

Platycodon grandiflorus saponin alleviates MSU-induced gouty arthritis based on NLRP3/ASC/caspase-1 pathway

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Research Article

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Abstract

Platycodon grandiflorus is a homologous material of traditional medicine and food. Besides a pickle, more importantly, it can also be used in traditional Chinese medicine as an alternative to modern western. Various studies have found that saponins in *Platycodon grandiflorus* (PGS) can play a role in different diseases (including liver cancer, lung cancer, cardiovascular and cerebrovascular diseases) as the main chemical constituents. However, studies on the treatment of gout arthritis by PGS are not reported, and the mechanism has not been speculated and elucidated systematically. This study describes the anti-inflammatory agent of PGS in treating gouty arthritis by characterizing the degree of joint swelling, inflammatory factors (IL-6, IL-1 β , TNF- α), peroxides (SOD, MDA, GSH-PX), histopathology and related proteins. The results show that when MSU is injected into the joint, it activates the NLRP3 protein to bind to caspase-1 via ASC to form the NLRP3 inflammatory conjugate, which undergoes a series of changes to promote the release of inflammatory factors causing joint swelling and pain. Therefore, the same as colchicine, PGS can effectively reduce the swelling degree, level of inflammatory factors, and related protein expression while diminishing oxidative stress levels. This study lays the foundation for treating gout arthritis by PGS.

1. Introduction

Gouty arthritis is caused by increased blood uric acid due to the disorder of purine metabolism or the abnormal excretion of uric acid, which leads to the precipitation of sodium urate crystals [1, 2, 3]. Sodium urate crystals continuously cause inflammatory reactions after deposition in the joints and the soft tissues around the joints [4]. Symptoms are mainly redness, heat, and pain in the joints. According to the survey report, gout arthritis patients have been increasing yearly in recent decades, and the proportion of young people is also gradually increasing [5]. Since the pain symptoms of gouty arthritis have different degrees of physical and mental damage to human beings, it significantly impacts the quality of human life [6]. Therefore, treating gouty joints is of great significance to the healthy development of human beings.

Platycodon grandiflorus is the dry root of *Platycodon grandiflorus* (Jacq.) A.DC of *Platycodonaceae*, a homologous variety for medicine and food, is mainly distributed in Northeast Asia [7, 8]. *Platycodon grandiflorus* saponin is the main chemical constituents of *Platycodon grandiflorus*. About 89 known saponins were extracted from *Platycodon grandiflorus*. The glycosyl ligands in *Platycodon* saponins are mainly composed of D-glucose, L-rhamnose, L-arabinose, D-xylose, and D-apiose and their derivatives [9, 10, 11]. It has been reported that *Platycodon grandiflorus* saponin can attenuate the specificity of NF- κ B and STAT1 by inhibiting Nrf2/ARE-mediated heme oxygenase-1 simultaneously [12]. Atopic dermatitis skin lesion. another study showed that saponins from *Platycodon grandiflorus* can improve non-alcoholic steatohepatitis induced by a high-fat diet [13]. thus indicating that *Platycodon grandiflorus* saponins has a particular role in anti-inflammatory.

In this study, the mechanism of Platycodon saponins in treating gouty arthritis was studied by establishing a mouse model of gouty arthritis. Based on swelling degree, inflammatory factor content, and expression of related proteins, we comprehensively evaluated the anti-inflammatory effect of Platycodon grandiflorus saponins.

2. Materials And Methods.

This section is detailed in the Supplementary Material (EMS₁).

