sodium, Methotrexate," "Methotrexate, Sodium Salt," "Methotrexate, Disodium Salt," "Methotrexate Hydrate," "Hydrate, Methotrexate," "Methotrexate, Dicesium Salt," and "Dicesium Salt Methotrexate." Supplementary Information 2 presents the detailed search

strategies employed in this study. Unpublished studies and clinical trial registries were also obtained from the databases of GreyNet International, Open Grey, Cochrane Library, and Chinese Clinical Trial Registry.

2.4. Study Selection and Data Extractions. The titles and abstracts of the searched results were independently assessed by two investigators (Q. Geng and B. Liu). The full texts of the potentially eligible studies were then screened for final inclusion in the current study. Disagreements were resolved by a third opinion (C. Lu). The extracted data included the study characteristics (authors, title, etc.), patient characteristics (number of patients, age, gender, etc.), intervention, control, and outcomes.

2.5. Risk of Bias in Individual Studies. The two investigators (Q. Geng and B. Liu) used the Cochrane Collaboration [22] "Risk of Bias" tool to assess the methodological quality of the each included studies. Seven items including random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases were assessed as low risk, high risk, or unclear risk. Each potential source of bias was graded as high, low, or unclear.

2.6. Statistical Analysis. Statistical analyses were performed using RevMan 5.3 software from the Cochrane Collaboration. The data were summarized using risk ratio (RR) at 95% confidence intervals (CIs) for dichotomous data. For continuous variables, the mean difference (MD) was used if the outcome measurement units of each study were the same, however, standardized MD (SMD) was used if the measurement units and methods were different among the studies. Statistical heterogeneity was tested by examining both the chi-square test and the I^2 statistic. The I^2 values ranged from 0 to 100% and were categorized as follows: I^2 <40%, might not be important; 50% < I^2 < 90%, moderate heterogeneity; 75% < I^2 < 100%, considerable heterogeneity [23]. A fixed-effect model was used to pool the estimates using the fixed effects model when $I^2 \leq 50\%, P \geq 0.1$. $I^2 > 50\%$ and $P < 0.1$ indicated the possibility of statistical heterogeneity, and random-effects model was adopted. Potential sources of heterogeneity were explored using subgroup and sensitivity analyses. We conducted a subgroup analysis of different courses and doses (three-month course of 30 mg/day, three-month course of 60 mg/day, six-month course of 30 mg/day, and six-month course of 60 mg/day). The results are presented as forest plots. Sensitivity analyses were also performed to test the stability of the results via the leave-one-out method. Funnel plots and Egger's test were used to assess for publication bias when there are at least 10 studies included in the meta-analysis [22].

2.7. Evidence Quality Evaluation. The results of the meta-analysis were evaluated using the GRADE method [24], and degradation was considered in terms of the risk of bias, inconsistency, indirectness, imprecision, and publication bias. The evidence quality was classified as "high quality," "moderate quality," "low quality," and "very low quality."

3. Results

3.1. Search Results. A total of 1020 articles were identified by literature search: SinoMed ($n = 258$), CNKI ($n = 330$), WanFang Data ($n = 352$), PubMed ($n = 19$), Cochrane Library ($n = 9$), and Embase ($n = 52$). Duplicate checking was conducted using NoteExpress 3.5.0, and 485 papers were ultimately selected. Furthermore, there were 16 reviews and 91 irrelevant studies. After reading through the full articles, the following were excluded from this review: Chinese and English language papers ($n = 7$), duplicate non-TG studies ($n = 201$), studies with primary effect points not meeting ACR20, ACR50, or ACR70 ($n = 101$), experimental studies ($n = 105$), and non-RA studies ($n = 7$). Two records were identified by manual searching. A total of nine RCTs [19, 25–32] were included in the meta-analysis (Figure 1). Supplementary Information 3 shows the list of excluded studies.

The nine trials involving 892 RA participants were all conducted in mainland China. These trials were published from 2013 to 2018. A total of 463 and 429 patients were enrolled in the TG adjuvant MTX therapy and MTX monotherapy groups, respectively. In terms of outcomes, two studies [26, 28] assessed ACR20, one study [32] focused on ACR50, three studies [25, 27, 30] focused on ACR20 and ACR50, and three studies [19, 29, 31] concentrated on ACR20, ACR50, and ACR70. For secondary outcomes, nine studies [19, 25–32] assessed SJC, TJC, ESR, and CRP, and four studies [25, 27, 29, 30] focused on RF (Table 1). TG is available in three- and six-month courses at doses of 30 and 60 mg/day. One study [32] focused on a three-month course at 30 mg/day, and four studies [25, 27, 30] focused on a three-month course at 60 mg/day. At six months of treatment, two studies [26, 28] assessed a dose of 30 mg/day, and two other studies (26, 28) concentrated on a dose of 60 mg/day.

3.2. Risk of Bias of the Included RCTs. Four studies [19, 25, 29, 30] had a low risk of bias for random sequence generation because their random number generation method uses a random number table or centralized randomization. The other studies were at unclear risk of random sequence generation because the method was not mentioned in any of these studies. All studies were rated as having an unclear risk for allocation concealment because it was unclear whether the allocation concealment researchers were third-party personnel. With regard to attrition bias, three studies [19, 25, 26] reported the results according to preset outcomes. Thus, these studies were rated as having low risk. The remaining studies failed to clarify whether the outcomes were established in advance. Thus, these studies were rated as having an unclear risk. Two studies [19, 31] were considered at low risk of detection bias, and the remaining studies did not provide blinding information and were

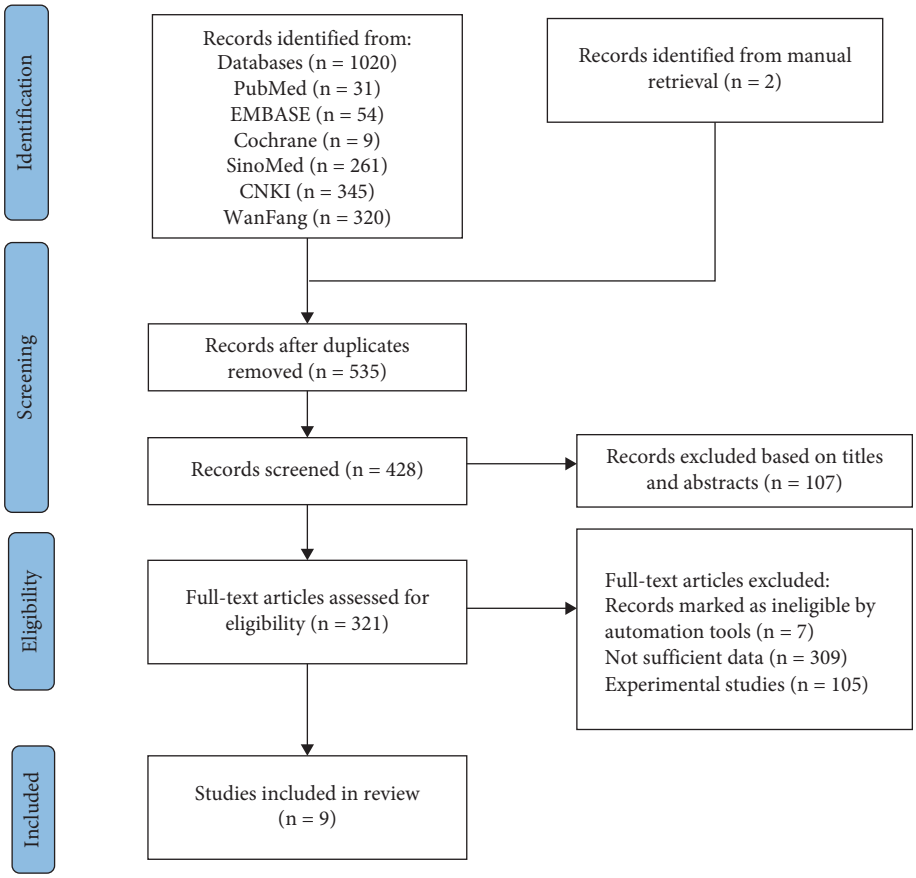


Figure 1: Flow diagram illustrating the process of identifying articles for selection study characteristics.

considered at unclear risk. Due to the specificity of the intervention, all included studies were considered to be at high risk for performance bias. For reporting bias, the current study had to check the Methods and Results sections of all trials on the bias of the information in the Methods section because of the unavailability of protocols in the included trials. After a rigorous assessment, there was a low risk of selective reporting and other biases in all studies (Figures 2 and 3).

3.3. Effects of Interventions with Different Courses and Doses of TG. Nine studies evaluated the effects and clinical safety of 892 patients in the TG adjuvant MTX therapy groups and MTX monotherapy therapy groups. For primary outcomes, our analysis revealed that TG adjuvant MTX therapy increased ACR20 (RR = 1.13; 95% CI: [1.04, 1.23]; $P = 0.005$) (figure 4(a)), ACR50 (RR = 1.28; 95% CI: [1.13, 1.46]; $P = 0.0001$) (figure 5(a)), and ACR70 (RR = 1.65; 95% CI: [1.18, 2.31]; $P = 0.004$) (figure 6(a)) responder rates compared with MTX monotherapy.

The response of the TG subgroups for different courses and doses showed that there was no significant improvement in ACR20 for a three-month course (RR = 0.99; 95% CI: [0.87, 1.12]; $P = 0.82$). The forest plot (figure 4(b)) results for a three-month course of 30 mg/day (RR = 1.10; 95% CI: [0.86, 1.40];$P = 0.46$) or 60 mg/day (RR = 0.94; 95% CI: [0.82, 1.09]; $P = 0.43$) showed no statistically significant difference. The results showed that the difference in ACR50 improvement was not statistically significant (RR = 1.10; 95% CI: [0.89, 1.35];$P = 0.39$) in a three-month course, and the results of the 60 mg dose were consistent with the above results (RR = 0.87; 95% CI: [0.66, 1.15]; $P = 0.32$). However, the difference was statistically significant when using a dose of 30 mg (RR = 1.45; 95% CI: [1.05, 2.01]; $P = 0.02$) (figure 5(b)).

Overall, ACR20 improvement after a six-month course of TG treatment (RR = 1.23; 95% CI: [1.09, 1.40];$P = 0.001$) was observed at doses of 30 (RR = 1.19; 95% CI: [1.04, 1.35]; $P = 0.010$) and 60 mg/day (RR = 1.32; 95% CI: [1.02, 1.70]; $P = 0.04$) (figure 4(c)).

The forest plot (figure 5(c)) results show the overall ACR50 efficacies (RR = 1.41; 95% CI: [1.20, 1.65]; $P < 0.0001$) for doses of 30 (RR = 1.46; 95% CI: [1.16, 1.84]; $P = 0.001$) and 60 mg/day (RR = 1.38; 95% CI: [1.10, 1.71]; $P = 0.004$). The results were all statistically significant in the overall ACR70 efficacies (RR = 1.65; 95% CI: [1.18, 2.31]; $P = 0.004$) of doses of 30 (RR = 2.00; 95% CI: [1.04, 3.83]; $P = 0.04$) and 60 mg/day (RR = 1.53; 95% CI: [1.03, 2.28]; $P = 0.03$) (figure 6(b)).

