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Research Article
Evaluation of Effects of a Chinese Herb Formula on Adjuvant Induced Arthritis in Rats

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Abstract

Background and Objective: QingFengTeng (*Sinomenii caulis*) and GuiZhi (*Cmnamomi mmulus*) were used together in the treatment of rheumatoid arthritis (RA) with high frequency in China. However, there are few researches on the therapeutic effects of the combination of the two herbs on RA. The present study was to investigate the anti-RA activities of different combination of the two herbs on adjuvant induced arthritis (AA) rats and further explore its possible mechanism. **Materials and Methods:** Four herb extracts were prepared with 70% ethanol from different combination of the two herbs. The AA rats were induced by intradermal injection of complete Freund's adjuvant (CFA) in left hind foot pad of rat. Sinomenone hydrochloride tablets (Sin) was chosen as the positive drug. Paw swelling and histopathological changes were evaluated. The serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α) were detected. **Results:** The results showed all the ethanol extracts of the formula ameliorated AA symptoms. Extract (4:1) and extract (3:1) significantly decreased the paw swelling, greatly elevated the levels of IL-10, got better results in improving pathological changes and their efficacies were slightly superior to the other treatment groups. **Conclusion:** For the first time, the suppressive mechanism of a formula composed of QingFengTeng and GuiZhi on AA rats were studied. These results indicated that the herb formula may be a potential candidate for the treatment of RA.

Key words: *Sinomenium acutum*, *Cinnamomum cassia* Presl, Chinese herb combination, adjuvant induced arthritis, serum levels

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The RA is a chronic inflammatory disease which affects the joints, causes pain, stiffness and inflammation of the synovial membrane, leading to articular destruction[1]. The conventional medication treatments for RA are non-steroid anti-inflammatory drugs (NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs). The former can relieve pain and symptoms, but cannot change the progression of the disease or prevent joint destruction[2]. The clinical efficacy of DMARDs is limited with many side effects such as gastrointestinal irritation, bone marrow suppression, liver and kidney damage, etc[3]. According to reports in the literature, RA affects about 0.24% of the global population and it has a higher disability rate which is just below malaria and just above iodine deficiency[4]. Therefore, the exploration of new anti-RA drugs with high efficacy and less toxicity is imminent.

QingFengTeng, the stem of plant *Sinomenium acutum* (Thunb.) Rehd et Wils (Menispermaceae), was first recorded in Ben Cao Tu Jing of the Song Dynasty with the functions of dispelling wind, eliminating dampness, dredging the channels and relieving pains[5]. Chinese people have been using the herb to treat RA for hundreds of years. In clinical, single QingFengTeng, or its main efficiency component, Sinomenine (a pure alkaloid isolated from QingFengTeng), or its complex prescriptions have been used for the treatment of RA mainly, as well as osteoarthritis, ankylosing spondylitis and arrhythmia[6,7]. QingFengTeng related preparations were also used as adjuvant agents combined with methotrexate to treat RA and achieved better efficacy and fewer adverse events[8,9].

GuiZhi, the tender twig of plant *Cinnamomum cassia* presl (Lauraceae), was initially recorded in Shen Nong Ben Cao Jing with the functions of expelling the evils in muscles by means of diaphoresis and warming channels and dredging collaterals. GuiZhi rarely used alone in clinical. It was often used in combination of Gancao (*Glycyrrhizae Radix Et Rrhizoma*), ChaiHu (*Bupleuri Radix*), FuZi (*Aconiti Lateralis Radix Praeparata*), ShaoYao (*Paeoniae Radix Alba*), FuLin (Poria), HuangQi (*Astragali Radix*), ZhiMu (*Anemarrhenae Rhizoma*) etc. Clinical application of GuiZhi GanCao Decoction focused on cardiovascular system such as arrhythmia, hypotension, cardiac neurosis treatment, etc.[10]. ChaiHu GuiZhi Decoction was mainly used for the treatment of colds, children recurrent respiratory infections, fever, gastritis, etc.[11]. Recently it was used as an anti-depressant[12]. GuiZhi FuZi Decoction, GuiZhi ShaoYao Decoction, GuiZhi ShaoYao ZhuMu Decoction[13] and GuiZhi HuangQi Decoction have been used to treat RA, acute gouty arthritis and osteoarthritis[14-16]. Some famous formulas were developed to new formulation instead of decoctions. For example, GuiZhi Fuling capsule (composed of *Cinnamomi Ramulus, Poria, Moutan Cortex*, *Persicae Semen* and *Radix Paeoniae Alba*) has achieved satisfactory effects in the treatment of uterine fibroids, dysmenorrheal, pelvic inflammatory disease, endometriosis, ovarian cysts, breast hyperplasia and other gynecological diseases[17].

