The Anti-Rheumatoid Arthritis Effects of *Rhododendron Molle* Roots on Collagen-Induced Arthritis in Rats

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Abstract

**Background:** The roots of *Rhododendron molle* G. Don have long been used to treat rheumatoid arthritis in Chinese folk medicine. Previous studies have suggested it may have anti-arthritic effects.

**Methods:** Collagen-induced arthritis (CIA) rats were established and administered with a water extract of *R. molle* roots (5.4 or 1.35 g/kg/day, for 28 days) to investigate its anti-arthritic effects. Subsequently, paw swelling, arthritis indices, and weight loss in CIA rats were examined, and blood was collected to determine the serum levels of IL-1β, IL-6, IL-17A, IFN-γ, and TNF-α.

**Results:** The results indicated that the water extract of *R. molle* roots inhibited paw swelling, reduced arthritis indices and prevented weight loss in CIA rats. Additionally, the water extract decreased the levels of IL-1β and IL-17A in CIA rats.

**Conclusion:** The results suggested that the roots of *R. molle* exert an anti-arthritic effect in CIA rats by inhibiting the release of certain pro-inflammatory cytokines.

**Keywords:** *Rhododendron molle*; Rheumatoid arthritis; Anti-arthritic effect; Collagen-induced arthritis

**Abbreviations:** RA: Rheumatoid Arthritis; CIA: Collagen-Induced Arthritis; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-17A: Interleukin-17A; IFN-γ: Interferon-γ; TNF-α: Tumor Necrosis Factor-α; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; IL-8: Interleukin-8; MTX: Methotrexate; CSA: Cyclosporine; AZA: Azathioprine; LEF: Leflunomide; CFA: Complete Freund’s Adjuvant; ELISA: Enzyme Linked Immunosorbent Assay; ANOVA: Analysis Of Variance.

Background

Rheumatoid arthritis (RA), an intractable and highly prevalent autoimmune disease, is a disabling condition that can lead to long-term joint damage, resulting in persistent pain and loss of function in affected areas, especially in the wrist and fingers. As a disease whose prevalence is related to advancing age, it affects more women than men [1-4]. Previous studies revealed that many proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokines such as IL-8 are increased abnormally in rheumatoid arthritis tissue [5]. Clinically, methotrexate (MTX), cyclosporine (CSA), azathioprine (AZA), leflunomide (LEF), and glucocorticoids are the conventional drugs for RA therapy. However, there are severe side effects associated with their long-term use, such as gastrointestinal ulcers, myelo-suppression, and cardiac diseases [6]. Meanwhile, natural monomers or extracts that have been isolated from traditional Chinese medicines, such as *Tripterygium wilfordii*, *Radix Aconiti*, and *Aconitum kusnezoffii Reichb*, have been demonstrated to treat RA effectively with relatively low toxicity.

*Rhododendron molle* G. Don, belonging to the Ericaceae family, is distributed widely in South China. It has been used to treat various diseases for thousands of years, such as rheumatism, traumatic injury, anal fistula, and ringworm sores [7]. Clinical applications and several simple pharmacological experiments have proven that the roots of R. molle have anti-RA effects [8,9]. Therefore, as a part of continuing efforts to identify novel plant-derived anti-RA candidate drugs, and to establish their efficacy by modern pharmacological methods, the present study was designed to evaluate the anti-arthritic effects of R.
Rhododendron molle systemically in an experimental animal model of RA.

Materials and Methods

Plant material

The roots of Rhododendron molle were collected from Lingui county, Guangxi province, in September 2008 and authenticated by Prof. Bao-Kang Huang in the Department of Pharmacognosy, Second Military Medical University. A voucher specimen (20080917) was deposited at the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai, and the People’s Republic of China.

Animals

Female wistar female rats (6 weeks old; 120 ± 10g) were purchased from the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine (Shanghai, China). They were acclimatized in the animal house conditions with a 12:12 h light: dark schedule and free access to a standard pellet diet and tap water.