For secondary outcomes, it was found that the TG adjuvant MTX therapy reduced the SJC (MD = -2.74; 95% CI: [−3.95, −1.54]; $P < 0.00001$), TJC (MD = −2.63; 95% CI: [−3.56, −1.69]; $P < 0.00001$), ESR (MD = -15.71; 95% CI: [−21.40, −10.01]; $P < 0.00001$), CRP (SMD = -1.00; 95% CI: [−1.58, −0.42]; $P = 0.0007$), and RF (MD = -45.72; 95% CI: [-74.86, -16.58]; $P = 0.002$) (Supplementary Figure 1).

Table 1: Characteristics of the included RCTs.

Study ID	Patients	Treatment					Control					Primary outcomes	Secondary outcomes	Course of treatment (months)
		Intervention	N	M/F	Age (Mean ± SD)	Dose(mg/d)	Intervention	N	M/F	Age(Mean ± SD)	Dose(mg/w)			
Wang 2013	Patients with RA	TG + MTX	76	—	43.4 ± 6.6	+TG (30)	MTX	50	—	43.4 ± 6.6	15	①②	④⑤⑥⑦⑧	3
Feng 2013	Patients with RA	TG + MTX	22	2/18	51 ± 4.2	+TG (60)	MTX	20	2/18	52 ± 3.8	10	①	④⑤⑥⑦	3
Yang 2013	Patients with EORA	TG + MTX	40	6/34	69.3 ± 6.9	+TG (30)	MTX	40	4/36	68.8 ± 7.4	10	①②	④⑤⑥⑦⑧	6
Zhang 2015	Patients with EORA	TG + MTX	66	6/54	65 ± 4.2	+TG (60)	MTX	60	6/54	65 ± 3.8	10	①	④⑤⑥⑦	3
Lin 2016	Patients with RA	TG + MTX	50	19/31	44.05 ± 4.9	+TG (30)	MTX	50	20/30	43.7 ± 5.3	10	①②③	④⑤⑥⑦⑧	6
Zhou 2017	Patients with RA	TG + MTX	56	—	45.7 ± 4.8	+TG (60)	MTX	56	—	45.7 ± 4.8	10	①②	④⑤⑥⑦⑧	3
Lv 2015	Patients with RA	TG + MTX	69	14/55	50.6	+TG (60)	MTX	69	10/59	51.0	7.5–12.5	①②③	④⑤⑥⑦	6
Zhou 2018	Patients with RA	TG + MTX	69	14/55	50.6	+TG (60)	MTX	69	10/59	51.0	7.5–12.5	①②③	④⑤⑥⑦	6
Wang 2018	Patients with RA	TG + MTX	15	7/8	55.5	+TG (60)	MTX	15	7/8	53.7	7.5–12.5	②	④⑤⑥⑦	3

Notes: EORA: elderly-onset rheumatoid arthritis ①: ACR20; ②: ACR50; ③: ACR70; ④: SJC; ⑤: TJC; ⑥: ESR; ⑦: CRP; ⑧: RF.

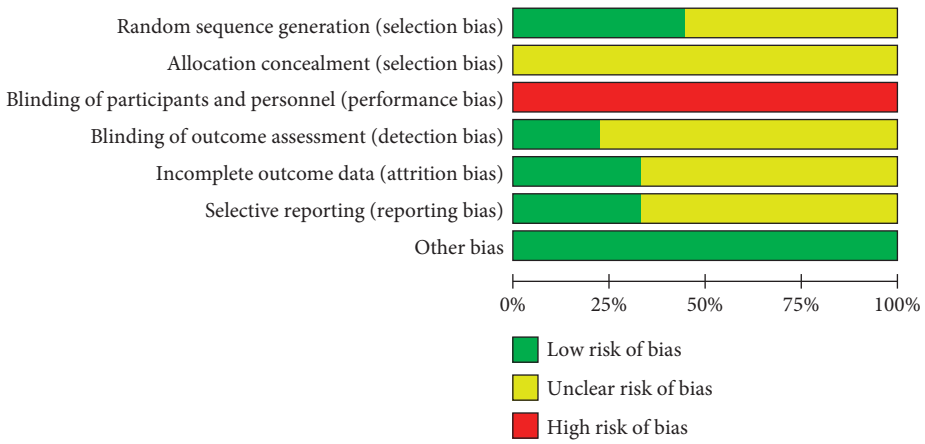


Figure 2: Risk of bias graph.

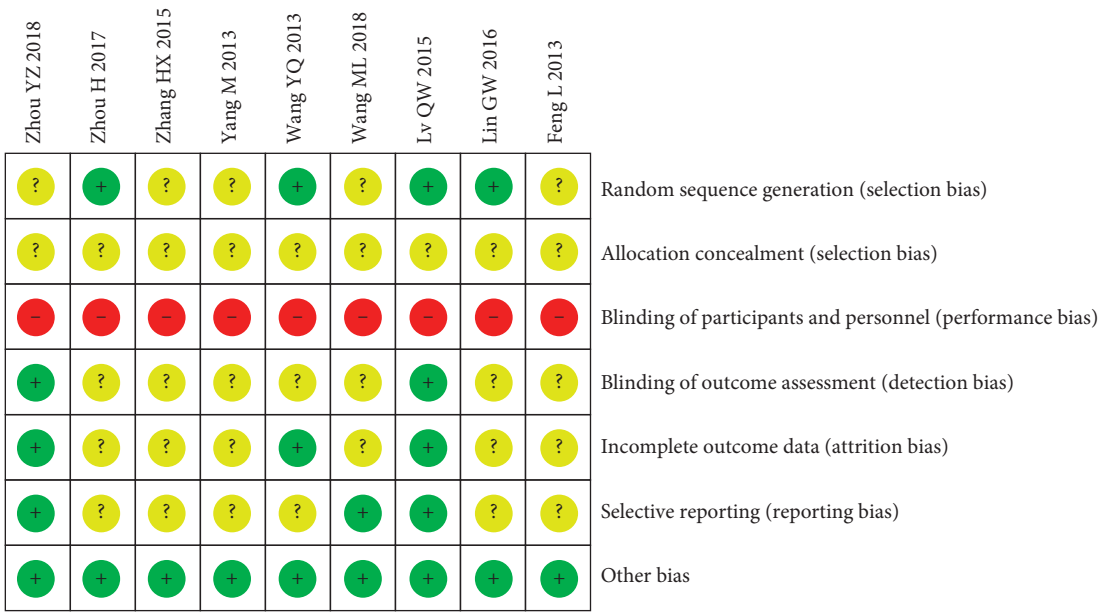


Figure 3: Risk of bias summary.

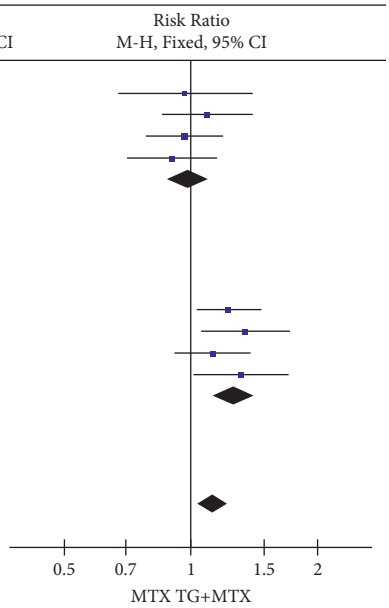
The overall reductions in SJC ($P < 0.00001$), TJC ($P < 0.00001$), ESR ($P < 0.00001$), CRP ($P = 0.009$), and RF ($P = 0.0002$) were statistically significant in a three-month course of TG treatment. Subgroup analysis showed statistically significant differences regardless of whether the dose was 30 or 60 mg/day (Supplementary Figure 2). After six months, SJC ($P = 0.004$), TJC ($P = 0.02$), ESR ($P = 0.0005$), CRP ($P = 0.005$), and RF ($P < 0.0001$) significantly improved, and the difference was statistically significant (Supplementary Figure 3).

3.4. Safety of Interventions. The forest plot (Supplementary Figure 4) showed that there was no significant difference between the safety of MTX monotherapy and TG adjuvant MTX in the occurrence of hepatic adverse effects (RR = 0.71; 95% CI: [0.38, 1.33]; $P = 0.28$), leukopenia (RR = 1.11; 95% CI: [0.55, 2.24]; $P = 0.78$), gastrointestinal adverse effects (RR = 0.83; 95% CI: [0.64, 1.08]; $P = 0.17$), cutaneous adverse effects (RR = 1.02; 95% CI: [0.57, 1.84]; $P = 0.94$), and irregular menstruation adverse effects (RR = 1.56; 95% CI: [0.69, 3.51]; $P = 0.29$). A heterogeneity test showed that the I^2 of each index was less than 50. Hence, the fixed-effects model was adopted.

After three months of treatment, the 30 mg/day dose was not statistically different in terms of safety, including hepatic adverse effects (RR = 0.44; 95% CI: [0.08, 2.53]; $P = 0.36$), leukopenia (RR = 0.33; 95% CI: [0.06, 1.73]; $P = 0.19$), gastrointestinal adverse effects (RR = 0.66; 95% CI: [0.04, 10.28]; $P = 0.77$), cutaneous adverse effects (RR = 3.31; 95% CI: [0.16, 67.57]; $P = 0.44$), 60 mg/day dose in terms of leukopenia (RR = 4.56; 95% CI: [0.55, 37.96]; $P = 0.16$), and gastrointestinal adverse effects (RR = 1.52; 95% CI: [0.21, 11.13]; $P = 0.68$) (Supplementary Figure 5).