3. Result And Discussion

3.1 Platycodon grandiflorus saponin fraction

PGS analysis was performed by high-performance liquid chromatography, and it was found that the saponin extract contained Desaccharide Platycodon saponin E; Platycodon saponin E; Platycodon saponin D₃; Desaccharide Platycodon saponin D; Platycodon saponin D; Acetyl Platycodon saponin D₂ (Fig. 1)

3.2 The effect of platycodon saponins on the degree of arthritis swelling caused by MSU

After MSU was deposited in the joint, the main symptoms of the appearance were redness and swelling of the joint [14]. A gouty arthritis model was established to observe the effect of PGS on the degree of swelling to acute gouty arthritis. The degree of swelling in the ankle was measured to verify the efficacy of PGS. It could be seen from Table 1 that compared with the control group, the model group had a higher degree of swelling. In contrast, the colchicine group had a significant inhibitory effect on the degree of swelling. Also, the difference between the groups and the drug effect could be seen by the appearance of the mouse ankle 24 h after the injection (Fig. 2). At the same time, it was found that the inhibitory effect of the middle-dose and high-dose groups of Platycodon grandiflorus saponin was also more apparent, indicating that PGS and colchicine also had the effect of reducing swelling (Table 1).

Table 1. The degree of swelling in mice at each time period, data are mean $\pm$ standard deviation n = 8, Statistical analysis of data was performed by one-way analysis .compared with normal group *P < 0.05, **P < 0.001, compared with model group #P < 0.05, ##P < 0.001

Table 1
The degree of ankle swelling in each group.

group	0h	2h	4h	24h	48h
blank group	2.77 ± 0.21	2.94 ± 0.25	2.87 ± 0.24	2.83 ± 0.14	2.80 ± 0.20
model group	2.85 ± 0.22	3.96 ± 0.17 ^{**}	3.90 ± 0.24 ^{**}	3.71 ± 0.20 ^{**}	3.49 ± 0.17 ^{**}
positive control group	2.74 ± 0.18	3.68 ± 0.28	3.58 ± 0.20 ^{**}	3.47 ± 0.16 ^{**##}	3.28 ± 0.22
low concentration group	2.96 ± 0.25	3.91 ± 0.43	3.73 ± 0.20 ^{**}	3.63 ± 0.19 ^{**##}	3.37 ± 0.27
Medium concentration group	2.84 ± 0.15	3.80 ± 0.24 ^{**}	3.68 ± 0.21 ^{**}	3.56 ± 0.15 ^{**##}	3.38 ± 0.22
high concentration group	3.08 ± 0.19	3.93 ± 0.303	3.76 ± 0.27	3.56 ± 0.30	3.41 ± 0.25

3.3 Effects of Platycodon grandiflorus saponins on the levels of inflammatory factors and peroxidative stress factors.

Gouty arthritis was often closely related to the release of inflammatory factors, and the increased expression of inflammatory factors aggravates gouty joints' symptoms [15, 16]. To investigate whether PGS can inhibit releasing inflammatory factors, an enzyme-linked immunosorbent assay determined the IL-6, IL-1 β , and TNF- α in serum. Firstly, by comparing the control group and the model group in Fig. 2, the differences were determined that the injection of MSU had an effect on the mice, indicating that the model was successfully established. Secondly, according to the expression of inflammatory factors of IL-6 (Fig. 3a), IL-1 β (Fig. 3b), and TNF- α (Fig. 3c) in the colchicine group and each group of Platycodon grandiflorus saponins in Fig. 1, the expression of these inflammatory factors was suppressed after MSU injection. In addition, the inhibitory effect of PGS on TNF- α expression was more obvious. It showed that PGS had an inhibitory effect on the release of inflammatory factors. Other biochemical indicators in serum detected the level of oxidative stress factors. Oxidative stress response was an essential link in the process of inflammatory factor expression. When oxidative stress response was activated, it accelerated cell damage [17]. In the present study, MDA (Fig. 3f) was found to be elevated, and SOD (Fig. 3d) and GSH-PX (Fig. 3e) were decreased in the model group, indicating that abnormal oxidative stress occurred. After drug treatment, MDA levels decreased and SOD and GSH-Px levels increased. It is suggested that

Kratom saponin may protect against tissue damage in the ankle caused by MSU by decreasing the level of oxidative stress factors.