The treatment rules of QingFengTeng were summarized by text mining techniques based on the related literatures about it from Chinese Biomedical Literature (CBM) Database[18]. The conclusion was that QingFengTeng was mainly used on anti-RA, involving rheumatoid diseases such as spondylitis, ankylosing spondylitis, systemic lupus erythematosus, gout, etc. Moreover, It was often used in combination with herbs such as *Angelicae Sinensis Radix*, *Radix et Rhizoma Tripteryg*, *Paeoniae Radix Alba*, *Angelicae Pubescentis Radix*, *Gentianae Macrophyllae Radix*, *Cinnamomi Ramulus* (GuiZhi), *Astragali Radix, Glycyrrhizae Radix et Rhizoma*. In fact, QingFengTeng and GuiZhi often appeared together in the complex prescriptions used for anti-RA clinically[19-22], involving those of famous professors[23-25]. However, there are few researches on the therapeutic effect of the combination of the two herbs on RA. Therein, in the present study, the anti-RA activities of different combination of the two herbs on AA rats were investigated and further its possible mechanism was explored.

MATERIALS AND METHODS

The study was carried out between January and July, 2017 at Pharmacology and Pharmaceutical Laboratory of School of Pharmacy at Anhui University of Traditional Chinese Medicine.

Reagents: Freund's complete adjuvant (FCA) was purchased from Sigma Chemical Co (St. Louis, Mo). The ELISA test kits were purchased from Sen Xiong Technology Industrial Co., Ltd. (Shanghai, China). Sinomenone hydrochloride tablets were purchased from Zhengqing Pharmacy Co., Ltd. (Hunan, China). Chromatographically pure reagent acetonitrile was purchased from Sigma Chemical Co. All other chemicals and reagents used for study were of analytical grade procured from approved organizations.

Plant material and preparation of ethanol extracts: Crude slices of these two herbs were purchased from TCM markets (Bozhou of Anhui, China) and authenticated by Prof. Yu, L.J. (Anhui University of Chinese Medicine, Hefei of Anhui, China). Two voucher specimens (HJ20150925-10, HJ20150925-11) have been deposited in the School of Pharmacy, Anhui University of Chinese Medicine, Anhui, China.

Dried raw material of two herbs was powdered and extracted with 70% ethanol (analytically pure) 3 times (1 h/time). These initial crude extracts were concentrated under reduced pressure and the concentrations were coded (about 0.3 g mL^{-1}) and weighed for the study. The different ratios of extracts were prepared by this method.

Qualitative analysis of extract: Chromatographic analysis was performed on the Waters Xevo G2 Q-Tof series UPLC-MS system (Waters Technologies, USA). Separation and detection was carried out on gradient UPLC system equipped with UV detector. The C_{18} reverse-phase UPLC column (ACQUITY UPLC BEH C_{18} 1.7 μm 2.1×100 mm) was utilized with acetonitrile and water (adjusted with 0.3% formic acid) as mobile phase A and B, respectively under a gradient program. The gradient profile was 0-3 min, 88-85% solvent B; 3-5 min, 85-82% solvent B; 5-10 min, 82-77% solvent B; 10-15 min, 77-70% solvent B; 15-22 min, 70-60% solvent B; 22-25 min, 60-50% solvent B and returned to initial 88% and held for 2 min. The flow rate and injection volume were 0.2 mL min^{-1} and 5 μL, respectively. The column temperature was set at 30°C. The chromatographic conditions for UPLC-TOF-MS analysis were the same as those used for UPLC-DAD fingerprint. After separation on Waters liquid chromatography system (Waters Corp., Milford, MA, USA), mass spectra in positive ion mode was acquired on AB Triple TOF 5600 plus System (AB SCIEX, Framingham, USA) with the following conditions: Source voltage 2 kV, source temperature 120-350, declustering potential (DP) 100 V and collision energy (CE), 10-30 V. For MS/MS acquisition mode, the parameters were almost the same except that the CE was set at 50±35 V. The IDA-based auto-MS2 was performed on the most intense metabolite ions in a cycle of full scan in the range of m/z 100-1200.

Quantitative analysis of extract: The HPLC system (Shimadzu, Kyoto, Japan) consisted of LC-16 solvent delivery module, SPD-16 UV-visible spectrophotometric detector, a CTO-16 column oven and a manual sampler. Separation was achieved on a C^{18} column (4.6 mm×250 mm, 5 μm, Luna, Phenomenex, Torrance, CA). The mobile phase was composed of acetonitrile (A) and 0.2% phosphoric acid (B). The flow rate was set at 1 mL min^{-1} and the column temperature was maintained at 30°C. The gradient profile was 0-10 min, 85-80% solvent B; 10-15 min, 80-75% solvent B; 15-16 min, 75-65% solvent B; 16-31 min, 65-50% solvent B and 31-45 min, 50-0% solvent B and held at 0% for 15 min, then the composition was returned to the initial condition in 1 min and held for 2 min. The mobile phase was filtered through a Millipore 0.45 μm filter and degassed prior to use. The peaks were detected at 254 nm and sinomenine, cinnamic alcohol, cinnamic acid and cinnamaldehyde were detected by comparing individual peak retention times with those of the standard materials.