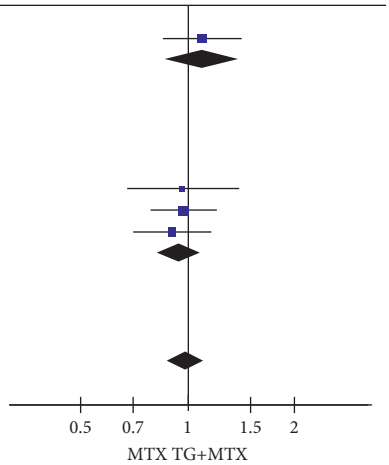
There was no statistical difference in hepatic adverse effects (RR = 1.25; 95% CI: [0.35, 4.50]; $P = 0.73$), leukopenia (RR = 1.00; 95% CI: [0.26, 3.87]; $P = 1.00$), gastrointestinal

Study or Subgroup	TG+MTX Events	TG+MTX Total	MTX Events	MTX Total	Weight (%)	Risk Ratio M-H, Fixed, 95% CI
1.1.1 Course of treatment-3 months						
Feng L 2013	16	22	15	20	5.4	0.97 [0.68, 1.39]
Wang YQ 2013	55	76	33	50	13.7	1.10 [0.86, 1.40]
Zhang HX 2015	48	66	45	60	16.2	0.97 [0.79, 1.19]
Zhou H 2017	37	56	41	56	14.1	0.90 [0.71, 1.15]
Subtotal (95% Cl)		220		186	49.4	0.99 [0.87, 1.12]
Total events	*156*		*134*			
Heterogeneity: chi^2 = 1.27, df = 3 (P = 0.74); I^2 = 0%						
Test for overall effect: Z = 0.23 (P = 0.82)						
1.1.2 Course of treatment-6 months						
Lin GW 2016	47	50	38	50	13.1	1.24 [1.04, 1.47]
Lv QW 2015	54	69	40	69	13.8	1.35 [1.07, 1.71]
Yang M 2013	35	40	31	40	10.7	1.13 [0.92, 1.38]
Zhou YZ 2018	50	69	38	69	13.1	1.32 [1.02, 1.70]
Subtotal (95% Cl)		228		228	50.6	1.27 [1.13, 1.42]
Total events	*186*		*147*			
Heterogeneity: chi^2 = 1.64, df = 3 (P = 0.65); I^2 = 0%						
Test for overall effect: Z = 4.11 (P < 0.0001)						
Total (95% CI)		*448*		*414*	*100.0*	*1.13 [1.04, 1.23]*
Total events	*342*		*281*			
Heterogeneity: chi^2 = 10.64, df = 7 (P = 0.16); I^2 = 34%						
Test for overall effect: Z = 2.81 (P = 0.005)						
Test for subgroup differences: chi^2 = 8.49, df = 1 (P = 0.004); I^2 = 88.2%						



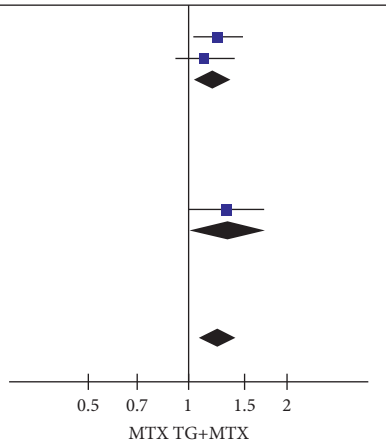
(a)

Study or Subgroup	TG+MTX Events	TG+MTX Total	MTX Events	MTX Total	Weight (%)	Risk Ratio M-H, Fixed, 95% CI
1.2.1 3 months+30 mg/d						
Wang YQ 2013	55	76	33	50	27.7	1.10 [0.86, 1.40]
Subtotal (95% CI)		76		50	27.7	1.10 [0.86, 1.40]
Total events	*55*		*33*			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.74 (P = 0.46)						
1.2.2 3 months+60 mg/d						
Feng L 2013	16	22	15	20	10.9	0.97 [0.68, 1.39]
Zhang HX 2015	48	66	45	60	32.8	0.97 [0.79, 1.19]
Zhou H 2017	37	56	41	56	28.5	0.90 [0.71, 1.15]
Subtotal (95% CI)		144		136	72.3	0.94 [0.82, 1.09]
Total events	*101*		*101*			
Heterogeneity: chi^2 = 0.22, df = 2 (P = 0.90); I^2 = 0%						
Test for overall effect: Z = 0.79 (P = 0.43)						
Total (95% CI)		*220*		*186*	*100.0*	*0.99 [0.87, 1.12]*
Total events	*156*		*134*			
Heterogeneity: chi^2 = 1.27, df = 3 (P = 0.74); I^2 = 0%						
Test for overall effect: Z = 0.23 (P = 0.82)						
Test for subgroup differences: chi^2 = 1.09, df = 1 (P = 0.30); I^2 = 8.2%						



(b)

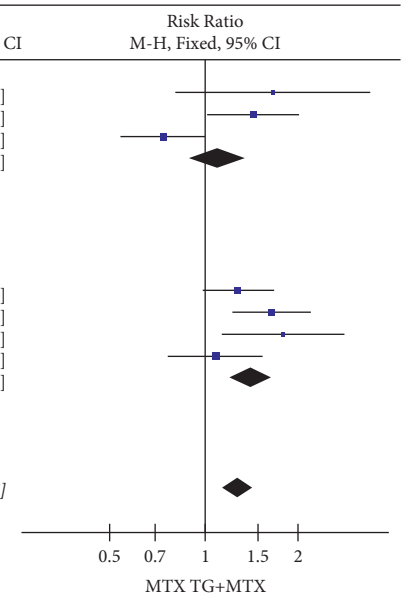
Study or Subgroup	TG+MTX Events	TG+MTX Total	MTX Events	MTX Total	Weight (%)	Risk Ratio M-H, Fixed, 95% CI
1.3.1 6 months+30 mg/d						
Lin GW 2016	47	50	38	50	35.5	1.24 [1.04, 1.47]
Yang M 2013	35	40	31	40	29.0	1.13 [0.92, 1.38]
Subtotal (95% CI)		90		90	64.5	1.19 [1.04, 1.35]
Total events	*82*		*69*			
Heterogeneity: chi^2 = 0.45, df = 1 (P = 0.50); I^2 = 0%						
Test for overall effect: Z = 2.58 (P = 0.010)						
1.3.2 6 months+60 mg/d						
Zhou YZ 2018	50	69	38	69	35.5	1.32 [1.02, 1.70]
Subtotal (95% CI)		69		69	35.5	1.32 [1.02, 1.70]
Total events	*50*		*38*			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.08 (P = 0.04)						
Total (95% CI)		*159*		*159*	*100.0*	*1.23 [1.09, 1.40]*
Total events	*132*		*107*			
Heterogeneity: chi^2 = 0.97, df = 2 (P = 0.62); I^2 = 0%						
Test for overall effect: Z = 3.27 (P = 0.001)						
Test for subgroup differences: chi^2 = 0.48, df = 1 (P = 0.49); I^2 = 0%						



(c)

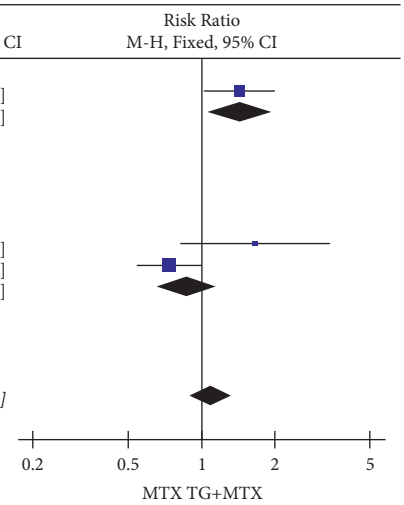
Figure 4: Forest plots for the ACR20 of the different courses and doses of TG adjuvant MTX therapy.

Study or Subgroup	TG+MTX Events	TG+MTX Total	MTX Events	MTX Total	Weight (%)	Risk Ratio M-H, Fixed, 95% CI
1.4.1 Course of treatment-3 months						
Wang ML 2018	10	15	6	15	3.3	1.67 [0.81, 3.41]
Wang YQ 2013	53	76	24	50	15.7	1.45 [1.05, 2.01]
Zhou H 2017	29	56	39	56	21.2	0.74 [0.55, 1.01]
Subtotal (95% Cl)		147		121	40.2	1.10 [0.89, 1.35]
Total events	*92*		*69*			
Heterogeneity: chi^2 = 10.39, df = 2 (P = 0.006); I^2 = 81%						
Test for overall effect: Z = 0.86 (P = 0.39)						
1.4.2 Course of treatment-6 months						
Lin GW 2016	40	50	31	50	16.9	1.29 [1.00, 1.67]
Lv QW 2015	53	69	32	69	17.4	1.66 [1.25, 2.20]
Yang M 2013	27	40	15	40	8.2	1.80 [1.14, 2.83]
Zhou YZ 2018	35	69	32	69	17.4	1.09 [0.78, 1.54]
Subtotal (95% Cl)		228		228	59.8	1.41 [1.20, 1.65]
Total events	*155*		*110*			
Heterogeneity: chi^2 = 4.88, df = 3 (P = 0.18); I^2 = 39%						
Test for overall effect: Z = 4.20 (P < 0.0001)						
Total (95% CI)		*375*		*349*	*100.0*	*1.28 [1.13, 1.46]*
Total events	*247*		*179*			
Heterogeneity: chi^2 = 19.32, df = 6 (P = 0.004); I^2 = 69%						
Test for overall effect: Z = 3.85 (P = 0.0001)						
Test for subgroup differences: chi^2 = 3.47, df = 1 (P = 0.06); I^2 = 71.2%						



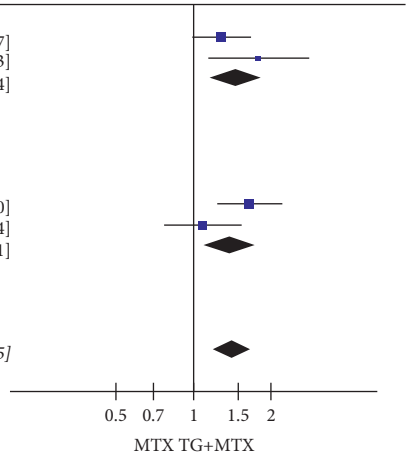
(a)

Study or Subgroup	TG+MTX Events	TG+MTX Total	MTX Events	MTX Total	Weight (%)	Risk Ratio M-H, Fixed, 95% CI
1.5.1 3 months+30 mg/d						
Wang YQ 2013	53	76	24	50	39.2	1.45 [1.05, 2.01]
Subtotal (95% Cl)		76		50	39.2	1.45 [1.05, 2.01]
Total events	*53*		*24*			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.26 (P = 0.02)						
1.5.2 3 months+60 mg/d						
Wang ML 2018	10	15	6	15	8.1	1.67 [0.81, 3.41]
Zhou H 2017	29	56	39	56	52.7	0.74 [0.55, 1.01]
Subtotal (95% Cl)		71		71	60.8	0.87 [0.66, 1.15]
Total events	*39*		*45*			
Heterogeneity: chi^2 = 4.17, df = 1 (P = 0.04); I^2 = 76%						
Test for overall effect: Z = 1.00 (P = 0.32)						
Total (95% CI)		*147*		*121*	*100.0*	*1.10 [0.89, 1.35]*
Total events	*92*		*69*			
Heterogeneity: chi^2 = 10.39, df = 2 (P = 0.006); I^2 = 81%						
Test for overall effect: Z = 0.86 (P = 0.39)						
Test for subgroup differences: chi^2 = 5.60, df = 1 (P = 0.02); I^2= 82.1%						



(b)

Study or Subgroup	TG+MTX Events	TG+MTX Total	MTX Events	MTX Total	Weight (%)	Risk Ratio M-H, Fixed, 95% CI
1.6.1 6 months+30 mg/d						
Lin GW 2016	40	50	31	50	28.2	1.29 [1.00, 1.67]
Yang M 2013	27	40	15	40	13.6	1.80 [1.14, 2.83]
Subtotal (95% Cl)		90		90	41.8	1.46 [1.16, 1.84]
Total events	*67*		*46*			
Heterogeneity: chi^2 = 1.69, df = 1 (P = 0.19); I^2 = 41%						
Test for overall effect: Z = 3.18 (P = 0.001)						
1.6.2 6 months+60 mg/d						
Lv QW 2015	53	69	32	69	29.1	1.66 [1.25, 2.20]
Zhou YZ 2018	35	69	32	69	29.1	1.09 [0.78, 1.54]
Subtotal (95% Cl)		138		138	58.2	1.38 [1.10, 1.71]
Total events	*88*		*64*			
Heterogeneity: chi^2 = 3.34, df = 1 (P = 0.07); I^2 = 70%						
Test for overall effect: Z = 2.85 (P = 0.004)						
Total (95% CI)		*228*		*228*	*100.0*	*1.41 [1.20, 1.65]*
Total events	*155*		*110*			
Heterogeneity: chi^2 = 4.88, df = 3 (P = 0.18); I^2 = 39%						
Test for overall effect: Z = 4.20 (P = 0.0001)						
Test for subgroup differences: chi^2 = 0.13, df = 1 (P = 0.72); I^2 = 0%						



(c)

Figure 5: Forest plots for the ACR50 of the different courses and doses of TG adjuvant MTX therapy.

