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# Triterpenoids alleviate DSS-induced colitis by inhibiting Th17 cell differentiation

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# Abstract

Demethylzeylasteral (Dem), the key component of *Tripterygium wilfordii* Hook F, has been considered to be a traditional Chinese medicine for the treatment of rheumatic diseases. In this study, a mouse colitis model induced by dextran sulfate sodium (DSS) was constructed to clarify the role of Dem in the inflammatory bowel model (IBD). Our results showed that Dem attenuates symptoms of IBD in mice, as evidenced by shortened colon length and weight loss, accompanied by a decrease in the proportion of Th17 cells. Moreover, Dem was shown to inhibit the activation of JAK2 and STAT3. In the IBD mouse model, we used siRNAs targeting either JAK2 or STAT3 to explain the anti-inflammatory effect of Dem. Knockdown of either JAK2 or STAT3 abolished the Th17 inhibition of Dem in vitro. Studies have found that Dem has a significant therapeutic effect on CIA by inhibiting the proliferation and activation of Th17 cells, which further verifies the anti-inflammatory mechanism of Dem. These results suggest that Dem exerts anti-inflammatory effects by inhibiting the differentiation and activation of Th17 cells. Its mechanism may be related to the regulation of the JAK2-STAT3 signaling pathway. Our study will provide a theoretical basis for the application of *Tripterygium wilfordii* Hook F.

# Introduction

Inflammatory bowel disease (IBD) is an immune-mediated chronic intestinal inflammatory disease that includes ulcerative colitis (UC) and Crohn's disease (CD) [1]. Its inflammation is limited to the intestinal mucosa [2]. Musculoskeletal complications are a common complication in patients with IBD [3]. The characteristics of arthritis in UC patients are similar to those of rheumatoid arthritis (RA) [4]. RA is a common inflammatory autoimmune disease affecting multiple organs and tissues [5]. It is mainly characterized by chronic inflammation and synovial infiltration of immune cells [6]. However, sporadic clinical data show that IBD is associated with RA [7]. However, the specific relationship between the two remains to be further studied.

In both diseases, the immune response is involved, and changes in $CD4^+$ T cells are common. $CD4^+$ T cells, as T-cell subsets, have been associated with the initiation and maintenance of immune diseases. Th17 cells are one of the cell subsets differentiated after activation of $CD4^+$ T cells [8]. Th17 cells are involved in the pathogenesis of IBD. There are also a large number of Th17 cells and their cytokines in the mucosa of IBD patients [8]. It not only protects the intestinal mucosa by maintaining the balance of the immune microenvironment but also intensifies the intestinal inflammatory response via proinflammatory cytokines [9]. In addition, Th17-cell subpopulations are also increased in the peripheral blood of patients with early RA [10].

The Th17-cell-specific transcriptional regulator is ROR-γt [11]. In previous studies, Th17 cells were considered to be closely related to immune diseases. Th17 cells secrete IL-17A, IL-17F, and IL-22, which can interact with other cytokines, such as TNF-α, IL-1β, and IFN-γ, to produce synergistic effects that mediate inflammatory responses [12, 13]. Th17 cells are affected by many factors, and one of the key upstream factors is signal transducer and activator of transcription (STAT3). The factors that affect the activation of STAT3 include Janus kinase signal transducers 2 (JAK2) and mammalian target of rapamycin (mTOR), which play a crucial role in the process of regulating immunity [14, 15].

*Tripterygium wilfordii* Hook F is a perennial vine of Celastraceae that contains alkaloids, diterpenoids, triterpenes and other chemical constituents. It is mainly used for the treatment of inflammatory-related diseases in the clinic [16-19]. Some studies have shown that it has anti-inflammatory, immunoregulatory, and antitumor activities [20-23]. Demethylzeylasteral (Dem) is a phenolic demethyltriterpene compound with a molecular weight of 480 and a molecular formula of $C_{29}H_{36}O_6$ extracted from

*Tripterygium wilfordii* Hook F [24]. It has been reported that Dem has anti-inflammatory and immunosuppressive effects [18, 25]. However, its specific anti-inflammatory and immune mechanisms remain unclear.

Studies have shown that Dem has an immunosuppressive effect [26] and can inhibit cell proliferation [27]. A recent study has been further verified in rectal cancer [28]. Some studies have shown that Dem inhibits the inflammatory signaling pathway and has antirheumatic arthritis activity [29]. Therefore, in this study, a mouse model of dextran sulfate sodium (DSS)-induced colitis was established to explore the anti-inflammatory and immunomodulatory mechanisms of Dem, and it was also discussed in a collagen-induced arthritis (CIA) mouse model.

# Materials and Methods

### Animals

C57BL/6, DBA/1J mice aged 8 weeks were housed under standard laboratory conditions with temperature (22 ± 2 °C) and a light/dark cycle of 12/12 h throughout the study. Mice had access to tap water and standard rodent pelleted chow ad libitum. All animal experiments were approved by the Animal Care and Use Committee of Nanjing University of Chinese Medicine (approval numbers ACU210305).

The UC mouse model was set up as follows:

Groups of C57BL/6 mice were randomly divided into four groups: the control group, the model group, the Dem-L group (1 mg/kg), and the Dem-H group (2 mg/kg). Mice in each group were intragastrically administered daily. In all groups, except the control group, 2.5% DSS (w/v) was dissolved in autoclaved water for the first 7 days, and from the 8th to the 14th day, all groups received autoclaved water. From the 15th through the 21st day, all but the control group drank 2.5% DSS (w/v) solution. At the same time, fresh solution was substituted every other day. A daily dose of Dem (1 and 2 mg/kg) or 0.5% CMC-Na solution was given to each group from the 8th to 21st day. The remaining water intake of each group was measured from those days. At the end of the experiment, all mice were sacrificed. Throughout the experiment, the body weight and fecal status of the mice were recorded and monitored daily.

The CIA mouse model was set up as follows:

Groups of DBA/1J mice were randomly divided into five groups: the control group, the model group, the Dem-L group (1 mg/kg), the Dem-H group (2 mg/kg), and the leflunomide (LEF) group (2 mg/kg). Mice in each group were intragastrically

administered daily.

With the exception of the control group, each of the other four groups was treated as follows:

Complete Freund's adjuvant (CFA; Chondrex-7001) and type II collagen were mixed in equal volumes, vortexed to make an emulsion and injected subcutaneously into mice on day 0 at a dose setting of 100 μL for initial immunization. A booster immunization was performed on day 14 using identical amounts of collagen and Freund's adjuvant (IFA; Chondrex-7002) mixed together in an emulsion and injected subcutaneously into mice.

**Macroscopic observation**

After the last administration of the drug, the mice were fasted for 12 h and given water. The spleen was carefully removed and weighed. The colon was also removed, and its length was measured. A spleen index was calculated based on the formula: the weight of the spleen compared to the weight of the body.

