Effects of dandelion tea on type II collagen induced arthritis in mice by regulation of ROR-γt/Foxp3 signaling

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Abstract

In this study, type II collagen was used to induce arthritis in mice to test the effect and mechanism of dandelion tea. The visual manifestations of arthritis, histopathological conditions of spleen, serum inflammatory factors and gene expression of synovium of foot and ankle joints in mice were detected by the methods of arthritis index (AI), spleen index, enzyme linked immunosorbent assay (ELISA), hematoxylin-eosin (H&E) staining and quantitative polymerase chain reaction (qPCR). The compound components of dandelion tea were determined by high performance liquid chromatography (HPLC). The experimental results show that dandelion tea can reduce the AI value, spleen index and paw swelling degree of arthritis mice, reduce the pathological damage of spleen, and increase the serum IL-10 and TGF-β levels, and can up-regulate Foxp3 expression and down regulate ROR-γt expression in the synovium of ankle joint. At the same time, the results of component analysis showed that the efficacy of dandelion tea came from the contents of gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, rutin, myricetin, isoquercitrin, isochlorogenic acid and luteolin. It can be seen that dandelion tea is a health drink with good intervention effect on arthritis.

Keywords: dandelion tea; ROR-γt/Foxp3; type II collagen; arthritis; immunity.

Practical Application: Dandelion tea is a traditional Chinese drink, but its role and mechanism are still lacking in scientific research. In this study, animal experiments confirmed that it has a good intervention effect on arthritis, and its mechanism of action was preliminarily determined to be related to ROR-γt /Foxp3 signaling. The research results are conducive to the further development and utilization of dandelion tea, and accumulate theoretical basis for developing it for better health care products.

1 Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by progressive destruction of synovium, cartilage and subchondral bone, resulting in joint deformation and high disability rate (Alghasham & Rasheed, 2014). The pathogenesis of RA has not been fully defined, but scientific studies have confirmed that inflammatory exudation of synovial tissue and recruitment of immune cells are the core links. Arthritis refers to the inflammatory disease that occurs in human joints and their surrounding tissues and is caused by inflammation, infection, degeneration, trauma or other factors. It can be divided into dozens of types. The clinical manifestations are joint redness, swelling, heat, pain, dysfunction and joint deformity. In severe cases, joint disability will be caused and the quality of life of patients will be affected. According to statistics, half of the people over the age of 50 suffer from osteoarthritis, 90% of the women and 80% of the men over the age of 65 suffer from osteoarthritis, and the life expectancy of those who are serious will be shortened by 10 to 15 years (Diogo et al., 2014). In the abnormal immune process of RA, the proportion of helper T cell 17 (Th17) and regulatory T cell (Treg) is unbalanced, which is considered to be an important factor in the occurrence and development of RA (Al-Megrin et al., 2022). T cell immunoglobulin and T cell immunoglobulin and

mucin domain-containing molecule-3 (Time-3) are expressed on the surface of immune cells to regulate cell proliferation, differentiation and apoptosis. In addition, galectin-9 (Gal-9) is its natural ligand (Song et al., 2015). In animal models of autoimmune diseases such as Crohn's disease (Li et al., 2010), multiple sclerosis (Kaneyama et al., 2014), autoimmune hepatitis (Ju et al., 2014), and type 1 diabetes, activating Gal-9/Tim-3 pathway can induce immune tolerance, and the mechanism may be related to Tim-3 inducing Th17 apoptosis, stimulating Treg proliferation, and reconstituting Th17/Treg balance. Two key expressions in the Gal-9/Tim-3 pathway are orphan nuclear receptor-γt (ROR-γt) and forkhead box P3 (Foxp3) (Li et al., 2010; Kaneyama et al., 2014; Ju et al., 2014; Shi et al., 2012). However, the role of this pathway in RA lesions has not been confirmed by randomized controlled trials.