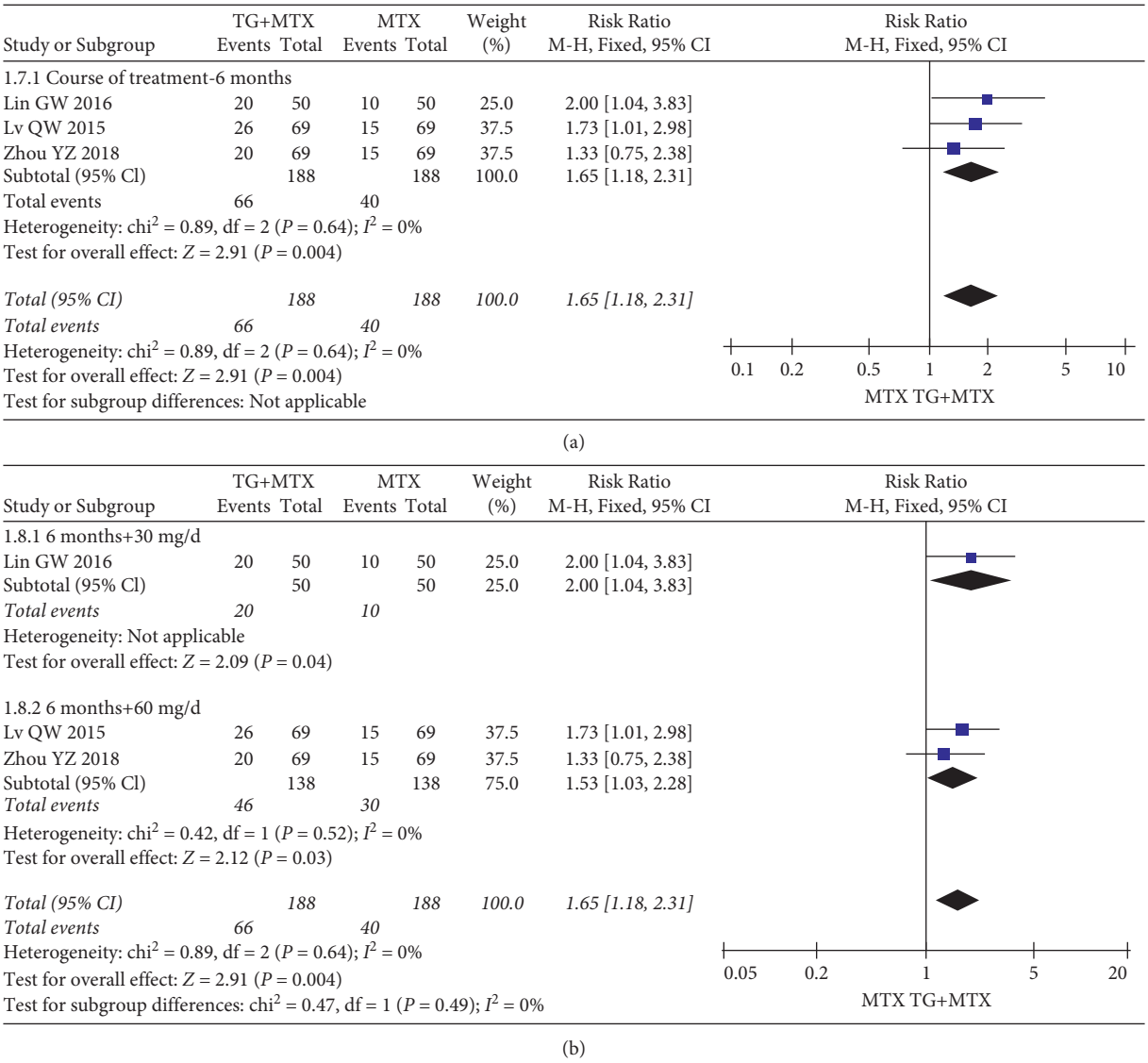


Figure 6: Forest plots for the ACR70 of the different courses and doses of TG adjuvant MTX therapy.

adverse effects (RR = 0.73; 95% CI: [0.26, 2.10]; $P = 0.56$), cutaneous adverse effects (RR = 0.75; 95% CI: [0.17, 3.25]; $P = 0.70$), and irregular menstruation adverse effects (RR = 4.00; 95% CI: [0.46, 34.54]; $P = 0.21$) between the six-month course and the 30 mg/day dose. Similar to the result for the 30 mg dose, the result for the 60 mg dose showed that there was no statistical difference in hepatic adverse effects (RR = 0.62; 95% CI: [0.27, 1.39]; $P = 0.24$), leukopenia (RR = 1.25; 95% CI: [0.35, 4.46]; $P = 0.73$), gastrointestinal adverse effects (RR = 0.83; 95% CI: [0.63, 1.09]; $P = 0.18$), cutaneous adverse effects (RR = 1.00; 95% CI: [0.52, 1.94]; $P = 1.00$), and irregular menstruation adverse effects (RR = 1.25; 95% CI: [0.51, 3.07]; $P = 0.63$) (Supplementary Figure 6).

3.5. Sensitivity Analysis. Sensitivity analysis was performed to evaluate the results of different studies. The secondary outcomes of SJC ($I^2 = 86\%$), CRP ($I^2 = 94\%$), ESR ($I^2 = 95\%$), and RF ($I^2 = 97\%$) heterogeneity were high. The robustness and variance of SJC, CRP, ESR, and RF were between 73% and 87%, 90% and 94%, 86% and 95%, and 97% and 98%, respectively. All results remained relatively stable according to the leave-one-out sensitivity analysis.

3.6. Publication Bias. Egger's tests were performed to evaluate the publication bias of the studies on the primary outcomes of ACR20 (Egger's test: $P = 0.513$) and ACR50 (Egger's test: $P = 0.539$) and on the secondary outcomes of SJC (Egger's test: $P = 0.555$), TJC (Egger's test: $P = 0.834$), CRP (Egger's test: $P = 0.217$), ESR (Egger's test: $P = 0.05$), leukopenia (Egger's test: $P = 0.250$), and gastrointestinal adverse reaction (Egger's test: $P = 0.844$). The symmetrical funnel plot indicated that there was no significant publication bias in this study. These results suggested that there was no significant publication bias in this meta-analysis. In addition, the publication bias could not be assessed for other outcomes because of the small number of studies.

3.7. Evidence Quality Evaluation. We used the GRADE Pro system to evaluate the quality of evidence for the primary outcomes of different courses and doses. The RCT was preset to the highest level of evidence in the GRADE evidence quality assessment and was processed according to five degradation factors. The results suggested that the quality of the evidence was low (Supplementary Information 4). The main reason for this result is that the study design is not rigorous, and the sample size is not sufficient.

4. Discussion

This study focused on 9 RCTs with 892 RA participants to evaluate the effects and clinical safety of different courses and doses of TG adjuvant MTX therapy in the treatment of RA in comparison with MTX monotherapy. The test results show that there were no significant differences in the baseline of patients and interventions in all evaluated studies. Most system baselines also showed no significant differences, thus conforming to the principle of meta-analysis. The results of this study showed that TG adjuvant MTX therapy is more effective than MTX monotherapy and is a safe strategy for RA treatment with different courses and doses. For effects, a six-month course of TG adjuvant MTX for RA increased the primary outcomes of ACR20, ACR50, and ACR70, which are the gold standard composite measures used in clinical trials of patients with RA [32]. For secondary outcomes, TG adjuvant MTX also reduced the expressions of SJC, TJC, ESR, CRP, and RF. SJC and TJC are indices for evaluating disease activity, severity, and curative effect in patients with RA [33]. ESR and CRP are often used in the clinical diagnosis of RA [34]. RF is a nonspecific detection indicator of RA with high sensitivity [35]. In terms of safety, TG adjuvant MTX therapy did not increase the incidence of adverse effects for three or six months compared with MTX monotherapy. Therefore, these clinical data suggest that a six-month course of treatment at 30 or 60 mg/day of TG adjuvant MTX therapy may be a more effective and safer strategy for RA treatment.

Currently, although the etiology and pathological mechanism of RA are not clear, a large number of studies have shown that the abnormality and interaction of cytokines play important roles in the pathogenesis of RA [36, 37]. Interleukin-6 (IL-6) is a type of proinflammatory cytokine that can promote the proliferation of B cells in RA disease, increase the biological effect of tumor necrosis factor-alpha (TNF-α), and promote the development of RA [38]. TNF-α is involved in the pathogenesis of RA by activating endothelial cells and promoting the synthesis and release of inflammatory cytokines [39]. Several studies have shown that both MTX monotherapy and TG adjuvant MTX therapy can reduce the expressions of IL-6 and TNF-α, however, the expressions of IL-6 and TNF-α decrease more significantly in the adjuvant therapy. It suggests that TG adjuvant MTX treatment can enhance the synergistic effect of the two drugs by inhibiting the activities of IL-6 and TNF-α and control the progression of RA [40]. This finding may explain the improvement in secondary outcomes at the three-month course even though no significant improvements were observed in ACR20, ACR50, and ACR70. It does not conflict with the absence of improvements in ACR20, ACR50, and ACR70, which is one of the secondary outcomes evaluated for ACR20, ACR50, and ACR70. Significant improvements in ACR20, ACR50, and ACR70 can only be evaluated if three additional indicators improve by more than 20%, 50%, and 70% on top of the improvement in SJC and TJC.

The heterogeneity of secondary outcomes was high. Although we performed subgroup analysis, we were unable to reduce the heterogeneity probably because of the fact that most secondary outcomes were laboratory measures with different reference ranges in different hospitals or probably because there was an uneven distribution among the subgroups in terms of the number of studies and cases.

This meta-analysis has some limitations. Firstly, random allocation principle, allocation concealment, and blinding were not described in detail in some of the included documents. Secondly, given that the sample of raw data in this study was small and because all involved trials were conducted in China, the results of this review might have introduced potential selection bias. It may have caused measurement bias in the implementation and outcome evaluation. Thirdly, the high heterogeneity of individual literature may be because of the low quality of the included literature, the difference in sample size, the difference in disease activity of patients, and the difference in the course of treatment. Although these limitations may undermine the level of evidence of this meta-analysis, the selected trials are highly comparable, and the documents were selected in strict accordance with the inclusion criteria.

5. Conclusion

According to the nine RCTs included, a six-month course of TG adjuvant MTX therapy at 30 or 60 mg/day is more effective than MTX monotherapy and is a safe strategy for treating RA. However, because of the low quality of GRADE evaluations and given the limitations of existing research, further high-quality multicenter RCT studies with large sample sizes are needed to confirm the clinical safety of TG combination therapy.

