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Anti-arthritic effect of total saponins from *Clematis henryi Oliv.* on collagen-induced arthritis rats

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Abstract

Background:

As a traditional herbal medicine, *Clematis henryi Oliv*. has been widely used in China for hundreds of years for the treatment of infectious and inflammatory disorders. Rheumatoid arthritis is a chronic systemic inflammatory disease lacks of effective therapeutic drugs.

Objective:

To investigate the anti-arthritic activity of total saponins extracted from *Clematis henryi Oliv*. (TSC) and the underlying mechanisms in collagen-induced arthritis (CIA) rats.

Methods:

The purified TSC were administrated to CIA rats at the dose of 150 and 50 mg/kg/d. Paw volume and claw pad thickness were measured every week. The levels of IgG, IL-1β, and TNF-α in serum were measured by ELISA kit, and histopathology of joint was examined by H&E staining.

Results:

Administration of TSC resulted in a significant decrease of paw volume and thickness in CIA rats. TSC also suppressed IgG, IL-1β, and TNF-α levels in serum of CIA rats. Histology revealed that TSC significantly inhibited joint inflammatory cells infiltration and reduced synovial hyperplasia.

Conclusion:

TSC have an anti-arthritic effect on CIA rats, and this effect is probably associated with downregulating the expression of inflammatory factors.

Keywords

Clematis henryi Oliv., inflammatory factor, rheumatoid arthritis, total saponins

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness, and destruction of synovial joints. It is also associated with extra-articular manifestations, mainly involving the heart, lung, and renal functions.[1] As a major cause of disability, RA affects approximately 1% of population across racial and ethnic groups.[2] The RA patients' synovial membranes is infiltrated by lymphocyte and granulocyte that produce chronic pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6), leading to the destruction of articular cartilage and bone.[3] It was reported that TNF-α inhibitors including infliximab and etanercept are effective in the treatment of RA.[4] Inhibiting IL-6 or TNF-α can ameliorate RA in animal models or humans.[5] Therefore, targeting pro-inflammatory cytokines will be benefit for RA therapy. However, the clinical need for effective treatment of RA remains unmet.

The *Clematis* species have been extensively used as the traditional medicines. The aerial parts and roots of various *Clematis* species are used in Europe and Eastern Asia in particular as a remedy to lessen the discomfort of rheumatic pain, eye infections, gonorrhea symptoms, bone illnesses, chronic skin disorders, gout and, varicosity. The roots of *Clematis henryi Oliv*. are specifically used by the traditional Tujia ethnic minority to treat inflammation-related diseases, such as gout, arthritis, and tetanus. Much research has shown that saponins are the major active components of the *Clematis* species.[6–8] However, little effort has been invested in researching about chemical composition and pharmacological activities of *Clematis henryi Oliv*. Furthermore, the anti-inflammatory mechanisms remain unknown. In the present study, we sought to investigate the anti-arthritic effects of total saponins extracted from *Clematis henryi Oliv*. (TSC) and the underlying mechanisms in collagen-induced arthritis (CIA) rats.

Materials and methods

Plant material and extract

The plant material was purchased from Xiangxi Liaoyuan Pharmaceutical Co., Ltd. (Qianzhou, Hunan Province, PR China), and identified by Dr. Da-Xiong Xiang (Department of Pharmacognosy, School of Pharmacy, Central South University, Changsha, PR China). A voucher specimen has been deposited in the Herbarium of Key Laboratory of Traditional Chinese Medicine (Second Xiangya Hospital of Central South University, Changsha, PR China).

The air-dried roots of *Clematis henryi Oliv*. were powdered. Two kilogram of powders were extracted three times with 70% ethanol (10 L) at 70°C for 2 h. The alcoholic extract was filtered and concentrated in a rotary evaporator under reduced pressure below 70°C to afford concentrated solution (2 L), which was subjected to D101 macroporous resin and then eluted by water and 60% ethanol to yield fraction 1 (134 g) and fraction 2 (206 g). The content of total saponins in the powder was determined by colorimetry of vanillin-glacial acetic acid.[9] The oleanic acid was used as control and detection wavelength was 550 nm. The concentration of saponins in fraction 1 is 13%, and that in fraction 2 is 71%. We chose fraction 2 for the following study.

