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•Research article•

Anti-rheumatoid arthritis potential of diterpenoid fraction derived from *Rhododendron molle* fruits

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[ABSTRACT] *Rhododendron molle* G. Don is first recorded in Shengnong's Herbal Classic, and its fruits, which are termed as *Liuzhouzi*, are often used to treat rheumatoid arthritis in Chinese folk. During our ongoing investigation to develop a safer and potential new arthritis therapy, a process for the preparation of diterpenoid fraction from *Rhododendron molle* fruits was established. In order to evaluate the main components and the anti-rheumatoid arthritis effect of the diterpenoid fraction, phytochemical and pharmacological experiments were used. As the result, the main components of diterpenoid fraction were identified as rhodojaponin III (**1**), rhodojaponin VI (**2**), 2-*O*-methylrhodojaponin (**3**), and 5′-*β*-D-glucopyranosy-loxyjasmonic acid (**4**). These four components constitute greater than 95% of diterpenoid fraction using area normalization method of HPLC-ELSD. The results of CIA rat experiment showed that high dose of diterpenoid fraction (0.6 $mg \cdot kg^{-1} \cdot d^{-1}$) significantly alleviated the symptoms of rheumatoid arthritis, similar to tripterygium polyglycosides, an effective RA therapy. Preliminary mechanism studies indicated that diterpenoid fraction significantly inhibited the abnormal proliferation of T and B lymphocytes, and remarkably reduced the levels of pro-inflammatory cytokines IL-6, IL-1*β* and TNF-*α*. Overall, our findings may provide a more effective and safe alternative treatment for RA using common clinical Chinese medicines like tripterygium polyglycosides.

[KEY WORDS] *Rhododendron molle*; Diterpenoid fraction; Anti-rheumatoid arthritis; Mechanism
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Introduction

Rhododendron molle G. Don, belonging to the Ericaceae family, was listed as a poisonous herb in Shengnong's Herbal Classic. The flowers, fruits and roots of *R. molle* are all used for the treatment of rheumatoid arthritis (RA) in Chinese medicine [1]. In Chinese clinics, the root of *R. molle* has been used to treat RA for decades [2-3]. Our previous studies confirmed that the water extract of *R. molle* roots could inhibit paw swelling, reduce arthritis indices, prevent weight loss, and decrease the levels of IL-1*β* and IL-17A in collagen-Induced arthritis (CIA) rats [4]. Phytochemical studies demonstrated that grayanane diterpenoids were important components of *R. molle*, and were also the main poisonous chemical constituents [5-7]. Rhodojaponin III, a representative grayanane diterpenoid, has a significant inhibitory effect on sodium channel current, and a pharmacological action in lowing blood pressure and slowing heart rate [8]. Additionally, rhodojaponin III exhibited antifeedant, growth inhibitory, and insecticidal activities against larvae of *Leptinotarsa decemlineata* and *Spodoptera frugiperda* [9]. Pharmacokinetic studies revealed cardiotoxicity effects of the extracts from *R. molle* flowers. The pharmacokinetic profiles of rhodojaponin-I, II and III are all indicative with symptoms of cardiotoxicity [10]. At the same time, improper use of *R. molle* led to many adverse events in Chinese clinics [11-12].

Three major factors, 1) our pilot experiments *in vitro* demonstrated that activity of *R. molle* fruits extract to inhibit T/B lymphocyte proliferation was stronger than that of flowers and roots extract; 2) the content of diterpenoids in *R. molle* fruits is much higher than that in its roots and flowers; 3) there is significant protection of traditional Chinese medicine resources, as part of continuing efforts for safer and more effective use of *R. molle*, led our team to establish a process for the preparation of diterpenoid fraction from fruits

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These authors have no conflict of interest to declare.

by resin and silica gel chromatography [13]. The prepared diterpenoid fraction meets the standards of the China NMPA (National Medical Products Administration) for Category 5 classification of Traditional Chinese Medicine. The contents of the index components, rhodojaponin III and VI, was between 50.0%–55.0%. The aims of the study described here are to identify the remaining components of diterpenoid fraction, further improve the safety of *R. molle*, and clarify the therapeutic effect of diterpenoid fraction on RA. Spectroscopic and chromatographic methods were used to investigate the remaining components of the fraction. The CIA rat model was established to reveal the effects of diterpenoid fraction on RA *in vivo*. Additionally, cytokines associated with RA, combined with T/B lymphocyte proliferation assays, were carried out to initially investigate the mechanism of diterpenoid fraction against RA *in vitro*. We hope all these studies lay a foundation for the further development and clinical use of *R. molle* in RA treatment.