Data Availability

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

Conflicts of Interest

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

Authors' Contributions

Qi Geng and Bin Liu contributed equally to this work. Cheng Lu conceived and designed the study and supervised the whole process with Yanping Wang, Yaolong Chen, and Jianfeng Yi. Qi Geng and Bin Liu performed the study search and literature selection and drafted the manuscript with the help of Yanfang Ma and Huizhen Li. Qi Geng and Bin Liu

extracted and analyzed the data. Guilin Ouyang and Zhixing Nie assessed the risk of bias. All authors read and approved the manuscript for submission.

Acknowledgments

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Supplementary Materials

Supplementary 1. Supplementary Information 1: items regarding the PRISMA checklist for network meta-analysis. Supplementary Information 2: detailed search strategies. Supplementary Information 3: a list of all excluded papers. Supplementary 2Supplementary Information 4: quality assessment using the GRADE approach. Supplementary Figure 1: forest plots for the secondary outcomes of TG adjuvant MTX therapy. Supplementary Figure 2: forest plots for the secondary outcomes of a three-month course of TG adjuvant MTX therapy at a dose of 30 mg/day. Supplementary Figure 3: forest plots for the secondary outcomes of the different courses and doses of TG adjuvant MTX therapy. Supplementary Figure 4: forest plots for the safety of TG adjuvant MTX therapy. Supplementary Figure 5: forest plots for the safety of a three-month course of TG adjuvant MTX therapy at the dose of 30 mg/day. Supplementary Figure 6: forest plots for the safety of the different courses and doses of TG adjuvant MTX therapy. (*Supplementary Materials*)

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Research Article

Jinwujiangu Capsule Treats Fibroblast-Like Synoviocytes of Rheumatoid Arthritis by Inhibiting Pyroptosis via the NLRP3/CAPSES/GSDMD Pathway

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Jinwujiangu capsule (JWJGC) is a traditional Chinese medicine formula used to treat rheumatoid arthritis (RA). However, whether its mechanism is associated with pyroptosis remains unclear. In this study, the ability of JWJGC to inhibit the growth of fibroblast-like synoviocytes of RA (RA-FLS) through pyroptosis was evaluated. The cells isolated from patients with RA were identified by hematoxylin and eosin (H&E) staining, immunohistochemistry, and flow cytometry. After RA-FLS were treated with different concentrations of JWJGC-containing serum, the cell proliferation inhibition rate, expression of caspase-1/3/4/5, NOD-like receptor protein 3 (NLRP3), gasdermin-D (GSDMD), and apoptosis-associated speck-like protein containing a CARD (ASC), concentrations of interleukin-1β (IL-1β) and interleukin-18 (IL-18), the activity of lactic dehydrogenase (LDH), and pyroptosis were evaluated. The results showed that JWJGC increased the proliferative inhibition rate, decreased the expression of caspase-1/3/4/5, GSDMD, NLRP3, and ASC, suppressed the expression of IL-1β and IL-18, induced the activity of LDH, and downregulated the number of double-positive FITC anti-caspase-1 and PI. Generally, our findings suggest that JWJGC can regulate NLRP3/CAPSES/GSDMD in treating RA-FLS through pyroptosis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic disease characterized by autoimmune functional disorders caused by genetic and environmental factors, which results in hyperplasia of synovial membranes, formation of vascular pannus, and destruction of the cartilage and bone [1]. Currently, the underlying mechanism of RA is not completely understood. However, it has been found that the synovial tissue of RA shows tumor-like growth characteristics accompanied by a high concentration of inflammatory factors related to the development of RA [2]. Fibroblast-like synoviocytes (FLS) constitute the main part of the synovial tissue and play an important role in the pathogenesis of RA [3]. Therefore, it is beneficial for RA patients to inhibit the migration of FLS and the expression of inflammatory factors.

Pyroptosis is programmed cell death initiated by inflammasomes [4]. Although pyroptosis can protect the host from microbial pathogens, its dysregulation leads to a variety of autoimmune and autoinflammatory conditions. After stimulating the compromised state signal originating from the host due to bacteria, fungi, and parasites, inflammasomes have different pattern-recognition receptors (PRRs) such as melanoma 2 (AIM2), pyrin, and NOD-like receptor protein 3 (NLRP3) [5]. Inflammasomes drive the activation of caspases, including caspase-1/3/4/5. On one hand, the activated caspase-1 promotes prointerleukin-1β (pro-IL-1β) and prointerleukin-18 (pro-IL-18) to be mature interleukin-1β (IL-1β) and interleukin-18 (IL-18), which can both trigger an inflammatory reaction [6]. On the other hand, activated caspase-1 cleaves gasdermin-D (GSDMD) to separate its N-terminal pore-forming domain (PFD) from

the C-terminal repressor domain (RD). PFD works on the membrane of cells to form pores that drive swelling and membrane rupture [7]. Meanwhile, inflammatory storms occur after IL-1β and IL-18 are released into the extracellular space.

Jinwujiangu capsule (JWJGC) is a hospital formula developed by Professor Wu-Kai Ma based on the theory of Miao medicine. In the past 20 years, this Chinese medicine prescription has been effective in improving the clinical symptoms of patients with few RA side effects. In previous studies, JWJGC decreased the ratio of joint swelling in a rat model and inhibited cell proliferation with low levels of inflammatory cytokines, including IL-1β and IL-18 in RA-FLS [8]. However, whether its mechanism of action is associated with pyroptosis remains unclear. The present study aimed to evaluate the regulatory effect of JWJGC on RA-FLS through pyroptosis via the NLRP3/CAPSES/GSDMD pathway. The flowchart of the study procedure is shown in Figure 1.

2. Materials and Methods

2.1. RA-FLS Isolation. We followed the methods described by Yi Ling et al. [9]. RA-FLS cells were isolated and cultured using the explant adherent culture method. Synovial tissue samples were obtained from three patients (two men and one woman: 0–70 years) during joint replacement surgery at the Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine. All patients were diagnosed with RA according to the 2010ACR/EULAR Classification Criteria for Rheumatoid Arthritis [10]. The experiments were approved by the Medical Ethical Committee of the Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine (PY2019104), and all patients provided written informed consent. The synovial tissues were washed five times with phosphate-buffered saline (PBS; 0626A18, BI, Israel) supplemented with 2% penicillin-streptomycin (J180027, HyClone, USA). After removing irrelevant tissues, the synovial tissue was cut into approximately one cubic centimeter pieces. All pieces were seeded in a 25 cm^2 culture flask for anchorage without a culture medium at 37°C in a humidified atmosphere of 5% CO_2 for 4 h. Then, 5 mL of the complete medium containing Dulbecco's modified Eagle's medium (DMEM; 8119424, Gibco, USA) supplemented with 20% fetal bovine serum (1948791, BI, Israel), and 1% penicillin-streptomycin (J180027, HyClone, USA) was added to the culture flask to harvest RA-FLS after approximately 3 weeks. Cells were subcultured at a ratio of 1 : 2 when they reached 90% confluence, and the cells at passages 3–6 were used for the experiments.

2.2. Cell Identification. We followed the methods described by Yi Ling et al. for cell identification [9]. Hematoxylin and eosin (H&E) staining and immunohistochemistry were used to identify RA-FLS. The third passage cells (3.5×10^5 cells/mL) were seeded on 10 mm × 10 mm coverslips in a 6-well plate for 24 h. After the cells were fixed on coverslips with 4% paraformaldehyde for 30 min, they were stained as per the manufacturer's instructions using an H&E staining kit (20190507, Solarbio, China). The fixed cells on coverslips were incubated with the primary rabbit antivimentin antibody (1 : 250; ab92547, Abcam, UK) at 37°C overnight. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (K195219C, ZSGB, China) served as the secondary antibody, and then, chromogen development was obtained with 3,3-diaminobenzidine reagent (K193328E, ZSGB, China). Subsequently, the FLS were counterstained with hematoxylin.

Flow cytometry was used to determine the purity of RA-FLS staining for the cell surface markers CD90 or VCAM-1. The third passage FLS (1×10^5 cells/mL) suspended in 1 mL of PBS in 12×75 mm^2 tubes were centrifuged at $200 \times g$ for 5 min at 4°C. The supernatant was then removed. The cells were incubated 30 min after 5 μL of FITC anti-CD90 (ab11155, Abcam, UK) or APC anti-VCAM-1 (ab103173, Abcam, UK) was added in the dark at 4°C. After three times of washing, the cells were resuspended in 500 μL of a buffer solution and analyzed using a flow cytometer (FACSCanto II, BD, USA).

2.3. Preparation of Drug-Containing Serum

2.3.1. Experimental Drug of JWJGC. JWJGC consisting of *Gardneria angustifolia* Wall. 10 g, *Zaocys dhumnades* 10 g, Curcumae Longae Rhizoma 15 g, Caulis Sinomenii 15 g, Paeoniae Radix Alba 15 g, Cibotii Rhizoma 15 g, *Homalomena occulta* 10 g, *Panax notoginseng* (Burk.) F. H. Chen 5 g, and licorice 3 g. JWJGC (batch number: 20181001) was obtained from the Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine. Drug quality control was also available.

2.3.2. Experimental Animals and Feeding Conditions. Nine male and nine female, healthy New Zealand White rabbits (3 ± 0.5 kg) were supplied by the Animal Experimental Center of Guizhou Medical University (batch number: SCXK (Guizhou) 2018-0001) and were housed in a standard 12-h light/dark cycle with water and food ad libitum at 20–25°C for 7 days. This experimental program was approved by the experimental animal ethics committee (ethical approval code: GK2019004).

2.3.3. Experimental Serum. Nine times the equivalent dose for rabbits was 3.645 g/1000 g of JWJGC according to the calculation by weight ratio between humans and rabbits, while 3.75 mg/1000 g of leflunomide (batch number: 190101, Suzhou Changzheng-Xinkai Pharmaceutical Co., Ltd., China) was the positive control. The rabbits were randomly divided into three groups, each having three males and three females. JWJGC (3.645 g/1000 g), leflunomide (3.75 mg/1000 g), and an equal volume of distilled water were orally administered to the rabbits twice daily at 9:00 am and 9:00 pm for 5 days. The rabbits were sacrificed by air injection through the auricular vein 2 h after the final administration. Blood was collected from the hearts of the rabbits under

