

Investigation of Chemical Compounds and Effect of *Astragalus Galactites* (Pall.) on MSU Crystal-Induced Acute Gouty Arthritis in Rats

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<https://dx.doi.org/10.13005/bpj/2880>

(Received: 28 September 2023; accepted: 01 December 2023)

The objective of the present investigation is to identify the biologically active components of *Astragalus galactites* (AG) and examine their effect on rats of acute gouty arthritis induced by crystals of the compound monosodium urate (MSU). Analyses of AG chemical constituents and their antioxidant activity were performed using both UV spectrophotometric and HPLC methods. Twenty-five adult Wistar rat males in total were assigned at random to a choice of five groups: AG 160 mg/kg, AG 330 mg/kg, MSU group, control group, and normal group. We reported the contents of Formononetin 1.97 µg/mL, Astragaloside IV 0.56 mg/mL, and total flavonoid 0.22 ± 0.07% in the aerial part of our sample AG. In the DPPH scavenging assay, methanol and ethanol extracts established antioxidant properties with an IC₅₀ concentration of 91.04 µg/mL and 93.13 µg/mL, respectively. In the ABTS scavenging assay, IC₅₀ concentrations of 387.2 µg/mL (methanol extract) and 436.2 µg/mL (ethanol extract) were also shown. This investigation also looked at the histopathological characteristics related to MSU-induced gouty arthritis in order to assess the preventive effects of the AG plant on inflammatory mediator levels. In the AG 330 mg/kg group, the level of PGE₂ significantly reduced ($p < 0.001$). Our results showed that the AG 330 mg/kg group was relatively effective in the treatment of gouty arthritis compared to other groups, which appears to be mediated by inhibiting the release of cytokines that pro-inflammation. The main compounds of the AG medicine plant, flavonoids and saponins, are acutely anti-gout due to the resulting decrease in PGE₂ levels. We have hypothesized that it is able to treat acute gouty arthritis by reducing levels of UA and PGE₂, providing an anti-inflammatory effect. This study contributes to the body of evidence that AG can be utilized in preventing and treating hyperuricemia.

Keywords: *Astragalus galactites* Pall; Antioxidant Activity; Gouty arthritis; MSU.

Astragalus is a large genus that is widely distributed in temperate regions throughout the world. *Astragalus galactites* (Pall.) (AG) is one of the genus *Astragalus* and belongs to the legume family Fabaceae¹. The native range of this species is the northern part of China, Mongolia, and Siberia².

A chronic disease called hyperuricemia is caused by a dysregulation of purine metabolism. In recent years, there has been a significant worldwide rise in the incidence of hyperuricemia, which seems to be related to changes in lifestyle and population aging³. The total prevalence of adults with hyperuricemia

was up to 14.6% in America⁴. There was a 16.6% prevalence of hyperuricemia in Australia⁵. In addition to these, a recent report stated that 17.4% of Chinese individuals had hyperuricemia⁶. Epidemiological studies of gout have not been conducted in Mongolia, but according to health statistics, the number of cases has increased every year: 357 cases in 2014, 456 cases in 2015, 619 cases in 2016, 1071 cases in 2017, and 1343 cases in 2018⁷. In order to address this problem, we must scientifically identify the medicinal plants of our country and find efficient and productive ways to control this illness through researching opportunities to obtain new medicines. The aerial parts of AG are used in traditional Mongolian medicine to treat illnesses related to inflammation or heat. Common biological diseases associated with this 'heat', such as myocardial ischemia, are traditionally addressed with treatment through heat reduction and improving cardiac blood supply. Aerial parts of AG are considered to be a rich source of a high concentration of bioactive compounds like flavonoids, alkaloids, polysaccharides, and saponins¹. Alkaloids, terpenoids, saponins, and flavonoids—particularly flavones and flavanols—have all been shown to have uric acid-lowering properties. By prohibiting xanthine oxidoreductase and controlling uric acid transporters, these compounds may lower blood uric acid levels⁸. One of the main active ingredients extracted from *Astragalus membranaceus* (Fisch) Bge, the saponin astragaloside IV is commonly used in traditional Chinese medicine for the treatment of immune system conditions, such as rheumatoid arthritis⁹. The total flavonoids of *Astragalus* (TFA) that have been extracted from *Astragalus membranaceus* Bunge exhibit strong anti-arthritic properties of arthritis induced by FCA in rats¹⁰. The pre-clinical studies for assessing the pharmacology, phytochemical, toxic, and biological properties of any herbal drug are essential before its clinical administration. The chemical analysis and pharmacological study of bioactive plants are of great importance in traditional medicine. Specifically, the flavonoid, and saponin examination, quantitative, found in the following scientific methodology and its comparison with standard compounds is very important for establishing AG's efficacy. A detailed analysis of the phytochemistry and pharmacology of AG, which is widely used in traditional

