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# Sophoricoside reduces inflammation in type II collagen-induced arthritis by downregulating NLRP3 signaling

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ABSTRACT

Immune responses, especially NLRP3 signaling in macrophages, play critical roles in rheumatoid arthritis (RA), an autoimmune and inflammatory disease. In this study, we aimed to identify novel therapies for RA. We focused on sophoricoside (SOP), an isoflavone glycoside isolated from *Sophora japonica*. We predicted the targets of SOP and performed a Gene Ontology analysis to assess its effects. The results suggested that SOP is related to inflammation regulation. We verified these findings by performing *in vitro* experiments with M1 macrophages differentiated from human peripheral blood monocytes (THP-1 cells). Sophoricoside administration reduced inflammatory activity and NLRP3, Caspase-1, and IL-1β protein levels in macrophages. In addition, SOP and triptolide (TP) was administered intragastrically to male SD rats (n = 40) in a collagen-induced arthritis model. We observed that SOP and TP reduced the inflammatory responses and symptoms of RA. Moreover, unlike TP, SOP showed no liver or kidney toxicity in rats. In conclusion, SOP reduces inflammation in type II collagen-induced arthritis by downregulating NLRP3 signaling and has potential for future clinical applications as an ideal therapy for RA.

## 1. Introduction

Rheumatoid arthritis (RA) is an autoimmune and inflammatory disease that causes progressive disability, premature death, and socio-economic burdens [1,2]. Immune responses play a critical role in RA [3], especially via macrophage activation [4,5], which triggers the entire immune response [6]. A better understanding of the mechanisms underlying the inflammatory activity in RA may provide new treatment methods [7]. Currently, RA therapies have undergone extensive development [1,8]. Triptolide (TP), a diterpenoid epoxide, exerts promising therapeutic effects in RA by regulating inflammatory activities [9,10]. However, treatment with TP causes toxicity in various organs, especially the liver and kidneys, limiting its clinical applicability [11,12]. Therefore, new therapies with fewer side effects for RA treatment are needed to treat RA.

Sophoricoside (SOP) is an isoflavone glycoside that has been isolated from *Sophora japonica* [13]. It is an immune modulator and has anti-oxidative, anti-obesity, and anti-hyperglycemic effects [14–16]. According to Chen et al., SOP reduces the expression of inflammatory factors such as TNF-α and IL-6 via the NF-κB signaling pathway [17–19]. Moreover, the administration of SOP decreased the hepatic cholesterol and triglyceride levels [20] without organ toxicity. Therefore, it may reduce inflammation and is expected to be a new therapy for RA.

In contrast, NLRP3 a supramolecular complex that activates Caspase-1, which regulates the production of IL-1β from pro-IL-1β, plays an important role in RA and other autoimmune diseases [21,22]. Li et al. reported that NLRP3 signaling is involved with NF-κB activation [23]. This suggests a correlation between SOP and NLRP3 activity.

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In this study, we performed target prediction for SOP and verified the cytotoxicity of SOP and its effect on NLRP3 signal suppression *in vitro*. In addition, using a rat model of collagen-induced arthritis (CIA) [24,25] we compared the effects of SOP and TP following gastric administration.

## 2. Materials and methods

### 2.1. SOP target prediction

Canonical SMILES were downloaded from the PubChem database and used for target prediction using Swiss Target Prediction [26]. The top 15 probability targets were used for Gene Ontology enrichment analysis using Metascape [27].

### 2.2. Transcriptional analysis of CIA mice

Transcriptional data were downloaded from the GEO dataset (Dataset Number: GSE61140) [28]. Then, data were analyzed using the Transcriptome Analysis Console and visualized using RStudio (version 2024.04.0 + 735). Gene Ontology analysis was performed using Gene Set Enrichment Analysis software (version 4.3.3).

### 2.3. Animal and experimental design

Eight-week-old male SD rats (n = 40 in total) were purchased from ExInVivo (Shijiazhuang, China), and divided into four groups: control, CIA, TP, and SOP. Rats in the CIA, TP, and SOP groups were injected with a 200 μL mixture (1:1) of collagen type II (COL2) (Chondrex Inc, Woodinville, USA) and complete Freund's adjuvant (Sigma, Burlington, USA) in the lower limbs. After 1 week, the rats were injected with another 100 μL of the mixture.

Three weeks after the first COL2 injection, the rats in the TP group were treated with 200 μg/kg TP (D21071904, ChemicalBook, Nanjing, China) dissolved in saline by intragastric administration daily, and rats in the SOP group were treated with 80 mg/kg SOP (D21012203, ChemicalBook) dissolved in saline by intragastric administration daily. The CIA and control groups were administered 1 mL saline daily. Clinical arthritis scores were assessed weekly, and the scoring criteria are listed in Supplementary Table 1. The four limbs were evaluated separately and the total score was summed; an arthritis score >4 was considered as successful model development and rats were processed for further analysis.

Seven weeks after the first COL2 injection, the rats were fasted for 12 h, euthanized by pentobarbital (80 mg/mL) injection, and blood was sampled from the abdominal aorta. The hind limb ankle joint, thymus, and spleen were washed with phosphate-buffered saline (PBS) and collected for analysis. The blood sample was kept at 20–25 °C for 1 h, then centrifuged at 3000 rpm for 15 min. The serum was collected and stored at −20 °C for further analysis.