**Colon damage assay**

Mouse colon damage was examined. Dark brown, ellipsoid, hard stool gets a score of 0, loose gets a score of 1, and thin, sloppy gets a score of 2. The benzidine method is used to determine occult blood in the stool as follows. A small amount of stool was evenly applied to white filter paper with a cotton swab and observed visually for the presence of blood, followed by a few drops of o-toluidine solution on the filter paper stool and then a few drops of hydrogen peroxide. If it does not show a blue–green color after 3 min, it is negative; otherwise, it is positive. Stool occult blood is scored as follows: 0 for no visual blood and a negative stool occult blood test result; 1 for no visual blood but a positive stool occult blood test result; 2 for visual blood. Scores for the above two items were added to the stool index.

The collected colon tissues were washed with PBS and fixed using 4% paraformaldehyde overnight. Tissues were dehydrated and embedded in paraffin. Five-micrometer-thick sections were cut and stained with hematoxylin and eosin (H&E).

**Isolation of T-cell subsets**

The mesenteric lymph nodes (MLNs) and spleens of mice were separated by sterile instruments on an ultraclean table, and then the MLN and spleen were placed into a culture dish containing precooled PBS to wash the surface. The MLN and spleen were placed on a 300 mesh filter with tweezers in a culture dish containing RPMI 1640 complete medium. The tissue was ground slowly with a 5 mL syringe needle core, the

cells were put into the culture medium by slightly lifting the filter, and there was no obvious tissue block in the filter. The cell suspension was collected into a 50 mL centrifuge tube after slowly blowing the mixed cells. After the cell suspension was prepared, it was centrifuged for five min at 4 °C and 2000 rpm, and the supernatant was discarded. Then, 3 mL Tris-$NH_4Cl$ red blood cell lysate was added. Blowing and mixing for five min was followed by the addition of 6 mL PBS to terminate the lysis, centrifugation for five min at 4 °C and 2000 rpm, and a discard of the supernatant. The cells were resuspended in PBS and counted, and the cell concentration was adjusted for subsequent experiments.

**Purification and culture of T-cell subsets**

Magnetic beads were used to label cells other than those initially labeled with $CD4^+$ T cells in mouse MLN and spleen suspensions, as instructed by the manufacturer (BD Bioscience, CA, United States), and then the LS separation column was put into the MACS magnetic field for purification to obtain the initial $CD4^+$ T cells with high purity. To induce the differentiation of Th17 cells, the anti-CD3 antibody was added to the 96-well plate at a concentration of 2 μg/mL one night before sorting the cells. The next day, the liquid in the plate was removed and discarded by a liquid transfer gun. The sorted initial $CD4^+$ T cells were inoculated into a 96-well plate coated with anti-CD3 antibody, and 1 μg/mL anti-CD28 antibody was added and cultured at 37 °C for 1 h. Then, $CD4^+$ T cells were treated with 20 ng/mL IL-6 and 3 ng/mL TGF-β. $CD4^+$ T cells in the Dem group were cultured in vitro with the indicated concentration of Dem for 5 days.

**Flow cytometry analysis**

To culture Th17 cells, they were first stimulated with a phorbol myristate acetate (PMA) and ionomycin mixture (Multi sciences Biotech, Hangzhou, China) for 4-6 h. Staining was terminated with fetal bovine serum after fixation with BD Horizon™ Fixable Viability Stain 660 for 15 min at 4 °C and protected from light. A 20-min incubation with anti-CD3 and anti-CD8 antibodies was then performed at 4 °C protected from light. Afterward, fixation and permeabilization were performed at 4 °C protected from light for 20 min. The cells were then stained with anti-IL-17 antibody for 30 min at 4 °C protected from light. Finally, the prepared samples were detected by flow cytometry. First, $CD3^+CD8^-$IL-$17^+$ cells were selected as Th17 cells by using Flow software to calculate the proportion of FVS-negative cells (live cells).

**Western blotting analysis**

The cell samples were lysed in RIPA buffer containing protease inhibitors and

centrifuged at 12,000 rpm for 20 min at 4 °C for FA-45-24-11 rotor models, Eppendorf. The colons of the sacrificed mice were quickly removed and washed twice in precooled saline before being used as tissue samples. Approximately 1 cm of mouse colonic tissue was broken into pieces and resuspended in 1 mL of ice-cold RIPA lysis buffer. It was then transferred to a precooled glass homogenizer. The mouse colonic tissue was cut into 1 cm lengths and resuspended in 1 mL ice-cold RIPA lysis buffer. The fluid was then transferred to a precooled glass homogenizer. Several strokes on ice homogenized the colonic tissue. It was then transferred into a 1.5 mL centrifuge tube and centrifuged for 15 min at 12,000 rpm (4 °C) (FA-45-24-11 rotor model, Eppendorf). For the total protein concentration measurement, the Beyotime Biotech BCA Protein Assay Kit (Shanghai, China) was used. The protein samples were then separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), the gel was cut, and the appropriate size polyvinylidene fluoride (PVDF) membrane was cut and transferred according to the size of the cut gel. The membranes were blocked with 5% skimmed milk prepared in TBST for 1 h at room temperature, followed by incubation of the closed membranes with primary antibody, washing of the membranes, incubation of secondary antibody and washing of the membranes. Finally, the A and B solutions from the enhanced chemiluminescence (ECL) chemiluminescent solution were mixed thoroughly on the membrane in a 1:1 ratio and transferred to the luminometer for luminescence imaging, which was quantified using ImageJ software.

**Enzyme-linked immunosorbent assay (ELISA)**

As soon as the samples were prepared, cytokine levels (IL-1β, IL-6, IL-17A, IL-17F, IL-22, and TNF-α) were measured using commercial ELISA kits (Signalway Antibody Co., College Park, MD, USA). A series of 96-well plates were coated with purified primary polyclonal antibodies against IL-1β, IL-6, IL-17A, IL-17F, IL-22 and TNF-α, followed by incubation and washing. The concentration of the sample was calculated by measuring the absorbance value of each well using an enzyme marker (Tecan Sunrise; Tecan, Männedorf, Switzerland) with the wavelength set at 450 nm, and the concentration was expressed as pg/mL. [30].

**Small interfering RNA transfection**

Small interfering RNAs (siRNAs) were transfected into cells with Lipofectamine™ RNAiMAX Transfection Reagent according to the manufacturer's instructions. SiRNAs were obtained from GenePharma (Shanghai). The sequences were as follows. SiSTAT3 sense: 5′-GGCGCAACCTAAGATTAAT-3′, 5′-

CGCCTGTGTATCATAATAT-3′, 5′-GGGCTAAATTCTGCAAAGA -3′
SiJAK2 sense: 5′-GGCGCAACCTAAGATTAAT-3′, 5′-CGCCTGTGTATCATAATAT-3′,
5′-GTCCACCCGTGGAATTTAT-3′.
A mixture of the three is used to improve knockdown efficiency.

