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Alleviation of Gouty Arthritis Based on NLRP3/ASC/caspase-1 Pathway by Saponins Extracted from *Platycodon grandiflorus*

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A B S T R A C T

Platycodon grandiflorus is a traditional food with excellent medicinal properties, such as promotion of lung health, expectorants, and cough suppression. Various studies have found that saponins, the main chemical component of *Platycodon grandiflorus*, are very effective against many inflammatory conditions, including mastitis, dermatitis, and laryngitis. However, the treatment of gouty arthritis with *Platycodon grandiflorus* saponins has not yet been reported and their mechanism of action have not been systematically investigated. In this study, a mouse model of gouty arthritis was established, and mice were treated with gavage for seven days in the blank, model, colchicine (1 mg/kg), and *Platycodon grandiflorus* saponin (12.5 mg/kg, 25 mg/kg, 50 mg/kg) groups. MSU was sterilized and injected into the joints on the fifth day. The anti-inflammatory effect of PGS in the treatment of gouty arthritis was illustrated by measuring the degree of joint swelling, serum inflammatory factors (IL-6, IL-1β, TNF-α), peroxides (SOD, MDA, GSH-PX), joint histopathology, and characterization of related proteins. The results show that PGS was effective in reducing swelling and lowering IL-1β (181.49 vs. 161.01), IL-6 (273.10 vs. 241.69), TNG-α (637.89 vs. 455.80), and peroxide levels, presumably by a mechanism related to the reduction of proteins on the NLRP3/ASC/caspase-1 pathway.

Introduction

Gouty arthritis is caused by a purine metabolism disorder or abnormal excretion of uricacid, resulting in the precipitation of sodium urate crystals. The main symptoms are redness, swelling, heat, and pressure pain in the joints, accompanied by general weakness, fever, and a headache According to survey reports, the number of patients with gouty arthritis has increased annually in recent decades, and the proportion of young people is gradually increasing (He *et al.*, 2022). As the painful

symptoms of gouty arthritis cause different degrees of physical and psychological damage to a person, it affects the quality of life (Zhang *et al.*, 2021). Therefore, the treatment of gouty arthritis is of great importance for the healthy development of human beings.

Sodium urate crystals cause inflammation after being deposited in the joints and the soft tissues surrounding them (Yao *et al.*, 2022). Thus, the deposition of Monosodium Urate Crystals (MSU) becomes a specific activator. When phagocytes engulf it, MSU activates the NLRP3 protein to bind to the pro-caspase-1 protease (Pro-caspase-1) *via* the bridging protein ASC to form NLRP3 inflammatory vesicles. At this point, the inflammatory vesicle conformation is altered to activate ASC protein from an oligomeric state to a macromolecular dimer called ASC spots.

ASC spots cleave Pro-caspase-1 into caspase-1, and the formation of caspase-1 can promote the release of inflammatory factor precursors, including interleukin 1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α) (Chitra *et al.*, 2016; Zhan *et al.*, 2021). The release of inflammatory factors can aggravate the inflammatory response in arthritis (Meng *et al.*, 2021). Therefore, reducing the inflammatory response and producing anti-inflammatory drugs are key to treating gouty arthritis (Yun *et al.*, 2020; Feng *et al.*, 2022). Currently, the main drugs for treating gouty arthritis are colchicine, non-steroidal anti-inflammatory drugs, and glucocorticoids (Gu *et al.*, 2021; Zhang *et al.*, 2022). These drugs have specific side effects in the human body that can cause discomfort and damage to liver and kidney function and the gastrointestinal tract. Identifying a cure with effective therapeutic effects and few side effects is essential for treating gouty arthritis (Jati *et al.*, 2022).

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 (Zhang *et al.*, 2020; Kim *et al.*, 2006). Countries such as China, North Korea, and South Korea have used *Platycodon grandiflorus* for thousands of years (Zhang *et al.*, 2015; Ke *et al.*, 2020). In the past, when there was a single method of consumption, the young seedlings or roots of *Platycodon grandiflorus* were made into kimchi or salad. With the development of modern technology, other edible forms of *Platycodon grandiflorus* have gradually become available.

Platycodon grandiflorus products such as noodles, preserved fruits, and healthy drinks have steadily appeared in the market (Ji *et al.*, 2020). *Platycodon grandiflorus*, as a traditional medicinal material, plays a role in expectorating phlegm, relieving coughs, antibacterial and anti-inflammatory processes, anti-oxidant, anti-tumor, and lowering blood sugar and blood pressure. In ancient times, *Platycodon grandiflorus* was used as a herbal remedy for many ailments (Li *et al.*, 2022).

For example, it was used with citrus peel and ginger to treat typhoid and laryngitis, peony and dioscorea to treat mastitis, measles, and dermatitis, thus indicating that *Platycodon grandiflorus* has a particular anti-inflammatory property (Su *et al.*, 2022).