Mongolian medicine, has not been reported. Therefore, there is a need to determine to reveal the biologically active compounds of ingredients of AG. This study will determine these constituents' chemical composition and evaluate their effect on gouty arthritis, and the anti-inflammatory effect of AG in traditional medicine establishing the scientific basis.

MATERIALS AND METHODS

Plant Materials

The plant sample was collected as a raw material in the Tahiltayn mountain area (47° 91' 44" N, 106° 70' 63" E), Songinokhairhan district, Mongolia, (May 2021). Professor E. Ganbold, Sc.D., of the National University of Ulaanbaatar, Mongolia, confirmed the taxonomic of the plant species' origin. The herbs were dried in the air and ground to a fine powder suitable for extraction.

Experimental Animals

A total of twenty-five adult Wistar rat males weighing between 220–250 grams were acquired from the Institute of Traditional Medicine and Technology of Mongolia's Experimental Animal Center. The temperature and humidity levels were kept under-regulated to 20±1c° and about 50–60%, with a 12-hour light/dark cycle and automated air conditioning system 8–15 times per hour. Rats were fed standard nutrients and allowed to drink ad libitum.

Reagents

Formononetin (e^{99.0%}), Astragaloside IV (e^{98.0%}), Monosodium urate (MSU) crystals (CAS 1198-77-2) were supplied by Sigma-Aldrich (USA). The cytokine immunoassay kits, Haematoxylin & Eosin (HE), and Masson's trichrome were purchased from MLBIO Biotechnology Co. Ltd. (Shanghai, China) and Sigma-Aldrich (Germany), respectively. Analytical grade included all other reagents.

Chemical analysis

Assessment of Total Flavonoid Content

Weigh 1.0 g of the dried and powdered sample accurately into a flask, add 50 mL of 70% ethanol, heat under reflux for half an hour, and then filtrate. The test solution was the supernatant. 1mL of 5% NaNO₂, 1mL of 10% Al (NO₃)₃, and 10 mL of 4% NaOH solution were added to the test solution. Measure the absorbance of the test

solution at 500 nm as directed under Ultraviolet-visible Spectrophotometry (UV-2102C, UNICO, China). Rutin equivalent was used to express the amount of total flavonoids present in the plant¹¹.

High-performance liquid chromatography (HPLC) method

The samples were homogenized with 80% methanol at 25-30°C, sonicated for half an hour, and centrifuged at 3500 rpm followed by filtration. The Welchrom C18 column (250 mm, 4.6 mm, 5 μm) was used for the reversed-phase HPLC. Acetonitrile (B) and 0.1% phosphoric acid (A) in distilled water were used for gradient elution (60:40%v/v, 95:5%v/v). In addition, there was a 0.5 mL/min flow rate. The wavelengths used for detection were 203 nm and 254 nm.

Antioxidant Activity

DPPH assay for free radical scavenging

The method with minor modifications^{12,13} was used to determine the radical scavenging activity of AG. In methanol (ethanol), a series of extracts were prepared at four distinct concentrations (0.025-0.2 mg/mL). After mixing the 1.5 mL extract and the 1.5 mL 3×10^{-4} M DPPH solution in methanol, they were left to sit in the dark at room temperature for half an hour. After that, a spectrophotometer was used to measure each plant extract containing DPPH's absorbance at 517 nm. As a blank and control, the plant extract solution (1.5 mL) plus methanol (1.5 mL) and the DPPH solution (1.5 mL) plus methanol (1.5 mL) were utilized, respectively. Every measurement was carried out three times. The sample's antioxidant capacity was shown as IC50.