All animal experiments were approved by the Ethics Committee of Hebei University of Chinese Medicine (Ethics Approval Number: DWLL202206008) and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.4. Paraffin section preparation and staining

The hindlimb ankle joint samples collected from rats were fixed in paraformaldehyde for 48 h at 4 °C. The samples were decalcified by incubation with 10 % EDTA for 3 months. After the decalcification, the samples were embedded in paraffin using a tissue embedding machine (EG11580, Leica, Wetzlar, Germany) and sectioned at a thickness of 4 μm.

After sectioning, hematoxylin and eosin staining was performed. Stained sections were observed under a light microscope (DM2550; Leica).

For NLRP3 staining, the sections were incubated with sodium citrate (pH 6.0) for 20 min at 95 °C for antigen retrieval. Sections were then washed with PBS and incubated with goat serum (Solarbio, Beijing, China) for 1 h at 37 °C followed by incubation with the primary antibody of NLRP3 (1:400, DF7438; Affinity Biosciences, Jiangsu, China) overnight at 4 °C. On the next day, the sections were washed with PBS and visualized using a rabbit enhanced polymer detection system (PV-9001DAB; ZSGB-BIO, Beijing, China).

### 2.5. Detection of liver and kidney function factors

Liver and kidney function factors were detected using an automatic biochemistry analyzer (iMagic-M7; Roche, Shanghai, China) using the rat serum samples (described in Section 2.3). Blood urea nitrogen (BUN), alanine aminotransferase (ALT), creatinine (Cr), and Aspartate transaminase (AST) were measured.

### 2.6. Culture of THP-1 cells and differentiation of M1 macrophages

Human peripheral blood monocytes (THP-1) were purchased from ATCC (number BNCC358410; Manassas, VA, USA). The cells were incubated with RPMI-1640 medium (Thermo Fisher Scientific, Waltham, USA) containing 10 % fetal bovine serum (Thermo Fisher Scientific) and 1 % penicillin-streptomycin (Sigma) at 37 °C and 5 % $CO_2$.

The THP-1 cells were divided into five groups: lipopolysaccharide (LPS), LPS + SOP low-concentration (LPS + SOP-L), LPS + SOP medium-concentration (LPS + SOP-M), LPS + SOP high-concentration (LPS + SOP–H), and control.

After 24 h of incubation, the medium was changed. For the LPS and LPS + SOP groups, 30 ng/mL of phorbol-12-myristate-13-acetate (Multi Sciences, Hangzhou, China) was added to induce the differentiation of M0 cells. After 24 h, phorbol-12-myristate-13-acetate was removed and LPS (1 μg/mL) (Solarbio) was added for another 24 h [29]. For the SOP-treated groups, 10, 30, and 100 μM SOP was added for the LPS + SOP-L, LPS + SOP-M, and LPS + SOP–H groups, respectively. Finally, adenosine triphosphate (5 mM) (Aladdin, Shanghai, China) was added for 30 min to induce differentiation into M1 macrophages.

The CCK8 (SB-CCK8S, Share-bio, Shanghai, China) was used to evaluate drug toxicity in THP-1 cells after 24 h administration of SOP with different concentrations (3, 10, 30, and 100 μM). Detection was performed according to the manufacturer's instructions.

### 2.7. Cell staining and identification

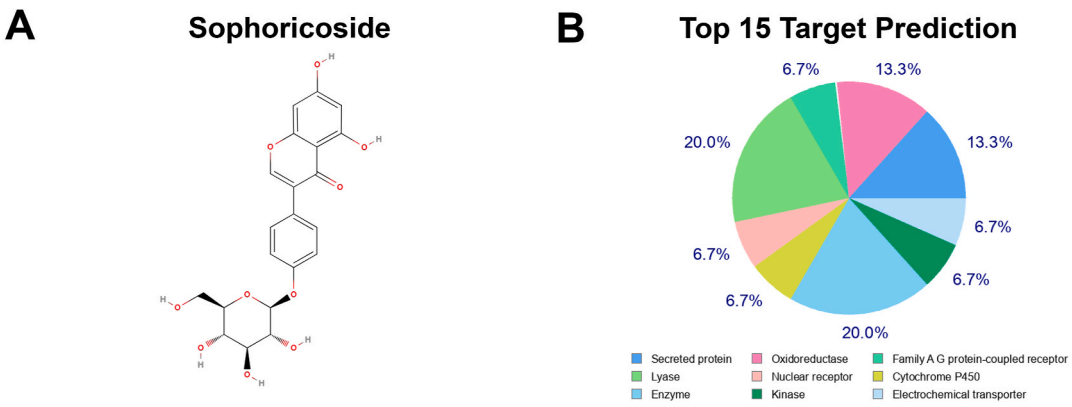
The M1 macrophages (described in Section 2.6) were washed with PBS and incubated with 10 % donkey serum (SL050; Solarbio) for 1 h, and subsequently incubated with a primary antibody against CD86 (1:400) (Boster, Wuhan, China) overnight at 4 °C. On the next day, cells were washed with PBS and incubated with a secondary antibody (1:400) (K0034D-Cy3; Solarbio) and DAPI (S2110; Solarbio) for 1 h at 20–25 °C. After another round of washing with PBS, M1 macrophage morphology was observed.