Materials and Methods

Plant materials

The *Rhododendron molle* G. Don (Ericaceae) fruits were purchased from Bozhou Medicinal Material Market, Anhui Province, China, and subsequently identified by Prof. HUANG Bao-Kang of the Second Military Medical University (SMMU). A voucher specimen (No. 20180422) was deposited at Shanghai University of Traditional Chinese Medicine.

Isolation and identification of the main remaining chemical constituents of diterpenoid fraction

The main chemical constituents of diterpenoid fraction were separated by chromatography (data not shown). The diterpenoid fraction was obtained by the previously reported method [13]. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX-400 spectrometer with TMS as the internal standard, operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C, respectively. Four main chemical constituents was isolated and identified as 5′-*β*-D-glucopyranosy-loxyjasmonic acid (1), rhodojaponin VI (**2**), rhodojaponin III (**3**), and 2-*O*-methylrhodojaponin VI (**4**).

CIA animal model preparation and experiment protocols

Animals

Male wistar rats (150 ± 20 g) were purchased from Shanghai Slaccas Laboratory Animals Co., Ltd. [License number: SCXK (Shanghai) 2017-000, Shanghai, China], and housed in the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine (Shanghai, China). All rats were fed in specific pathogen-free conditions (temperature: 24 ± 2 °C; relative humidity: 60% ± 5%; day/night cycle of 12 hours). All animal experimental procedures were carried out in strict accordance with the Chinese legislation on the use and care of laboratory animals.

Chemicals and reagents

Bovine CII collagen and incomplete freund's adjuvant (IFA) were bought from Chondrex (USA); 1 mL syringe was purchased from Becton, Dickinson Co., Ltd. (USA); high speed homogenizer was purchased from IKA Co. (Germany). Pure water was prepared from Milli-Q (18.2 MΩ) ultra-pure water systems (Millipore, France). Tripterygium glycosides were purchased from Huitian Biological Pharmaceutical Co., Ltd. (Fujian, China). Glacial acetic acid and sodium chloride injection were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and rat serum IL-1*β*, IL-6, IL-10, and TNF-*α* enzyme-linked immunosorbent assay (ELISA) kits were purchased from Bender (Germany).

Diterpenoid fraction was self-made in our lab (In diterpenoid fraction, content of rhodojaponin III was 41.6%; content of rhodojaponin VI was 13.6%. The contents were determined by linear curve method). Tripterygium polyglycosides were purchased from Shanghai Forward Pharmaceutical Co., Ltd..

Dose groups and administration

To investigate the potential effect of diterpenoid fraction on RA, 48 rats were divided into the following six groups (n = 8) and orally treated with a) saline (blank), b) saline (model), c) tripterygium glycosides (positive drug, 50 $mg \cdot kg^{-1} \cdot d^{-1}$), d) diterpenoid fraction (Low dosage, 0.06 $mg \cdot kg^{-1} \cdot d^{-1}$), e) diterpenoid fraction (Moderate dosage, 0.20 $mg \cdot kg^{-1} \cdot d^{-1}$) and f) diterpenoid fraction (High dosage, 0.60 $mg \cdot kg^{-1} \cdot d^{-1}$).

Experimental model: Preparation and experiment protocols

The CIA rat model was established referring to the previous literature [14], with minor modifications. Briefly, 2 $mg \cdot mL^{-1}$ bovine CII collagen was dissolved in 0.05 $mol \cdot L^{-1}$ glacial acetic acid. The CII solution was then mixed with the same volume of IFA and completely emulsified. Rats were injected intradermally with the CII emulsion at the tail root (200 μg each). After one week, the rats received a second immunization by the subcutaneous injection of 100 μg CII emulsion into their tail root. After approximately two weeks, the immunized rats exhibited obvious symptoms of RA, such as inflammation, erythema, and swelling at their toe joints. At 2 weeks after the initial immunization, the rats were administered orally with saline, tripterygium glycosides, or different doses of diterpenoid fraction. The paw volumes and body weights of the rats were measured every four days during the experiment. The severity of arthritis was measured every four days according to 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 [15]. Since the incidence of inflammation in the forepaw was very low and the hind foot joint was more prone to severe swelling, we used the sum of the two hind feet to evaluate the pathogenesis of the rats. After four weeks of treatment, blood samples were collected by the abdominal aortic method, and the rats were then sacrificed humanely *via* decapitation.