Experimental animals: Male Sprague-Dawley rats (180±20 g, Grade, Certificate SYXK2015-002) were purchased from the Experimental Animal Center of Anhui Medical University (Hefei, China). Rats were kept in plastic cages at 25±1 and 55±5% relative humidity under a 12 h light/12 h dark cycle environment and standard food and water were given *ad libitum*. Animals were acclimated in the laboratory for at least 1 week prior to experiment. All experimental protocols were approved by the Committee on Ethics of Animal Experiments, Anhui University of Chinese Medicine (Hefei, China) and were conducted in compliance with the Guidelines for Animal Experiments of Anhui University of Chinese Medicine.

Experimental protocol: The AA model was induced by the chemical reagents method as previously described[26]. Before the onset of arthritis, forty-eight rats with AA were divided into 6 groups randomly with eight in each group: Four ratios of extract groups (2:1, 3:1, 4:1, 5:1, respectively, 1.5 g kg^{-1}), Sin group (positive control group, 25 mg kg^{-1}) and AA group (an equal volume of CMC-Na). Sin was chosen as a positive drug. Additionally, as normal control group, eight rats were given an equal volume of vehicle (CMC-Na) at the same time. AA rats were treated intragastrically once a day from day 17-23 after immunizations.

Evaluation of paw swelling: Paw swelling was determined by measurement of the paw volume using a Volume Meter (PV-200, TaiMeng Technology and Science Development Co., Ltd., China). Measurements were obtained just before CFA injection on day 0 and thereafter continued at every 3 days from day 11-23.

Detection of cytokines: At the end of the experiment, all the animals were sacrificed using a dose of 30 mg kg^{-1} of pentobarbital. Blood samples were obtained from carotid artery and centrifuged at 3000 rpm for 15 min and then the supernatants were collected and stored at -80°C for cytokine analysis. The levels of IL-1β, IL-6, IL-10 and TNF-α were evaluated by ELISA kits in accordance with the manufacturer's instructions.

Histological analysis: Rats were sacrificed on day 24 after drawing blood from carotid artery. Left hind paws were harvested from the knee joint, fixed in 10% (V/V) neutral buffered formalin for 24 h, paraffin-embedded and cut into 4 μm thicknesses for hematoxylin-eosin (HE) staining and histopathological assessments. The observation of cell infiltration, pannus formation and edema was conducted under a 130 common light microscope.

Statistical analysis: All the results were expressed as mean±standard deviation (SD) and carried out by Graphpad Prism version 6.0. One-way ANOVA was used for determining the statically significant differences between the values of various experimental groups. The minimal level of statistical significance was considered at p-values less than 0.0001 ($p<0.0001$).

RESULTS

Analysis of HPLC-TOF/MS: The total ion flow chart was obtained by HPLC-TOF/MS analysis and it was shown in Fig. 1. The precise molecular weight of the chemical constituents obtained using TOF/MS was compared with the theoretical molecular weight of the known compounds in the self-built chemical composition database. With the initial qualitative analysis of chemical composition, a total of 14 kinds of ingredients were identified. In the mixture, the active ingredients of *Cassia Twig* were mostly volatile oils, which were not detected here. As shown in Fig. 1, there was no difference in chemical compositions between the extracts of single *Caulis Sinomenii* and two herbs combination.

In order to form better peak shape and avoid trailing, formic acid was added to elution phases, both in water and acetonitrile and better resolution was obtained. High response of $[M+H]^+$, $[M-H]^+$, $[M+K]^+$, $[M-CH_3]^+$ ions of determined analytes were detected in the sample solution. The elution gradient was also optimized to get the best resolutions for all peaks as far as possible.

Compared with the database collected[27-32], the protonated molecule ions $[M+H]^+$ of Compound 1, 3 at m/z 330, 356 identified as Sinomenine and Tetrahydropalmatine. Compound 2 and 14 yielded product ions at m/z 342, 684. They were both identified as Magnoflorine. According to the $[2M+2H]^+$ ions at m/z 566, Michelalbine was distinguished with the identical $[M+H]^+$ ions at m/z 282. Neobavaisoflavone gave the product ions at m/z 679 $[2M+H]^+$, 453 $[2M+H]^+$, 509 $[2M+H]^+$ corresponding to $C_{20}H_{21}NO_4$(L-diversine), $C_{16}H_{34}$ (Hexadecane) and $C_{18}H_{38}$ (X-pentadecane). In the MS analysis,