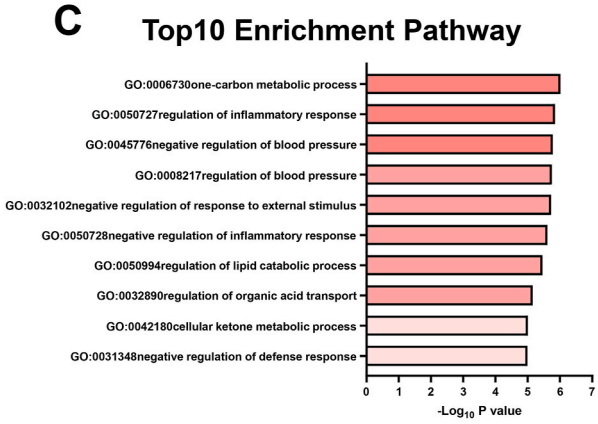
### 2.8. Enzyme-linked immunosorbent assay (ELISA) detection

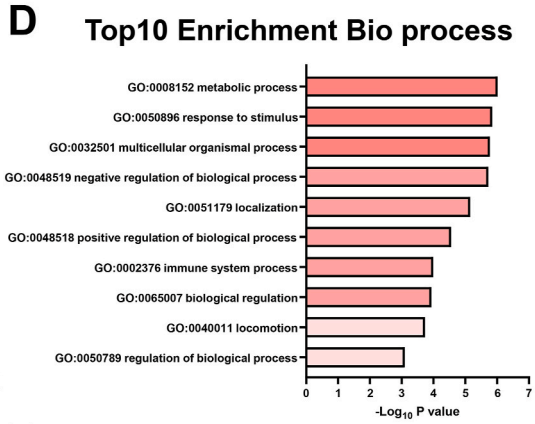
Rat serum samples (described in Section 2.3) were used for anti-CCP detection (CSB-E13830r; Cusabio, Wuhan, China). Rat serum and the M1 macrophage (described in Section 2.7) culture supernatant were used for IL-6 (EK0412; Boster) and TNF-α (EK382HS; Lianke Biotech, Hangzhou, China) detection. All ELISA were performed in accordance with the manufacturer's instructions.

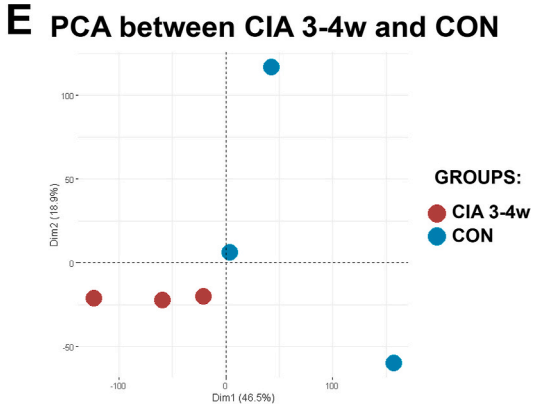
### 2.9. Realtime-qPCR

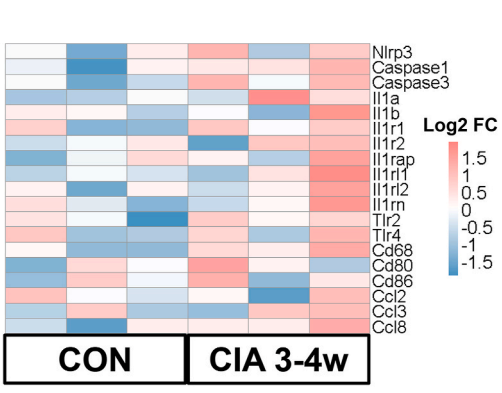
The M1 macrophages (described in Section 2.6) were washed three times with PBS, and then, mRNA was isolated using the Eastop Super Total RNA ExtractionKit (LS1040; Promega, Madison, USA) as per the

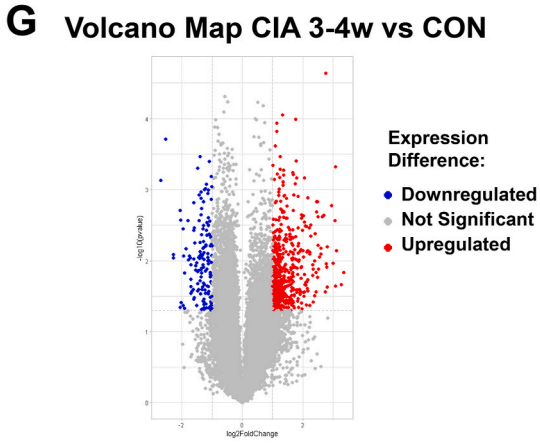


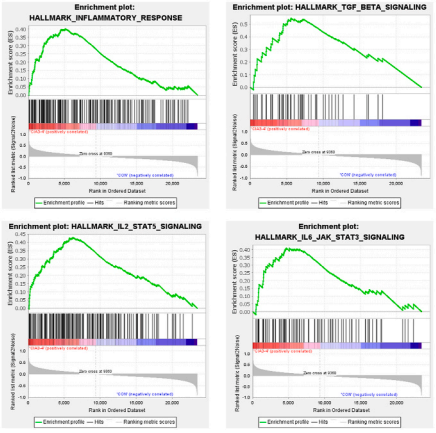




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**Fig. 1.** Target Prediction Indicated SOP Might Relate to Metabolism and Immune Regulation.
A. Pharmaceutical chemical formula of SOP.
B. Top 15 possible predicted targets.
C. Top 10 GO enrichment pathways of the predicted targets.
D. Top 10 GO enrichment biological processes of the predicted targets.
E. Principal component analysis (PCA) between the CIA and CON groups.
F. Heat map of changes in NLRP3-related gene and representative inflammatory gene expression levels between the CIA and CON groups.
G. Volcano map expressing the overall changes in the transcriptome between the CIA and CON groups. (Red, upregulated genes in the CIA group; Blue, downregulated genes in the CIA group) (Thresholds: $p < 0.05$, as per the Student's *t*-test; Fold change >2)
H. Gene Set Enrichment Analysis (GESA) results of the inflammation-related pathways. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

manufacturer's instructions. The mRNA was reverse transcribed using the GoScrpt Rverse transcription System (A5001; Promega).