ABTS free radical scavenging assay

In methanol and ethanol solution, a series of extracts were prepared at four various concentrations (0.025-0.2 mg/mL), respectively. By reacting 2.45 mmol/L potassium persulfate with 7 mmol/L ABTS solution, ABTS radical cations were created. For 16 hours, the mixture was kept at room temperature in the dark. After that 3.0 mL of ABTS solution was combined with 0.3 mL of each sample, at different concentrations, and thoroughly mixed. After 6 minutes, measure the absorbance at 734 nm¹⁴.

Effect of AG on MSU Crystals-induced Acute Gouty Arthritis in Rats

Ethics statement

The research was conducted in compliance

with the Health Ethics Guidelines that the Ministry of Health in Mongolia (2018) issued. The Institute of Traditional Medicine and Technology of Mongolia (ITMTM) and members of "The Research Ethics Committee" approved the study protocol (101/13-10-2022).

MSU crystals-induced acute gouty and treatment

In this study, twenty-five adult Wistar rat males weighing between 220-250 grams were used. MSU crystals-induced acute gouty arthritis animal model: MSU injections carried out intra-articularly to rats caused the gouty model¹⁵. Rats were given 1.25 mg MSU (in 100 μL phosphate-buffered saline) injected into the left tibiotarsal joint (ankle), and they were given 90 mg/kg of ketamine hydrochloride. The experiment's injection method was carried out in the same manner as the Jiexi (ST41) intra-articular acupuncture technique, and the injection point corresponds to the Jiexi (ST41) in traditional Chinese, Korean, and Mongolian medicine. Animals were divided into 5 groups: control (n=5), MSU (n=5), Allopurinol (n=5), AG330 mg/kg (n=5), and AG160 mg/kg (n=5), treated groups were orally administered once daily (decoction water extract) for 5 days before MSU crystal injection. The edema was photographed after 24 hours. Ankle tissue and blood samples were taken 24 hours later for further analysis (Figure 1).

Enzyme-linked immunosorbent assay (ELISA)

Afterwards collection the blood was allowed to be kept at room temperature for 15 minutes. Then, the samples were centrifuged for 10 minutes at 3000 rpm in order to distinguish the serum. Using a microplate reader (ChroMate-4300, Awareness Technology Co., USA), the levels of IL-1b, IL-6, IL-10, TNF-a, and PGE2 were estimated by ELISA kits following compliance with the manufacturer's instructions (Shanghai MLBIO Biotechnology Co. Ltd).

Histopathological examination

Specimens of ankle joints were decalcified in a 10 percent formic acid working solution and stabilized in a 10 percent neutral buffered formalin solution¹⁶⁻¹⁸.

After the completion of the decalcification process, the sample is washed under running water for a full day, dehydrated using progressively stronger alcohols, encased in paraffin wax, sectioned (3-5 μm thick), and stained with Masson's

trichrome and hematoxylin and eosin (HE). Histological images were observed with a light microscope (Nikon Eclipse Ci, Japan)^{19,20}.

The aerial parts of AG are considered to be a rich source of an abundance of bioactive compounds like polysaccharides, flavonoids, alkaloids, and saponins¹. We have used aerial parts of AG in all of these experiments.

Statistical analysis

Data was represented as the average \pm SD, and the significance levels were established through a one-way analysis of variance (ANOVA) and Tukey's post hoc test. P-values less than 0.05 were regarded as statistically significant.

RESULTS

Chemical study

Total flavonoid

The flavonoid contents of the extract in terms of rutin equivalent (the standard curve equation: $y=0.095x - 0.0008$ $R^2 = 0.9999$) were between 4.0 to 40.0. The flavonoid content was determined $0.22 \pm 0.17\%$ in the ethanol extract of the AG plant.

HPLC identification of Formononetin and Astragaloside IV

We have determined Formononetin, Astragaloside IV contained in the AG's methanol extract based on the studies that determined some isoflavone and triterpenoid saponin contained in other species of Astragalus by the method of HPLC. 20 μ L of each of them were injected into

the HPLC system according to the chromatographic conditions given in the section on the HPLC method and the chromatograms were recorded. The retention time was 8.56 minutes and 6.53 minutes for formononetin, and Astragaloside IV, respectively. The chromatogram of the AG indicated the presence of Formononetin, and Astragaloside IV with a retention time of 8.37 minutes and 6.37 minutes compared with their standard substances. The chromatograms of Astragaloside IV and Formononetin are displayed in Figures 2 and 3. The regression equation obtained from the calibration curves ($Y=8452.83x+177959.6$ ($R^2=0.9964$)) and ($Y=1809520+971.99$ ($R^2=0.9849$)) can be used to determine the formononetin and Astragaloside IV contents in the AG (Figures 4 and 5). From the results of these calculations, it can be seen that the formononetin and Astragaloside IV content in the AG is 0.002 ± 0.004 mg/mL, 0.56 ± 0.005 mg/mL.