Platycodon grandiflorus saponins are the main chemical constituents of *Platycodon grandiflorus*. It has been reported that *Platycodon grandiflorus* saponins can attenuate the specificity of NF-κB and STAT1 by inhibiting Nrf2/ARE-mediated heme oxygenase-1. Thereby reducing Atopic dermatitis skin lesion (Choi *et al.*, 2013; Jae *et al.*, 2017). Another study showed that saponins from *Platycodon grandiflorus* could improve non-alcoholic steatohepatitis induced by a high-fat diet (Han *et al.*, 2021; Zhang *et al.*, 2022).

In addition, platycodon saponins can inhibit IL-13-induced expression of inflammatory cytokines and mucus in nasal epithelial cells by inhibiting the NF-κB and MAPK signaling pathways (Ju-Suk *et al.*, 2017). Based on previous studies, we found that

Platycodon grandiflorus saponins could alleviate the inflammatory response by inhibiting the expression of related inflammatory pathways, indicating that it does have certain anti-inflammatory effects. However, the anti-inflammatory effects and mechanisms of gouty arthritis have not been well studied; therefore, we speculate that *Platycodon grandiflorus* saponins may have similar mechanisms in the treatment of gouty arthritis.

In this study, we established a mouse model of gouty arthritis to investigate the mechanism of *Platycodon grandiflorus* saponin for the treatment of gouty arthritis. We comprehensively evaluated the effects of *Platycodon grandiflorus* saponin on gouty arthritis based on the degree of ankle swelling, cellular changes in tissue sections, the content of inflammatory factors such as IL-1β and IL-6 and oxidative stress factors such as MDA and SOD, and the expression of related proteins. It provides data support for the development and utilization of *Platycodon grandiflorus* in the field of anti-inflammation while expanding the development of *Platycodon grandiflorus* saponin in food and drug.

Materials and Methods

Preparation of *Platycodon grandiflorus* saponin

The roots of *Platycodon grandiflorus* were first crushed and sieved through a 60 mesh sieve. Then microwave extraction method was used to extract *Platycodon grandiflorus* saponins. After extraction, the purification was carried out by macroporous adsorption resin method to improve the content of Platycodon saponins and the purified Platycodon saponins were collected.

Experimental animals

The 56 KM male mice (Shandong Pengyue Laboratory Animal Co., Ltd.) weighed approximately 25g ± 2g at temperature 22–23° and humidity of 35%–40%. The production license number is SCXK (Lu) 20190003. The light/dark cycle was 12 h, and the mice were acclimated to feeding for one week under conditions of free drinking and feeding. All experiments were conducted according to the guidelines set by the European Community (Directive 2010/63/EU). This study was approved by the Qilu University of Technology and Sci-Tech Quality Testing Co., Ltd. (experimental animal use permit number: SCXK (Lu) 20210015).

Effect of Platycodon saponins on the behavioral evaluation of gait

The gait condition of the mice was observed at 12, 24 and 48 h after modeling and the gait index was evaluated. The evaluation index was scored as 1, normal gait, fast crawling, no lameness; 2, more normal gait, fast crawling, no lameness; 3, abnormal gait, slow crawling, lameness; 4, abnormal gait, very slow crawling, severe lameness, and the comprehensive examination of the effect of orris saponin on the gait of mice with gouty arthritis

Modeling and treatment of acute gouty arthritis

The mice were divided into a blank group, model group, colchicine group (1mg/kg, Xishuangbanna Pharmaceutical Co.), saponins low dose group (12.5mg/kg), saponins medium dose group (25mg/kg), and saponins high dose group (50mg/kg). Eight mice in each group were gavaged for seven days, the mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium on the fifth day, after which they were placed in a supine position, and were disinfected at the ankle with 75% alcohol.

Except for the blank group, all groups were injected with 50 μl of MSU (25 mg/ml, Sigma Life Sciences and High Tech Group, Inc.) at the ankles, and the blank group was injected with 50ul of saline. After one hour of gavage on the seventh day, the mice were anesthetized. After removing the skin and muscle tissues from the ankles, the ankle tissues were divided into two parts: one was put into tissue fixative for pathological section observation, and the other was put into a -80 ° refrigerator.

Experimental design

Degree of swelling of the ankle joint

The ankle diameters of mice were measured with Vernier calipers at the same position before and 1, 4, 24, and 48 h after MSU injection. Five measurements were performed to observe changes in the degree of ankle distension over 48 h.

Detection of inflammatory factors and oxidative stress factors in serum

Mice were anesthetized and sacrificed on the seventh day, and blood was collected. After incubating at room temperature (approximately 25 °C) for 1 h, the mice were centrifuged at 4 °C and 3000 r/min immediately.

The supernatant was collected and stored in a -80℃ refrigerator immediately before the assay was done. The assay was performed using ELISA (Herpen (Shanghai) Biotechnology Co.) and the levels of IL-1β, TNF-α, IL-6, MDA, SOD, and GSH-PX factors in the serum were detected according to the ELISA kit instructions.