A perennial plant called dandelion has long been employed in Chinese traditional medicine. Acute mastitis, lymphadenitis, malignant boils, acute conjunctivitis, cold fever, acute tonsillitis, acute bronchitis, gastritis, hepatitis, cholecystitis, and urinary tract infection are among the conditions that the dandelion plant body may cure (Gargouri et al., 2012; González-Castejón et al., 2012; Davaatseren et al., 2013; Ghaima et al., 2013; Ovadje et al.,

2012). Dandelion is a fresh bud that grows every spring. It can be dried to make health tea for people to drink. Dandelion tea can supplement the human body with rich nutrition, clear away heat, detoxify, lose weight and delay aging. Now research shows that health tea can prevent and regulate arthritis (Li et al., 2015). Through the effect of natural health tea on arthritis, the quality of life caused by arthritis can be effectively avoided. In this study, a mouse model of collagen induced arthritis (CIA) was established to simulate human RA, and through the effect and possible mechanism of dandelion tea, it provides a theoretical basis for looking for natural health tea to interfere with RA.

2 Materials and methods

2.1 Dandelion tea extraction

The 200 g of dandelion tea (Zhejiang Yifutang Tea Industry Co., Ltd, Hangzhou, Zhejiang, China) that had been freeze-dried were ground into a fine powder, 4 L of ethanol at a concentration of 70% (v/v), were added, and the mixture was extracted at 60 °C for three hours. To produce the dandelion extract, the extract was filtered and the solvent was evaporated in a rotary evaporator.

2.2 Establishment of animal model

Type II collagen (R&D Systems, Inc., Minneapolis, MN, USA) was added with 0.1 mol/L glacial acetic acid, stirred and dissolved in an ice bath to prepare a 2 mg/mL solution. After being refrigerated at 4 °C overnight, the type II collagen solution was mixed with complete Freund's adjuvant (CFA) in an equal volume of 1:1 to prepare an emulsifier. Six week old BALB/c mice, half male and half female, totally 40, were purchased from Shanghai Wuhua Biotechnology Co., Ltd, Shanghai, China. Mice were randomly divided into four groups, namely, normal group, model group, dandelion tea low concentration (DTL) group and dandelion tea high concentration (DTH) group. After the start of the experiment, under sterile conditions, mice in other groups except the normal group were intradermally injected with type II collagen emulsifier at the base of the tail of the mice at a dose of 0.1 mL/mice. (Yang et al., 2016). At the same time, the mice in the normal group and the model group were gavaged with 0.2 mL of distilled water every day, and the mice in the DTL and DTH groups were gavaged with dandelion tea extract at a dose of 50 and 100 mg/kg for three weeks. After 21 days, the model group, DTL and DTH groups were given the same dose of enhanced immunity, and type II collagen was injected once again. The mice in each group continued to be gavaged according to the previous gavage dose for 14 days. Finally, the orbital blood was taken from the mice, and the visceral tissues were taken for later use. At the same time, vernier calipers were used to measure the thickness of the base of the mouse paws and toes, and the arthritis index was calculated according to the joint swelling. The spleen of mice was weighed and the spleen index was calculated, spleen index = (spleen mass, mg/mouse body mass, g) × 10.

2.3 Arthritis index (AI) determination

AI is a standard for measuring the swelling of the paw, and the swelling of the limbs is scored on a scale of 0 to 4: 0 means no redness, 0 points; 1, slightly swollen toe joints, 1 point; 2, toe joints and toes Joint swelling was scored as 2; grade 3 was swelling of the paw below the ankle joint, scored 3; and grade 4 was swelling of the entire paw including the ankle joint, scored 4. The AI was the sum of the joint swelling scores of the limbs, with a minimum of 0 and a maximum of 16 for each mouse. The higher the AI index, the more severe the joint symptoms.

2.4 Enzyme linked immunosorbent assay (ELISA)

The collected mouse whole blood was placed in a 37 °C incubator for 1 h, placed in a centrifuge tube, centrifuged at 2000 r/min for 15 min, the supernatant was aspirated Long et al. (2022), and the concentrations of IL-10 and TGF-β were measured according to the instructions of the ELISA kit (R&D Systems, Inc.).

2.5 Hematoxylin-eosin (H&E) staining

The spleen tissue from the dissected mice was preserved in 10% (v/v) formalin by volume fraction after being washed three times with saline. The fixed tissue was dehydrated at 4 °C for 48 hours, paraffin-embedded, sliced into 5-10 μm slices, and stained with H&E dyes. Finally, a light microscope was used to assess tissue pathology (BX53, Olympus, Tokyo, Japan).