Method validation

The HPLC method for the Estimation of Formononetin and Astragaloside IV were developed and validated according to ICH Q2 (R1) guideline (Table 1). In the HPLC method, the results of the validation parameters provided above are suitable²¹.

Antioxidant activity

The DPPH and ABTS assay were used to evaluate antioxidant activity, and the results were expressed as a percentage of inhibition. Table 2 shows the results of the analysis. The results were contrasted with gallic acid as the standard. For each extract, the concentration that resulted in 50%

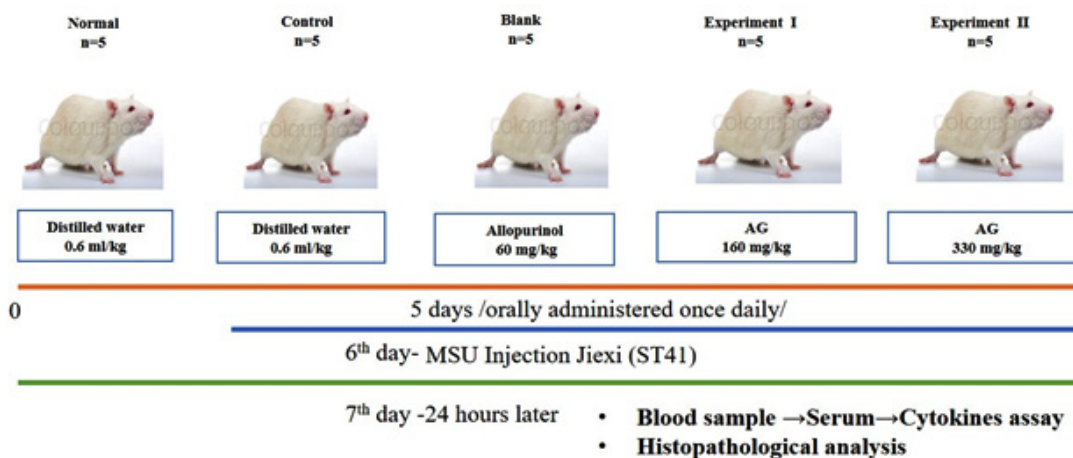


Fig. 1. Animal experimental design

inhibition (IC₅₀) was found. AG methanol extract had the DPPH and ABTS scavenging effects (IC₅₀ = 91.04 µg/mL, 387.2 µg/mL), AG ethanol extract had the DPPH and ABTS scavenging effects (IC₅₀ = 93.14 µg/mL, 436.3 µg/mL), respectively.

Pharmacology study

Tumor necrosis factor (TNF)- α , interleukin (IL)-1 α , IL-6, and PGE₂ are among the inflammatory mediators created by blood cell infiltration after MSU injection. In the control group, the level of TNF α increased in a gouty model. Allopurinol (Allo) is one of the clinical pharmaceuticals used for the treatment of gouty arthritis. Significant differences ($p=0.032$) were observed between the AG160 mg/kg and Allo groups or treatment groups. In the current investigation, all treatment groups had a decrease in IL-10 level, while the control group had increased levels ($p<0.032$). The PGE₂ level was significantly reduced in the AG330 mg/kg group ($p<0.001$) (Figure 6). Through intra-articular injection into the tibiotarsal joint, a model for gout was successfully developed, and the

study's evaluation timeline was established from our preliminary investigation into alterations in the level of uric acid.