Histological and pathological examination

After the rats were killed, ankle joints, knee joints and

surrounding tissues were taken and fixed with 4% paraformaldehyde for 24 h. Decalcification with 10% EDTA-2Na was performed every other day for 1 month, followed by dehydration with different concentration gradient ethanol, paraffin embedding, sectioning (4 μm) and HE staining. The histopathological changes of the ankle and knee joints were observed under the microscope and photographed.

Measurement of serum cytokine levels

The serum was obtained after incubation under ice-cold conditions, and was centrifuged (3000 $r \cdot min^{-1}$) for 15 min. Isolated serum samples were stored at −80 °C until use in cytokine expression experiments. The levels of IL-1β, IL-6, IL-10, and TNF-α in the serum were then measured using commercial ELISA kits according to the manufacturer instructions.

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons tests (between different groups) and are expressed as the mean ± standard deviation. GraphPad Prism 7 for Windows (Graphpad Software Inc., USA) was used for statistical analysis. P values < 0.05 were considered significant.

Inhibition of abnormal proliferation of T/B lymphocytes

Chemicals and reagents

Diterpenoid fraction isolation was conducted using the same method as reported above. Rhodojaponin III and VI were derived in our lab at purities greater than 98% as determined by HPLC-ELSD area normalization method. The fruits of *R. molle* were extracted using the 70% ethanol reflux method in our laboratory. Tripterygium polyglycosides and methotrexate were purchased from Shanghai Forward Pharmaceutical Co., Ltd. and Shanghai Sine Pharmaceutical Factory Co., Ltd., respectively.

Cells

C57BL/6 mice were sacrificed by cervical dislocation, and the spleen of the mice were sterilly separated and washed in pre-cooled PBS (pH 7.4, Gibco). The collected spleen cells were made into a single cell suspension and centrifuged for 5 min at 1500 $r \cdot min^{-1}$ (5810R centrifuge, Eppendorf, Germany). Cells were precipitated by the addition of 1 mL Tris-NH_4Cl buffer, lightly mixed, and placed at room temperature for 2 min. After incubation 9 mL of medium was added and the samples were centrifuged for 5 min at 1000 $r \cdot min^{-1}$. Cell precipitates were washed once with PBS, centrifuged, and the supernatant was discarded. Spleen cells were resuspended with RPMI-1640 (Gibco) containing 10% FBS (Gibco) and counted.

Cytotoxic experiment

The purpose of the cytotoxicity assay is to compare the toxicity of each sample and determine the dose for subsequent experiments. $CD3^+$ T cells and $CD19^+$ B cells were separated from spleen cell suspension by magnetic beads, respectively. The cytotoxicity of the samples to spleen cells and T/B lymphocytes were evaluated by Cell Counting Kit-8 (CCK-8) assay. Experiments were carried out using previously reported methods [16]. The results of the cytotoxic experiments are reported in the supplementary information.

T lymphocytes proliferation assay

$CD3^+$ T lymphocytes were inoculated into 96-well plates and then treated with medium containing various concentrations of samples indicated by group. The control group was treated with solvent (DMSO, 0.5% *V*/*V*) only. CD3/28 antibodies (2 $\mu g \cdot mL^{-1}$, Thermo Fisher Scientific, USA) were administered to stimulate T cell receptors (TCRs) to promote the proliferation of T lymphocytes. After 48 h in culture, 5 μL of CCK-8 solution (Beyotime Biotechnology) was added to each well of the plate. The plate was returned to the incubator (37 °C/5% CO_2) for 4 h. The absorbance at 450 nm was measured using a spectrophotometric plate reader (DNM9602, Beijing Perlong New Technology Co., Ltd.). All values were corrected by cell-free controls. The experiment was repeated three times.

B lymphocytes proliferation assay

B lymphocytes proliferation was measured using the same method as the T lymphocytes studies detailed above. To induce $CD19^+$ B lymphocytes proliferation LPS (lipopolysaccharide, final concentration, 1 $\mu g \cdot mL^{-1}$, Thermo Fisher Scientific, USA) was used to stimulate T lymphocytes.

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons tests (between different groups). SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. P values < 0.05 were considered significant.