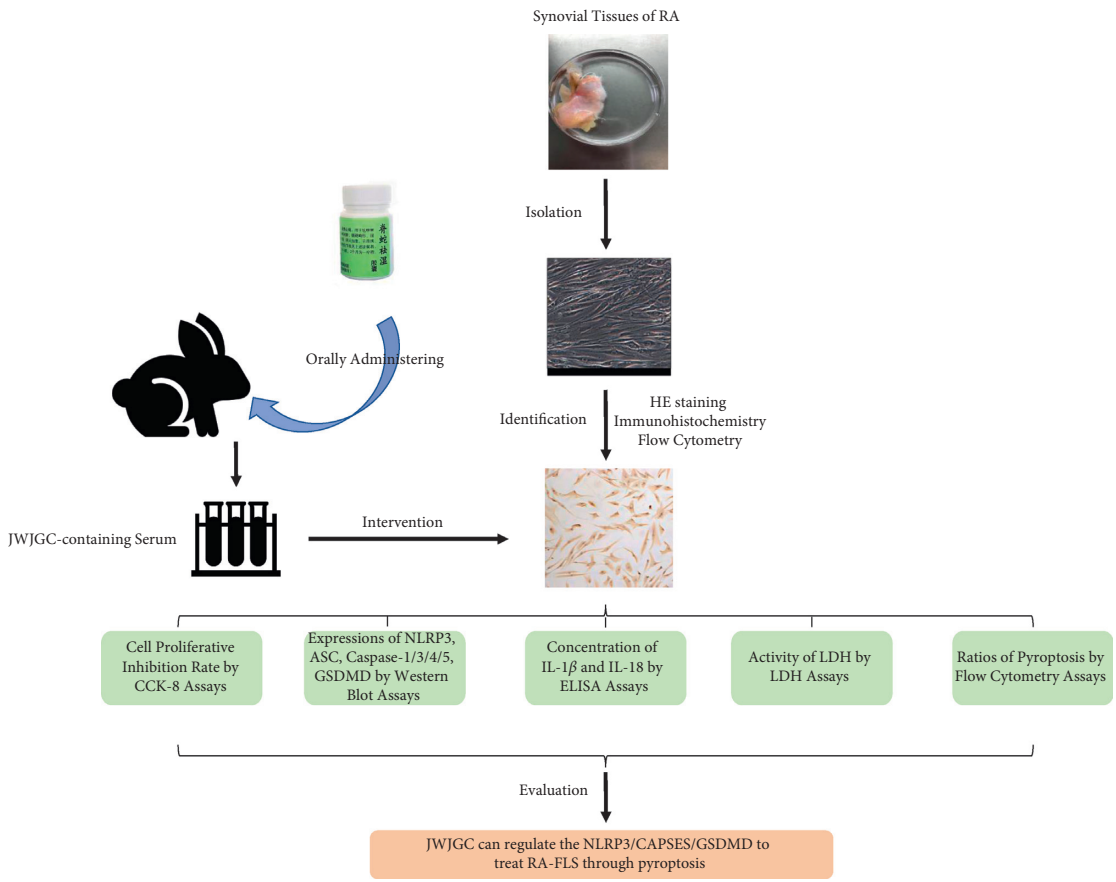

Figure 1: The work flowchart of this study.

aseptic conditions. The serum was separated by centrifugation at 3000 rpm for 20 min, inactivated at 56°C for 30 min, sterilized with 0.22 μm millipore filters, and stored separately at −20°C for the follow-up study. The dose per mg administered to the rabbits was calculated as follows: $M_1 = M_2 \times 3.08$, where M_1 is the dose per kg rabbit per day and M_2 is the dose per kilogram of adults per day.

2.3.4. RA-FLS Treatments. The cells were divided into six groups according to the treatment. The blank serum control group was treated with DMEM supplemented with 20% fetal bovine serum and 1% penicillin-streptomycin, whereas the rabbit serum control group was treated with DMEM supplemented with 20% rabbit serum and 1% penicillin-streptomycin. The JWJGC low-dose group (JWJGC-L) was treated with DMEM supplemented with 5% JWJGC-containing serum, 15% rabbit serum, and 1% penicillin-streptomycin. The JWJGC medium-dose group (JWJGC-M) was treated with DMEM supplemented with 15% JWJGC-containing serum, 5% rabbit serum, and 1% penicillin-streptomycin. The JWJGC high-dose group (JWJGC-H) was treated with DMEM supplemented with 20% JWJGC-containing serum and 1% penicillin-streptomycin. The leflunomide-positive control group was treated with DMEM supplemented with 20% leflunomide-containing serum and 1% penicillin-streptomycin.

2.4. Cell Counting Kit-8 (CCK-8) Assay. The CCK-8 assay (CCK-8; NW595, Dojindo, Japan) was used to detect the cell proliferative inhibition rate according to the manufacturer's protocol. Briefly, the third passage cells were seeded in 96-well plates (6×10^3 cells/well) and incubated at 37°C and 5% CO_2 for 24 h. After the cells were subjected to 180 μL of different treatments for 24 h or 48 h, 20 μL CCK-8 solution was added to each well and further incubated for 2 h at 37°C. The absorbance values were measured at a wavelength of 450 nm using a microplate reader (1510, Thermo Fisher Scientific, Waltham, MA, USA). Cell proliferative inhibition rate = (OD value of contrast well − OD value of experimental well)/(OD value of contrast well − OD value of blank well) × 100%.

2.5. Western Blot Assays. The third passage cells (1.2×10^6 cells) underwent different treatments in a 75 cm^2 culture flask for 24 h. The supernatants were preserved at −20°C for the succeeding assays. Total proteins were extracted from cells with RIPA lysis buffer (20170510, Solarbio, China), followed by quantification using a BCA protein concentration determination kit (20190921, Solarbio, China) according to the manufacturer's protocol. Cell lysates containing 40 μg total protein were loaded onto 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels (1610185, Bio-Rad, USA). The separated protein bands were

transferred onto nitrocellulose membranes. After blocking with 5% skim milk in Tris-buffered saline-Tween (TBST) for 1.5 h, membranes were incubated overnight at 4°C with different primary antibodies, including those against NLRP3 (ab210491, Abcam, UK; 1 : 1000), caspase-1 (ab62698, Abcam, UK; 1 : 500), caspase-3 (ab13847, Abcam, UK; 1 : 500), caspase-4 (ab238124, Abcam, UK; 1 : 1000), caspase-5 (ab40887, Abcam, UK; 1 : 1000), GSDMD (ab210070, Abcam, UK; 1 : 1000), and apoptosis-associated speck-like protein containing CARD (ASC) (ab151700, Abcam, UK; 1 : 1000). The membranes were washed five times with TBST, and then, peroxidase-conjugated goat anti-rabbit IgG antibody (019189, Pumei, China; 1 : 10000) was used as a secondary antibody and incubated for 1 h. After five more washes with TBST, an enhanced chemiluminescence substrate (170-5060, Bio-Rad, USA) was used to detect the amount of target proteins. ImageJ software was used to quantify the relative intensity following normalization with β-actin (4970S, Cell Signaling, USA; 1 : 1,000).

2.6. ELISA Assays. The supernatants originated from "2.5" thawed at room temperature, and concentrations of IL-1β (ab214025, Abcam, UK) and IL-18 (ab215539, Abcam, UK) were determined by ELISA kits according to the manufacturer's protocol.

2.7. LDH Assays. Lactic dehydrogenase (LDH), a key feature of cells undergoing apoptosis, necrosis, and other forms of cellular damage, is rapidly released into the cell culture supernatant when the plasma membrane is damaged. The activity of LDH was tested using LDH assay kits (20200630, Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. From "2.5," 25 μL of supernatant was moved to a 96-well plate, and 25 μL of reconstituted substrate mix was added to each well. The plate was then incubated for 15 min in a dark room at 37°C. Then, 25 μL of 2,4-dinitrophenylhydrazine was added to each well and incubated for 5 min in a dark room at 37°C. Moreover, 250 μL NaOH (0.4 mol/L) was added to each well and incubated for another 5 min in a dark room at 37°C. Absorbance was recorded at 450 nm using a microplate reader (1510, Thermo Fisher Scientific, Waltham, MA, USA). Activity of LDH (U/L) = (OD value of experimental well − OD value of contrast well)/(OD value of standard well − OD value of blank well) × 0.2 μM.

2.8. Flow Cytometry Assays. After the third passage cells (1.2×10^6 cells) underwent different treatments, the cells were incubated in 5 μL of FITC anticaspase-1 (SC-392736, Santa Cruz Biotechnology, USA) in a dark room at 37°C for 1 h, washed with 2 mL of apoptosis buffer, and centrifuged. Then, 5 μL of PI was added to the tube, followed by another incubation for 15 min. Consequently, the cells were resuspended in 500 μL of apoptosis buffer and analyzed by flow cytometry (FACSCanto II, BD, USA).

2.9. Statistical Analysis. Statistical analysis was performed using the SPSS 17 software program (SPSS Inc., USA). The one-way analysis of variance was used to compare the means of the different groups. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. H&E Staining, Immunohistochemistry, and Isolation Purity. The third passage RA-FLS suffered from H&E staining and immunohistochemistry after being purified by passage culture. The cytoplasm was red and the nucleus was blue in H&E staining (Figures 2(a) and 2(b)), while the cells were spindle-shaped with blue nuclei and brown cytoplasm in immunohistochemistry (Figures 2(c) and 2(d)). Immunohistochemistry results indicated that vimentin was positive. Tissue origin, cell shape, and positive immunohistochemistry results proved that the observed cells were RA-FLS. The purity of the isolated cells was 96.6 ± 1.44% ($n = 4$) marked with FITC anti-CD90 or 97.1 ± 0.39% ($n = 4$) with APC anti-VCAM-1 (Figures 2(e), 2(f), 2(g), and 2(h)).

3.2. JWJGC Affected Cell Proliferation. Compared with the blank serum control group, there was no significant difference in the cell proliferative inhibition rate in the rabbit serum control group ($P > 0.05$). However, it was significantly elevated in the JWJGC-L, JWJGC-M, JWJGC-H, and leflunomide-positive control groups, relative to the rabbit serum control group ($P < 0.05$). Moreover, the cell proliferative inhibition rate was proportional to the concentration of JWJGC-drug serum and the time of the intervention (Figure 3(a)).

3.3. JWJGC Affected the Expressions of NLRP3, ASC, Caspase-1/3/4/5, and GSDMD. There was no significant difference in the expression of NLRP3, ASC, caspase-1/3/4/5, and GSDMD between the blank serum control group and the rabbit serum control group ($P > 0.05$). However, compared with the rabbit serum control group, caspase-1/3/4/5 and GSDMD displayed decreased expression in the JWJGC-L, JWJGC-M, JWJGC-H, and leflunomide-positive control groups ($P > 0.05$), NLRP3 expression was reduced only in the JWJGC-H group, and ASC declined in the JWJGC-M, JWJGC-H, and leflunomide-positive control groups ($P < 0.05$) (Figure 4).

3.4. JWJGC Affected IL-1β and IL-18 Secretion. JWJGC did not affect the secretion of IL-1β and IL-18 after treatment in both the blank and rabbit serum control groups ($P > 0.05$). Nevertheless, the concentrations of IL-1β and IL-18 were both lower in JWJGC-L, JWJGC-M, JWJGC-H, and leflunomide-positive control groups than those of the rabbit serum control group ($P < 0.05$) (Figures 3(b) and 3(c)).