Myeloperoxidase (MPO) activity study

Quantitative analysis of neutrophils in mouse paw tissue was performed using an enzyme-linked immunosorbent assay (ELISA). An appropriate amount of normal saline was added to the ankle joints of mice for crushing treatment. The tissue homogenate was centrifuged at 4 °C for 10 min at 3000 R/min to obtain the supernatant, and the MPO level in the ankle tissue was measured according to the ELISA kit instructions.

Tissue section of gouty arthritis model

After the mice were anesthetized and sacrificed, their ankle joints were collected and placed in a tissue fixative. The ankle joints of the mice were then placed in an embedding box for decalcification, and further paraffin sections were prepared, stained with H&E, and magnified at 200x under a light microscope. Changes in the cell morphology were observed.

Western blot

To verify the therapeutic effect of Platycodon saponins on gouty arthritis, the expression of proteins involved in related pathways was studied. First, protein extraction was performed on the collected tissues. The tissues were crushed, and lysate and protease inhibitors were added to extract them. The ratio of 50:1 was lysed on ice for half an hour, centrifuged at 4 °C, and 12000 r/min for 10 min to remove the supernatant, and 20 μL of the supernatant was taken for quantitative analysis of BCA (Seville (Wuhan) Biotechnology Co.) protein. Second, the remaining portion was evenly mixed according to the ratio of adding 1 μL of 5× protein loading buffer for every 4 μL of protein sample solution, followed by boiling in water for 4–5 min.

The obtained protein samples were first subjected to SDS-PAGE protein electrophoresis, and the electrophoresed proteins were transferred to PVDF membranes, which were blocked with nonfat milk powder for 2.5 h.

The PVDF membranes were then placed in the primary antibody diluted 1:1000. Incubate at 4 °C on a shaker. After incubation, the membranes were washed three times with TBST for 15 min each. After washing, the PVDF membranes were placed back into the incubation box, the secondary antibody was added to the incubation box at a dilution of 1:3000 for 1 h, and then the membrane was washed after incubation. After washing, equal volumes of ECLA (Biosharp Biotechnology Co.) and ECLB were added for color development and the protein bands were imaged using a gel imager.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 (I.B.M., U.S.A.), all data were expressed as mean ± standard deviation (S.E.M.), and statistical analysis

was performed using one-way analysis of variance (ANOVA) to determine the level of significance ($P<0.05$).

Results and Discussion

Effect of Platycodon saponin on gait behavior index in mice

Observation of the model group showed that there was redness, swelling and heat at the ankle after successful modeling, and with the extension of time, this phenomenon became more and more obvious, peaking at 24 h. At this time, the mice moved slowly and lameness appeared, and this phenomenon continued for a longer period of time, and there were different degrees of improvement for the colchicine group and the mice in the low, medium and high dose groups of *Platycodon grandiflorus* saponin, of which the colchicine and *Platycodon grandiflorus* saponin high dose groups showed better improvement, with mild sloping feet and even no sloping feet in individual mice.

The effect of *Platycodon grandiflorus* saponins on the degree of arthritis swelling caused by MSU

As shown in Table 3, the model group had a higher degree of swelling than the control group. In contrast, colchicine had a significant inhibitory effect on the degree of swelling. In addition, the difference between the groups and the drug effect could be observed by the appearance of the mouse ankle 24 h after the injection (Figure 4a). At the same time, it was found that the inhibitory effect of the middle- and high-dose groups of *Platycodon grandiflorus* saponin was also more apparent, indicating that PGS and colchicine also reduced swelling.

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

As shown in Figure 1, inflammatory factors (IL-6, IL-1β, and TNF-α) differed significantly between groups, with the highest effect in the model group and the lowest effect in the blank group. After treatment of the modeling group with drug interference, inflammatory factors were significantly reduced in both the colchicine and *Platycodon grandiflorus* saponin groups.

The greatest degree of change was observed in the TNF-α inflammatory factor model group compared to that in the drug-treated group. In addition to the three dose groups of *Platycodon grandiflorus* saponins, the high-dose group showed the most significant swelling reduction, but was less effective than the colchicine group. Moreover, peroxide expression levels were measured and found to be increased in serum MDA (Figure. 1f) and decreased in SOD (Figure. 1d) and GSH-PX (Figure. 1e) levels after modeling; and decreased in MDA and increased in SOD and GSH-PX levels after colchicine and *Platycodon grandiflorus* saponin treatment.

Inhibitory effect of *Platycodon grandiflorus* saponins 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, 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, indicating noticeable pathological changes in arthritis.

In the colchicine and *Platycodon grandiflorus* saponin groups, there were different degrees of improvement compared with the model group, especially in the high-dose group of *Platycodon grandiflorus* saponins, membrane cell proliferation and inflammatory cell infiltration were reduced, vascular proliferation was decreased, and cell membrane boundaries became clear (Figure 2b). In addition, we studied the effect of *Platycodon grandiflorus* saponins on neutrophil infiltration by

measuring MPO concentration in the ankle joint. The results showed that neutrophil infiltration in the joint tissues of mice treated with colchicine and *Platycodon grandiflorus* was significantly reduced (Figure 2c).