Animals

All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals revised in 2010. Female Sprague-Dawley (SD) rats (aged 10 weeks, 190 ± 10 g) were purchased from Silaikejingda Laboratory Animals Co. Ltd. (Changsha, PR China). The animals were housed in a controlled environment (24°C) throughout the experiment under a 12:12 h light-dark cycle (lights on 07:00–19:00), and fed with a standard rodent diet *ad libitum* (Animal Center of the Second Xiangya Hospital, Central South University, Changsha, PR China). Water was freely available. All rats were acclimated to the surroundings for 1 week before the experiment. The experimental protocols related to animals were approved by the Central South University Animal Experimentation Ethics Committee.

Collagen-induced arthritic model

Collagen-induced arthritis (CIA) is an animal model that widely used to study pathogenesis and to validate therapeutic effect of RA.[10,11] The animal model of RA was established by injected bovine collagen type II in all the groups except normal control group. Bovine collagen type II (2 mg/mL, Chondrex, Redmond, WA, USA) was dissolved in 0.05 M acetic acid and emulsified with an equal volume of incomplete Freund's adjuvant (IFA, 10 mL/jug, Sigma,

USA). A total of 0.3 mL of emulsion (1 mg/mL) was injected intradermally into the base of the tail. At day 14 after primary immunization, rats were given a booster inject intradermally on the other side of the tail with 0.3 mL emulsion of collagen-II and IFA. Normal control group received the injection of an equal volume of 0.3 M acetic acid at the same location. The swelling scope and extent of limb joints was observed every week. At day 21 the paw volume was significantly increased and the claw pad was obviously thickened. According to arthritis index score, animal model was successfully established.[11]

Design of treatment

The CIA rats were used to assess the anti-arthritic effect of TSC. Forty-eight SD rats were randomly divided into five groups: group 1 (0.9% saline, normal control, n = 8), group 2 (0.9% saline, model control group, n = 10), group 3 (Methotrexate, 1 mg/kg/w, positive drug control group, n = 10), group 4 (high dose group, TSC, 150 mg/kg/d, n = 10), and group 5 (low dose group, TSC, 50 mg/kg/d, n = 10).

TSC was prepared in a dose of 150, 50 mg/kg body weight for the anti-arthritic studies and dissolved in physiological saline prior to the experiment. Methotrexate (Shanghai Fosun Pharmaceutical Co. Ltd., PR China.) was prepared in a dose of 1 mg/kg, dissolved in physiological saline prior to the experiment. The drugs and saline were administrated intragastrically. All of the test solution was administered in A volume of 10 mL/kg. Rat's paw volume and claw pad thickness were measured every week by water plethysmometer and vernier caliper, respectively.

Histological evaluation

Tissue sections were taken from rat ankle joint and were fixed in 4% formaldehyde over 24 h at 4°C, decalcified with 10% EDTA at 4°C, then dehydrated in ethanol, cleared with dimethyl benzene, embedded in paraffin blocks, and cut serially into slice of 4 μm thickness. Routine hematoxylin and eosin staining (H&E staining) was performed.

Anti-inflammatory assay

After sacrificing the rats, blood was collected from abdominal aorta. The blood samples were centrifuged at 3000 rpm/min for 15 min, and then the supernatant was stored at 4°C. The concentration of IgG, IL-1β, and TNF-α in supernatant were detected using ELISA kits (Research & Diagnostics System, USA) according to the manufacturer's instruction. Optical density value was detected at 450 nm.

Statistical analysis

Statistical results were expressed as the means ± the standard error of mean (SEM). The one-way ANOVA test with Student-Newman-Keuls was used to analyze and compare the data. Values of P <0.05 were considered to be statistically significant.