2.6 qPCR experiment

Synovium of mouse left forefoot ankle joint weighing 0.1 g was homogenized with 1.0 mL of RNAzol (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and then washed once more with saline to extract the RNA from the tissue. The RNA content was corrected after the OD_{260}/OD_{280} was computed at 1 μg/L. Following cDNA synthesis, the qPCR reaction was conducted using 1 μL of cDNA, 10 μL of SYBR Green PCR Master Mix, 1 μL of primers (ROR-γt, forward primer: 5'-CATTCAGTATGTGGTGGAGTTTGC-3', reverse primer: 5'-GACTA GGACGACTTCCATTGCTC-3'; FoxP3, forward primer: 5'-GTACACCCAGGAAAGACAGCAAC-3', reverse primer: 5'-CTCGAAGACCTTCTCACAACCA-3'), and 7 μL of sterile distilled water. After preparing the reaction solution, it was inserted in the real-time fluorescence quantitative PCR apparatus (StepOnePlus, Thermo Fisher Scientific, Waltham, MA, USA). The mRNA was amplified at the defined conditions. β-actin (forward primer: 5'-CACGATGGAGGGGCCGGACTCATC-3', reverse primer: 5'-TAAAGACCTCTATGCCAACACAGT-3') was utilized as the internal parameter, and the relative expression intensity of each gene was computed using the $2^{-\Delta\Delta Ct}$ technique (Hu et al., 2022).

2.7 High performance liquid chromatography (HPLC)

To make a 1 mg/mL solution, weigh precisely 2 mg of the dried standard and 2 mL of methanol at a constant volume. A disposable needle and filter membrane were also used to aspirate 0.5–1.0 mL of the sample solution into an injection bottle. The chromatographic conditions were as follows: Agilentzorbax SB-C18 column (5 μm, 4.6 mm × 250 mm); column temperature of 30 °C; mobile phase of methanol, acetic

acid water, acetonitrile, and clean water; flow rate of 0.5 mL/min; detection wavelength of 359 nm; and injection volume of 10 μL (1260 liquid chromatograph, Agilent, Santa Clara, CA, China). The chromatogram was then used to assess the compound's composition.

2.8 Statistical analysis

The final experimental findings are shown as the standard deviation of the mean after data were gathered for each mouse. The one-way variance technique's results were tested to see if they were significant at the $P < 0.05$ level using SPSS software (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Mouse spleen index

Arthritis had a certain effect on the body weight of mice. The body weight of the normal group mice was significantly higher than that of the model group, which induced a corresponding decrease in the body weight of the key mice (Table 1). Dandelion tea could inhibit the weight loss. The spleen index showed the opposite trend. The normal group had the lowest spleen index and the model group had the highest. Dandelion tea could reduce the spleen index of arthritic mice. The normal group had the lowest spleen index and the model group had the highest. Dandelion tea could reduce the spleen index of arthritic mice.

3.2 Mouse paw swelling

The normal group had the thinnest footpad (1.49 ± 0.22 mm), the model group had the thickest (2.12 ± 0.25 mm), and the model group showed the highest degree of swelling (Figure 1). Dandelion tea can make the thickness of the foot pads of arthritic mice thinner and reduce the degree of foot swelling, and the higher the concentration (1.69 ± 0.17 mm), the better the effect.

3.3 Mouse AI value

There was no redness and swelling in the feet of the mice in the normal group, and the AI value was always 0 during the experiment (Figure 2), while the AI index of the mice in the model group reached 12.1 ± 1.7, which was significantly different from that of the mice in the normal group ($P < 0.05$). Compared with the model group, the arthritis index of mice in the dandelion

Table 1. Body weight and spleen index of arthritis mice.