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

The results showed that the relative expression of IL-1β (Figure 3a), ASC (Figure 3b), and caspase-1(Figure 3c) proteins in the model group was significant compared with other groups during the gavage of *Platycodon grandiflorus* saponins. The expression of related proteins was significantly attenuated in all groups, indicating that *Platycodon grandiflorus* saponins could alleviate the inflammatory response in gouty arthritis by inhibiting the expression of associated proteins in the inflammatory pathway.

Owing to the changes in modern dietary habits, people consume foods that contain excessive purine, some of which is not excreted during metabolism, which in the long run increases the concentration of uric acid in the blood and eventually forms urate crystals around joints (Zamudio-Cuevas *et al.*, 2015; Udhaya *et al.*, 2022). The deposition of uric acid crystals further causes swelling, pain in the joints, and fever (Wu *et al.*, 2022). The symptoms can recur, from acute to chronic gouty arthritis, and in severe cases, can lead to stiff and deformed joints and functional impairment (Qu *et al.*, 2016; Xu *et al.*, 2022; Han *et al.*, 2016). This has a serious impact on human quality of life. There is an urgent need to develop safe and efficient natural active ingredients for the current clinical treatment of gouty arthritis, which are associated with certain unpleasant side effects (Tulsi *et al.*, 2021; Chen *et al.*, 2022). Therefore, in this study, we used saponins, which are the main chemical components of the medicinal and food ingredients of *Platycodon grandiflorus*, and based on previous studies, we can recognize that *Platycodon grandiflorus* saponins can achieve anti-inflammatory effects by inhibiting certain inflammatory responses or inflammatory pain pathways; therefore, we verified whether *Platycodon grandiflorus* saponins are effective by establishing a mouse model of gouty arthritis.

In this study, we analyzed the effects of *Platycodon grandiflorus* saponins on MSU-induced acute gouty arthritis. Visually, the degree of swelling was significantly higher in the model group that did not receive total saponins from *Platycodon grandiflorus* than in the other groups. Furthermore, the H&E staining of bone tissue revealed that the cell morphology was significantly improved by the gavage of colchicine and *Platycodon grandiflorus* saponins compared with the model group. In addition, we found that *Platycodon grandiflorus* saponins had significant effects on various inflammatory factors and oxidative stress factors, similar to the inhibitory effects of colchicine on proinflammatory factors. The rise in proinflammatory factors, such as IL-6, IL-1β, and TNF-α, has a significant impact on the onset and exacerbation of gouty arthritis, especially the release of IL-1β. IL-1β is found in an active form in synovial, cartilage, and other joint tissues, and is the initiator of inflammatory regulation and the most classical inflammatory regulator. IL-1β binds to leukocyte factors (IL-1R1 and IL-1RAcP), leading to altered chemokine and neutrophil recruitment, which triggers gouty arthritis (Lin *et al.*, 2020). IL-6 is an inflammatory factor that promotes bone damage and has been used as an indicator of the activity and severity of gouty arthritis (Galozzi *et al.*, 2021).

Another inflammatory factor, TNF-α, is also important in gouty arthritis and has been reported to be involved in the treatment of gouty joints with TNF-α antagonists (Emre *et al.*, 2016). TNFand IL-1 are important cytokines that mediate inflammatory responses. Therefore, the reduction of proinflammatory factors can significantly reduce the occurrence of inflammatory responses in the body. In this study, PGS effectively reduced the expression of inflammatory factors. In addition, we investigated the effect of oxidative stress response on gouty arthritis.

Table.1 Experimental animal groups

Group	Experimental protocol
Control group (n=8)	Sanitary saline
Model group (*n*=8)	Sanitary saline + MSU
Colchicinegroup (*n*=8)	Colchicine + MSU
12.5mg/kg PGS (*n*=8)	12.5 mg/kg PGS + MSU
25mg/kg PGS (*n*=8)	25 mg/kg PGS + MSU
50mg/kg PGS (*n*=8)	50 mg/kg PGS + MSU

Table.2 Gait behavior index of mice in each group, data are mean±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

group	6h	12h	24h
Control group	1.43±0.404$^{\#\#}$	1.23±0.252$^{\#\#}$	1.03±0.058$^{\#\#}$
Model group	3.57±0.208**	3.80±0.20**	3.47±0.252*
Colchicine group	2.20±0.436$^{*\#\#}$	2.57±0.306$^{**\#\#}$	2.17±0.208$^{**\#\#}$
12.5mg/kg PGS	3.03±0.153$^{**\#}$	3.23±0.231$^{**\#}$	2.77±0.252$^{**\#}$
25mg/kg PGS	2.70±0.20$^{**\#\#}$	2.90±0.30$^{**\#\#}$	2.63±0.252$^{**\#\#}$
50mg/kg PGS	2.47±0.153$^{**\#\#}$	2.77±0.153$^{**\#\#}$	2.37±0.208$^{**\#\#}$

Table.3 The degree of swelling in mice at each time period, data are mean±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

group	0h	2h	4h	24h	48h
Control 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**
Colchicine group	2.74±0.18	3.68±0.28	3.58±0.20**	3.47±0.16$^{**\#\#}$	3.28±0.22
12.5mg/kg PGS	2.96±0.25	3.91±0.43	3.73±0.20**	3.63±0.19$^{**\#\#}$	3.37±0.27
25mg/kg PGS	2.84±0.15	3.80±0.24**	3.68±0.21**	3.56±0.15$^{**\#\#}$	3.38±0.22
50mg/kg PGS	3.08±0.19	3.93±0.303	3.76±0.27	3.56±0.30	3.41±0.25

Fig.1 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$^{\#}$ $P< 0.05$,$^{\#\#}$ $P < 0.001$.