After reverse transcription, real-time qPCR analysis was conducted using the CFX Duet Real-TA6002; Promega). The primers used in the study were designed using NCBI Primer-BLAST, and the primer sequences are listed in Supplementary Table 3.

### 2.10. Western blotting

The M1 macrophages (described in Section 2.7) were cultured in 6-well plates at a density of $5 \times 10^5$ cells/well. Cells were lysed on ice for 30 min using RIPA buffer (Share-Bio) and centrifuged at 12000 rpm for 15 min. The protein was collected from the supernatant, and the concentration was adjusted after bicinchoninic acid analysis (Thermo Fisher Scientific).

Twenty micrograms of protein in each well were separated by 10 % SDS-PAGE (Epizyme, Shanghai, China) at 80 V for 35 min and 120 V for 60 min. Proteins were transferred to a PVDF membrane (Merck Millipore, Burlington, USA) at 300 mA for 1 h. After blocking with 5 % skim milk in Tris-buffered saline with 0.1 % Tween-20 for 1 h at 20–25 °C, the membranes were separated and incubated with 1:1000 of rabbit primary antibodies against IL-1β (Affinity Biosciences), NLRP3 (Abcam, Jiangsu, China), Caspase-1 (Abways Biotechnology, Shanghai, China), and GAPDH (Abbkine, Wuhan, China) overnight at 4 °C.

On the next day, after being washed with Tris-buffered saline with 0.1 % Tween-20, the membranes were incubated with goat anti-rabbit secondary antibodies (1:5000) (20000135; Proteintech, Wuhan, China) for 2 h at 20–25 °C. Blot imaging was performed using chemiluminescence (Fusion FX5 Spectra; Collégien, France) with an ECL solution (BL520A; Biosharp, Beijing, China). Calculations were performed using Fiji ImageJ version 1.54f (FIJI, NIH, USA). GAPDH was used as a control reference.

### 2.11. Data visualization and statistical analysis

The data were visualized using GraphPad version 10.0.2 (GraphPad Software, San Diego, CA, USA) and RStudio version 494 (Posit Software, Boston, USA). Statistical analysis was performed using GraphPad, and the quantitative variables are expressed as the mean ± standard deviation (SD). Differences were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's correction, and were considered significant at $p < 0.05$.

## 3. Results

### 3.1. Target prediction of SOP

To identify the drug targets of SOP, we performed target prediction using the pharmaceutical chemical formula of SOP (Fig. 1A). The targets of SOPs were mostly related to lyases and electrochemical transporters (Fig. 1B), and the top 15 targets are listed in Supplementary Table 2. We then performed Gene Ontology enrichment analysis on the predicted targets and observed that the one-carbon metabolic process pathway and regulation of the inflammatory response pathway were highlighted (Fig. 1C), suggesting that SOP regulates inflammation. At the biological process level, the metabolic process and response to the stimulus process were indicated (Fig. 1D). Therefore, target prediction indicated that SOP administration might be related to metabolism and immune regulation, suggesting that SOP may reduce the inflammation caused by RA.

### 3.2. Representative markers of CIA obtained by transcriptome analysis

To filter out the indicators to evaluate the effectiveness of SOP, we analyzed the transcriptome results (GSE61140) between the CIA model 3–4 weeks (CIA 3–4w) and control (CON) groups (Fig. 1E and F). In total, 544 genes were significantly (Fold change over 2, $p < 0.05$) upregulated in the CIA 3–4w group, whereas 154 genes were downregulated (Fig. 1G). By summarizing the representative changes in inflammatory factors, we found that IL-1β and its related gene expression was enhanced in the CIA 3–4w group. In addition, a few M1 macrophage markers, such as CD68 and CD86, were increased in the CIA 3–4w group, indicating that M1 macrophage infiltration may be present in the CIA model.

Results of the Gene Set Enrichment Analysis showed that the gene set of the inflammatory response (Fig. 1H), TGF-β, IL-2/STAT5, and IL6/JAK/STAT3 signaling, were all upregulated in the CIA 3–4w group compared with the CON group (Fig. 1G). These findings suggest that Interleukin signaling plays an important role in CIA and may be considered as an indicator of CIA.

### 3.3. SOP downregulated NLRP3 signaling in M1 macrophages

Subsequently, we performed *in vitro* experiments focusing on essential regulators of Interleukin signaling by differentiating THP-1 cells into M1 macrophages (Fig. 2A). First, to confirm whether the differentiation of M1 macrophages was successful, we performed CD86 staining on M1 macrophages after inducing differentiation. We observed that these M1 macrophages were CD86-positive, indicating that the differentiation of M1 macrophages from THP-1 cells was successful (Fig. 2B). Next, we evaluated drug toxicity after 24-h administration of SOP at different concentrations. Although the survival rate of THP-1 cells was significantly reduced at 100 μM SOP, the average survival rate remained higher than 80 % (Fig. 2C).

The results of ELISA detection revealed that the IL-1β and TNF-α levels increased significantly in the supernatant of the LPS group compared to those in the CON group ($p < 0.0001$), whereas they reduced significantly in the LPS + SOP–H group compared with the CON group (IL-1β $p < 0.0001$; TNF-α $p < 0.01$). In addition, the LPS + SOP-M group revealed a mild decrease in IL-1β ($p < 0.01$) (Fig. 2D). These results suggest that the administration of 100 μM SOP reduced the inflammatory response of the M1 macrophages *in vitro*.