Group	Body weight (g)	Spleen weight (mg)	Spleen index
Normal	32.54 ± 2.11[a]	89.5 ± 6.6[d]	27.65 ± 3.06[d]
Model	26.89 ± 3.02[b]	223.3 ± 10.5[a]	83.96 ± 10.04[a]
DTL	29.89 ± 2.68[a]	171.6 ± 8.9[b]	57.77 ± 5.47[b]
DTH	31.16 ± 2.47[a]	123.8 ± 7.7[c]	39.94 ± 4.18[c]

DTL: 50 mg/kg dandelion tea treatment, DTH: 100 mg/kg dandelion tea treatment. The English letters in each column are the marks of statistical analysis. Different letters indicate that there is a significant statistical difference between the corresponding two groups ($P < 0.05$), and the same letter indicates that there is no significant statistical difference between the corresponding two groups ($P > 0.05$).

tea group was significantly improved, and the effect of high concentration (DTH: 3.8 ± 0.4) was more obvious ($P < 0.05$).

3.4 Anti-inflammatory factors

The serum anti-inflammatory factor levels of mice were detected by ELISA (Figure 3), and it was found that the levels of IL-10 and TGF-β in the normal group were the highest, while those in the model group were the lowest. Compared with the model group, dandelion tea can increase the serum levels of IL-10 and TGF-β in arthritic mice.

3.5 Histopathological observation of mouse spleen

The spleen tissue section showed that the spleen tissue cells of the normal mice were intact (Figure 4). The spleen tissue cell
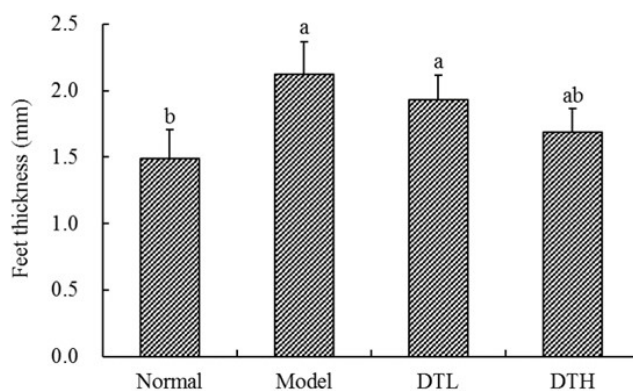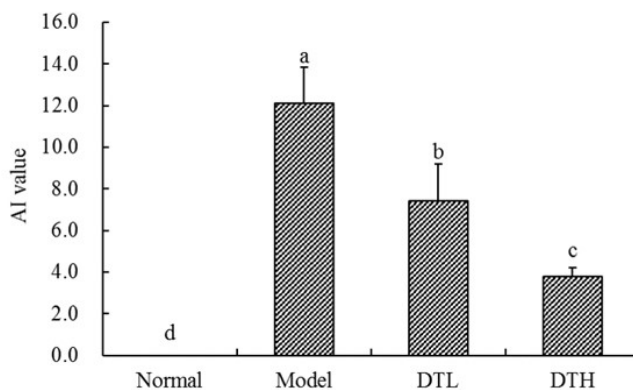

Figure 1. Feet thickness of arthritis mice. DTL: 50 mg/kg dandelion tea treatment, DTH: 100 mg/kg dandelion tea treatment. The English letters are the marks of statistical analysis. Different letters indicate that there is a significant statistical difference between the corresponding two groups ($P < 0.05$), and the same letter indicates that there is no significant statistical difference between the corresponding two groups ($P > 0.05$).