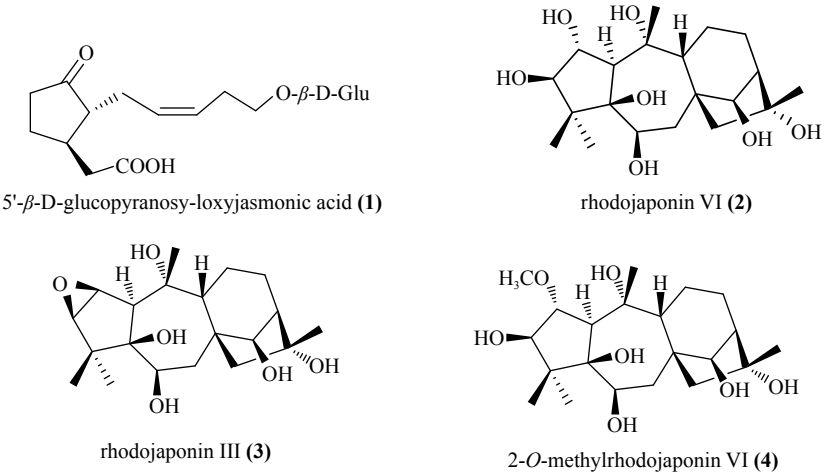
Results and Discussion

The main remaining chemical constituents of diterpenoid fraction from R. molle fruits

The chemical constituents of diterpenoid fraction were studied by chromatographic and NMR methods. From these results, it was determined that the fraction was mainly composed of 5′-β-D-glucopyranosy-loxyjasmonic acid (**1**), rhodojaponin VI (**2**), rhodojaponin III (**3**), and 2-*O*-methyl-rhodojaponin VI (**4**) (Fig.1). In the HPLC-ELSD spectrum, the total content of the four compounds was more than 95% by the area normalization method, which suggested that **1** and **4** were the main components remaining to be identified in this fraction (Fig. 2). Diterpenoids in *R. molle* are both the main active and toxic ingredients, therefore the identification of chemical composition of diterpenoid fraction will not only increase the quality and stability, but also improve the safety of its use in therapeutics.

Anti-RA studies of diterpenoid fraction of R. molle fruits

Tripterygium polyglycosides, a famous Chinese medicine extract of *Tripterygium wilfordii*, was widely used to treat RA in the clinic [17], so it was chosen as a positive control to compare the anti-RA activity of other traditional Chinese medicines. The results of CIA rat experiment showed that high dose of diterpenoid fraction (0.6 $mg \cdot kg^{-1} \cdot d^{-1}$) significantly alleviated symptoms and de-



5'-*β*-D-glucopyranosy-loxyjasmonic acid **(1)**

rhodojaponin VI **(2)**

rhodojaponin III **(3)**

2-*O*-methylrhodojaponin VI **(4)**

Fig. 1 Structures of the main chemical constituents in diterpenoid fraction

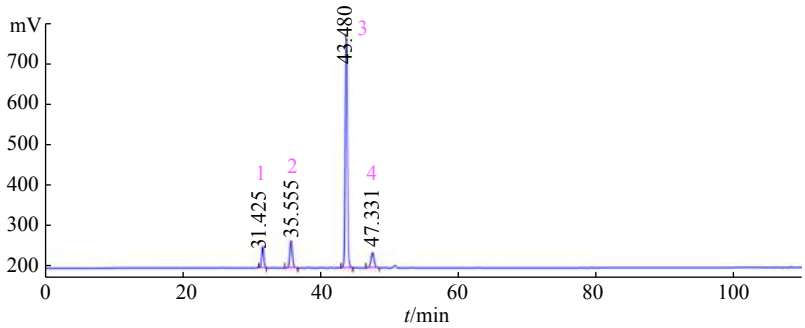


Fig. 2 The HPLC-ELSD chromatography of diterpenoid fraction

creased the arthritis index score (Figure S4 in Supplementary Information and Fig. 3). The treatment reduced the level of pro-inflammatory cytokines (IL-1*β*, IL-6 and TNF-*α*), inhibited the infiltration of inflammatory cells, and protected the synovial membrane as well (Fig. 4). These effects are similar to the positive therapeutic outcome already demonstrated with tripterygium polyglycosides treatment (50.0 $mg \cdot kg^{-1} \cdot d^{-1}$). However, from the perspective of weight changes, diterpenoid fraction was less effective than the positive drug, implying stronger toxicity (Fig. 5). Consequently, the therapeutic window range of diterpenoid fraction was relatively wider (LD_{50} of diterpenoid fraction to KM mice was 4.0 $mg \cdot kg^{-1}$, and the high dose in this experiment was 0.6 $mg \cdot kg^{-1}$. LD_{50} of tripterygium polyglycosides to KM mice was 100.0 $mg \cdot kg^{-1}$, and the experimental dose was 50.0 $mg \cdot kg^{-1}$). Furthermore, high dose of diterpenoid fraction (1.2 $mg \cdot kg^{-1} \cdot d^{-1}$) demonstrated good anti-inflammatory and analgesic effects in mice auricle swelling test, writhing body test, and hot plate test (see supplementary information). These results are similar to that of all positive drugs, further indicating the potential therapeutic effects of diterpenoid fraction on RA.