3.5. JWJGC Affected LDH Activity. The activity of LDH in the rabbit serum control group did not change significantly compared with that in the blank serum control group ($P > 0.05$). The activity of LDH in the JWJGC-L, JWJGC-M,

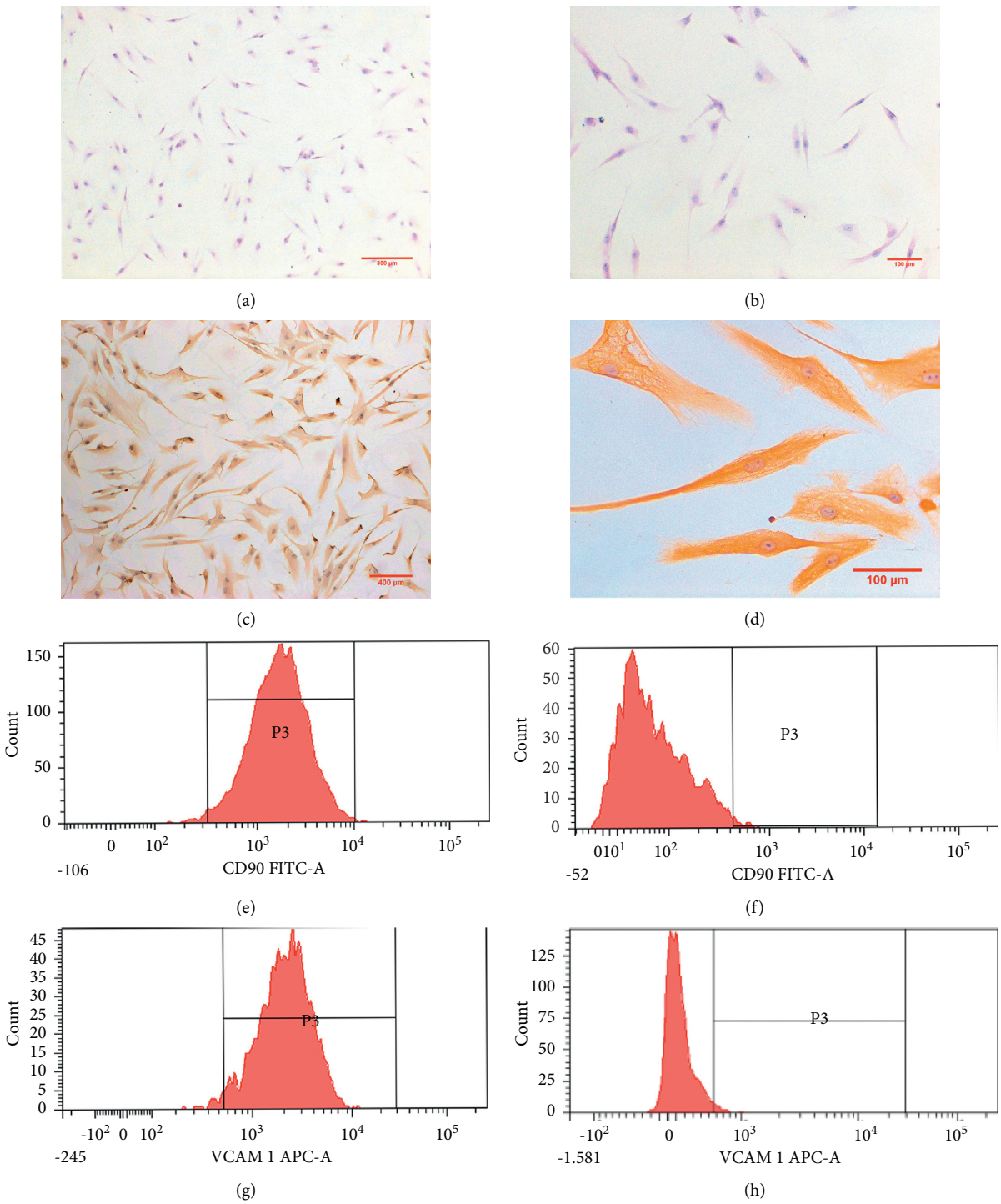

Figure 2: (a) (×100) and (b) (×200) showing the H&E staining results. Immunohistochemistry results are shown in (c) (×100) and (d) (×200). (e) The purity of the isolated cells marked with FIDC anti-CD90 (96.6 ± 1.44%; $n = 4$). (f) The control. (g) The purity of the isolated cells marked with APC anti-VCAM-1 (97.1 ± 0.39%; $n = 4$). (h) The control.

JWJGC-H, and leflunomide-positive control groups was inhibited, compared with the rabbit serum control group ($P < 0.05$) (Figure 3(d)).

3.6. JWJGC Affected Pyroptosis in RA-FLS. The pyroptosis of the RA-FLS was marked with double positivity for FITC antcaspase-1 and PI, which was shown in the Q2 of every image. There was no significant difference between the blank and rabbit serum control groups ($P > 0.05$). However, the ratios of pyroptosis were significantly blocked in the JWJGC-L, JWJGC-M, JWJGC-H, and leflunomide-positive control groups, compared with the rabbit serum control group ($P < 0.05$) (Figure 5).

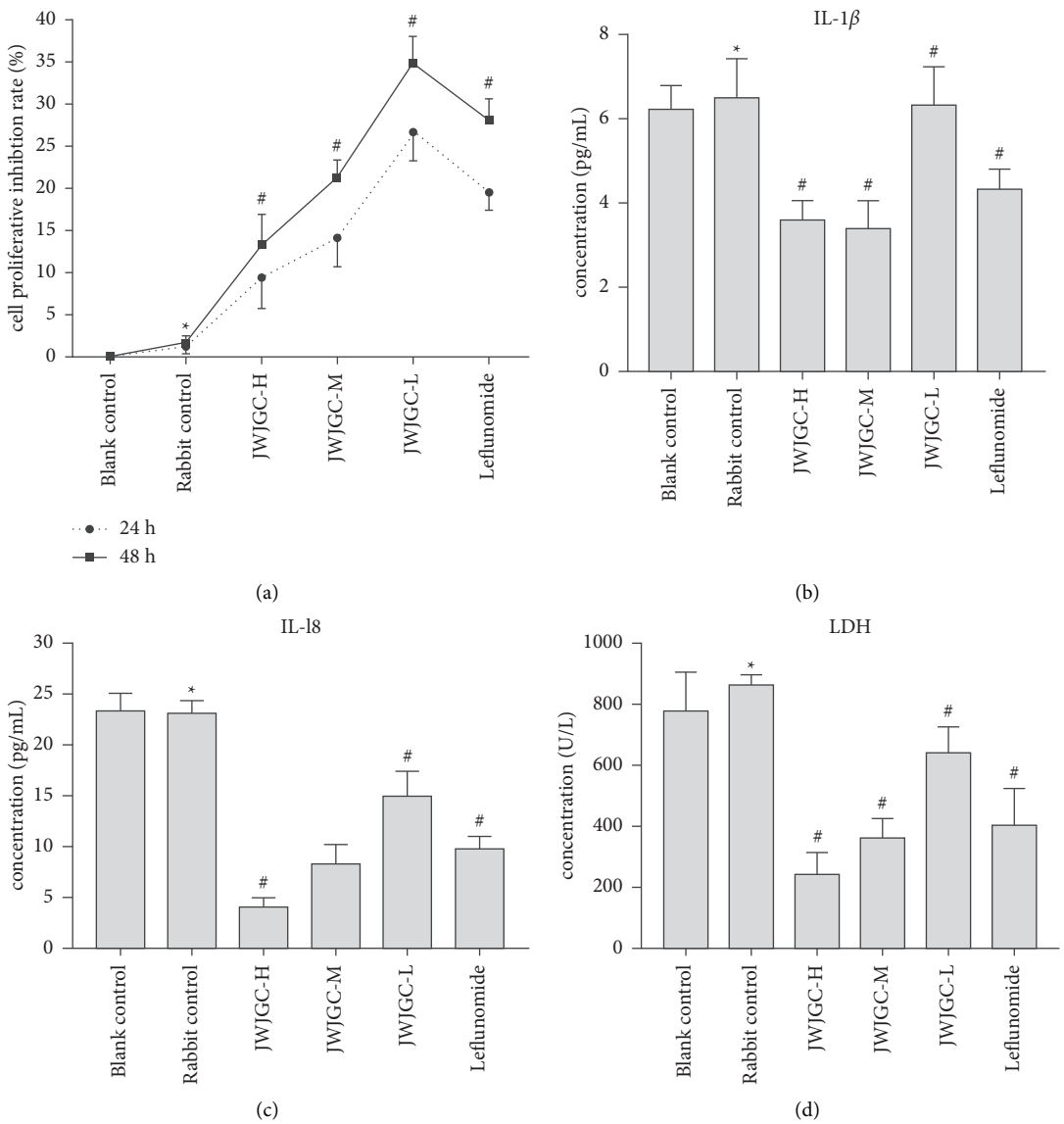

Figure 3: (a) The cell proliferative inhibition rate after the different treatments for 24 h or 48 h. (b)-(c) The concentrations of IL-1β and IL-18. (d) The concentrations of LDH. Data are expressed as mean ± SEM ($n = 5$). $^{*}P > 0.05$, compared with blank control; $^{\#}P < 0.05$, compared with the rabbit group. Blank control, blank serum control group; rabbit control, rabbit serum control group; JWJGC-H, JWJGC high-dose group; JWJGC-M, JWJGC medium-dose group; JWJGC-L, JWJGC low-dose group; Leflunomide, leflunomide-positive control group.

4. Discussion

RA is an autoimmune disease characterized by hyperplastic synovial tissue that eventually destroys the cartilage and bone. In its pathological process, the synovium is transformed from a relatively acellular structure to a hyperplastic and invasive tissue, and the lining layer expands from 1 to 2 cells to a depth of approximately 10–20 cells [11]. The resident cells in the synovial membrane lining layer are FLS (type B synovial cells) and synovial tissue macrophages (type A synovial cells) [12]. FLS are the target cells in the development of destructive joint inflammation of synovial tissue in RA with an overproduction of enzymes and infiltration of immune cells [13]. RA-FLS show unique invasive characteristics, which are autonomous and vertically transmitted, and these cells function as the main promoters of inflammation [14]. However, the ability of RA-FLS to proliferate in joints is limited, and its abnormal proliferation is brought about by the change in programmed cell death accompanied by increased expression of cytokines to some extent [15]. In addition, its ability to secrete cytokines, chemokines, and angiogenic factors is enhanced, and the unbalanced cytokine network leads to the enhancement of the inflammatory response, which eventually develops into RA [16]. In the present study, the cell proliferative inhibition rate changed with the time of intervention and concentrations of JWJGC-drug serum, indicating that JWJGC improved the tumor-like growth characteristics of RA-FLS to inhibit proliferation in the treatment of RA.