Figure 2. Arthritis index (AI) of arthritis mice. DTL: 50 mg/kg dandelion tea treatment, DTH: 100 mg/kg dandelion tea treatment. The English letters are the marks of statistical analysis. Different letters indicate that there is a significant statistical difference between the corresponding two groups ($P < 0.05$), and the same letter indicates that there is no significant statistical difference between the corresponding two groups ($P > 0.05$).
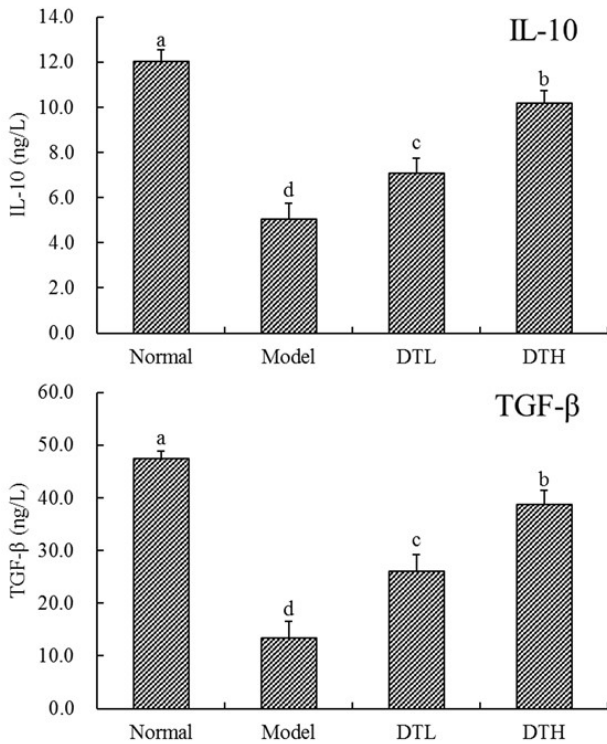
Figure 3. Serum IL-10 and TGF-β levels of arthritis mice. DTL: 50 mg/kg dandelion tea treatment, DTH: 100 mg/kg dandelion tea treatment. The English letters are the marks of statistical analysis. Different letters indicate that there is a significant statistical difference between the corresponding two groups ($P < 0.05$), and the same letter indicates that there is no significant statistical difference between the corresponding two groups ($P > 0.05$).

structure of the mice in the model group was destroyed, and a large number of cell necrosis occurred. The spleen tissue of the dandelion tea group also had some bad cells, especially under the action of high concentration of dandelion tea, the necrosis of spleen tissue cells was greatly reduced, and the cell structure was basically intact.

3.6 Relative expression of ROR-γt and FoxP3 in mouse synovial tissue

The relative mRNA expression of ROR-γt in the synovial tissue of mice in the model group was the highest (Figure 5), and the FoxP3 expression was lowest. Compared with the model group, dandelion tea could down-regulate the relative expression of ROR-γt and up-regulate the FoxP3 in synovial tissue. The expression levels were the closest to the normal group.

3.7 Composition of dandelion tea

The test results show that dandelion tea contains 10 compounds (Figure 6), which are gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, rutin, myricetin, isoquercitrin, isochlorogenic acid and luteolin.

4 Discussion

The etiology of rheumatoid arthritis is still unknown. At present, it is believed that various factors such as environment, genetics, and immune disorders play an important role in the pathogenesis of RA. In particular, immune disorders are considered to be the main mechanism of RA pathogenesis. The abnormal immune balance of CD4^{+} T cell subsets such
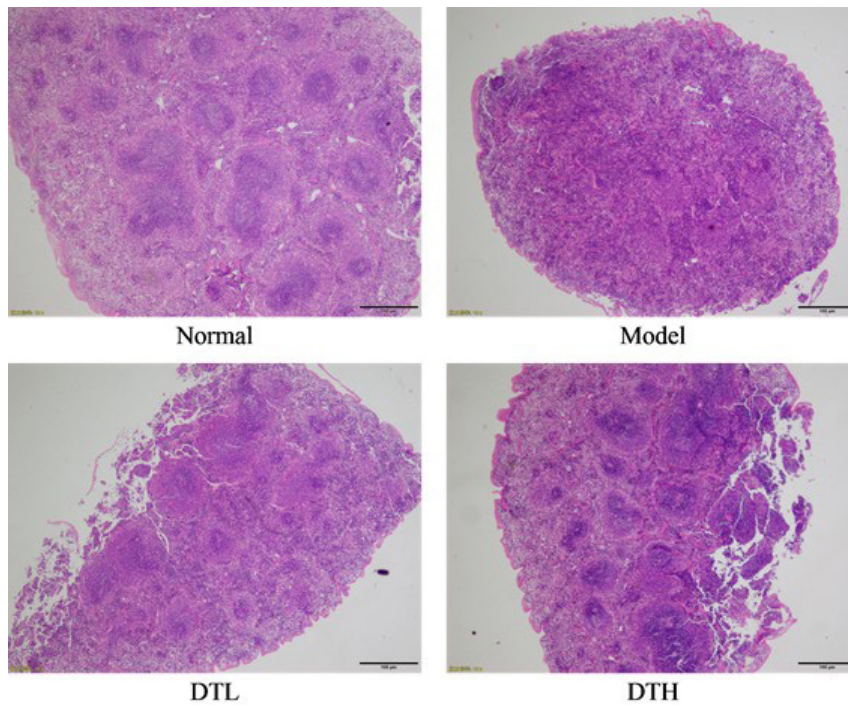