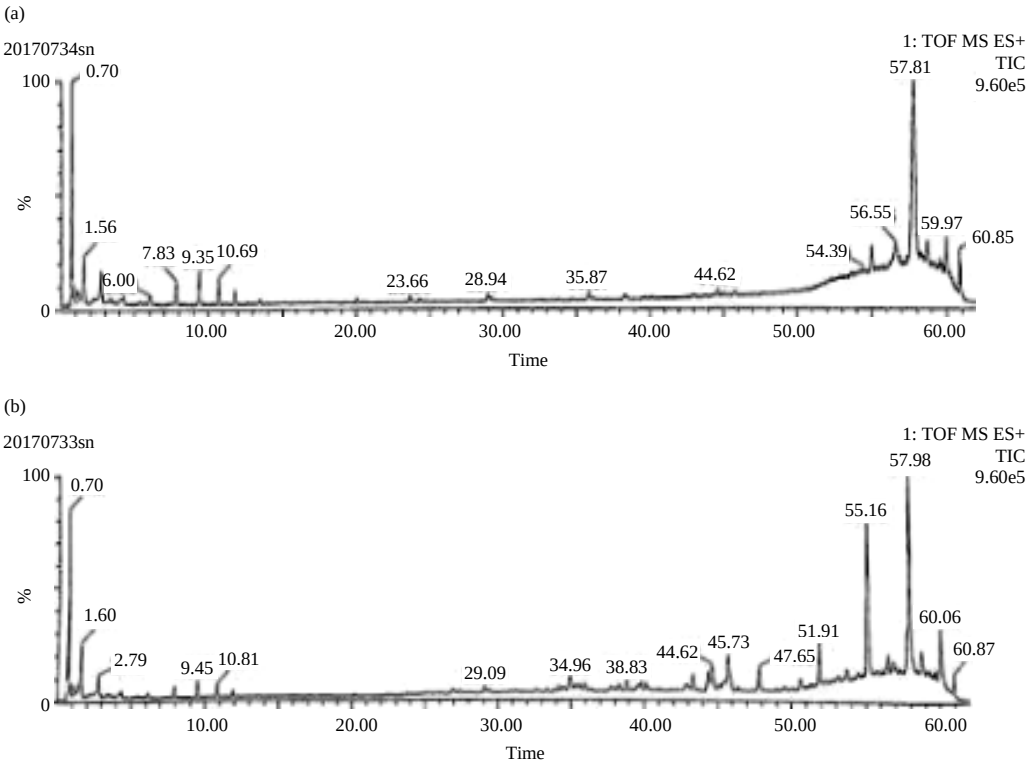

Fig. 1(a-b): Total ion flow chart of HPLC-TOF/MS TIC of extracts, (a) Extracts of *Caulis Sinomenii* and (b) Extracts of *Caulis Sinomenii* and *Cassia twig*