Chemicals and reagents

A water extract of Rhododendron molle roots was obtained in our laboratory. CII collagen was purchased from Chondrex (WA, USA); complete Freund’s adjuvant (CFA) was purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China); tripterygium glycosides were purchased from Huitian Biological Pharmaceutical Co., Ltd. (Fujian, China); MTX was purchased from Shanghai Pharmaceutical Group Co., Ltd. (Shanghai, China); glutaraldehyde and sodium chloride injection were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); and rat serum IL-1β, IL-6, IL-17A, IFN-γ, and TNF-α enzyme linked Immuno sorbent assay (ELISA) kits were purchased from Bender (Germany).

CII-Induced arthritis (CIA) animal model preparation and experiment protocols

To evaluate the potential anti-arthritis effects of a water extract of Rhododendron molle roots, a total of 48 rats were randomly divided into the following six groups (n=8):

- a) Normal: normal rats treated with saline (1 mL/100 g);
- b) Control: CIA rats treated with saline (1 mL/100 g);
- c) Tripterygium glycosides: CIA rats treated with tripterygium glycosides (positive drug, 40 mg/kg);
- d) MTX (1 mg/week): CIA rats treated with MTX (positive drug, 1 mg/week);
- e) Water extract of Rhododendron molle roots (5.4 g/kg): CIA rats treated with the water extract at a dose of 5.4 g/kg; and
- f) Water extract of Rhododendron molle roots (1.35 g/kg): CIA rats treated with the water extract at a dose of 1.35 g/kg.

The CIA rat model was prepared as previously described [10], with minor modifications. In brief, CII was dissolved in 0.04 M acetic acid to a final concentration of 4 mg/mL. Then, the CII solution was emulsified with an equal volume of CFA. Rats were initially immunized by a subcutaneous injection of the CII emulsion into the tail root (250 μL/200 g). After one week, the rats received a second immunization by the subcutaneous injection of the CII emulsion with an equal volume of the CII emulsion into their backs (125 μL/200 g). After approximately two weeks, the immunized rats showed clear symptoms of RA at their toe joints, including inflammation, erythema, and swelling (Figure 1). At 14 days after the initial immunization with CII, the rats were treated orally with saline, tripterygium glycosides, MTX, or different doses of water extracts of Rhododendron molle roots. During the experiment, the body weights and paw volumes of the rats were measured every 3 days. In addition, the arthritis indices of the rats were measured every three days using the following ordinal scale: Grade 0 = No sign of arthritis; Grade 1 = Redness and swelling in the paw; Grade 2 = Deformity of the paw; Grade 3 = Ankylosis in the paw; Grade 4 = Maximal swelling and deformity with ankylosis [11]. After the treatments were administered for 4 weeks, blood samples were collected by the abdominal aortic method, and the rats were then sacrificed via decapitation.

Figure 1: The paws and tails of normal and CIA rats.
Determination of Serum Cytokine Levels

Serum samples were prepared after they were incubated under ice-cold conditions and then centrifuged for 15 min (3000 rpm). The samples were stored at -80°C until analysis. The serum levels of IL-1β, IL-6, IL-17A, IFN-γ, and TNF-α were then determined using commercial ELISA kits according to the manufacturer’s protocols and instructions.

Statistical Analysis

The data were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparisons tests (between different groups) and are presented as the mean ± SD. The statistical significance of the observed differences was determined using SPSS software (SPSS for Windows 17.0, SPSS Inc., Chicago, IL, USA). Differences were considered significant at p < 0.05.