3.4 Inhibitory effect of platycodon grandiflorus saponin on tissue injury and neutrophil infiltration

H&E staining revealed that the tissue morphology and arrangement in the blank group were normal, with clear cell morphology and cell membrane boundaries and no cell infiltration. In contrast, the model group showed prominent cell infiltration, disorganized tissue structure, detached synovial epithelial tissue, and altered cell morphology, showing noticeable pathological changes in arthritis[18, 19, 20]. In the colchicine group and the Platycodon grandiflorus saponin group, there were different degrees of improvement compared with the model group, especially in the high dose group of Platycodon grandiflorus saponin, membrane cell proliferation and inflammatory cell infiltration were reduced, vascular proliferation was decreased, and cell membrane boundaries became clear (Fig. 3a). In addition, we studied the effect of platycodon grandiflorus saponin on neutrophil infiltration by measuring the concentration of MPO at the ankle joint. The results showed that neutrophil infiltration in the joint tissues of mice treated with colchicine and platycodon grandiflorus was significantly reduced (Fig. 4b).

3.5 Effects of Platycodon grandiflorus saponins on the expression of related inflammatory proteins

To further investigate the therapeutic effect of Platycodon grandiflorus saponin on gouty arthritis, protein blotting experiments were performed to detect the expression of related proteins on the NLRP3/ASC/caspase-1 signaling pathway. The results showed that the relative expression of ASC (Fig. 5a), IL-1 β (Fig. 5b), and caspase-1 (Fig. 5c) proteins in the model group was significant compared with other groups, during gavage of Platycodon grandiflorus saponin. The expression of related proteins was significantly attenuated in all groups, indicating that origanum saponin could alleviate the inflammatory response of gouty arthritis by inhibiting the expression of associated proteins on the inflammatory pathway.

4. Conclusions

- When MSU is absorbed, it can accelerate the release of IL-1 β , and IL-1 β can bind to leukotrienes (IL-1R1, IL-1RAcP), resulting in changes in chemokines, recruitment of neutrophils, and gouty arthritis [21, 22, 23]. Therefore, reducing pro-inflammatory factors can significantly reduce the occurrence of inflammatory responses in the body[24]. and in this study, PGS can effectively reduce the expression of inflammatory factors. In addition, we investigated the effect of the oxidative stress response on gouty arthritis. It is known that when NLRP3 inflammasome induces caspase-1 to release inflammatory factors, an inflammatory reaction occurs [25, 26].
- According to the experimental results, on the one hand, PGS could reduce the inflammatory factors IL-6, IL-1 β , and TNF- α in serum. On the other hand, PGS improved the oxidative stress response and

reduced the inflammatory reaction that MSU caused. It also plays a suppressive role in expressing related proteins, which relieve the swelling and pain caused by gouty arthritis. In conclusion, we experimentally validated that PGS can treat gouty arthritis by inhibiting the NLRP3/ASC/caspase-1 inflammatory pathway(Figure. 6). In this study, for the first time, we carried out the mechanism study of PGS on gouty arthritis, which was helpful for the treatment of gout. The drug research of gouty arthritis has provided a new substance, which might become a new method for treating gouty arthritis.

Declarations

5.1 Declaration of interests

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

5.2 Ethical Approval

This experiment was approved by the Qilu University of Technology and Sci-Tech Quality Testing Co., Ltd (experimental animal use permit number: SCXK (Lu) 20210015).All experiments described below followed the National Institutes of Health guidelines for the Care of Use of Laboratory Animals.

5.3 Data Availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

5.4 Consent to participate

not applicable

5.5 Consent for publication

not applicable

5.6 Authors' contributions

PingSun: performing experiment, data analysis, manuscript writing. YangLiu and Tingting Zhan:Examination of experimental design and manuscript. HuiXu and Jianjun Liu: supervision, data interpretation. Zerun Li and Yupeng Nie: design of the sample preparation. Shanshan Wang and Yanhong Huang manuscript reviewing and editing.