Results

Anti-arthritic activity of TSC

A previous study reported that *Clematis mandshurica* Ruprecht root extract alleviated hind paw edema of rats induced by carrageenan.[12] Can TSC protect SD rats from paw injury induced by collagen? We assessed the paw volume and claw pad thickness in CIA rats with TSC treatment and without. Compared with the control group, paw volume and thickness in CIA rats were significantly increased (Figures 1 and 2). Methotrexate was chosen as the positive drug in consideration of its frequent use in RA.[13] No significant change of paw volume and thickness was observed in CIA rats treated with 0.9% saline. Compared with the model group, methotrexate was able to ameliorate the pad injury induced by collagen. Both doses of TSC have a similarity effect to methotrexate, and TSC exerted significant anti-arthritic activity in a dose-dependent manner. Three weeks after high dose (150 mg/kg) administration, paw volume and thickness of CIA rats were decreased. Paw volume and thickness were also decreased in low dose group (50 mg/kg) after 4 weeks.

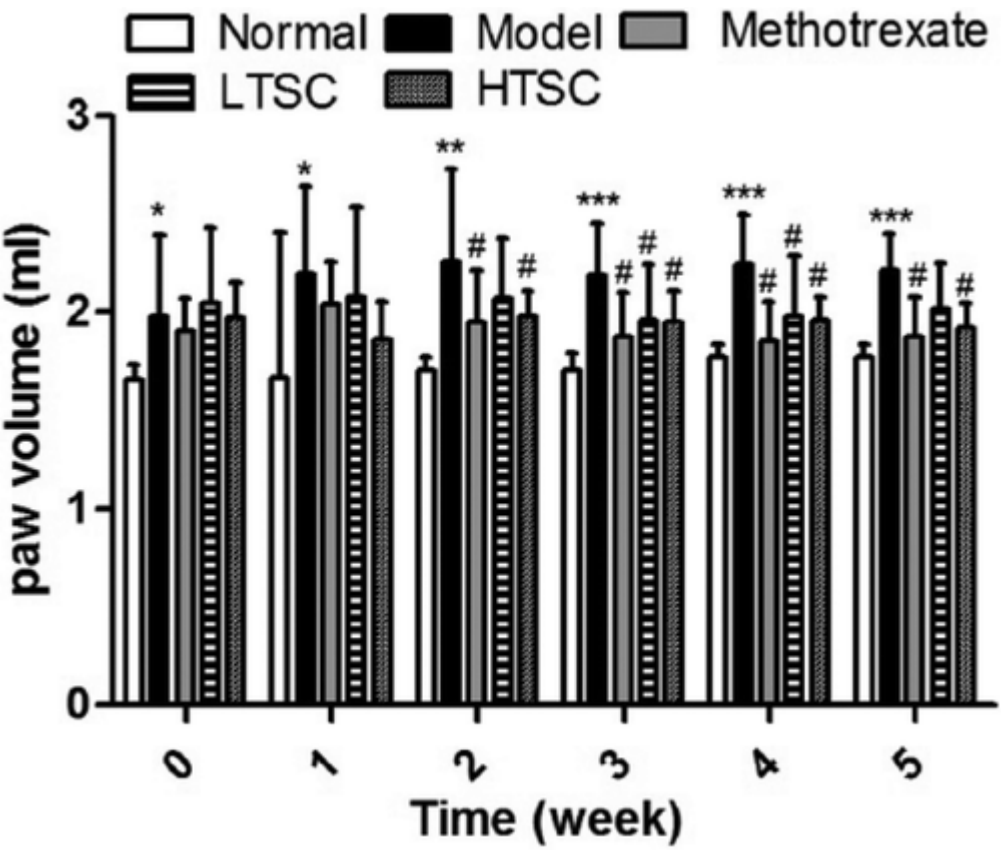

Figure 1. Effects of TSC on paw swelling volume in CIA rats. Values are presented as mean ± SEM. LTSC: low dose of TSC, HTSC: high dose of TSC. *P <0.05, **P <0.01 compared with normal control, $^{\#}P$ <0.05, $^{\#\#}P$ <0.01 compared with model control group.