Moreover, mRNA expression levels of *TNF-α* ($p < 0.01$), *Caspase-1* ($p < 0.001$) were significantly upregulated in the LPS group (Fig. 2E). However, the expression of these genes was reduced in the LPS + SOP-M group compared to that in the LPS group.

In addition, we used western blotting to detect NLRP3 signaling proteins. The levels of NLRP3 ($p < 0.0001$), Caspase-1 ($p < 0.05$), and IL-1β ($p < 0.01$) increased in the LPS group compared to those in the CON

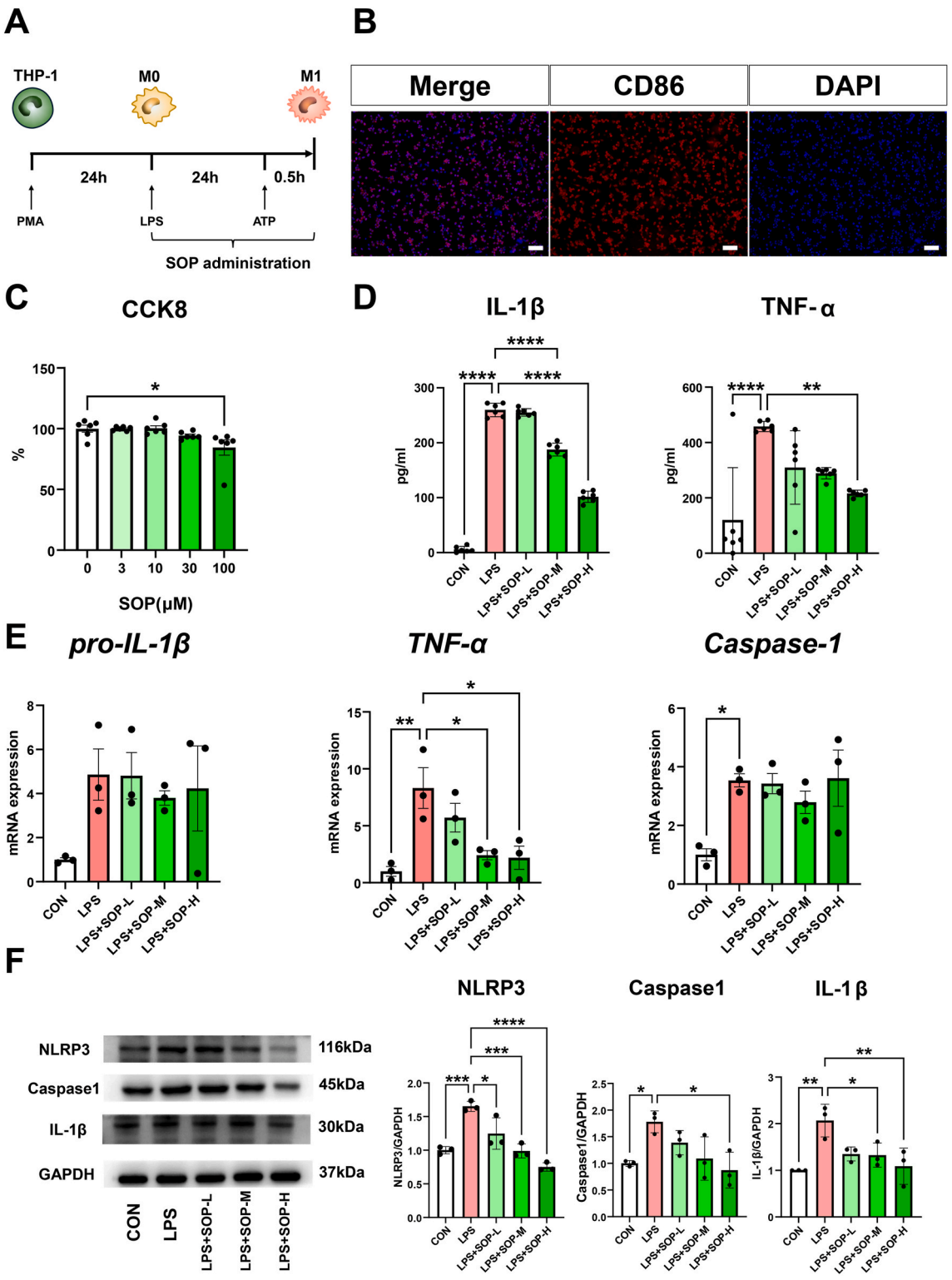


**Fig. 2.** SOP Downregulated NLRP3 Signaling of M1 Cells in *vitro*.

A. Overall *in vitro* experimental design.

B. Representative immunocytochemistry images. Blue, DAPI; Red, CD86. Scale bar = 100 μm

C. Results of CCK8 analysis of THP-1 cells treated with different concentrations of SOP. (n = 6 in each group).

D. Quantification of IL-1β and TNF-α levels in the supernatant of each group using ELISA. (n = 6 in each group).

E. Changes in mRNA expression of pro-IL-1β, TNF-α, and Caspase-1 in each group, detected by real time-qPCR. (n = 3 in each group).