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Figures

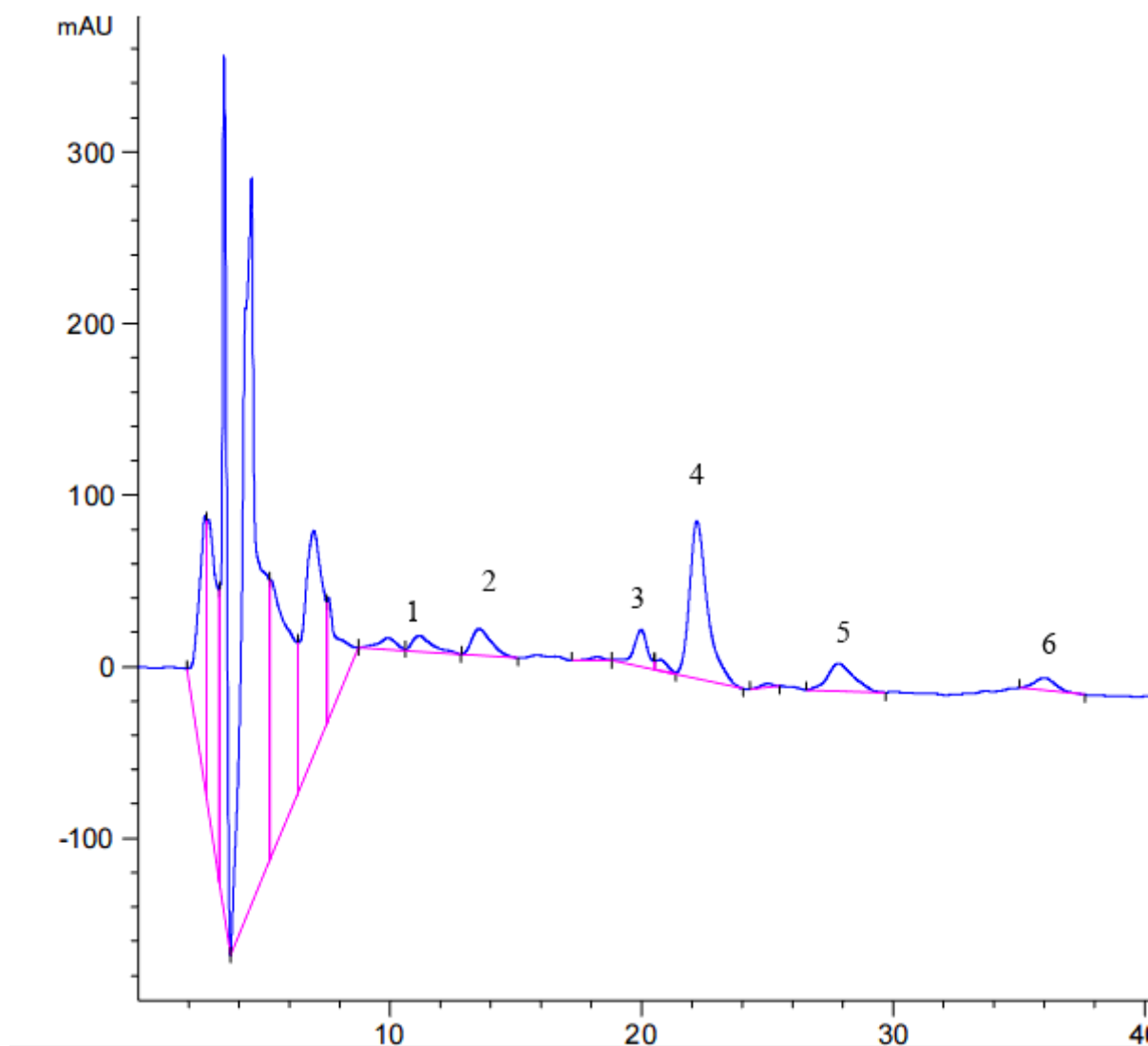


Figure 1

HPLC chromatogram of Platycodon saponin



Figure 2

The degree of swelling at the joint in each group 24h after MSU injection

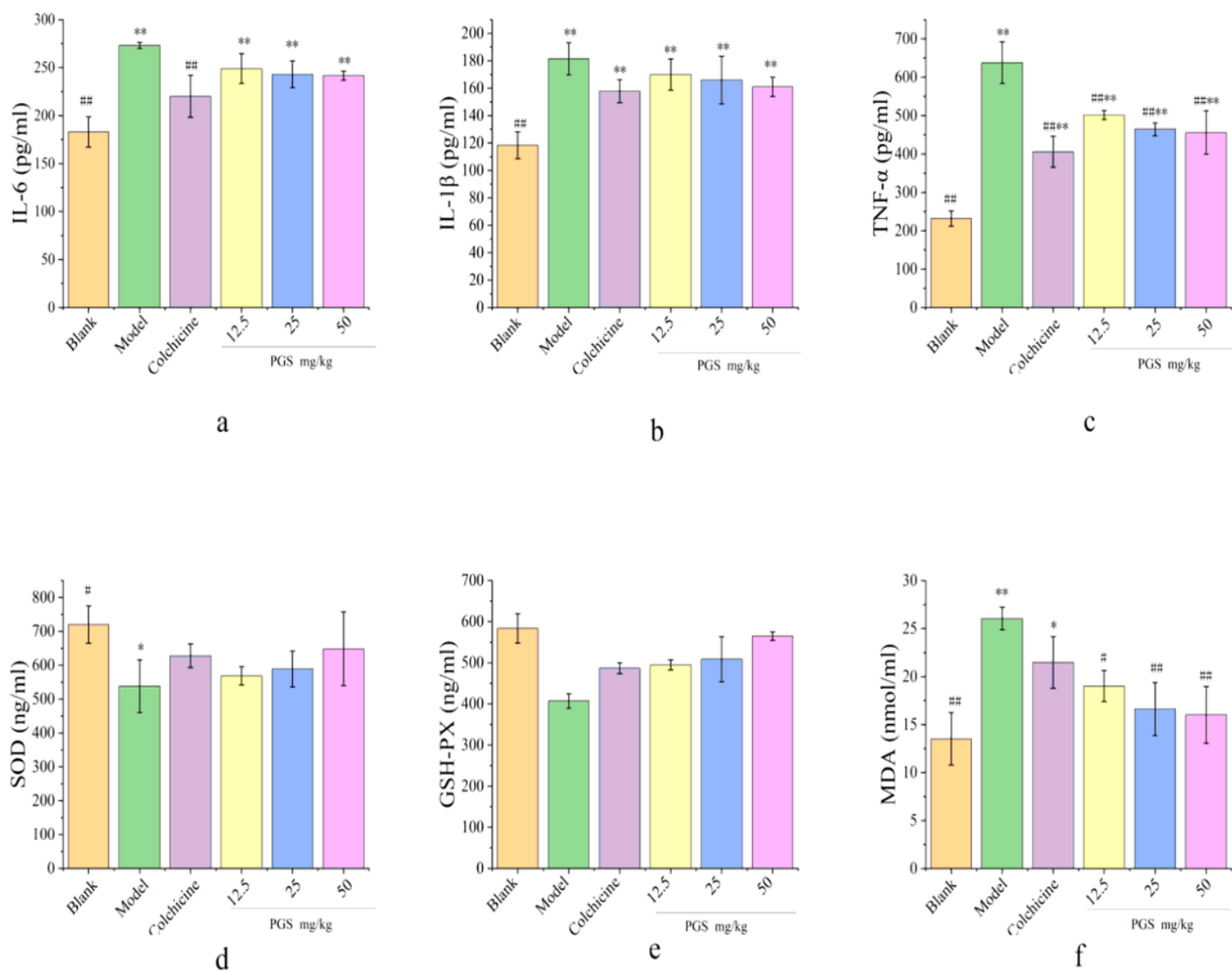


Figure 3

Serum levels of IL-6, IL-1β, TNF-α, SOD, GSH-PX, and MDA, and supernatant was obtained by centrifugation of blood taken 48 h after MSU injection. Data are mean ± standard deviation, and statistical analysis of data was performed using one-way analysis of variance .compared with normal group* P < 0.05, ** P < 0.001, compared with model group# P < 0.05, ## P < 0.001.