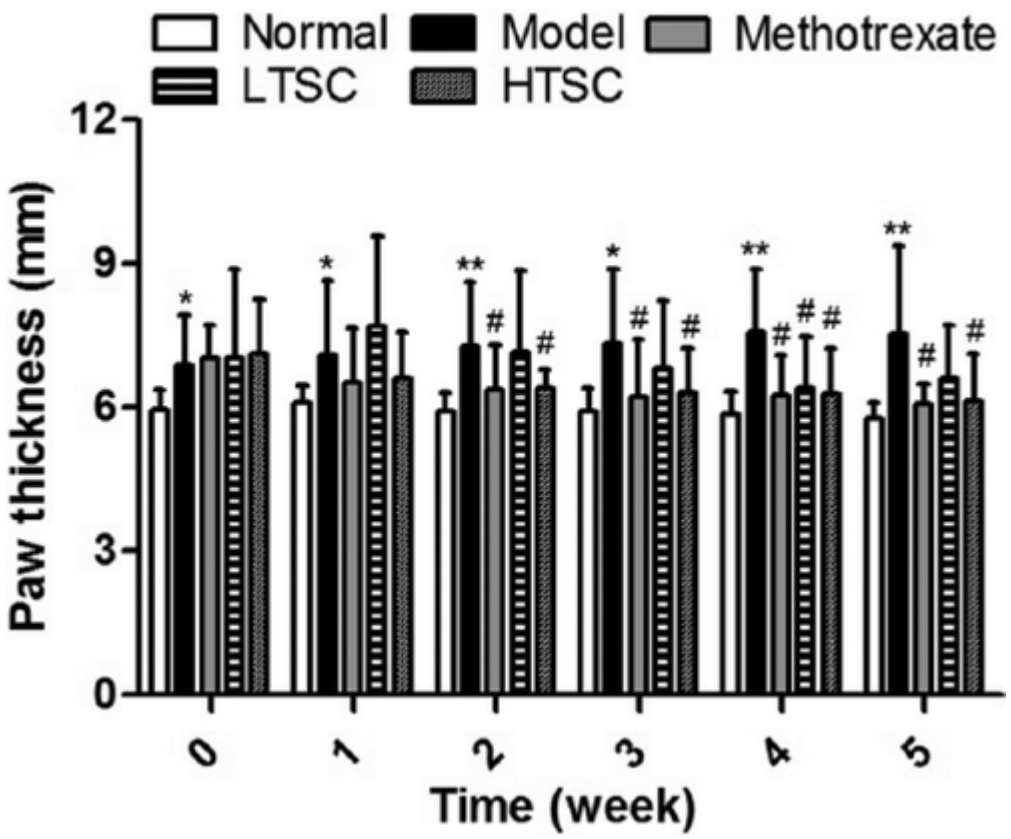

Figure 2. Effects of TSC on paw swelling thickness in CIA rats. Values are presented as mean ± SEM. LTSC: low dose of TSC, HTSC: high dose of TSC. *P <0.05 compared with normal control, $^{\#}P$ <0.05 compared with model control group.

Inhibitory effects of TSC on IgG, IL-1β, and TNF-α levels in CIA rats

Pro-inflammatory cytokine system has been well-recognized and it is important in destruction of articular structure. Many studies revealed that inhibiting IL-6 or TNF-α can ameliorate RA in animal models or humans.[5] Our results also showed that IgG, IL-1β, and TNF-α levels in the model control group were significantly raised (P <0.01) compared with the normal control group. Interestingly, the levels of IL-1β and TNF-α were reduced in a dose-dependent manner in CIA rats treated with TSC. These results suggested that the anti-arthritic effect of TSC is probably associated with the downregulation of IL-1β and TNF-α expression. IgG, a rheumatoid factor, which was previously reported to be associated with RA,[14] was also measured in serum of CIA rats. As shown in Figure 3, TSC treatment efficiently suppressed IgG production in serum of CIA rats.