Consequently, significant differences ($p<0.032$) were observed between AG330 mg/kg and AG160 mg/kg and the treatment groups or Allo. As a result of this study, the uric acid levels were increased in control groups and decreased in treatment groups ($p<0.001$). Therefore, Figure 6A shows that the Uric acid level significantly reduced ($p<0.001$) in the treatment groups. We examined the edema of the ankle-hind paws of the rats in order to figure out the effects of AG in an animal model of gouty. The images of the hind paws of the rat ankle were taken 24 hours after the MSU injection. The result suggests that AG mitigates MSU crystal-induced paw edema (Figure 7). It can be seen from the edema images of the rear ankle of the rats that the redness and edema in the treatment group indicated the anti-gouty and anti-inflammatory properties of AG. In contrast to the control group, the allopurinol

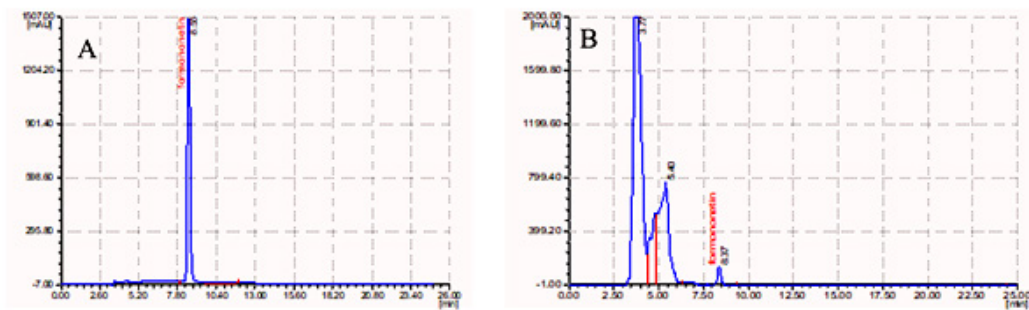


Fig. 2. HPLC Chromatograms of Formononetin in the AG

A. Chromatogram of Formononetin standard, B. Chromatogram of the methanol extract of AG

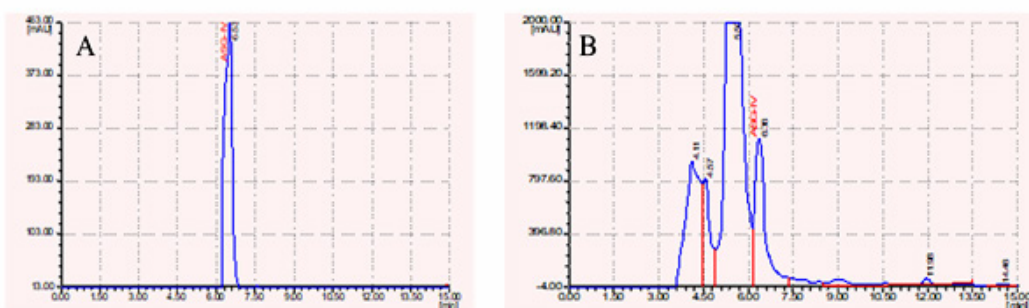


Fig. 3. HPLC Chromatograms of Astragaloside IV in the AG

A. Chromatogram of Astragaloside IV standard, B. Chromatogram of the methanol extract of AG

groups showed obvious edema, redness, and an inflammatory process in the ankle joint. In contrast to the allopurinol groups, edema, and redness of the ankle were slightly reduced in the AG160 mg/kg groups, while the AG330 mg/kg groups had a significant anti-inflammatory effect. The results were confirmed by histopathology analysis.

Edema of the experimental animal's paws was used to measure by using a plethysmometer. However, there were no discernible statistically major variances between the groups.

Histopathological results of HE staining

Histopathological observation of ankle joint tissues structures in all experimental groups

is illustrated in Figure 8. Ankle joint tissues of the control group showed dense inflammatory cell infiltration and new vascularization. Dominant infiltrated cells were macrophage, plasma and multinucleated giant cells. There was edema and an MSU crystal-like structure in the synovial membrane. The synovial lining cell proliferation was observed. The normal group showed that normal histological structure of ankle joint tissue. Allopurinol treated group showed that there was little inflammatory cell infiltration and new vascularization. Dominant infiltrated cells were macrophages and lymphocytes. There was edema and MSU crystal-like structure in the synovial