F. Representative blots and quantification of the protein levels of NLRP3, Caspase-1, and IL-1β in M1 macrophages. (n = 3 in each group)

All data are expressed as mean ± SD. Differences were evaluated using a one-way ANOVA followed by Tukey's correction. "*" indicates a comparison between the groups. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, and ****$p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

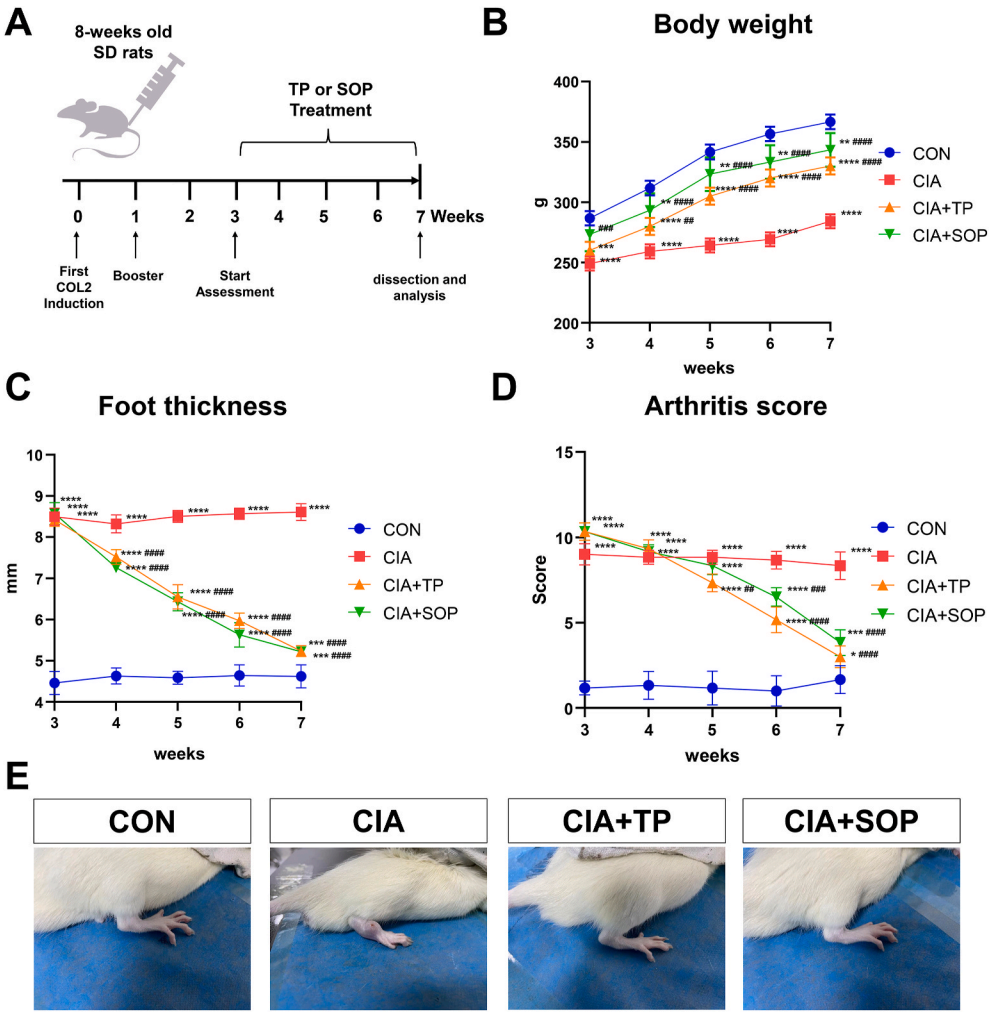


**Fig. 3.** SOP Reduced Arthritis Symptoms *in vivo*.
A. Experimental design of the rat model of CIA.
B. Changes in body weight in the CON, CIA, CIA + TP, and CIA + SOP groups. (3–7 weeks; n = 6 in each group).
C. Changes in foot thickness in the CON, CIA, CIA + TP, and CIA + SOP groups. (3–7 weeks; n = 6 in each group).
D. Arthritis score of the CON, CIA, CIA + TP, and CIA + SOP groups. (3–7 weeks) (n = 6 in each group).
E. Photos of the arthritic right hind limb induced by COL2 in the CON, CIA, CIA + TP, and CIA + SOP groups.
All data are expressed as mean ± SD. Differences were evaluated using a one-way ANOVA followed by Tukey's correction. "*" and "$^{\#}$" indicate a comparison with the CON and CIA groups, respectively. *, $^{\#}p < 0.05$; **, $^{\#\#}p < 0.01$; ***, $^{\#\#\#}p < 0.001$, and ****, $^{\#\#\#\#}p < 0.0001$.

group (Fig. 2F). After SOP administration, the levels of these proteins decreased with increasing SOP concentration. All protein levels significantly decreased in the LPS + SOP–H group (NLRP3, $p < 0.0001$; Caspase-1, $p < 0.05$; IL-1β, $p < 0.01$) compared to those in the LPS group. This indicated that SOP blocked NLRP3 signaling *in vitro* and might have a positive effect on RA.

### 3.4. SOP reduced RA symptoms in the CIA model

To verify the effectiveness of SOP on RA, we developed a rat model of CIA (Fig. 3A). In total, 40 rats were used in the study, and after the scoring assessment, arthritis was determined to be successfully induced 24 rats that were used for further analysis. Three weeks after COL2 injection, body weight was significantly reduced in the CIA group ($p < 0.0001$) compared to that in the CON group (Fig. 3B). After 1 week of SOP or TP administration, the body weights of both the CIA + TP ($p < 0.01$) and CIA + SOP ($p < 0.0001$) groups started to increase significantly compared with the CIA group. We measured foot thickness to evaluate RA severity. The foot thickness of the CIA group ($p < 0.0001$) was significantly greater than that of the CON group 3 weeks after the first injection (Fig. 3C). The foot thicknesses of CIA + TP group ($p < 0.01$) and CIA + SOP group ($p < 0.0001$) decreased significantly at 4 weeks. After 3 weeks of TP or SOP administration (6 weeks after the first COL2 injection), the arthritis scores of the CIA + TP and CIA + SOP groups decreased significantly (Fig. 3D), and the redness and swelling in the hind legs of rats disappeared after 4 weeks of SOP or TP administration (Fig. 3E). These results indicate that SOP relieved the symptoms of RA similar to that with TP.