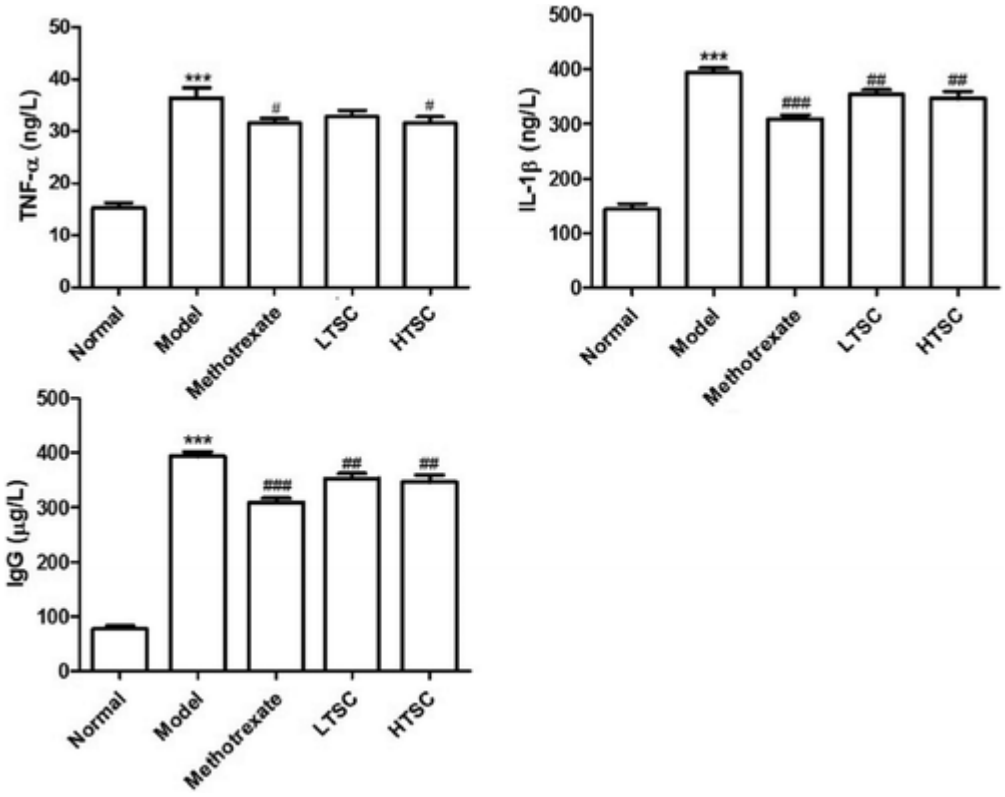

Figure 3. Effects of TSC on IgG, IL-1β, and TNF-α levels in CIA rats. Values are presented as mean ± S.E.M. LTSC: low dose of TSC, HTSC: high dose of TSC.*P <0.05, **P <0.01 compared with normal controls, $^{\#}P$ <0.05, $^{\#\#}P$ <0.01 compared with the model control group.

Inhibitory effects of TSC on synovium hyperplasia and inflammatory cell infiltration

Normal rat synovial tissue, showing two to three layers of synovial cells lying on fibro-fatty tissue and loosely organized, are partly not continuous. The synovial membrane in RA becomes severely inflamed and thickened, with numerous infoldings on its surface. Meanwhile, the synovial membrane is invaded by a variety of inflammatory cell.[15] In this study, the histopathology of joint was evaluated by H&E staining after sacrificing the rats. The joint tissue in the model group rats was markedly abnormal with serious injury on tissue structure, inflammatory cells infiltration, and synovial hyperplasia (Figure 4). The CIA rats treated with TSC inhibited the histological damage, synovial hyperplasia, and inflammatory cells infiltration.