**Assessment of arthritis severity**

The evaluation method and standard of arthritis index for this experiment are based on our previous research. Mice were monitored and evaluated for arthritis severity one time per week. A four-point scale was used to evaluate each paw. The score ranged from 0 to 4. For each mouse, the maximum score was 16:

0 - no redness or swelling;

1 - a little red and swollen;

2 - marked redness and swelling.

3 - severe redness and swelling.

4 - deformity, rigidity, or dysfunction.

Two observers independently scored the project. [31, 32].

Foot swelling was measured at the same time. The hind paw edema volumes of all mice were measured by a plethysmometer once every two days. The difference between the foot volume at the above time points and that before inflammation (mL) indicates the degree of foot swelling.

**Statistical analysis**

Statistical analysis was conducted using SPSS software, and figures were created with GraphPad Prism. Parametric tests for normality, expressed as the mean ± standard deviation (SD), were performed using one-way analysis of variance (ANOVA) to test for differences in overall means, followed by an LSD (homogeneous variance) or Games-Howell (heterogeneous variance) analysis. In the statistical analysis, P values lower than 0.05 were considered significant.

# Results

**Dem improves DSS-induced colitis**

To further demonstrate the anti-inflammatory mechanism of Dem, a DSS-induced IBD mouse model was employed in this study. Compared with the control group, the body weight of mice in the model group and the stool index were significantly increased. Dem treatments attenuated the symptoms of IBD, including the stool index, colon

length and weight loss (Fig. 1A-C). Pathological observation revealed obvious inflammation in the colon mucosa in untreated IBD mice, which manifested as edema of the intestinal epithelium, infiltration of inflammatory cells, and ulceration. However, Dem treatment improved the pathological changes in colon tissue in IBD mice (Fig. 1D). Compared with the model group, the serum levels of TNF-α, IL-1β, and IL-6 after Dem treatment were significantly decreased (Fig. 1E).

**Dem significantly inhibits the Th17-cell proportion and attenuates symptoms of IBD through the JAK2-STAT3 pathway**

Inflammation models can easily lead to the activation and proliferation of immune cells. We next examined Th17 cells in mouse MLN and found that Dem significantly inhibited the differentiation and activation of Th17 cells (Fig. 2C). We also examined the levels of cytokines by ELISA (Fig. 2A). The phosphorylation of STAT3 and JAK2 was also examined. Dem inhibited the phosphorylation of both JAK2 and STAT3 (Fig. 2B). Dem exerted a therapeutic effect on the IBD model by inhibiting the phosphorylation of STAT3 and JAK2 and decreasing the proportion of Th17 cells.

**Dem inhibits Th17-cell differentiation through the JAK2-STAT3 pathway**

Previous studies have shown that reduced phosphorylation of STAT3 and JAK2 alleviates the progression of IBD in mice, suggesting that inhibition of STAT3 and JAK2 expression could be used as a means of treating IBD. SiRNAs were used to test the dependence of the Th17 inhibition effect of Dem. Nontargeting siRNA (siControl), which does not target any known human gene, was used as a negative control. In addition, siRNAs for JAK2 and STAT3 were selected for further investigation. Cells were transfected with the indicated siRNAs based on the manufacturer's instructions. The STAT and JAK2 genes were silenced using STAT3 siRNA and JAK2 siRNA transfection, respectively, and the results showed that transfection alleviated the inhibitory effect of Dem on Th17 cells, as evidenced by the proportion of $CD4^+$IL-$17A^+$ Th17 cells and the level of associated transcription factors in $CD4^+$ T cells. In addition, Dem significantly reduced the expression levels of STAT3 and JAK2. The results indicated that Dem inhibited the differentiation and activation of Th17 cells through the JAK2-STAT3 pathway (Fig. 3A-G).

**Inhibitory effects of Dem on Th17-cell differentiation and activation in vitro**

To investigate whether Dem can regulate Th17-cell differentiation, $CD4^+$ T cells were induced in vitro under Th17 differentiation conditions in the presence or absence of Dem for 5 days. The results showed that the proportion of $CD4^+$IL-$17A^+$ Th17 cells in $CD4^+$ T cells and the related transcription factor levels gradually increased from day 3 to day 5 (Fig. 4A, B). The IL-17A level in the culture supernatant was increased along with the increased Th17 cells (Fig. 4C). Five days after the Th17-induced condition, Dem significantly inhibited Th17 differentiation (Fig. 4D). Dem also inhibited the levels of ROR-γt and pSTAT3 but not mTOR (Fig. 4E). Furthermore, Dem markedly decreased the levels of IL-17A in the culture supernatant (Fig. 4F). These findings demonstrated that Dem can inhibit the differentiation and activation of Th17 cells.

**Dem improves CIA-induced arthritis**

After the second week of the first immunization of collagen, mice were orally administered Dem for 14 days. Compared with Model group mice, Dem (2 mg/kg) showed significantly lower arthritic scores, similar to LEF group (Fig. 5A-C). Compared with the model group, the arthritis symptoms of CIA mice treated with Dem and LEF were obviously milder (Fig. 5D). Dem treatment resulted in a significant increase in the splenic index (Fig. 5E). The serum levels of three critical proinflammatory cytokines (IL-1β, TNF-α, and IL-6) were increased significantly in the Model group. Dem decreased all of them prominently (Fig. 5F).

**Alleviation of clinical symptoms, inflammatory cytokine production and Th17-cell proportion in CIA in mice by Dem**

The proportion of Th17 cells in the spleen and the levels of transcription factors in the spleen of mice were then examined. Mice with high-dose Dem markedly decreased the Th17-cell proportion in $CD4^+$ T cells (Fig. 6A). The level of ROR-γt in spleen tissue was decreased compared to that in the model group (Fig. 6B). To further test the inhibitory effect of Dem on Th17-cell activation, specific cytokines produced by Th17 cells were also tested. The results showed that Dem significantly decreased the levels of IL-17A, IL-17F and IL-22 (Fig. 6C). The above data suggest that Dem alleviated CIA symptoms by inhibiting Th17-cell proliferation and activation.

# Discussion

DSS-induced IBD is a classic animal model in the exploration of IBD. Its clinical

features are similar to those of human IBD, with distinct chronic inflammatory features such as inflammatory cell infiltration, mucosal erosion, loss of crypts and malformations, thickening of the colonic wall, ulceration and granulation tissue formation [1]. The gut and bones are closely linked, and arthritis is a common complication of UC. Inflammation and immune cell infiltration are prominent aspects of RA [6]. From autoimmunity, it leads to local inflammation, finally leading to bone and joint destruction [33]. The hallmark of RA is the development of synovitis [34]. The sensitivity of mice to CIA is consistent with that of humans to RA, and the CIA model is one of the most widely used animal models for evaluating treatment strategies for RA [10].