Figure 4. Pathological observation of spleen tissue in arthritis mice. DTL: 50 mg/kg dandelion tea treatment, DTH: 100 mg/kg dandelion tea treatment.
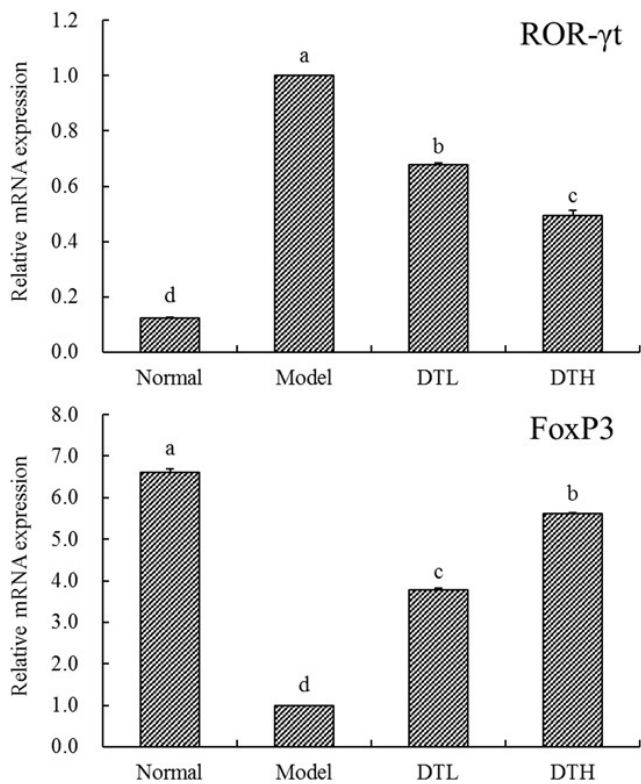
Figure 5. ROR-γt and FoxP3 mRNA expression in mouse synovial tissue of arthritis mice. DTL: 50 mg/kg dandelion tea treatment, DTH: 100 mg/kg dandelion tea treatment. The English letters are the marks of statistical analysis. Different letters indicate that there is a significant statistical difference between the corresponding two groups ($P < 0.05$), and the same letter indicates that there is no significant statistical difference between the corresponding two groups ($P > 0.05$).

as Treg plays an important role in the pathogenesis of RA (Yang et al., 2022). There are many factors related to the differentiation and regulation of Th17 and Treg cells. Each factor presents a complex network relationship, which is relatively independent and promotes and restricts each other. The mutual inhibition between subpopulations is realized through the interaction between key differentiation transcription factors in the cell (Hueber et al., 2010). ROR-γt is a specific transcription factor of Th17 cells. If ROR-γt deficiency, the expression of Th17 and its secreted IL-17 was significantly decreased, and the incidence of autoimmune diseases was also decreased, while ROR-γt overexpression, CD4$^+$ T cells can express a large amount of IL-17 without foreign cytokines. ROR-γt mainly promotes the differentiation of Th17 cells and the expression of IL-17 through STAT3 mediated signaling pathway. IL-17-driven inflammation is typically regulated by regulatory T cells and the anti-inflammatory cytokines IL-10 and TGF-β. However, if dysregulated, the IL-17 response can contribute to the immunopathology of infection or autoimmunity. The inflammatory response induced by Th17 cells and their secreted cytokine IL-17 plays an important role in RA synovial joint injury (Yang et al., 2013).