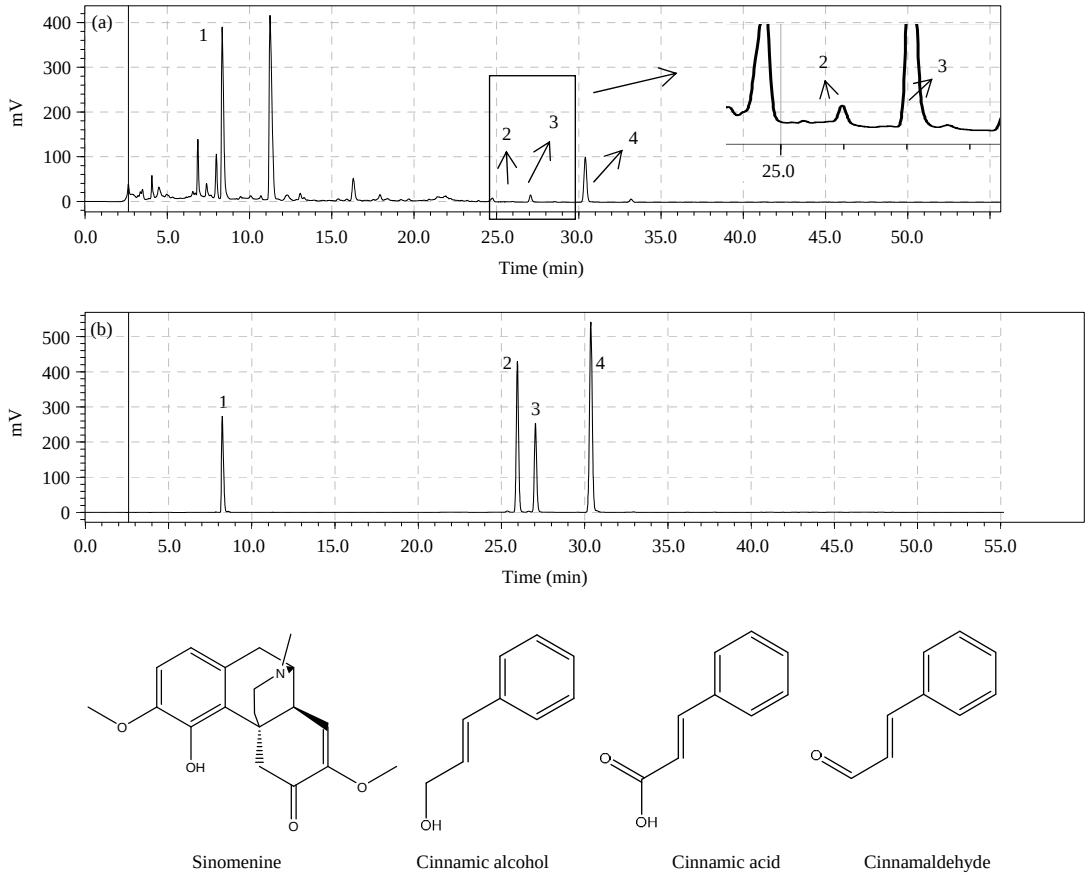

Fig. 2(a-b): HPLC analysis of chemical constituents in the extracts of the herb formula, (a) HPLC chromatogram of the sample used in the experiment and (b) HPLC chromatogram of standard reference. Peaks were detected at 254 nm (1: Sinomenine, 2: Cinnamic alcohol, 3: Cinnamic acid, 4: Cinnamaldehyde)

Table 1: Qualitative analysis of chemical constituents

RT/TR (min)	Identification	Formula	Selection ion	m/z
1.56	Sinomenine	$C_{19}H_{23}NO_4$	$[M+H]^+$	330.17
2.74	Magnoflorine	$C_{20}H_{24}NO_4$	$[M+H]^+$	342.17
4.25	Tetrahydropalmatine	$C_{21}H_{25}NO_4$	$[M+H]^+$	356.19
6.00	Michelalbine	$C_{17}H_{15}NO_3$	$[2M+2H]^+$	566.43
7.83	L-diversine	$C_{20}H_{21}NO_4$	$[2M+H]^+$	679.51
9.37	Acutu-mine	$C_{20}H_{21}NO_4$	$[2M-2H]^+$	792.60
10.69	Hexadecane	$C_{16}H_{34}$	$[2M+H]^+$	453.34
11.82	X-pentadecane	$C_{18}H_{38}$	$[2M+H]^+$	509.88
23.66	Unknown	$C_{24}H_{31}O_6$	$[M+H]^+$	415.21
28.94	Sinactine	$C_{20}H_{21}NO_4$	$[M+H]^+$	149.02
35.87	X-morphinan	$C_{19}H_{25}NO_4$	$[M-CH_3]^+$	317.23
55.01	Acetyl-oleanolic acid	$C_{32}H_{50}O_4$	$[M+H+K]^+$	536.16
56.55	Acetyl-oleanolic acid	$C_{32}H_{50}O_4$	$2[M+H+K]^+$	1072.3
59.97	Magnoflorine	$C_{20}H_{24}NO_4$	$2[M+H]^+$	684.2

Table 2: Quantitative analysis of the mixture by HPLC (n = 3)

Number	Sinomenine (mg)	Cinnamaldehyde (mg)
2:1	10.74±0.91	2.23±0.22
3:1	6.28±0.61	2.01±0.15
4:1	4.41±0.47	1.40±0.18
5:1	2.90±0.20	1.13±0.12

product ions at m/z 792 and 317 by loss of 2H and CH_3 were found to be Acutu-mine and X-morphinan. Compound 10 with the $[M+H]^+$ ions at m/z 149 produced the fragment of Sinactine. Acetyl-oleanolic acid produced ions $[M+H+K]^+$ at m/z 536,356. These qualiative analysis of chemical constituents were listed in Table 1.

HPLC analysis of chemical constituents in extracts: The HPLC analysis of extracts showed that the extracts contained several kinds of compounds as shown in Fig. 2.

The result of quantitative analysis of the mixture by HPLC was shown in Table 2. In the case of the total amount of mixed herbs remained unchanged, different proportions of the two herbs were screened. The leaching rate of both cinnamaldehyde and sinomenine in the extraction was higher with increasing amount of GuiZhi shown in Table 2. It was speculated that the acide active ingredients in GuiZhi, such as, cinnamic acid, improved the leaching of alkaloid components.

Effects of extracts on paw swelling: Paw swelling was an external objective indicator evaluating the severity of inflammation in RA model. A remarkable increase in the left hind paw swelling was observed in AA rats compared with the normal rats. All the treatment groups diminished swelling in the left hind paw (Fig. 3). While a significant decrease in paw swelling was observed when the AA rats were treated with extract (4:1), extract (3:1) and Sin group (Fig. 3).