Drugs such as aminosalicylates, immunomodulators and biologics are currently used to treat IBD, but are not as effective. *Tripterygium wilfordii* Hook F is a traditional Chinese herbal medicine mainly used for the treatment of related inflammatory diseases. Dem, an important active ingredient of *Tripterygium wilfordii* Hook F, has anti-inflammatory and immunosuppressive effects. Our findings revealed that the anti-inflammatory effects of Dem were mediated through inhibition of Th17-cell differentiation and activation. The mechanism may involve the regulation of the JAK2-STAT3 signaling pathway.

The proinflammatory factors IL-1β, IL-6, and TNF-α play a central role in chronic inflammation, such as UC and RA [35, 36]. It was found in the experimental results that Dem can significantly improve UC and RA symptoms and reduce the content of serum TNF-α, IL-1β, and IL-6 in the IBD mouse model. The effect of Dem on the levels of systemic proinflammatory factors provides a preliminary theoretical basis for its therapeutic effects.

Th17 cells play an important role in the occurrence and development of inflammatory responses. Under the stimulation of IL-6 or IL-23, naïve T cells differentiate into Th17 cells, mainly secreting proinflammatory cytokines such as IL-17 and IL-22, which can mediate proinflammatory functions, induce tissue damage, and lead to systemic and local inflammation [8]. The inhibition of IL-17 and Th17 differentiation-related cytokines (such as IL-1β, IL-6 and TNF-α) has an anti-inflammatory effect [37]. Therapeutic effects can be achieved by inhibiting signaling pathways and related inflammatory factors and regulating Th17-cell-related immune balance[38-40]. We found in UC and RA animal models that Dem could significantly reduce the proportion of Th17 cells, the expression of ROR-γt, and the levels of IL-17A, IL-17F and IL-22,

suggesting that the efficacy of Dem was closely related to Th17 cells.

The activation of inflammatory signaling pathways can lead to the phosphorylation of STAT3, an upstream factor of Th17 cells. Phosphorylated STAT3 can induce the expression of ROR-γt, thereby promoting the differentiation of naïve T cells to their subset Th17 cells [41, 42]. To this point, we constructed siRNA specific for STAT3 and transfected lymphocytes to examine the change in the effect of Dem on inhibiting the differentiation of naïve T cells into Th17 cells after transfection. After STAT3 was silenced, Dem was added, and the results showed that the Dem did not influence the proportion of Th17 cells, the expression of ROR-γt, or the amount of IL17A. It is speculated that the inhibitory effect of Dem on Th17 cells is STAT3 dependent.

Previous studies have shown that mTOR and JAK2 can regulate STAT3, and mTOR is a key signaling regulatory molecule in the cell that mediates a variety of intracellular and extracellular signals on cell metabolism, growth, proliferation, and major human diseases such as multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis, all of which involve the dysregulation of the mTOR signaling pathway. JAK is a nonreceptor soluble tyrosine protein kinase in the cytosol [43]. After JAK2 is activated, the tyrosine residues on the receptor are phosphorylated, which plays a crucial role in the regulation of immune and inflammatory processes. After examining the close relationship between JAK2 and Dem, we continued to construct a specific siRNA for JAK2 and added Dem after transfection to compare the effect of Dem on inhibiting the differentiation of naïve T cells into Th17 cells after transfection. The results show that after JAK2 was silenced and mixed with Dem, Dem could not exert its inhibitory effect on the differentiation and activation of Th17 cells. It can be assumed that JAK2 is a key to the efficacy of Dem. We validated this conclusion with a UC animal model.

From the experimental results, it can be demonstrated that Dem is an important effective component of *Tripterygium wilfordii* Hook F and can inhibit the differentiation and activation of Th17 cells by blocking the JAK2-STAT3 pathway. These results provide a theoretical basis for the development of drugs for *Tripterygium wilfordii* Hook F.

From the experimental results, it can be demonstrated that Dem is an important active ingredient of *Tripterygium wilfordii* Hook F, which can inhibit the differentiation and activation of Th17 cells by blocking the JAK2-STAT3 pathway, and has a relieving effect on IBD and CIA-induced arthritis. These results provide a theoretical basis for

the drug development of *Tripterygium wilfordii* Hook F.

## Declarations

**Ethical Approval:** All animal experiments were approved by the Animal Care and Use Committee of Nanjing University of Chinese Medicine (approval numbers ACU210305).

**Competing interests:** The authors declare no competing interests.

**Authors' contributions:** Yong Bian, Jin Li, Data curation, Formal analysis, Investigation, Methodology, Software, Writing and original draft; Rong Miao, Wei Wang, Data curation, Formal analysis, Investigation, Project administration; Jianxin Shi, Le Shi, Tao Liang, Yifan Zhang, Data curation, Formal analysis; Yun Yu, Dongping Yuan, Conceptualization, Funding acquisition, Project administration, Resources, Supervision.

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# Figure legends

Fig. 1. Dem improves DSS-induced colitis.

The administration groups were given Dem at doses of 1 mg/kg and 2 mg/kg and both the control and model groups were given 0.5% CMC-Na once daily. A. Changes in body weight in mice; B. Stool index; C. Representative pictures of colon and colon length in each group; D. Representative H&E staining of colon tissue sections from each group, scale bar = 50 μm; E. Levels of TNF-α, IL-1β and IL-6 in mouse serum.

Fig. 2. Dem significantly inhibited the Th17-cell proportion and attenuated symptoms of IBD through the JAK2-STAT3 pathway.

Th17 cells were isolated from the MLNs of mice, A. Proportion of $CD4^+IL\text{-}17^+$ cells in mouse MLNs; B. Levels of ROR-γt, phosphorylated (p)-STAT3, STAT3, p-JAK2 and JAK2 in Th17 cells; C. Levels of IL-17 and IL-22.

Fig. 3. Dem inhibits Th17-cell differentiation via the JAK2-STAT3 pathway.

STAT and JAK2 genes were silenced using STAT3 siRNA and JAK2 siRNA transfection, respectively, A. Proportion of $CD4^+IL\text{-}17^+$ cells after siRNA transfection of STAT3; B. Levels of ROR-γt; C. Levels of IL-17; D. Proportion of $CD4^+IL\text{-}17^+$ cells after siRNA transfection of JAK2; E. Levels of p-STAT3, STAT3, p-JAK2, JAK2, p-

mTOR and mTOR levels; F. Levels of ROR-γt; G. Levels of IL-17.