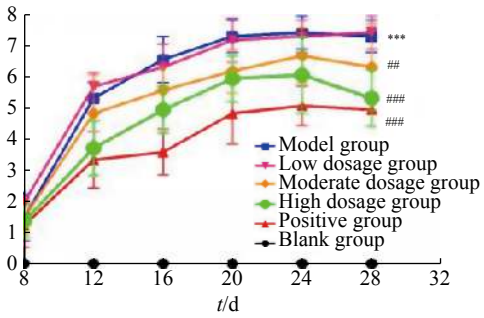


Fig. 3 The arthritis index of CIA rats. All data are presented as means ± SEM (n = 8). $^{***}P < 0.001$ *vs* blank group; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs* model group

Previous studies indicated that many pro-inflammatory cytokines, such as TNF-*α*, IFN-*γ*, GM-CSF, IL-1, IL-6 and IL-17 are increased abnormally in RA affected joints. The significance of cytokines in the pathogenesis of RA has been confirmed by the successful use of biologic agents that suppress the actions of TNF-*α*, IL-1 and IL-6 in RA [18]. In this study, the content of pro-inflammatory cytokines in the serum of model group was significantly increased with the development of RA, indicating that the secretion of pro-inflammatory cytokines is closely related to the occurrence and development of RA (Fig. 6). With this understanding, diterpenoid fraction may alleviate and inhibit the disease of CIA rats by reducing the level of pro-inflammatory cytokines. In the serum of model group, the level of anti-inflammatory cytokines

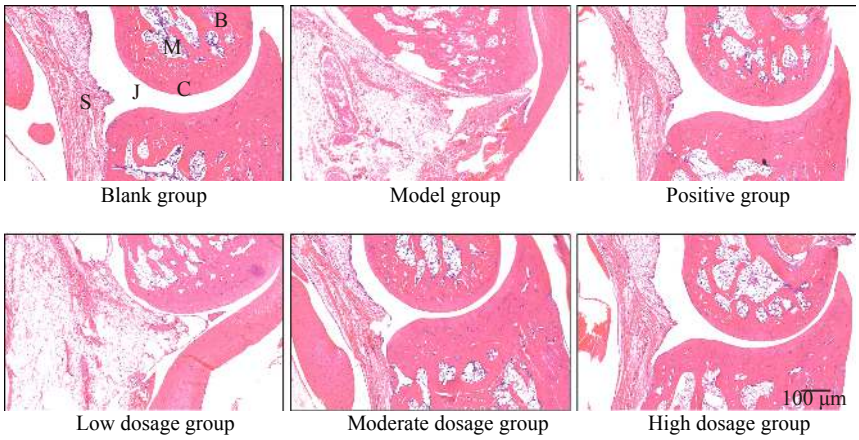


Fig. 4 The histopathological examinations of CIA rats B, bone; C, cartilage; J, joint-space; M, marrow; S, synovium

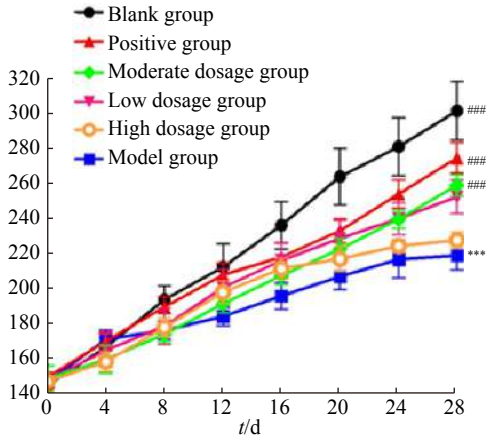


Fig. 5 The weight changes of rats. All data are presented as means ± SEM (n = 8). $^{***}P$ < 0.001 *vs* blank group; $^{###}P$ < 0.001 *vs* model group

also increased significantly, which showed that the rats were responding to the disease by secreting anti-inflammatory cytokines from their own immune systems. However, both the positive control tripterygium polyglycosides and experimental diterpenoid fraction had little effect on the cytokine levels, suggesting that diterpenoid fraction may not play an anti-RA role by promoting the secretion of anti-inflammatory cytokines. Additionally, the serum levels of IL-1β, IL-6 and TNF-α in model group were significantly increased, which indicated that IL-1β, IL-6 and TNF-α played a certain role in the pathogenesis of RA (Fig. 6).