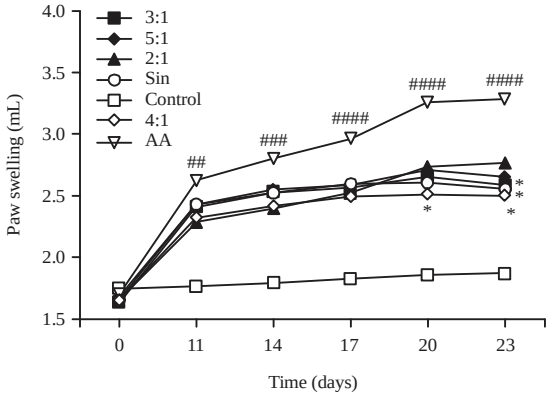

Fig. 3: Effects of treatment of rats with extracts on paw volume after single intradermal injection of FCA. Values are expressed as means. Compared to the control group: ##p<0.01, ###p<0.001, ####p<0.0001, compared to the AA group: *p<0.05

Effects of extracts on serum cytokines: Compared with control group, Fig. 4 shows that there was an obvious increase in the production of IL-1β (30.1 pg mL^{-1}), IL-6 (156.4 pg mL^{-1}), TNF-α (297.7 pg mL^{-1}) and a marked decrease in the level of IL-10 (30.9 pg mL^{-1}) in the serum of AA model group ($p<0.0001$). Compared with AA group, all the treatment groups greatly declined the serum levels of IL-1β, IL-6 and TNF-α ($p<0.0001$). Extract (4:1) and extract (3:1) groups greatly elevated the levels of IL-10 ($p<0.0001$) and their efficiencies were slightly superior to extract (2:1) ($p<0.001$), extract (5:1) ($p<0.01$) and Sin group ($p<0.05$).

Effects of extracts on histopathology in AA rats: The control group showed normal articular cartilage, absence of synovial membrane hyperplasia and open articular cartilage (Fig. 5a). While the ankle joints of the AA group rats displayed prominent destructive inflammation of the articular bone and extra-articular tissues, joint swelling with synovial hyperplasia, cellular infiltration and narrowing joints space with severe pannus formation alone with the thinning of the cartilage plate (Fig. 5b). The suppressive effects of extracts on AA rats were further supported by histological analysis (Fig. 5d-g). The extracts treated rats revealed a marked decrease in synovial inflammatory cell infiltration and synovial hyperplasia. These pathological changes were evidently reduced by the administration of extract (3:1) and extract (4:1) (Fig. 5e, 4f).

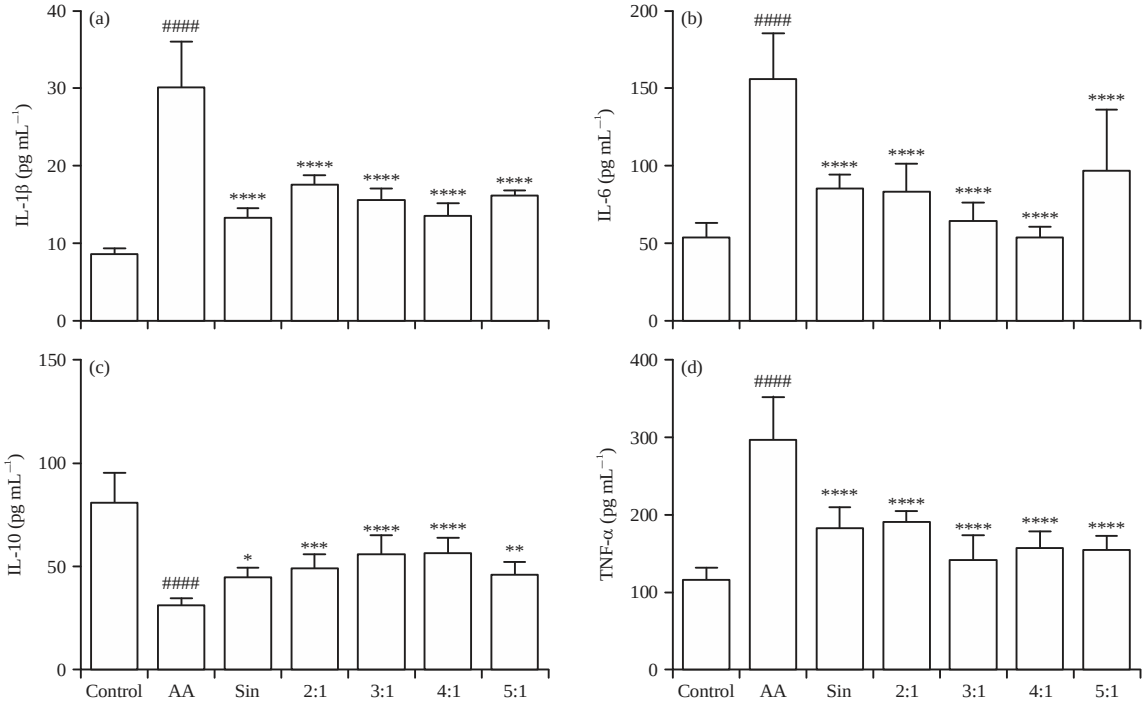

Fig. 4(a-d): Effect of extracts on production of IL-1β, IL-6, IL-10 and TNF-α in AA rats. Values are expressed as Mean±SD. Compared with control: ####p<0.0001, Compared with model: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