Fig. 4. Inhibitory effects of Dem on Th17-cell differentiation and activation in vitro.
In vitro induction of $CD4^+$ T cells for 5 days under Th17 differentiation conditions in the presence or absence of Dem, A. Proportion of $CD4^+IL\text{-}17^+$ cells during induction of $CD4^+$ T-cell differentiation in vitro; B. Levels of ROR-γt; C. Levels of IL-17 in supernatant; D. Proportion of $CD4^+IL\text{-}17^+$ cells after 5 days of induction; E. Levels of ROR-γt, p-STAT3, STAT3 levels; F. IL-17 levels in the supernatant.

Fig. 5. Dem improves CIA-induced arthritis.
The administration groups were given Dem at doses of 1 mg/kg and 2 mg/kg, the control and model groups were given 0.5% CMC-Na and the positive control group was given 2 mg/kg of LEF. A. Changes in body weight of mice; B. Arthritis index scores; C. Degree of swelling of paws; D. Representative pictures of swelling in each group of mice; E. Representative pictures of spleen and spleen index in each group; F. Levels of TNF-α, IL-1β and IL-6 in serum of mice;

Fig. 6. Effect of Dem on mouse CIA clinical symptoms, inflammatory cytokine production, and Th17-cell proportion.
Th17 cells were isolated from the spleens of mice, A. Proportion of $CD4^+IL\text{-}17^+$ cells in the spleens of mice; B. Th17-cell levels of ROR-γt; C. Levels of IL-17 and IL-22.

# Figures

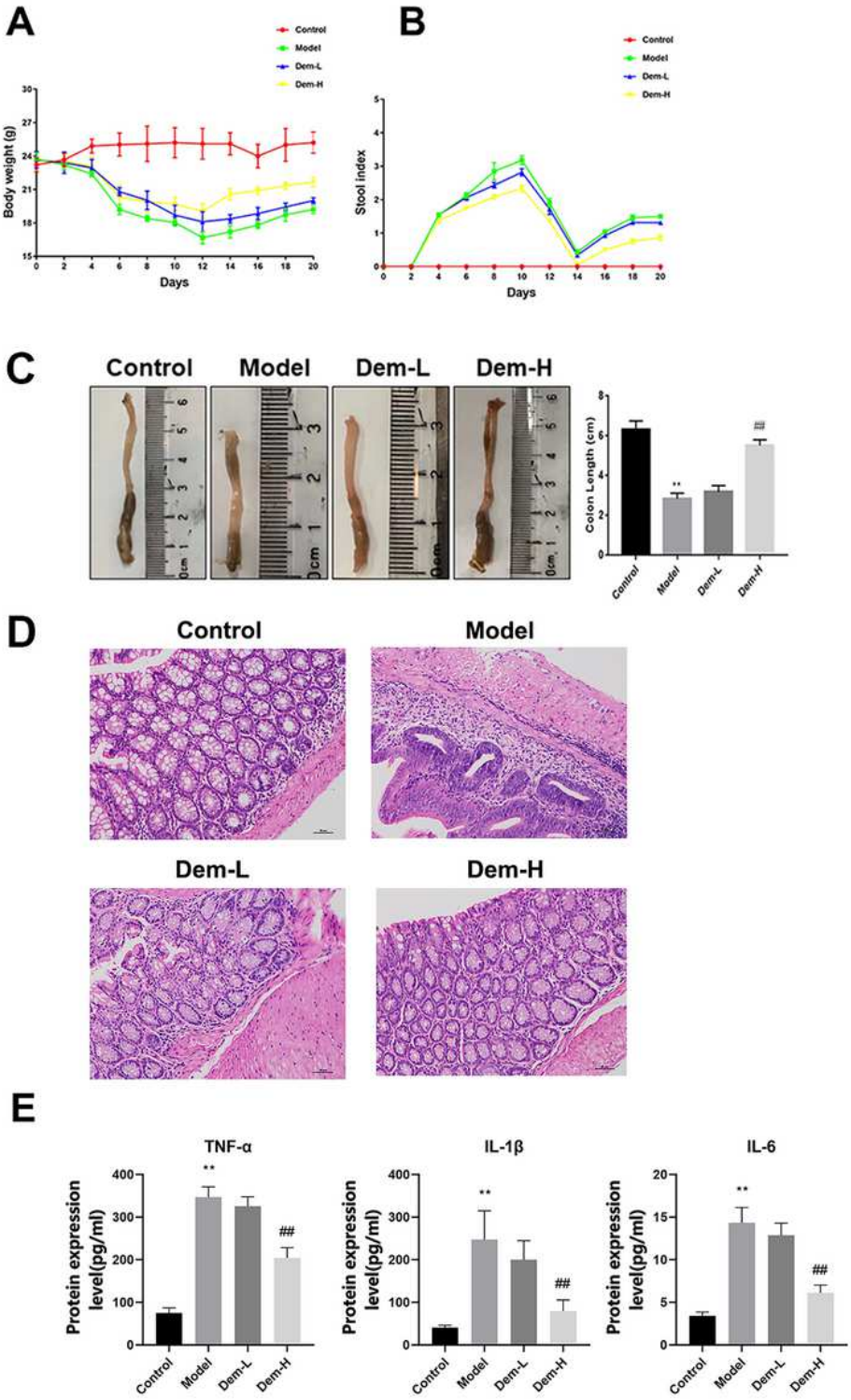
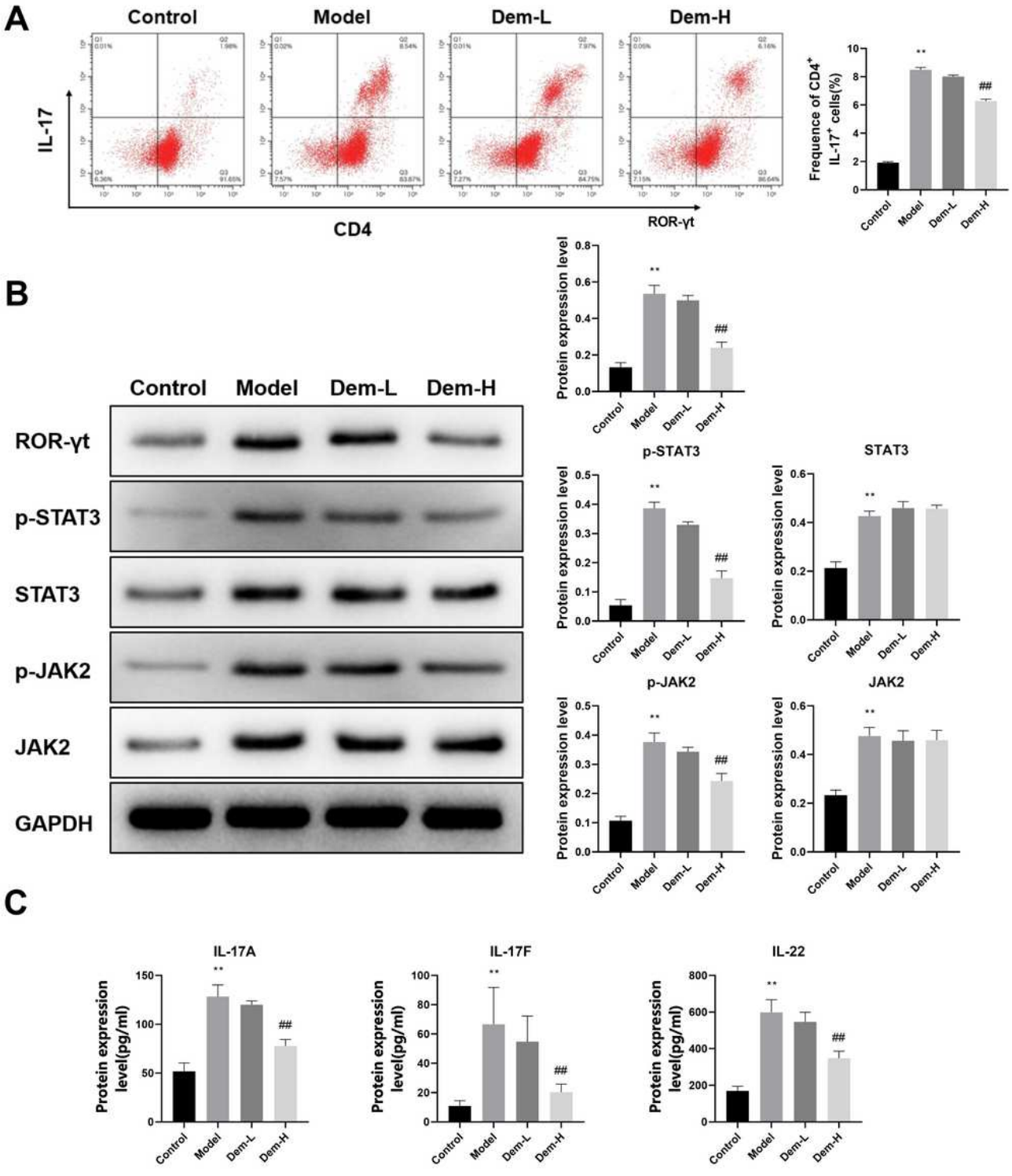