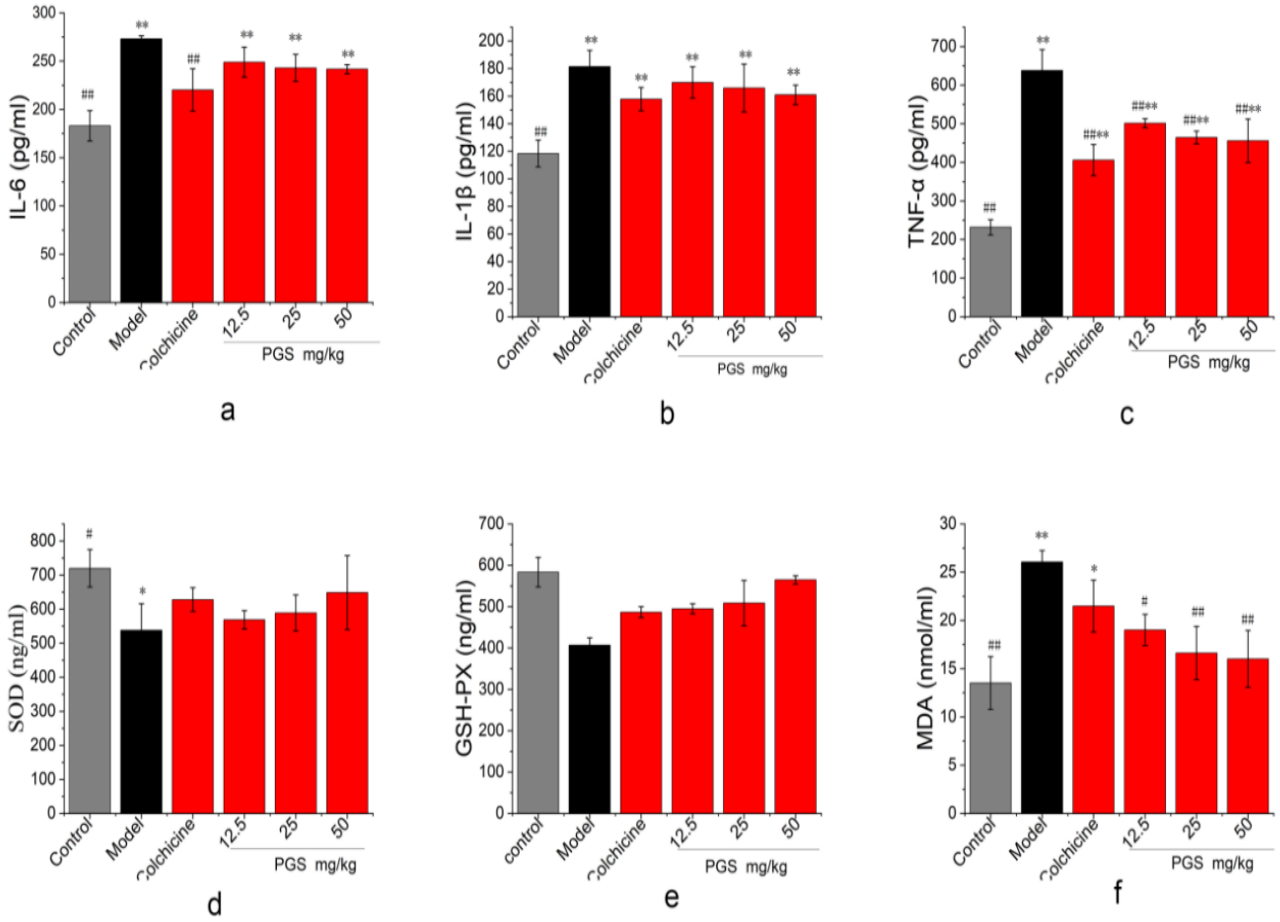


Fig.2 The degree of swelling at the joint in each group 24h after MSU injection(a). Effect of *Platycodon grandiflorus* saponins on inflammatory cell infiltration in ankle joint tissue in a model of MSU-induced acute gouty arthritis. H&E staining of the ankle joint (×200) (b). Measurement of myeloid oxidase (MPO) activity in ankle joint tissues (c).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$^{\#}$ $P< 0.05$,$^{\#\#}$ $P< 0.001$.