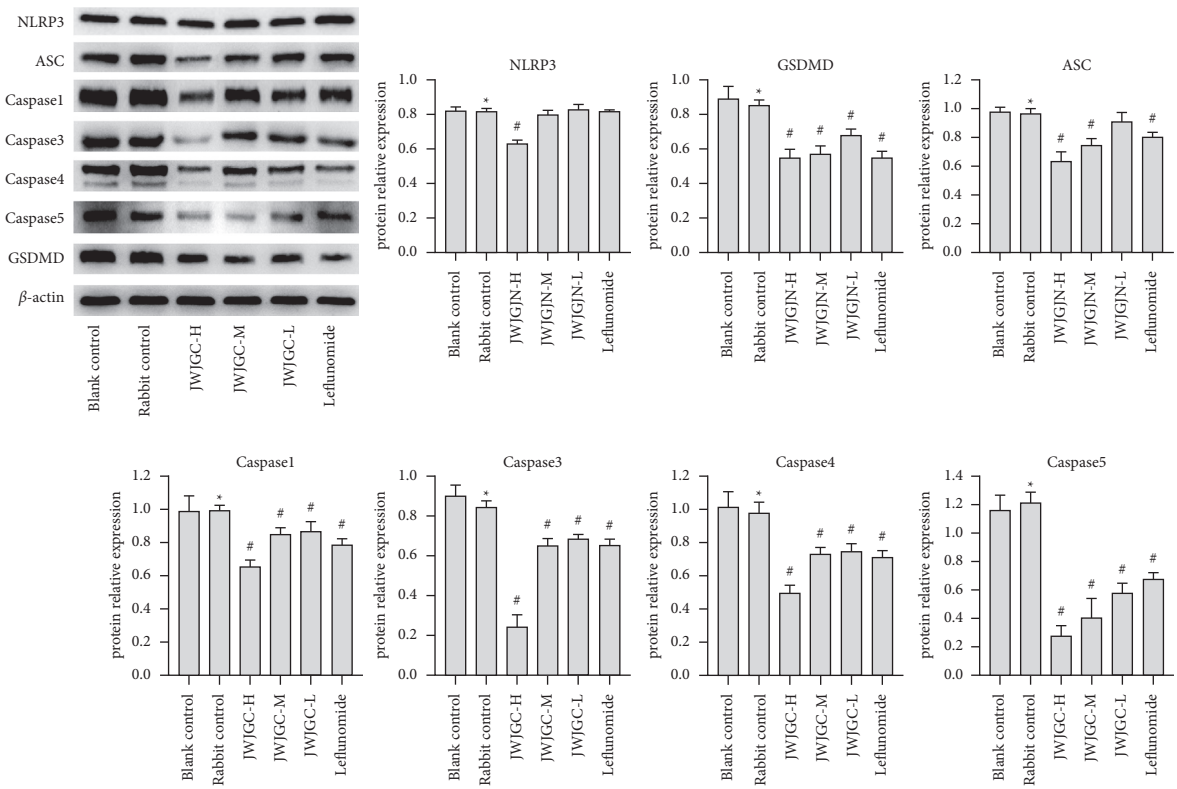

Figure 4: Western blot bands of the expression of NLRP3, ASC, GSDMD, and caspase-1/3/4/5 after the different treatments. Data are expressed as mean ± SEM ($n = 3$). $^{*}P > 0.05$ compared with blank control; $^{\#}P < 0.05$ compared with the rabbit group. Blank control, blank serum control group; rabbit control, rabbit serum control group; JWJGC-H, JWJGC high-dose group; JWJGC-M, JWJGC medium-dose group; JWJGC-L, JWJGC low-dose group; Leflunomide, leflunomide-positive control group.

NLRP3 is a cytosolic signaling complex that mediates inflammatory responses and is often involved in the pathogenesis of noninfectious diseases. It responds primarily to danger signals, such as aging, overnutrition, or environmental changes [17]. It is mainly expressed in monocytes, macrophages, dendritic cells, and neutrophils; however, elevated levels of NLRP3 expression are also observed in RA patients [18]. ASC connects NLRP3 and procaspase-1, which is involved in the priming phase of pyroptosis [19]. Caspase-1 not only matures IL-1β and IL-18 but also induces membrane perforation through GSDMD [20]. Caspase-3/4/5 are unable to activate pro-IL-1β and pro-IL-18, but they can induce pyroptosis by GSDMD or GSDME [21,22]. In this study, the expression of NLRP3, caspase-1/3/4/5, ASC, and GSDMD declined after treatment with JWJGC-drug serum, implying that GWJGC could downregulate the expression of NLRP3, caspase-1/3/4/5, ASC, and GSDMD.

IL-1β is one of the primary explicit members of the IL-1 family and is produced by myeloid cells. It initiates innate immunity and forms an adaptive immune response during acute inflammation [23]. However, if IL-1β is overactivated, chronic oxidative stress, oxidative damage of DNA sequence, epigenetic changes, and autoimmune diseases may occur [24, 25]. IL-18 belongs to the IL-1 family and promotes the differentiation and production of cytokines based on specific receptors on the cell membrane [26]. For example, IL-18 mediates the expression of IL-1, TNF-α, and IL-6 via the NF-κB pathway [27]. IL-1β and IL-18 are present in the cytoplasm at rest, without biological function; however, when caspases are activated by inflammasomes, IL-1β and IL-18 are activated. Most of the cytokines are secreted from cells through the endoplasmic reticulum to exert biological effects, while IL-1β and IL-18 cannot be released into the extracellular environment through the endoplasmic reticulum pathway because of the absence of an N-terminal secretion signal sequence. However, they can be transported outside with the assistance of the GSDMD protein related to perforation [28]. In these experiments, the concentrations of IL-1 and IL-18 were decreased by treatment with JWJGC-drug serum, indicating that JWJGC could reduce the secretion of IL-1 and IL-18.

Programmed cell death is the main mechanism of the cellular metabolism that maintains growth and immune homeostasis. Human programmed cell death patterns mainly include apoptosis, necrosis, autophagy, and pyroptosis, which are both morphological and biochemical [29]. Pyroptosis was discovered in the 1990s and was formally named in the early 21st century to distinguish it from cell necrosis and apoptosis [4]. Pyroptosis is divided into classical and nonclassical pathways according to the caspases [30]. In the classical pathway, inflammasomes are polymeric protein complexes composed of PRR, procaspase-1, and

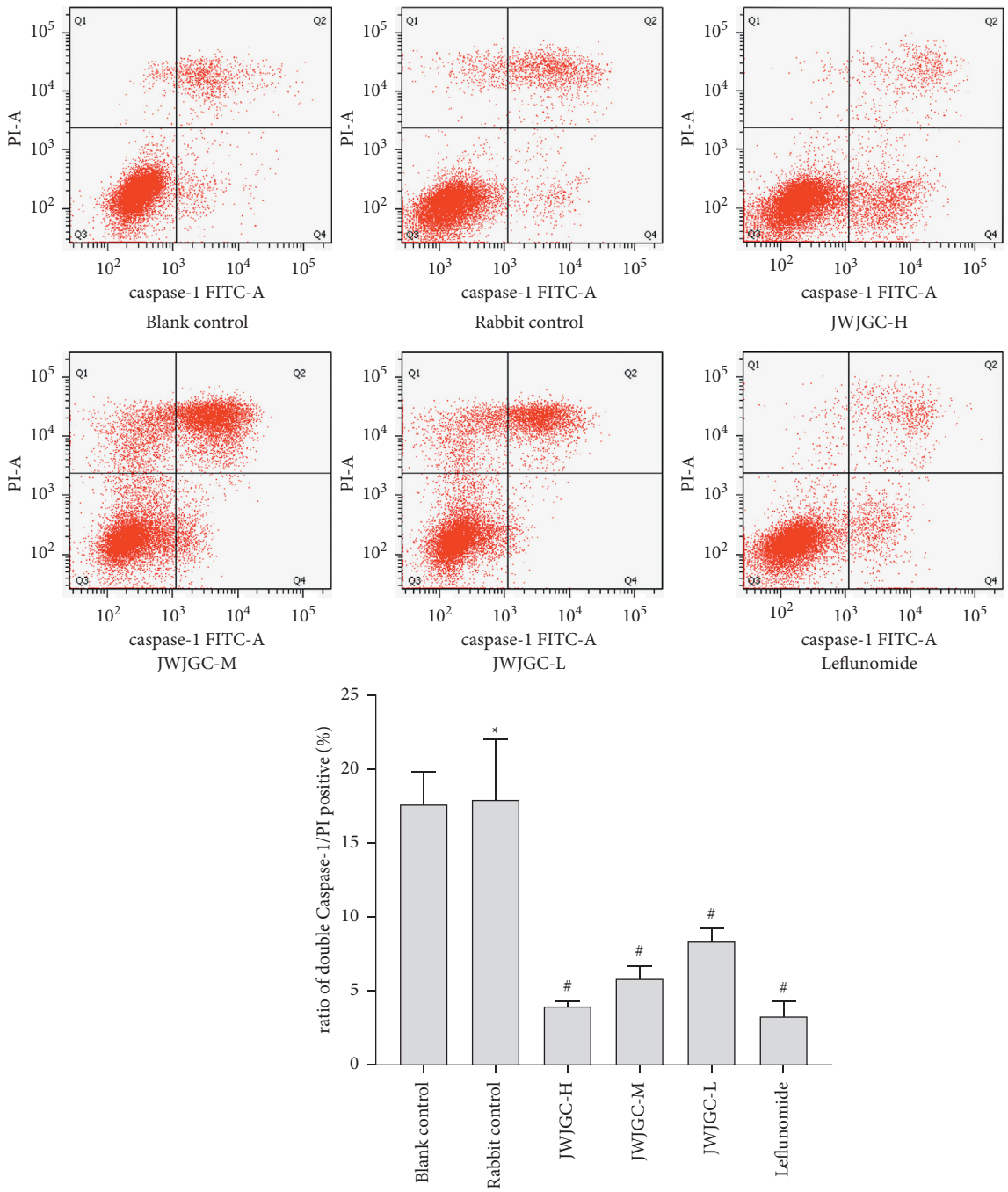

FIGURE 5: Double-positive caspase-1/PI is shown in the Q2 of every image. Data are expressed as mean ± SEM ($n = 3$). $^{*}P > 0.05$ compared with blank control; $^{\#}P < 0.05$ compared with the rabbit group. Blank control, blank serum control group; rabbit control, rabbit serum control group; JWJGC-H, JWJGC high-dose group; JJWJGC-M, JWJGC medium-dose group; JWJGC-L, JWJGC low-dose group; Leflunomide, leflunomide-positive control group.

ASC, which activate caspase-1 [31–34]. Subsequently, active caspase-1 converts pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18 [35]. Meanwhile, the N-terminus of GSDMD forms pores via active caspase-1. GSDMD p30 is localized to the lipid bilayer, whereas GSDMD p20 remains in the water environment. In liposomes, p30 exists as a high-level oligomer with a circular structure to recognize phospholipid molecules on cell membranes. Eventually, 18 nm channels were formed, which means pyroptosis occurred [36–38]. In the nonclassical pathway of pyroptosis, humans are mediated by caspase-3/4/5, and mice are mediated by caspase-11 [39, 40]. LDH activity reflects the content of the damaged cell membrane because LDH can only be released into the extracellular space through such membrane [41]. The results showed that the number of pyroptotic cells and the activity of LDH were lower with the treatment of JWJGC-drug serum, which demonstrated that GWJGC could prevent pyroptosis.

5. Conclusions

JWJGC inhibited the proliferation of RA-FLS by down-regulating the expression of NLRP3, ASC, caspase-1/3/4/5, and GSDMD, reducing the secretion of IL-1β and IL-8, repressing the activity of LDH, and decreasing the double-positive FITC anticaspase-1 and PI. Collectively, our results demonstrate that JWJGC can treat RA-FLS by regulating pyroptosis via the NLRP3/CAPSES/GSDMD pathway. Further studies on the regulation of genes need to explore the therapeutic mechanisms underlying JWJGC effects in RA.

Data Availability

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

Ethical Approval

The study protocol was approved by the Animal Management Center and the Medical Ethical Committee of the Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, China.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yi Ling, Mao Xiao, and Zhao-Wei Huang contributed equally to this work.

Acknowledgments

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