**Figure 1**

Dem improves DSS-induced colitis. The administration groups were given Dem at doses of 1 mg/kg and 2 mg/kg and both the control and model groups were given 0.5% CMC-Na once daily. A. Changes in body weight in mice; B. Stool index; C. Representative pictures of colon and colon length in each group;

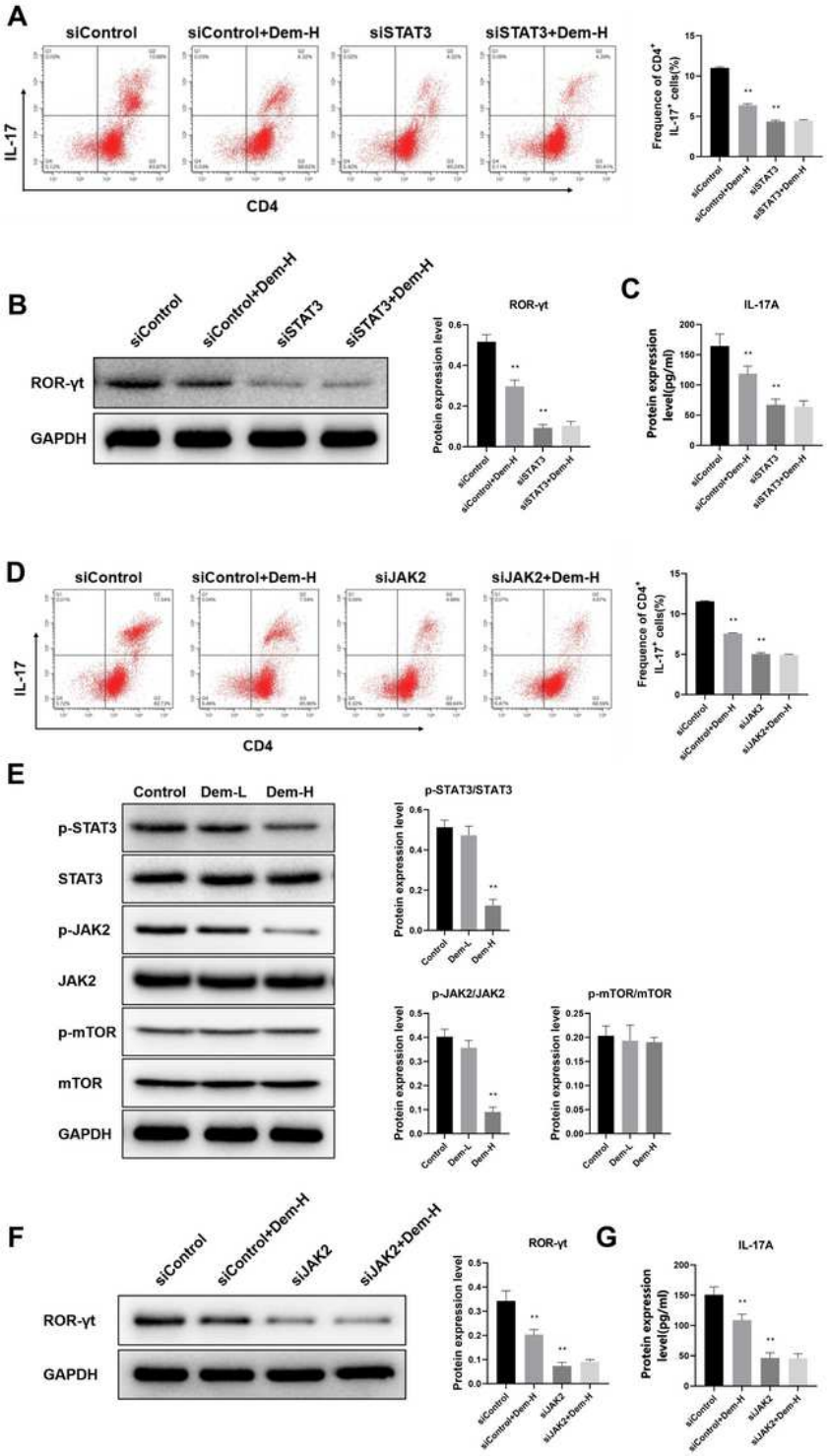
D. Representative H&E staining of colon tissue sections from each group, scale bar = 50 μm; E. Levels of TNF-α, IL-1β and IL-6 in mouse serum.