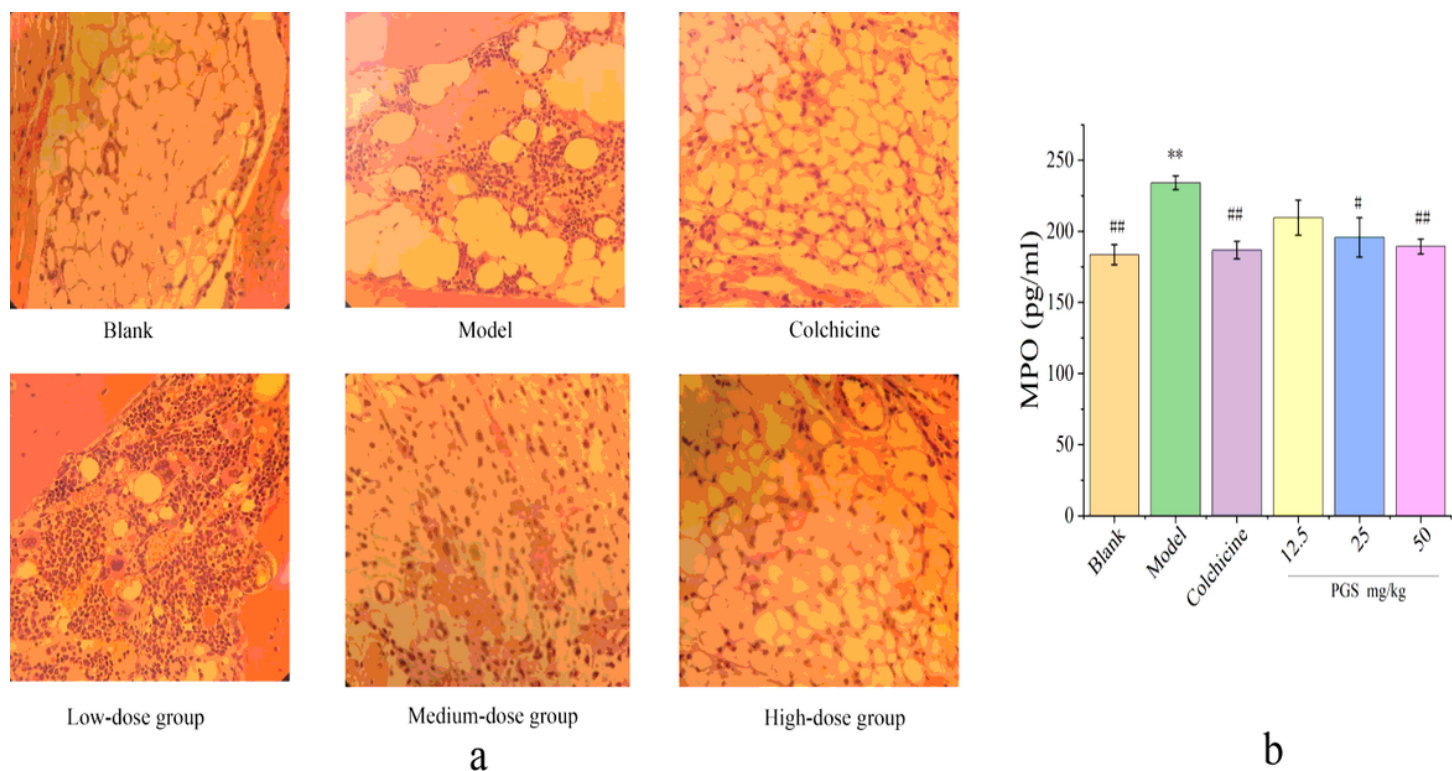


Figure 4

Effect of *Platycodon grandiflorus* saponin on inflammatory cell infiltration in ankle joint tissue in a model of MSU-induced acute gouty arthritis. H&E staining of the ankle joint (×200) (a). Measurement of myeloid oxidase (MPO) activity in ankle joint tissues (b).and statistical analysis of data was performed using one-way analysis of variance .compared with normal group* $P < 0.05$, ** $P < 0.001$, compared with model group# $P < 0.05$, ## $P < 0.001$.