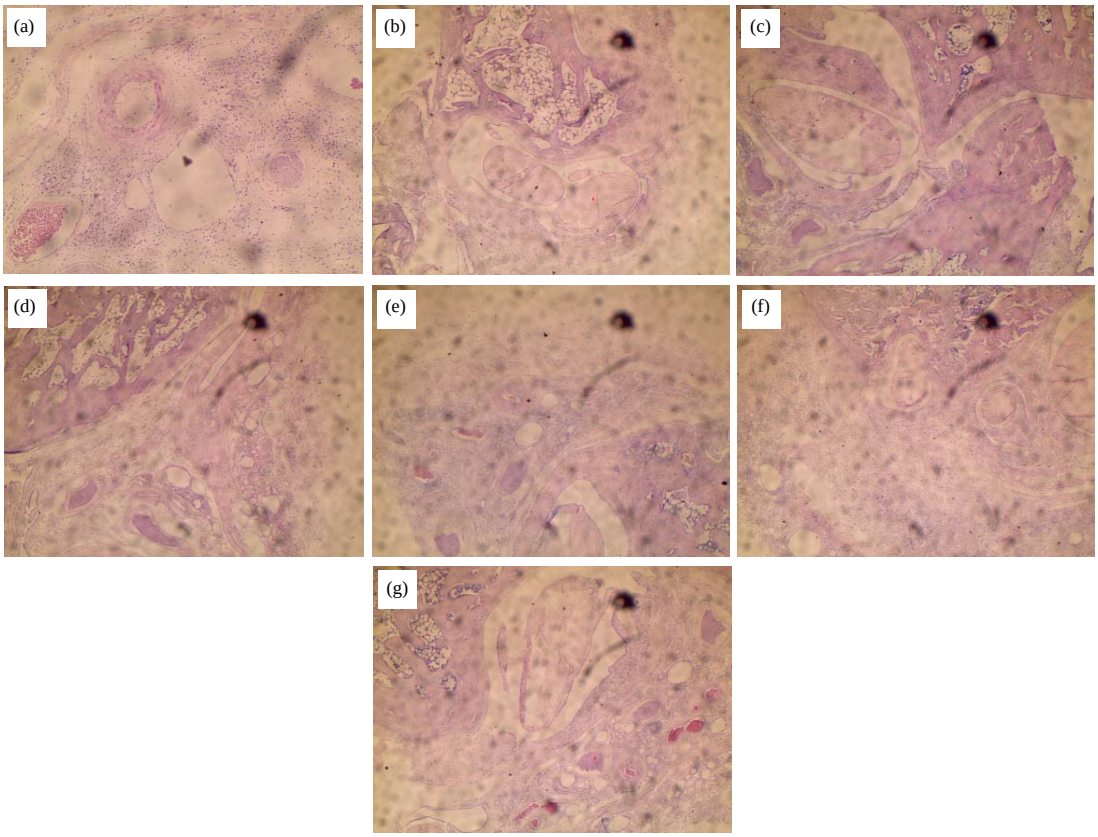

Fig. 5(a-g): Effect of extracts on histopathology changes. The representative examples of each group, (a) Control group, (b) AA group, (c) Sin group, (d) Extract (2:1) group, (e) Extract (3:1) group, (f) Extract (4:1) group and (g) Extract (5:1) group. (HE staining, magnification 40X, scale bar, 500 μm)

Particularly, there was only mild synovial infiltration with few inflammatory cells and no obvious damage in cartilage bone erosion in the extract (4:1) (Fig. 5f). However, extract (5:1, 2:1) and Sin group showed slight improvement in pathological changes (Fig. 5c, d, g) and the effect was not as good as the other two treatment groups.

DISCUSSION

The RA is a common autoimmune disease, which is characterized by joint swelling and pain, joint stiffness, deformity and serious functional damage. The AA could act as an experimental model in present study to demonstrate the effects of extract on human RA because of its similarity to human RA in both clinical and histopathologic features.

A considerable number of studies have showed that proinflammatory cytokines, such as IL-1β, IL-6 and TNF-α, play an important role in initiation and maintenance of acute and chronic inflammation[33-35]. Furthermore, it has been demonstrated that TNF-α is an important inducer to the IL-1β, which is necessary to promote the beginning of the chronic states of the RA[36]. On the contrary, IL-10 has been generally recognized as a potent anti-inflammatory cytokine via inhibiting the releases of pro-inflammatory cytokines[37,38], which could also protect the joints integrity in RA progression[39,40]. This study demonstrated that the extracts of the herb formula could significantly decrease the releases of IL-1β, IL-6 and TNF-α in serum of AA rats, while increase that of IL-10. Combined with the results of paw swelling and histopathology changes, extract (4:1) and extract (3:1) groups showed better efficacy than other treatment groups.