## Figure 2

Dem significantly inhibited the Th17-cell proportion and attenuated symptoms of IBD through the JAK2-STAT3 pathway. Th17 cells were isolated from the MLNs of mice, A. Proportion of CD4+ IL-17+ cells in

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## Figure 3

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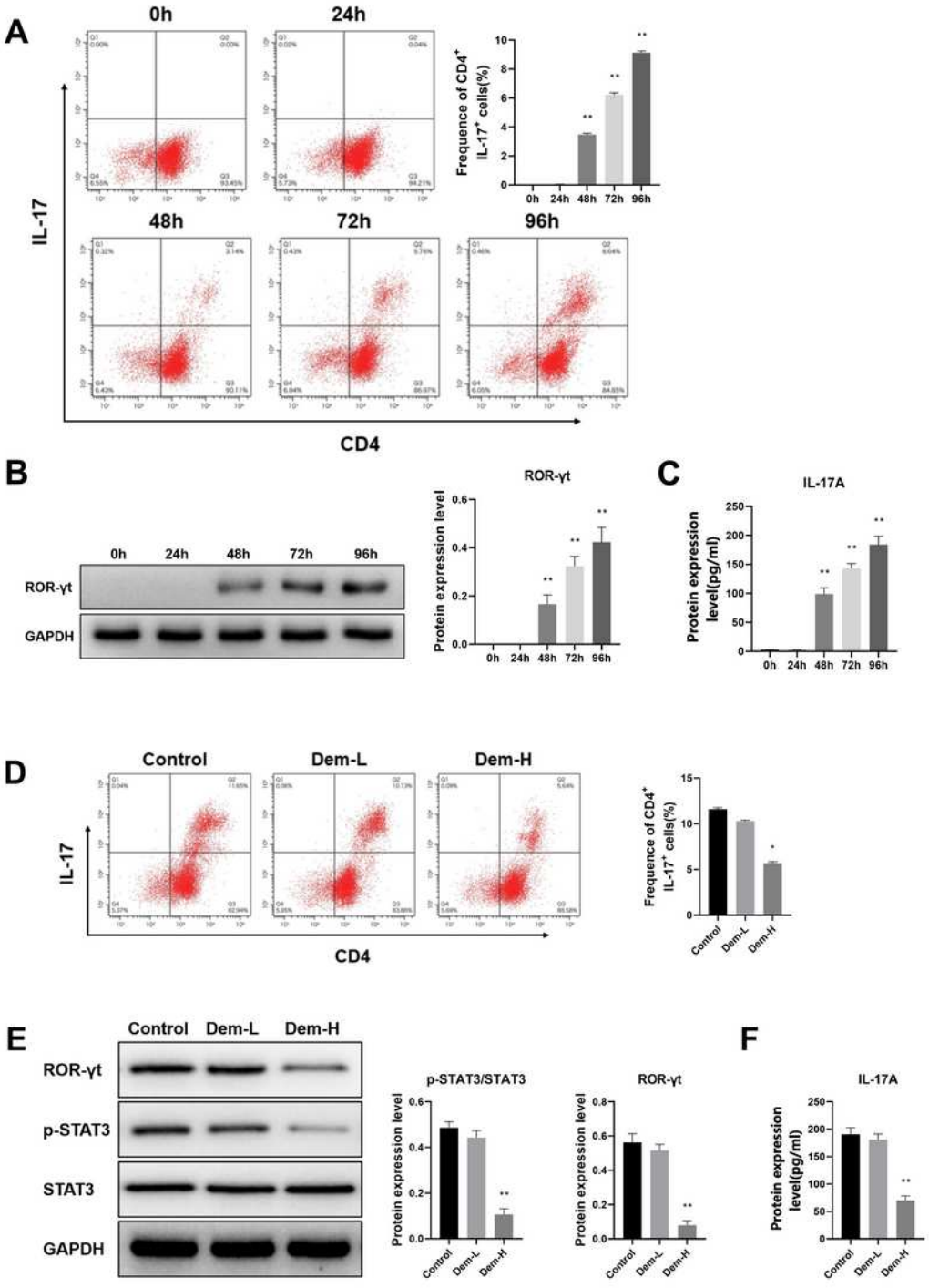



Figure 4

Inhibitory effects of Dem on Th17-cell differentiation and activation in vitro. In vitro induction of CD4+ T cells for 5 days under Th17 differentiation conditions in the presence or absence of Dem, A. Proportion of CD4+ IL-17+ cells during induction of CD4+ T-cell differentiation in vitro; B. Levels of ROR-γt; C. Levels of IL-17 in supernatant; D. Proportion of CD4+ IL-17+ cells after 5 days of induction; E. Levels of ROR-γt, p-STAT3, STAT3 levels; F. IL-17 levels in the supernatant.