Inhibition of T/B lymphocytes proliferation

In general, abnormal proliferation of T/B lymphocytes is the main cellular characteristic of the pathogenesis of RA [19]. As shown in Fig. 7 and Fig. 8, medium and high doses of diterpenoid fraction and rhodojaponin III had significant ef-

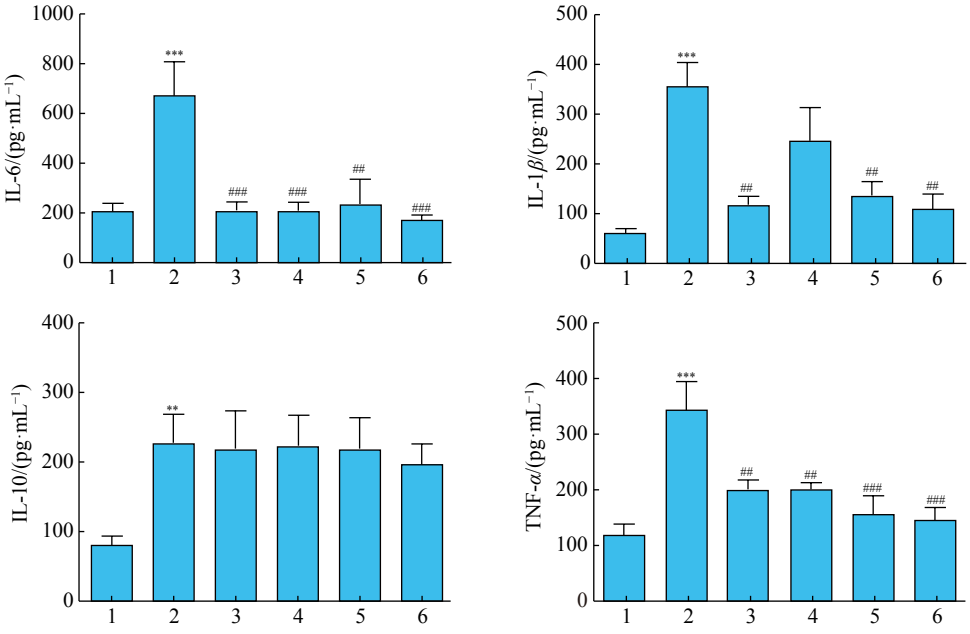


Fig. 6 The levels of cytokines of CIA rats. 1, Blank group; 2, Model group; 3, Positive group; 4, Low dosage group; 5, Moderate dosage group; 6, High dosage group. All data are presented as means ± SEM (n = 8). $^{**}P$ < 0.01, $^{***}P$ < 0.001 *vs* blank group; $^{##}P$ <0.01, $^{###}P$ < 0.001 *vs* model group

fects on inhibiting T and B lymphocyte proliferation ($P < 0.01$), which was similar to that of the positive control drugs (tripterygine polyglycosides and methotrexate). At the same time, rhodojaponin VI and total extract of *R. molle* fruits at high doses had a strong effect on inhibiting T and B lymphocyte proliferation ($P < 0.05$). The results of T/B lymphocyte proliferation experiments together with the results of serum cytokine assay revealed the mechanism of anti-RA effects of diterpenoid fraction may be related to its immune effect and inhibition of pro-inflammatory cytokines, as shown in Fig. 9 [20].

Conclusion

In conclusion, the remaining components of diterpenoid fraction are mainly composed of 5′-*β*-D-glucopyranosy-loxy-jasmonic acid and 2-*O*-methylrhodojaponin VI, except for the

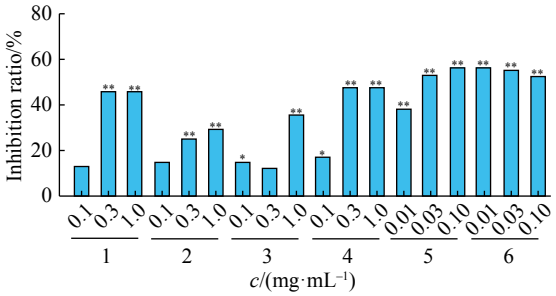


Fig. 7 The result of T lymphocytes proliferation test. 1, diterpenoid fraction; 2, total extract of *R. molle* fruits; 3, rhodojaponin VI; 4, rhodojaponin III; 5, tripterygine polyglycosides; 6, methotrexate. All data are presented as means ± SEM ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* CD3/28 antibodies induced group