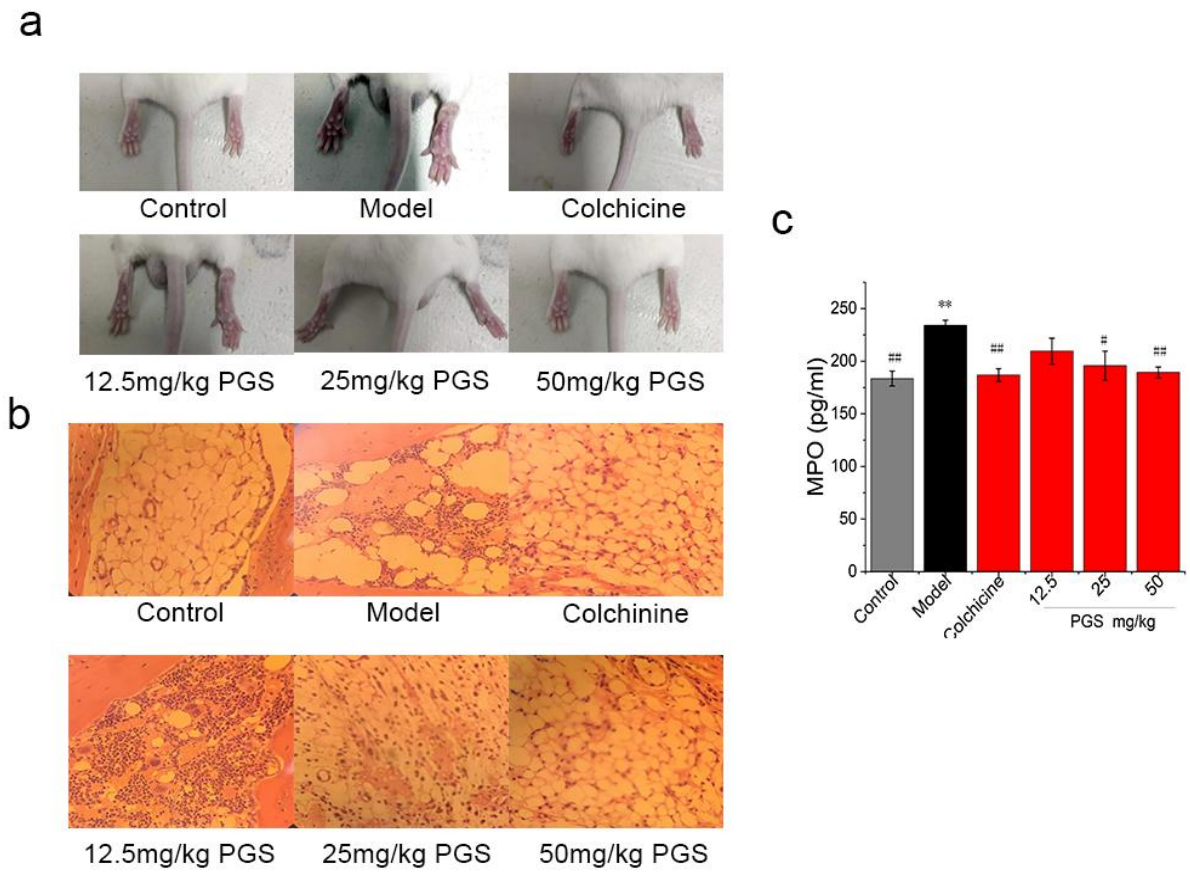


Fig.3 Determination of relative protein levels of caspase-1, IL-1β and ASC in the ankle joint by western blot analysis, Data are mean ± standard deviation, and statistical analysis of data was performed using one-way analysis of variance. compared with normal group* $P< 0.05$, ** $P < 0.001$, compared with model group$^{\#}$ $P < 0.05$,$^{\#\#}$ $P< 0.001$.