### 3.5. SOP reduced inflammatory factors in serum with less toxicity than did TP

To further verify the effectiveness of SOP, we detected IL-6, TNF-α, and anti-CCP levels in rat serum. Serum of the CIA group showed increased levels of IL-6, TNF-α, and anti-CCP ($p < 0.0001$) (Fig. 4A), which decreased after TP or SOP treatment ($p < 0.0001$). Hematoxylin and eosin-stained sections of the ankle joints were observed. The CIA group showed severe joint damage, which was relieved by TP and SOP treatment (Fig. 4B). In addition, we stained for NLRP3 and focused on the damaged area, which showed strong NLRP3 positivity; the CIA + TP

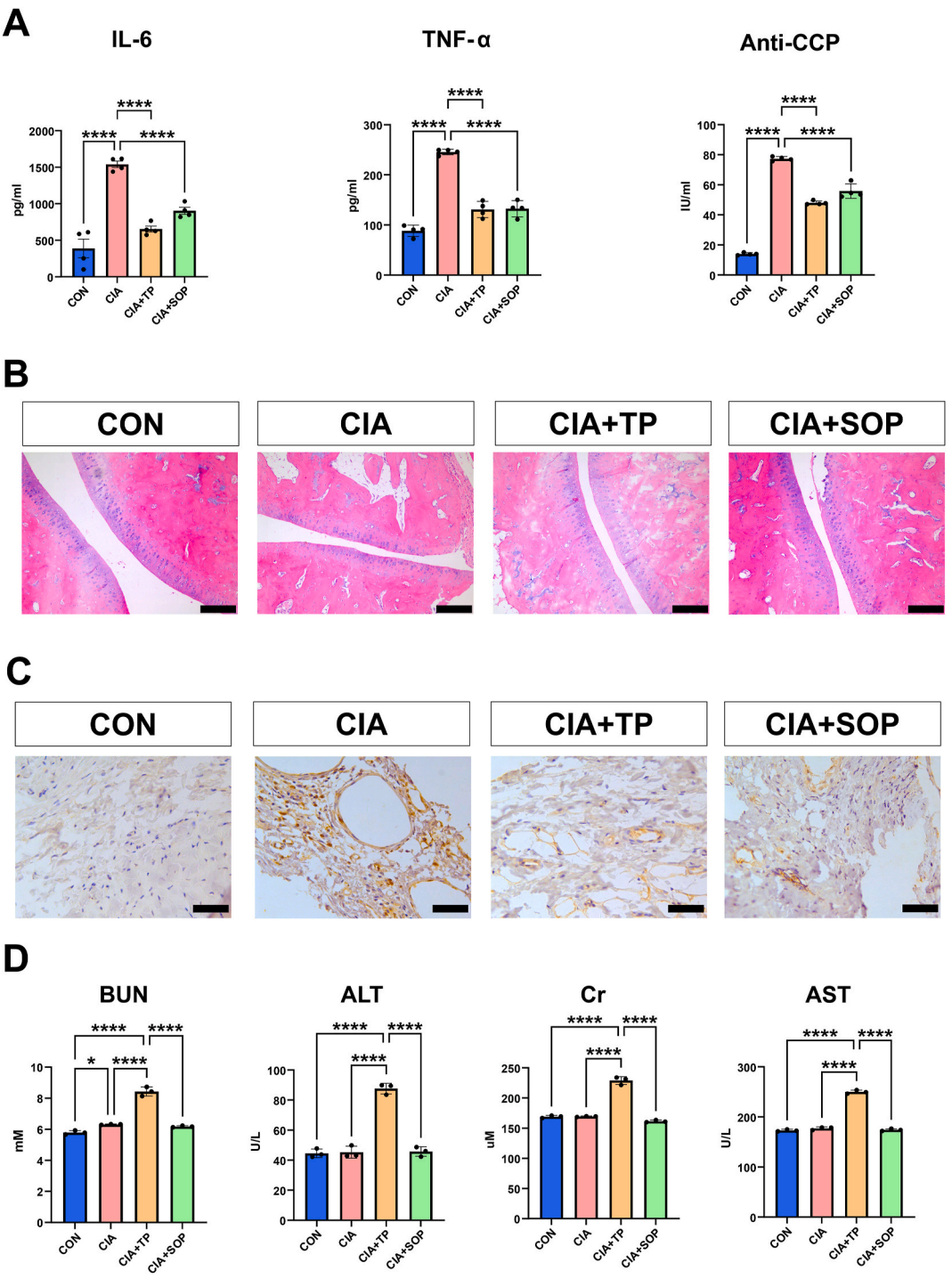


**Fig. 4.** SOP Reduced Serum Levels of Inflammatory Factors Without Liver or Kidney Toxicity.
A. Quantification of IL-6, TNF-α, and Anti-CCP serum levels in the CON, CIA, CIA + TP, and CIA + SOP groups by ELISA. (n = 4 in each group)
B. Representative hematoxylin and eosin stain images of the CON, CIA, CIA + TP, and CIA + SOP groups. Scale bar = 200 μm
C. Representative NLRP3 immunostaining images of the CON, CIA, CIA + TP, and CIA + SOP groups. Scale bar = 50 μm.
D. Quantification of serum BUN, ALT, Cr, and AST levels in the CON, CIA, CIA + TP, and CIA + SOP groups to assess liver and kidney functions. (n = 3 in each group)
All data are expressed as mean ± SD. Differences were evaluated using a one-way ANOVA followed by Tukey's correction. "*" indicates a comparison between the groups. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, and ****$p < 0.0001$.

and CIA + SOP groups showed slight positive staining (Fig. 4C). This suggests that SOP has an effect similar to that of TP and reduces inflammation and infiltration of macrophages into the joints.