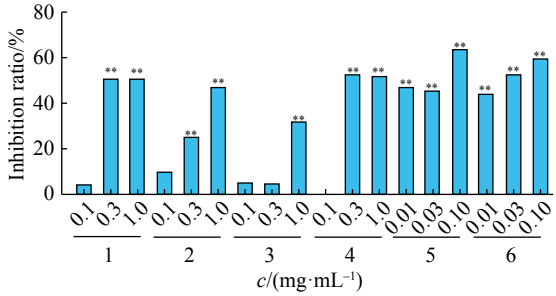


Fig. 8 The result of B lymphocytes proliferation test. 1, diterpenoid fraction; 2, total extract of *R. molle* fruits; 3, rhodojaponin VI; 4, rhodojaponin III; 5, tripterygine polyglycosides; 6, methotrexate. All data are presented as means ± SEM ($n = 3$). $^{**}P < 0.01$ *vs* CD3/28 antibodies induced group

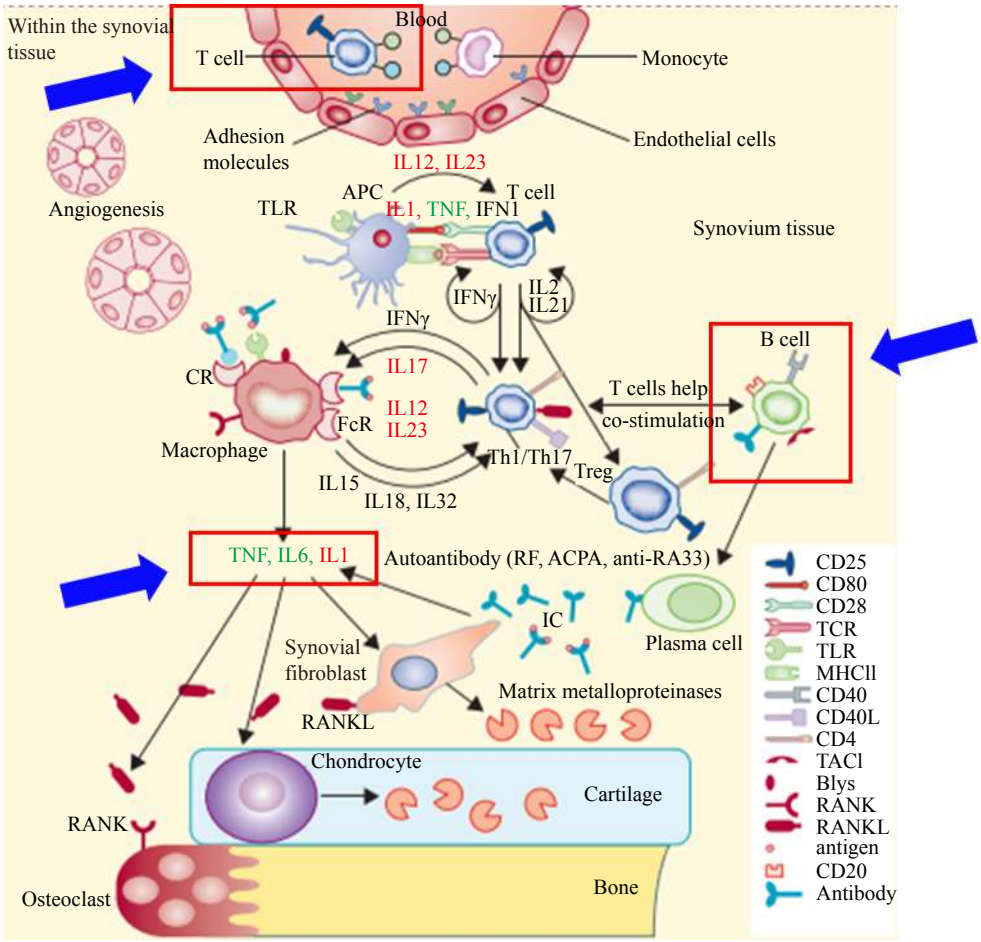


Fig. 9 Mechanism of diterpenoid fraction in the treatment of RA

two index compounds, rhodojaponin III and VI. The results of the pharmacodynamic evaluation showed that diterpenoid fraction had therapeutic effects on CIA rats, improved the symptoms of RA in CIA rats, and possessed anti-inflammatory and analgesic effects (see supplementary information). The mechanism of anti-RA in diterpenoid fraction was related to its immune function and inhibition of pro-inflammatory cytokines. In addition, cytotoxic experiments showed that diterpenoid fraction was less toxic to spleen cells than tripterygine polyglycosides and methotrexate, but the anti-RA effect was equivalent. These results indicate that diterpenoid fraction has good prospects for further development. Considering the similarity in the mechanism of treating RA, diterpenoid fraction may be developed as a clinical supplement or alternative to tripterygium polyglycosides in the future.