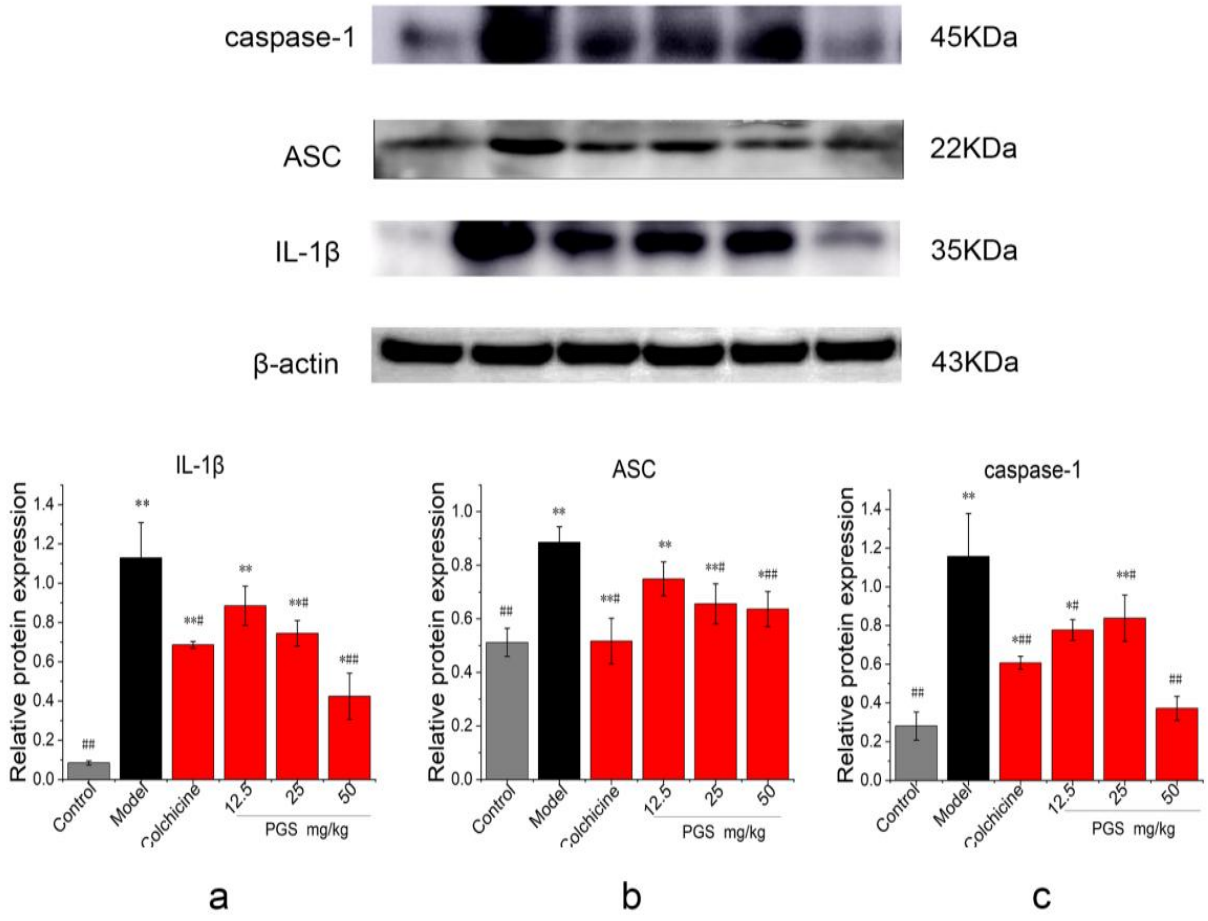
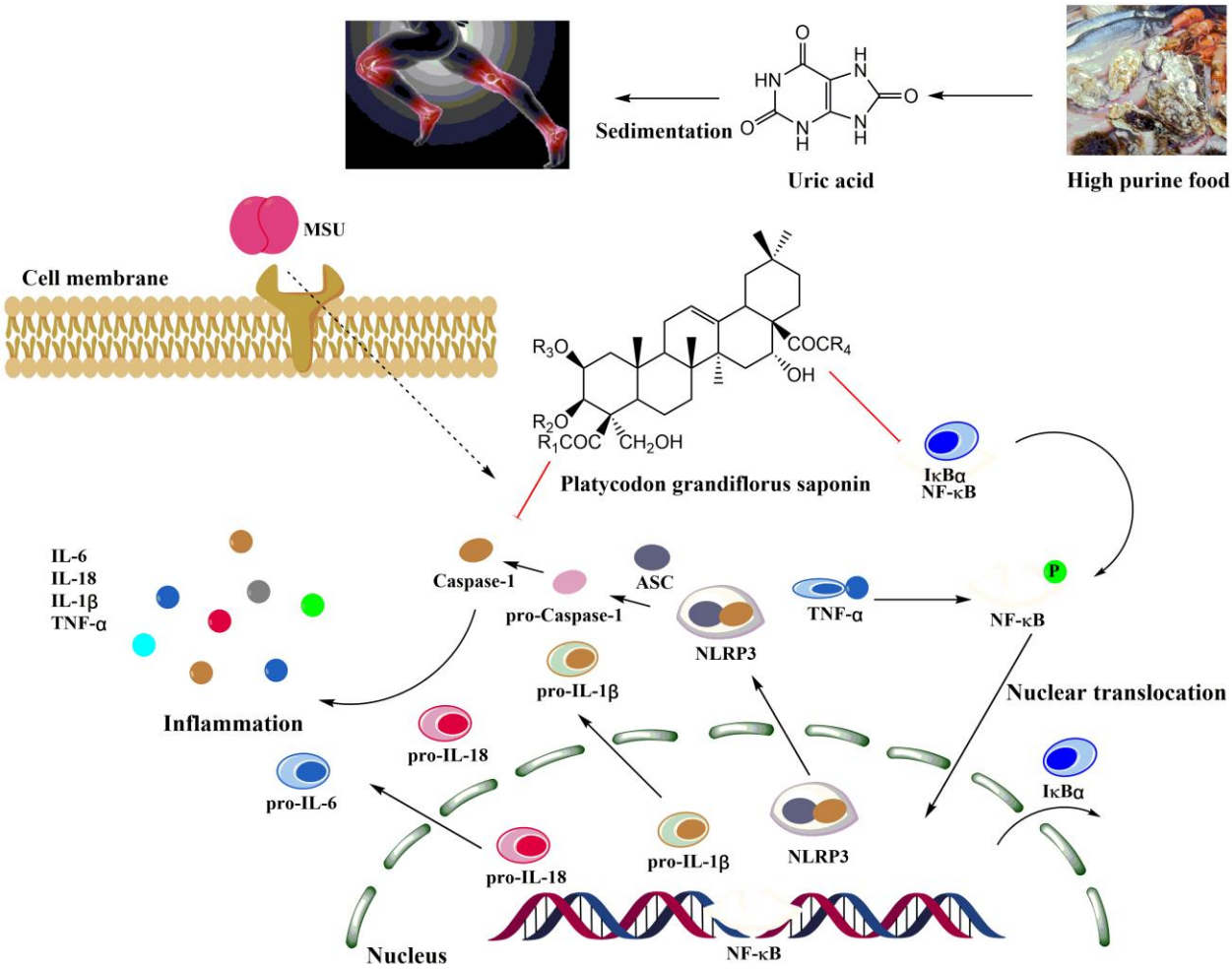