In contrast, serum of the CIA + TP group showed significantly increased blood urea nitrogen, alanine aminotransferase, creatinine, and aspartate transaminase levels compared to those of the other treatment group ($p < 0.0001$) (Fig. 4D), indicating that TP treatment exhibits organ toxicity in rats. No abnormalities were observed in the CIA + SOP group indicating that SOP, unlike TP, does not cause strong liver or kidney toxicity in rats.

## 4. Discussion

To identify new therapies for RA, we focused on SOP, which has a lower organ toxicity than TP. However, the relationship between SOP and RA remains unclear. Therefore, we performed target prediction for SOP, which indicated that the targets of SOP are related to metabolism and inflammation regulation. This conclusion is supported by findings of Chen et al. [17–19], suggesting that SOP treatment may reduce inflammation in RA. However, the relationship between SOP and specific inflammatory factors remained unclear. Owing to the limitations of target prediction, inflammatory markers such as NLRP3 and Caspase-1 were not listed in the results.

Therefore, to determine the representative inflammatory factors in RA, we analyzed the results from other groups' CIA models [28]. Found macrophage markers and interleukin factors were upregulated in the joints affected by RA. This suggests that blocking the macrophage immune response in RA may have a positive therapeutic effect and that upregulated inflammation markers might be used to evaluate the effect of SOP. However, the trends observed for a few Interleukin factors were not clear in the dataset, which might be caused by individual differences that need to be verified by experiments.

To verify these findings, we analyzed the effects of SOP on macrophages *in vitro*, specifically the effect on NLRP3, a supramolecular complex that regulates IL-1β production [21,22]. We observed that SOP reduced the levels of IL-1β and TNF-α in the supernatant of cultured M1 macrophages. Realtime-qPCR results indicated that SOP suppressed the expression of TNF-α and Caspase-1 in M1 macrophages, but the expression of pro-IL-1β unchanged. Moreover, the NLRP3, Caspase-1, and IL-1β protein levels decreased as the concentration of SOP increased. The results demonstrate that SOP downregulates NLRP3 signaling, reduces the production of IL-1β but not the pro-IL-1β. Such regulation by SOP might have a therapeutic effect on RA.

To evaluate the effect of SOP on RA, we developed a rat model of CIA and administered rats with TP or SOP. Both TP and SOP reduced immune factor levels and joint damage resulting from CIA. This result demonstrated that SOP has a similar therapeutic effect as TP in CIA [30,31]. Moreover, according to liver and kidney function factors, SOP did not cause liver or kidney toxicity, which is conducive to its practical clinical application in RA treatment.

Our experimental results indicated that SOP has the positive effect of suppressing inflammatory reactions in RA and downregulated NLRP3 signaling in M1 macrophages. However, the detailed intracellular molecular interactions following SOP administration remain unclear, and the involvement of cells other than M1 macrophages has not yet been studied. Therefore, these topics require further investigation.

In conclusion, SOP reduced inflammation in type II collagen-induced arthritis by downregulating NLRP3 signaling in macrophages, eventually relieving the symptoms of RA. This may serve as an ideal therapy for RA in the future.

## CRediT authorship contribution statement

**Youyang Liu:** Validation, Methodology, Investigation. **Yunlu Zhao:** Writing – original draft, Conceptualization. **Qi Guo:** Writing – original draft, Conceptualization. **Pengfei Wang:** Visualization, Data curation. **Peixuan Li:** Visualization, Data curation. **Qingqing Du:** Visualization, Data curation. **Huazhou Xu:** Investigation, Methodology, Validation. **Qingyin Yu:** Investigation, Methodology, Validation. **Xiaoyi Zhao:** Investigation, Methodology, Validation. **Weiya Zhang:** Investigation, Methodology, Validation. **Shengjun An:** Funding acquisition. **Shuhui Wu:** Writing – review & editing, Supervision, Project administration.

## Explanation of authorship rearrangement

Dear editors of Biochemistry and Biophysics Reports.

As the corresponding author, I am sorry for I am not explaining the reason of authorship rearrangement.

Because first author Youyang Liu, has already graduated from our university and the other authors were busy on their own research project. The additional experiments were conducted by other members in our laboratory, these members were added as new authors to the paper titled "Sophoricoside Reduced Inflammation in Arthritis Induced by Type II Collagen by Downregulating NLRP3 Signaling".

Detail new authors' contribution information are as follows:

Huazhou Xu, performed reverse transcription from RNA, and conducted realtime-qPCR with cDNA.

Qingyin Yu, conducted THP-1 cell culture and collected RNA from the cells.

Xiaoyi Zhao, Weiya Zhang conducted THP-1 cell culture.

All authors including the new authors added in the manuscript, have been notified of the rearrangement of the authorship. And all agreed with the rearrangement.

We hope this statement will clarify the reason of the authorship rearrangement, and I hope the request of authorship change could be approved.

Thank you.

Shuhui Wu.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101867.

## Data availability

Data will be made available on request.

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