As the main active constituent of QingFengTeng, Sinomenine (SN) has been used in traditional medicine in Asia for its beneficial effects on auto immune diseases, especially RA[41,42]. Its efficiency used in RA patients has been confirmed in open clinical trials[43]. The SN is also selected as quality control component in Chinese phamacopoeia. According to reports in the literature, SN decreases the mRNA expression of TNF-α and IL-1β by inhibiting the NF-κB binding activity, which is mediated through up-regulating the NF-κB expression of PMs

and synoviocytes in AA rats[42]. The effects on lymphocyte proliferation are part of the anti-inflammatory and anti- arthritic mechanisms of SN obvious in clinical trials. Several authors suggested that SN exerted anti-RA action probably through modulating the frequencies of Treg cells and Th17 cells in intestinal lymph nodes and yielding a trafficking of lymphocytes[44] from gut to joint[45]. Recent studies showed SN is a promising analgesic and anti hyperalgesic for pain and hypersensitivity in RA[46]. Above all, SN, as the main active constitute of the Chinese herb formula, played a major anti-RA function.

It has been shown that the volatile oils of GuiZhi perform well in cellular anti-inflammation assays[47,48]. Furthermore, in many of the famous ancient books about Traditional Chinese Medicine (TCM) such as JinGuiYaoLue, GuiZhi was described as a guiding herb which was able to deliver medicine into the joints. Thus, a formula containing QingFengTeng and GuiZhi might bring better effects from the point of GuiZhi's anti-inflammatory activity and drug-guiding functions.

A TCM formula usually composes of several herbs to achieve satisfactory therapeutic efficacy and fewer side effects. Modern pharmacological research focuses on the single compound, while ignoring the interaction between the TCMs which may lead to the enhancement of the efficacy or reduction of side effects.

According to TCM theoretical system, a good formula usually composed of four parts which is monarch herb, minister herb, assistant herb and guiding herb, respectively. This is a highly characteristic drug classification method so as to design a suitable multi-component formula including different herbs[49,50]. The guide drug refers to the ingredient leading the other drugs in the prescription to the affected part, or the ingredient regulating the properties of other drugs.

For example, Borneol is a classical romantic refreshing TCM and commonly used as a guiding component which is called an "upper guiding drug". It was reported that Borneol can enhance the BBB permeability and improve the transportation of Kaempferol to brain[51,52]. As another example, vinegar-baked Radix Bupleuri (VBRB), a guiding herb of liver, is usually used to focus the effect of other drug on liver[53]. It was reported that VBRB could enhance the distribution of resveratrol in liver and reduce the distribution in other tissues.

As a famous guiding herb, GuiZhi was described to delivery medicine into the joints where are the target tissues for RA patients. In the respective of paw swelling, histological changes and up-regulating levels of IL-10, extract (4:1) and extract (3:1) showed better effects than possive drug. Moreover, doses of SN were much lower than that of possive drug. Assuming a rat weighed 0.2 kg, the oral dose of possive drug was 25 mg kg^{-1}, that meaned the total dose of SN was 5 mg. Extract (4:1) group was given at a dose of 1.5 g kg^{-1} and the dose of SN was only 0.4 mg determined by HPLC. Obviously, the dose of herb formula was about 12 times lower than that of Sin group in terms of SN. Overall, present study formulas, extract (4:1) and extract (3:1) obtained similar efficacy with the positive drug using a much lower dose.

The enhancement on efficacy of anti-RA by GuiZhi may result from synergistic effect of two herbs on anti-inflammation, enhancement on absorption of active fractions, or improvement on drug tissue distribution. In order to verify above hypothesis, the study of pharmacokinetics *in vivo* must be carried out, that is author's next research plan.

CONCLUSION

The therapeutic effect of the combination of QingFengTeng and GuiZhi with different ratios on AA rats were investigated. The results showed all the ethanol extracts of the formula ameliorated AA symptoms. Extract (4:1) and extract (3:1) significantly decreased the paw swelling, greatly elevated the levels of IL-10 and their efficacies were slightly superior to the other treatment groups (2:1 and 5:1). The exploration exhibited good anti-RA effects of a Chinese herb formula based on a combination of QingFengTeng and GuiZhi in different ratios and revealed possible mechanism. This study will help the researchers to uncover a new formula which may be a potential candidate for the treatment of RA.

SIGNIFICANCE STATEMENT

This study discovered the effects of a Chinese herb formula based on a combination of QingFengTeng and GuiZhi in different ratios on AA rats and revealed possible mechanism. This study will help the researchers to uncover a new formula which may be a potential candidate for the treatment of RA that many researchers have not explored. Thus, a new theory on effects of two herbs combination may be arrived at.

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