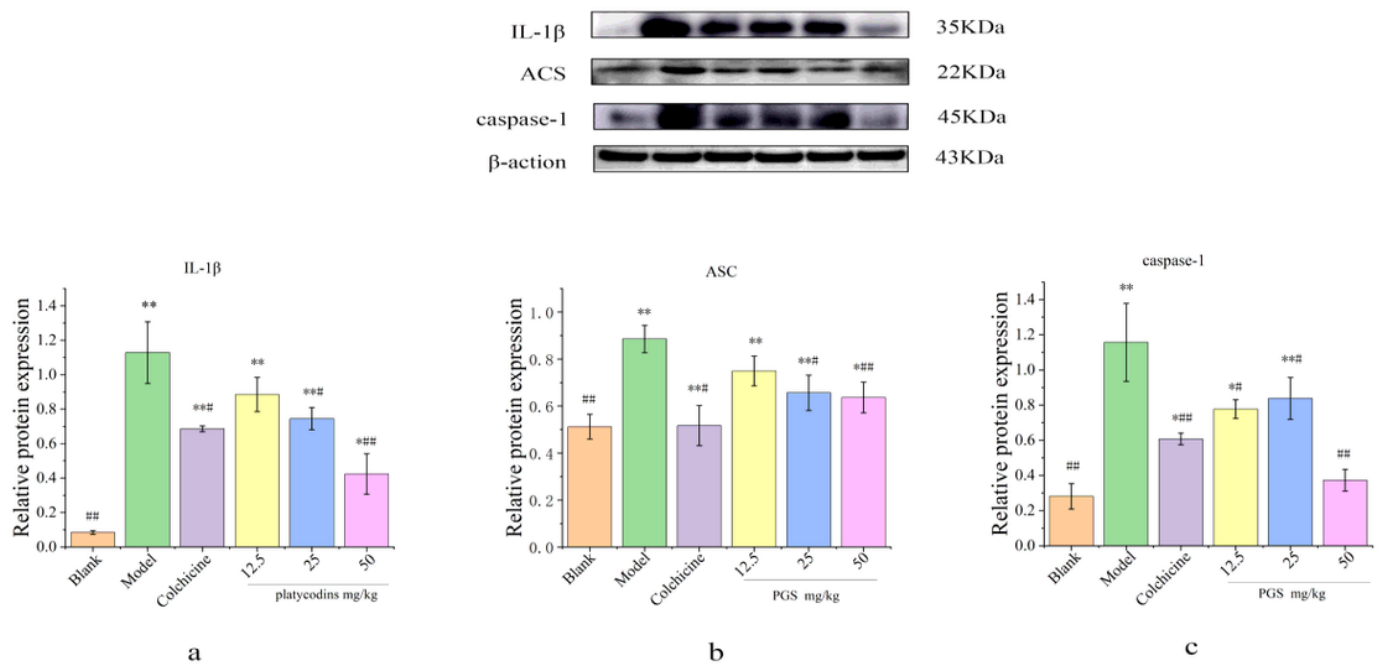


Figure 5

Determination of relative protein levels of caspase-1, IL-1 β and ACS in the ankle joint by western blot analysis, Data are mean $\pm$ standard deviation, and statistical analysis of data was performed using one-way analysis of variance. compared with normal group* $P < 0.05$, ** $P < 0.001$, compared with model group# $P < 0.05$, ## $P < 0.001$