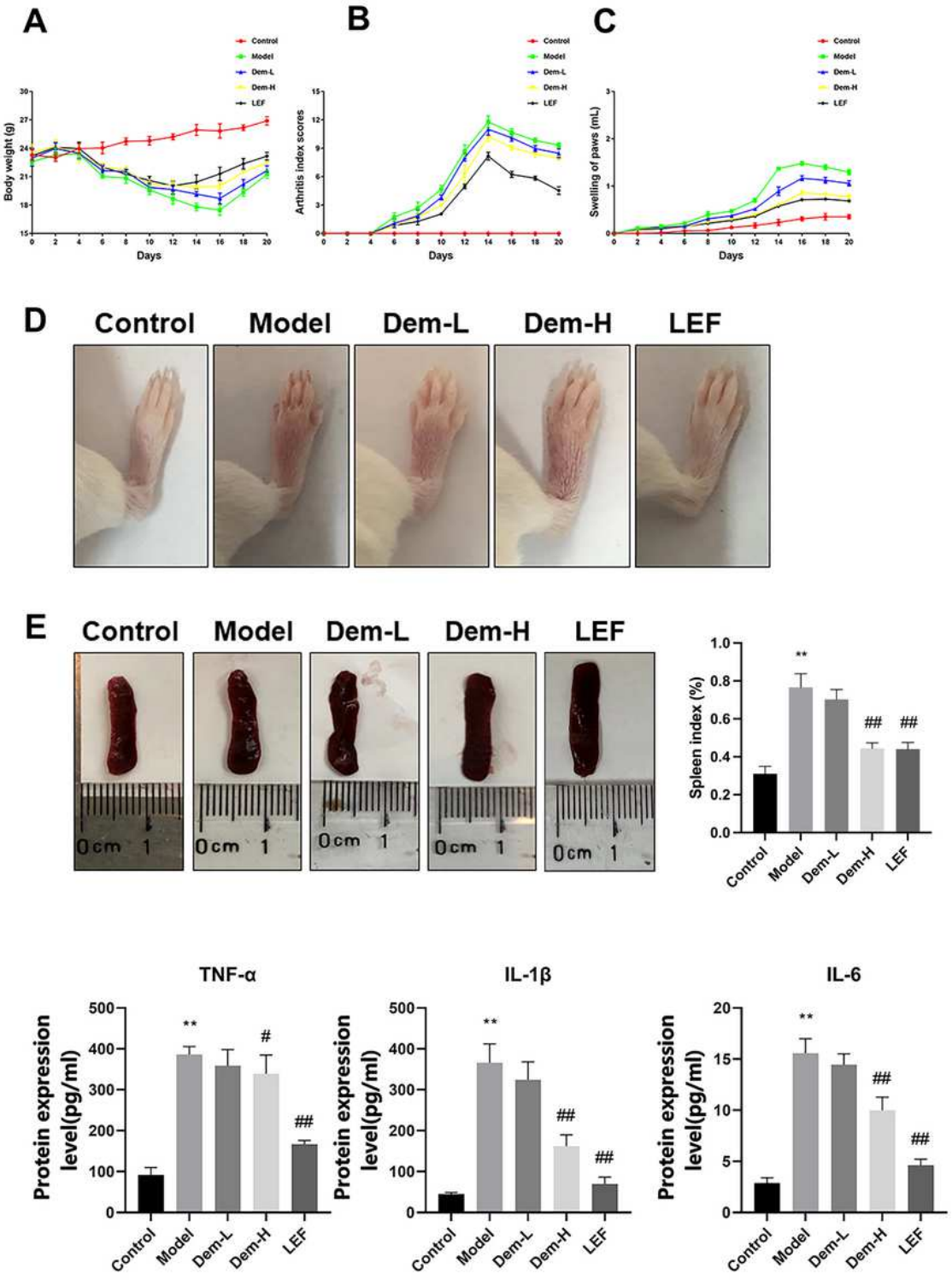
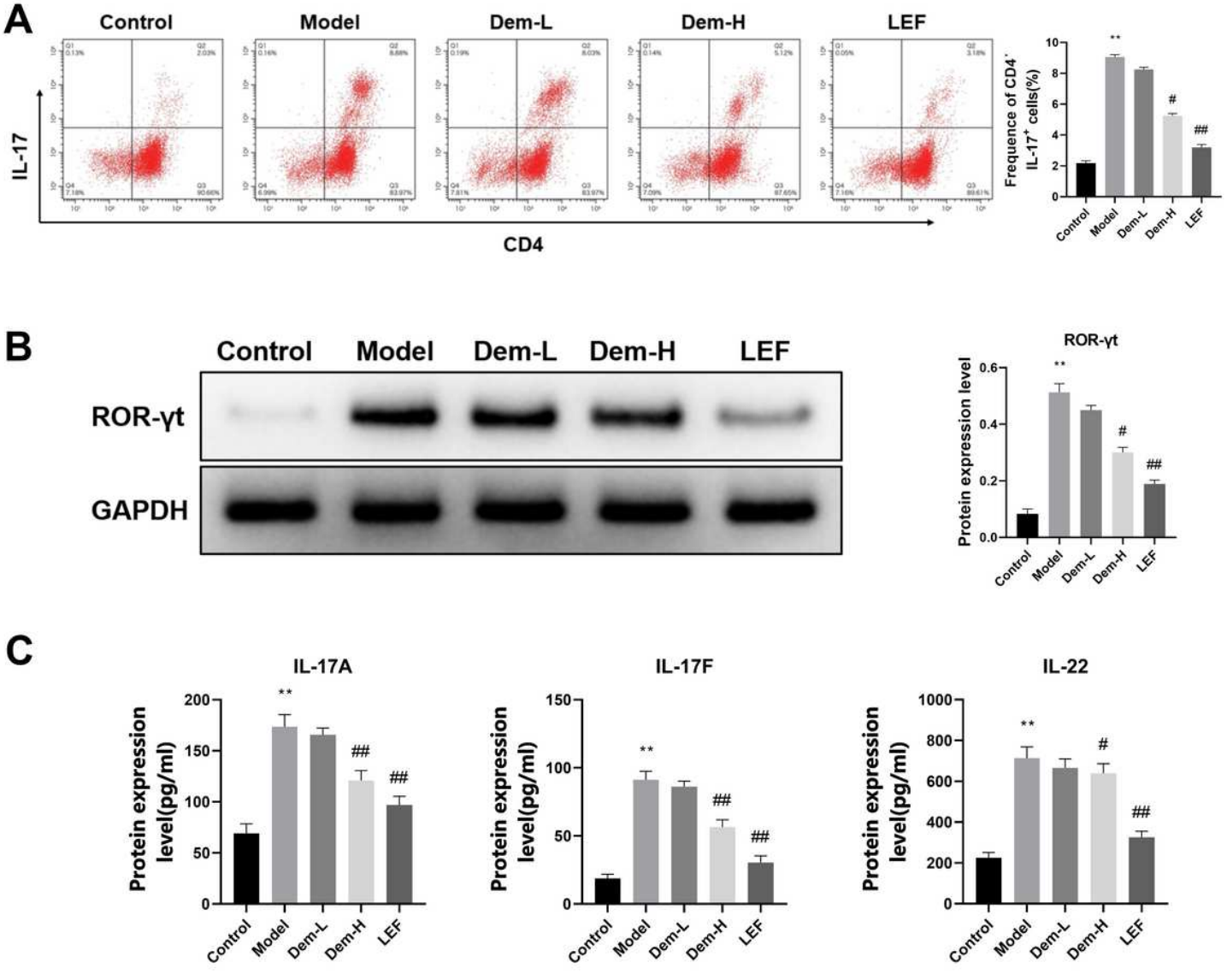



Figure 5

Dem improves CIA-induced arthritis. The administration groups were given Dem at doses of 1 mg/kg and 2 mg/kg, the control and model groups were given 0.5% CMC-Na and the positive control group was given 2 mg/kg of LEF.A. Changes in body weight of mice; B. Arthritis index scores; C. Degree of swelling of paws; D. Representative pictures of swelling in each group of mice; E. Representative pictures of spleen and spleen index in each group; F. Levels of TNF-α, IL-1β and IL-6 in serum of mice;




## Figure 6

Effect of Dem on mouse CIA clinical symptoms, inflammatory cytokine production, and Th17-cell proportion. Th17 cells were isolated from the spleens of mice, A. Proportion of CD4+ IL-17+ cells in the spleens of mice; B. Th17-cell levels of ROR-γt; C. Levels of IL-17 and IL-22.