Fig.4 Schematic diagram of the treatment of gouty arthritis *with Platycodon grandiflorus* saponin. PGS reduces the symptoms of gouty arthritis by inhibiting the secretion of IL-6, IL-1β, TNF-α and inhibiting NLRP3 signaling



An inflammatory reaction occurs when the NLRP3 inflammasome induces caspase-1 to release inflammatory factors (Cho *et al.*, 2020). Antioxidant enzymes are produced in the body to reduce oxidative stress response.

Oxidative stress destabilizes the inflammasome to accelerate the inflammatory response due to the interaction between oxidative stress and the inflammatory response. Therefore, a significant increase in oxidative stress factors inhibits the inflammatory response (Matosinhos *et al.*, 2022). This study showed that PGS can increase SOD and GSH-PX activity to reduce oxidative stress, thereby decreasing NLRP3 inflammasome-mediated inflammatory responses (Yuko *et al.*, 2018). To further illustrate the effect of *Platycodon grandiflorus* saponins on the mechanism of gouty arthritis, we detected protein expression by western blotting and found that the expression of related proteins in the joints of mice in the *Platycodon grandiflorus* saponin group was significantly lower than that in the model group, indicating that *Platycodon grandiflorus* saponins could inhibit the expression of NLRP3/ASC/caspase-1 inflammatory pain road-related proteins and further inhibit the release of inflammatory factors, thus achieving the effect of reducing the inflammatory response.

The current method of establishing a gouty arthritis

model is mainly through MSU injection. MSU injected into the ankle and absorbed by the body activates a series of reactions caused by NLRP3 protein, which eventually releases inflammatory factors. The NLRP3/ASC/caspase-1 inflammatory pathway was revealed by studying inflammatory factors and related proteins. ASC is an important linker protein between NLRP3 protein and pro-caspase-1 protease in acute gouty arthritis. When NLRP3 protein binds to caspase-1 through ASC, it becomes an NLRP3 inflammatory vesicle, which can promote the conversion of pro-caspase-1 to active caspase-1, which can release mature IL-1β and trigger a series of inflammatory responses (Figure 6). NLRP3 inflammatory vesicles have been linked to many diseases, and it has been shown that inhibition of NLRP3 inflammatory vesicles improves renal injury in diabetic nephropathy and that activation of inflammatory vesicles is associated with cardiac injury (Wei *et al.*, 2022). Thus, inflammatory vesicles play an important role in the inflammatory response and activation of NLRP3 inflammatory vesicles, resulting in vasodilation and a marked degree of edema, along with upregulation of leukocyte recruitment chemokines and proinflammatory cytokine expression.

Therefore, inhibiting the binding of NLRP3 inflammatory vesicles to this inflammatory pathway can reduce the occurrence and exacerbation of the inflammatory response (Shi *et al.*, 2016). Based on our experimental results, it is known that PGS can reduce the expression of ASC protein and caspase-1 protein, blocking the binding of inflammatory vesicles, which is consistent with previous findings.

According to the experimental results, PGS could reduce the inflammatory factors IL-6, IL-1β, and TNF-α in serum. In contrast, PGS improved the oxidative stress response and reduced the inflammatory reaction caused by MSU. Furthermore, PGS plays a suppressive role in the expression of related proteins, which relieves swelling and pain caused by gouty arthritis. In conclusion, we have experimentally validated that PGS can treat gouty arthritis by inhibiting the NLRP3/ASC/caspase-1 inflammatory pathway. In this study, we performed a mechanistic analysis of PGS in gouty arthritis, which is helpful for the treatment of gout. Drug research on gouty arthritis has provided a new substance that may become a novel method for treating gouty arthritis; however, clinical trials have not been conducted and its use should be further validated to ensure the safety and efficacy of PGS in the treatment of gouty arthritis.

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