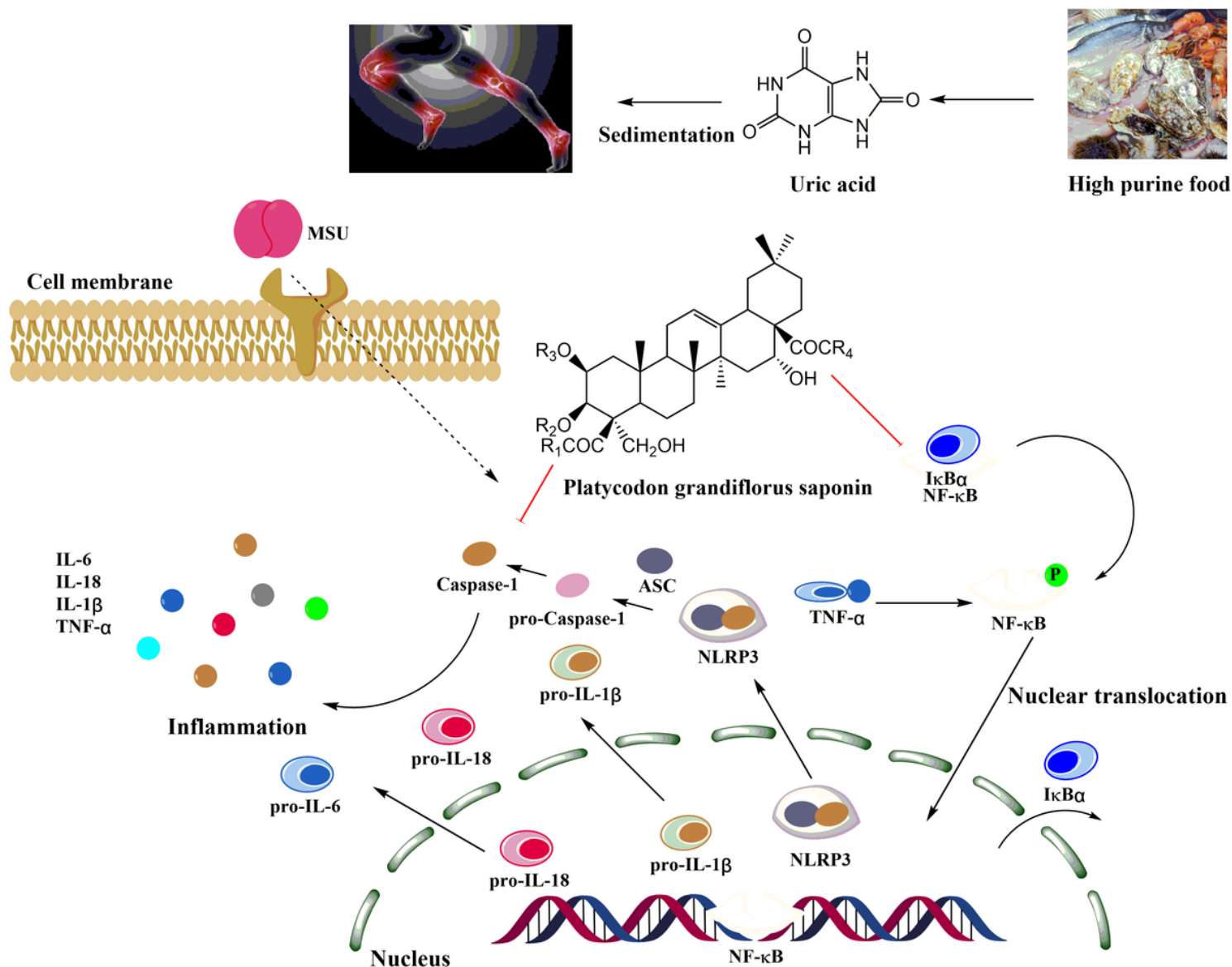


Figure 6

Schematic diagram of the treatment of gouty arthritis with *Platycodon grandiflorus* saponin. PGS reduces the symptoms of gouty arthritis by inhibiting the secretion of IL-6, IL-1β, TNF-α and inhibiting NLRP3 signaling

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