Foxp3 is a specific transcription factor of regulatory T cell and Treg. CD4$^+$ CD25$^+$ Tregs specifically express the transcription factor Foxp3 and secrete inhibitory cytokines (such as IL-10, TGF-β). Other pathways play the role of anti-inflammatory and maintenance of autoimmune tolerance, and are CD4$^+$ T cells with immune negative regulatory function. Treg can inhibit the function of T cells and antigen-presenting cells, reduce the production of proinflammatory cytokines and antibody secretion, etc. Treg cells can not only inhibit the occurrence of autoimmune diseases, but also play an important role in maintaining the balance of the immune internal environment (Wang et al., 2022). ROR-γt/Foxp3 balance can determine the differentiation of initial T cells to Th17 or Treg after stimulation by antigen. Th17 cells mainly secrete the proinflammatory factor IL-17 to promote the inflammatory response, while Treg cells inhibit the overactivation of effector T cells, maintain autoimmune tolerance, and suppress the inflammatory response. Therefore, ROR-γt/Foxp3 balance fundamentally affects the balance of Th17/Treg and the occurrence and development of RA (Chen et al., 2011).

Splenomegaly is the clinical manifestation of rheumatoid arthritis, a severe type of rheumatoid arthritis with splenomegaly and leukopenia. At the same time, experimental spleen lesions can be verified by spleen index and spleen pathological observation (Feng et al., 2015). In this study, the arthritic mice also had typical elevated spleen index and spleen cell structure damage, and dandelion tea could inhibit these spleen lesions, suggesting that dandelion tea could improve arthritis by regulating spleen function. In addition, the joint swelling degree and AI value of mice in the dandelion tea group were significantly different from those in the model group, confirming that dandelion tea can inhibit the occurrence and development of joint inflammation in CIA mice to a certain extent, and reduce the symptoms of joint inflammation. The increased levels of IL-10 and TGF-β can antagonize the effect of inflammatory mediators and reduce tissue lesions. This conclusion is also confirmed. Dandelion tea may regulate Th17 apoptosis through multiple pathways, activate Treg, and trigger a strong immune tolerance effect, thereby increasing the levels of IL-10 and TGF-β and inhibiting arthritis. In mice treated with dandelion tea, ROR-γt was significantly decreased compared with the model group, and Foxp3 was significantly increased compared with the model group, which eventually led to the differentiation of naive T cells into Treg cells, inhibited the intensity of inflammatory response, and had a therapeutic effect on CIA mice.

Gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, rutin, myricetin, isoquercitrin, isochlorogenic acid and luteolin are all good immunoregulatory and anti-inflammatory substances, which can inhibit the inflammatory response in various diseases, and also have a certain effect on immune regulation (Wang et al., 2014; Shi et al., 2013; Xie et al., 2014; Kandemir et al., 2022; Shen et al., 2020). The combined action of these active substances can interfere with arthritis and are the key substances in the action of dandelion tea.
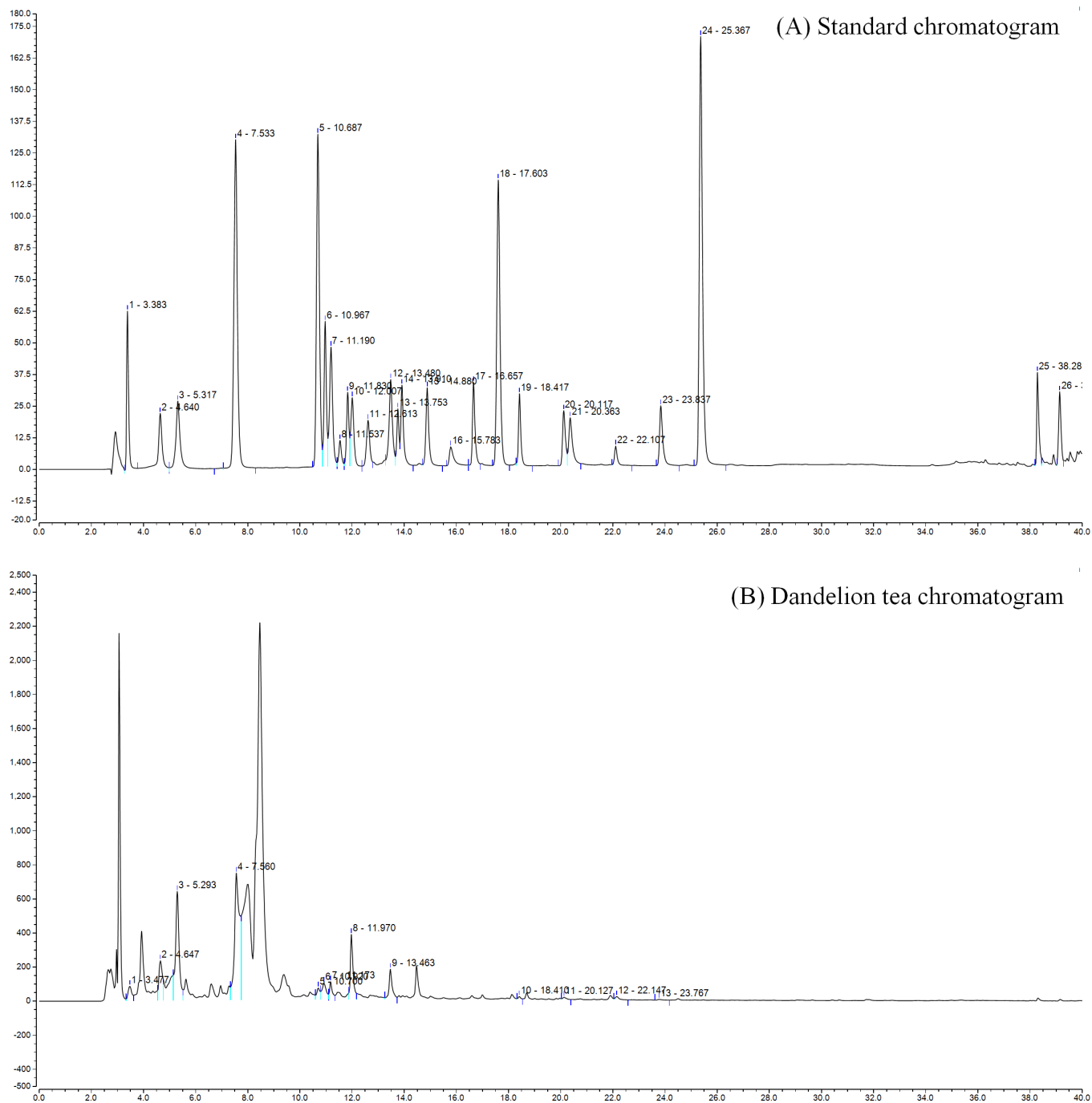
Figure 6. Compound composition of dandelion tea, in (A) standard chromatogram: (1) gallic acid, (2) protocatechuic acid, (3) chlorogenic acid, (4) caffeic acid, (5) p-coumaric acid, (6) rutin, (7) myricetin, (8) unknown, (9) isoquercetin, (10) ferulic acid, (11) dihydroquercetin, (12) isochlorogenic acid A, (13) astragaloside, (14) quercetin, (15) hesperidin, (16) myricetin, (17) unknown, (18) phlorin, (19) neohesperidin dihydrochalcone, (20) luteolin, (21) quercetin, (22) unknown, (23) apigenin, (24) hesperidin; in (B) dandelion tea chromatogram: (1) gallic acid, (2) protocatechuic acid, (3) chlorogenic acid, (4) caffeic acid, (5) p-coumaric acid, (6) rutin, (7) myricetin, (8) isoquercetin, (9) isochlorogenic acid A, (10) unknown, (11) luteolin, (12) unknown, (13) unknown.

5 Conclusion

In conclusion, this study proves that gavage of dandelion tea can improve the general condition of CIA mice and significantly inhibit the occurrence and development of joint inflammation. Its therapeutic effect may be related to regulating the balance of T lymphocyte subset-specific expression of transcription factors ROR-γt and Foxp3, and regulating the balance of Th17/Treg. The specific immune regulation mechanism still needs to increase

the sample size for further research. At the same time, it is also confirmed that dandelion tea is a kind of health tea that can act on arthritis, and has great development and utilization value.

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