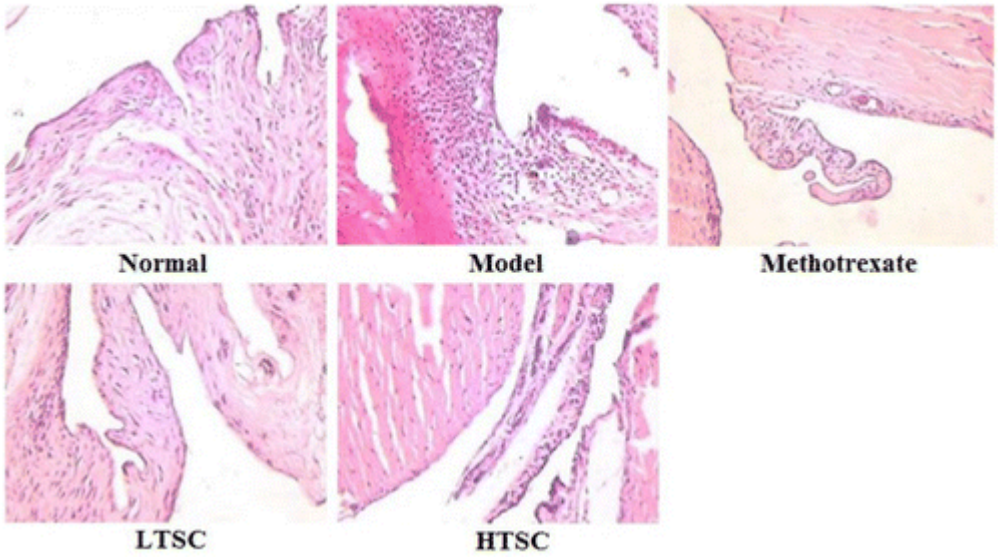

Figure 4. Pathohistology of joint tissue sections of CIA rats treated with different dose of TSC by H&E staining. Original magnification ×250. LTSC: low dose of TSC, HTSC: high dose of TSC.

Discussion

RA is a complex immune mediated inflammatory disease which attacks the soft tissue of joints and leads to inflammation, swelling, and pain. Many factors are involved in the development of this disease. RA patients usually require lifelong treatment with medications. Therapeutic approaches used for RA are traditional non-selective non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), corticosteroids, and biologics, which are not always effective and are associated with significant side effects.[16] Therefore, the medicines with high efficiency and low toxicity should be selected preferentially in RA treatment.

The genus *Clematis* is a large genus within dicotyledons. About 300 species are known worldwide.[17] *Clematis* extract have a variety of biological activities, such as anti-inflammatory, anti-nociceptive, anti-myocardial ischemia, and anti-cancer effects.[17–19] *Clematis henryi Oliv*. belongs to the *Clematis* genus; however, there are few reports about its chemistry component and pharmacology activity. Our preliminary research has shown that the anti-inflammation effect of fraction 2 is better than that of the others (fraction 1 and crude extract). In the current study, we evaluated anti-arthritics activity of TSC (fraction 2) in CIA rats. Our results showed that high doses of TSC reduced paw volume and claw pad thickness of RA rats. Histopathologic examination indicated that TSC significantly eased joint inflammation and reduced synovial hyperplasia.

Inflammation is closely linked to the pathogenesis of RA. Numerous studies have demonstrated that many pro-inflammatory cytokines are secreted by synovial cells and monocytes and/or macrophages that infiltrate the synovial tissue. Among them, TNF-α, IL-6, and IL-1β play an important role in the initiation and development of RA.[20,21] Accordingly, pro-inflammatory cytokines are attractive therapeutic targets for RA treatment. For example, TNF-α is now targeted in the standard treatment of patients with RA. Other cytokines are being tested as therapeutic targets in clinical studies, with promising result.[22,23] In our research, IgG, IL-1β, and TNF-α levels in RA rats were higher than that in normal rats. It was consistent with the results of previous studies. The production of TNF-α, IgG, and IL-1β was obviously decreased in serum of CIA rats treated with TSC. These results confirmed that the anti-arthritic effect of TSC is associated with the downregulation of IgG, IL-1β, and TNF-α levels. Fibroblast-like synoviocytes (FLS) are resident mesenchymal cells of the synovial joint. Activation of FLS plays a crucial role in pathogenesis of RA by regulating the secretion of inflammatory cytokines.[24] The effect of TSC on FLS function needs to be further investigated.

Conclusion

In conclusion, our study indicated that TSC has strong therapeutic effect on CIA rats. This anti-arthritic activity of TSC is associated with its inhibitory effects on IgG, IL-1β, and TNF-α levels. The *Clematis henryi Oliv*. has potential clinical value in RA patients. Further studies are needed to identify the active components of TSC.

Declaration of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

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