References

[1] Jiangsu New Medical College. *Chinese Materia Medica Dictionary* [M]. Shanghai: Shanghai People's Publishing House, 1986: 1448.

[2] Luo YY, Li YL, Li YQ, *et al*. Observation on 70 cases of rheumatoid arthritis treated by *Rhododendron molle* root tablets [J]. *Chin J Integr Tradit West Med*, 1990, **6**: 376-377.

[3] Luo YY, Zha JQ, Li YQ, *et al*. Treatment of 114 cases of rheumatoid arthritis with *Rhododendron molle* roots [J]. *Chin J Integr Tradit West Med*, 1993, **13**(8): 489-490.

[4] Fang X, Xiong Z, Li J, *et al*. The anti-RA effects of *Rhododendron molle* roots on collagen-induced arthritis in rats [J]. *J Complem Med Altern Health*, 2017, **3**(1): 1-5.

[5] Li Y, Zhu YX, Zhang ZX, *et al*. Diterpenoids from the fruits of *Rhododendron molle*, potent analgesics for acute pain [J]. *Tetrahedron*, 2018, **74**(7): 693-699.

[6] Zhou SZ, Yao S, Tang CP, *et al*. Diterpenoids from the flowers of *Rhododendron molle* [J]. *J Nat Prod*, 2014, **77**(5): 1185-1192.

[7] Zhou SZ, Tang CP, Ke CQ, *et al*. Three new dimeric diterpenes from *Rhododendron molle* [J]. *Chin Chem Lett*, 2017, **28**(6): 1205-1209.

[8] Li CJ, Liu H, Wang LQ, *et al*. Diterpenoids from the Fruits of *Rhododendron molle* [J]. *Acta Chim Sin*, 2003, **61**(7): 1153-1156.

[9] Klocke JA, Hu MY, Chiu SF, *et al*. Grayanoid diterpene insect antifeedants and insecticides from *Rhododendron molle* [J]. *Phytochemistry*, 1991, **30**(6): 1797-1800.

[10] Dong LC, Zhang XH, Ma J, *et al*. The integrated pharmacokinetics of major rhodojaponins correlates with the cardiotoxicity after oral administration of *Rhododendri Mollis* Flos extract in rats [J]. *J Ethnopharmacol*, 2014, **157**: 69-78.

[11] Liu CX. *Rhododendron molle* poisoning cause Ⅱ degrees sinus conduction block in 1 case [J]. *Zhejiang J Tradit Chin Med*, 2012, **47**(2): 84.

[12] Wang ZY. A case report of *Rhododendron molle* poisoning [J]. *Chin J Clin*, 1988, **5**: 287.

[13] Yao YM, Fang X, Zhang JQ, *et al*. Preparation of diterpenoid fraction from fruits of *Rhododendron molle* G. Don by macroporous resin combined with normal-phase silica gel column chromatography [J]. *Chin J Pharm*, 2019, **50**(12): 1444-1449.

[14] Setoguchi C, Tsuji F, Katsuta O, *et al*. Combined effects of bucillamine and etanercept on a rat type II collagen-induced arthritis model [J]. *Mod Rheumatol*, 2010, **20**(4): 381-388.

[15] Brand DD, Latham KA, Rosloniec EF. Collagen induced arthritis [J]. *Nat Protoc*, 2007, **2**: 1269-1275.

[16] Huang HY, Liu JJ, Xi RR, *et al*. An investigation of hormesis of trichloroethylene in L-02 liver cells by differential proteomic analysis [J]. *Mol Biol Rep*, 2008, **36**(8): 2119-2129.

[17] Canter PH, Lee HS, Ernst E. A systematic review of randomised clinical trials of *Tripterygium wilfordii* for rheumatoid arthritis [J]. *Phytomedicine*, 2006, **13**(5): 371-377.

[18] Isomaki P, Junttila I, Vidqvist KL, *et al*. The activity of JAK-STAT pathways in RA: constitutive activation of STAT3 correlates with interleukin 6 levels [J]. *Rheumatology*, 2015, **54**(6): 1103-1113.

[19] Karouzakis E, Neidhart M, Gay RE, *et al*. Molecular and cellular basis of rheumatoid joint destruction [J]. *Immunol Lett*, 2006, **106**(8): 8-13.

[20] Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis [J]. *Lancet*, 2016, **388**(10055): 2023-2038.

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