Results

Water extract of R. molle roots decreases paw swelling, arthritis indices, and weight loss in collagen-induced arthritis (c ia) rats

A CIA rat model was prepared to evaluate the therapeutic effects of a water extract of R. molle roots in rats with RA. As shown in Figures 2-4, compared with the normal rats, significant RA symptoms were observed when the experimental rats were immunized with type II collagen (CII) twice, including paw swelling and erythema, higher arthritis indices, and obvious weight loss. These symptoms improved significantly after treatment with tripterygium glycosides and MTX. The results showed that starting on the 6th day after the initial tripterygium glycosides and MTX administration, paw swelling was decreased in CIA rats compared with that in the control group (Figure 2). Additionally, the arthritis indices in the tripterygium glycosides and MTX-treated CIA rats were also decreased significantly compared with those in the control group (Figure 3). Meanwhile, it was clear that the administration of tripterygium glycosides and MTX could reverse the body weight loss observed in the CIA rats (Figure 4). Similar to tripterygium glycosides and MTX treatment, the water extract of R. molle roots decreased paw swelling and arthritis indices significantly when administered at doses of 5.4 g/kg, and decreased these symptoms moderately when administered daily at a dose of 1.35 g/kg (Figure 2 & 3). Interestingly and importantly, the body weight loss observed in the CIA rats was reversed by daily treatment with the water extract of R. molle roots to a greater degree than that observed for treatment with tripterygium glycosides.

Water extract of R. molle roots decreases the release of il-1β, il-6, il-17a, ifn-γ, and tnf-α into the serum of CIA rats

The results shown in Figure 4 indicate that there was a clear increase in the levels of IL-1β, IL-6, IL-17A, IFN-γ, and TNF-α in CIA rats compared with normal rats. However, only the release of IL-1β and IL-17A decreased significantly in rats treated with...
Discussion

In recent years, scholars worldwide have begun to focus on traditional Chinese medicine to treat RA, and some natural compounds, such as tripterygium glycosides, and total glucosides of paonia and sinomenine, have been confirmed to be potential medicines to treat RA. It is recognized generally that rheumatoid arthritis (RA) is an immune-mediated disease with chronic progressive inflammation [12,13]. Furthermore, RA animal models have been investigated extensively, and the CIA model is considered a classical model for use in the study of RA [14]. In addition, CIA is a well-described RA animal model that induces immunological and pathological features similar to those in human RA [14-16].

In this study, we established a CII-induced arthritis rat model successfully, and used these CIA rats to evaluate the anti-arthritic activity of a water extract of *R. molle* roots. Our results demonstrated that treatment with the water extract of *R. molle* roots decreased paw swelling, arthritis indices, and weight loss in CIA rats. This result suggested that the water extract of *R. molle* roots have therapeutic effects on CIA.

While inflammatory reactions are not the only features of RA, they are the main problem in RA patients. Therefore, controlling inflammatory reactions represents a feasible RA treatment method [14,17,18]. Our results demonstrated that the water extract of *R. molle* roots decreased inflammatory responses during the development of RA, including alleviating paw swelling, decreasing arthritis indices, and reducing inflammation in the joints. To explore the pharmacological mechanism of the effects of the water extract of *R. molle* roots, we investigated the release of pro-inflammatory cytokines, such as IL-1β, IL-6, IL-17A, IFN-γ, and TNF-α, which are considered potential therapeutic targets for RA [19-23]. The results showed that daily treatment with a water extract of *R. molle* roots for 4 weeks suppressed the levels of IL-1β and IL-17A in the serum of CIA rats.

Conclusion

The results suggested that the roots of *R. molle* have anti-arthritic effects on RA to some extent, and the pharmacological mechanism might be based on the inhibition of the release of certain pro-inflammatory cytokines.

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Availability of Data and Materials

The datasets supporting the conclusions of this article are included within the article.

Authors’ Contributions

All authors participated in the conception and design of this research. XF carried out the experiments, the data analysis and wrote the first draft of the manuscript; ZX contributed to the acquisition and interpretation of data; JL and QM coordinated the study; KFR and SL supervised the work and reviewed the final draft of the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

Ethics Approval and Consent to Participate

All the procedures were conducted in strict accordance with the China legislation on the use and care of laboratory animals, and according the guidelines established by Institute for Experimental Animals of Shanghai University of Traditional Chinese Medicine; the procedures were approved by the University ethical committee for animal experiments.

References