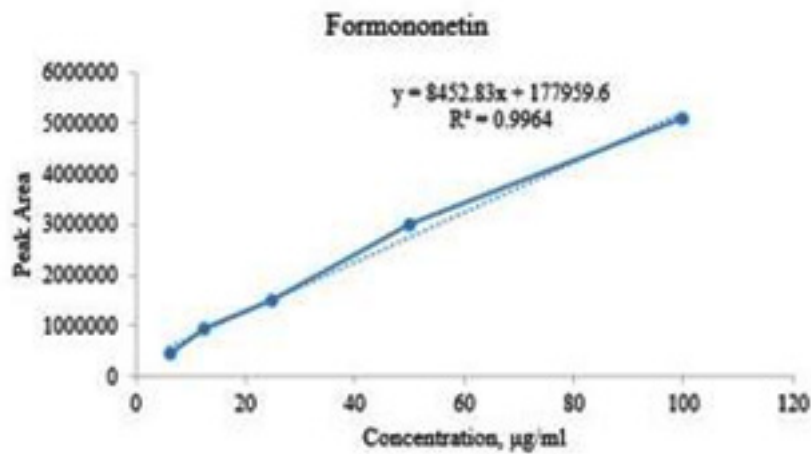


Fig. 4. Calibration curve of Formononetin

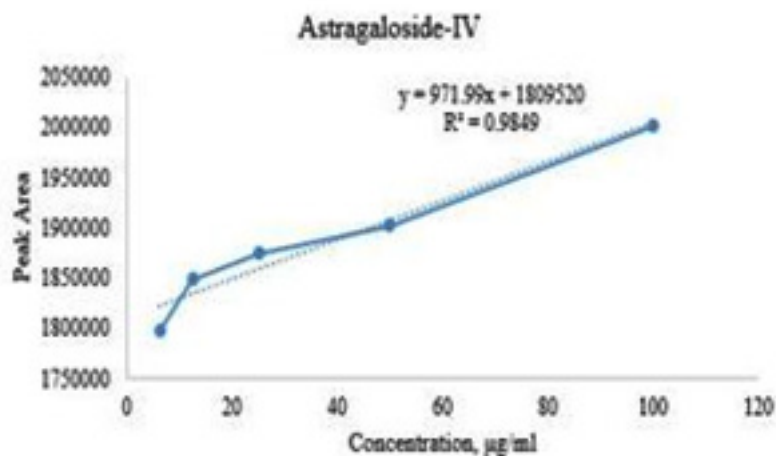


Fig. 5. Calibration curve of Astragaloside IV

membrane. The synovial lining cell proliferation was observed. AG160 mg/kg treated group showed that less of inflammatory cell infiltration and new vascularization. Mainly infiltrated cells were macrophages, plasma and lymphocytes. There was edema and MSU crystal-like structure in the synovial membrane. The synovial lining cell proliferation was observed. AG330 mg/kg treated group showed that few of inflammatory cell infiltration and new vascularization. Mainly infiltrated were the plasma cells, macrophage and lymphocytes. The synovial lining cell proliferation and numerous capillaries were observed in the joint soft tissue. Histopathological results of HE staining of experimental MSU-induced gouty arthritis are displayed in Figure 8.

Histopathological results of Masson's trichrome staining

Histopathological observation of the control group can be seen as a blue color which has infiltrated deep layers of soft tissue and the density of the tissue structure was low. Histopathological examination showed that blue color infiltrated

which was significantly weak by allopurinol, AG160 mg/kg, and AG330 mg/kg groups. Inflammatory infiltration was seen in the outer and mid-layer of soft tissue in allopurinol and AG160 mg/kg treated groups. AG330 mg/kg treated group was inflammatory infiltration observed outer layer of the tissue. Histopathological results of Masson's trichrome staining of experimental MSU-induced gouty arthritis are displayed in Figure 9.

As can be seen from the evaluation table above, the group treated with AG330 mg/kg reduced inflammatory cell infiltration compared to the MSU group, and the MSU crystal-like structure was brought to the same level as the control group. The results of the AG160 mg/kg group were similar to those of the MSU+Allo group.

DISCUSSION

Flavonoids are one of the main compounds in AG, the content was determined at $0.22 \pm 0.17\%$. These are a class of polyphenolic compounds with anti-inflammatory, and antioxidant properties.

Table 1. Validation parameters of the proposed HPLC method

Parameters	Formononetin	Astragaloside IV
Wavelength of detection	254 nm	203 nm
Retention time	8.37 min	6.37 min
Beer's law limit ($\mu\text{g/mL}$)	6.25-100	6.25-100
Regression formula ($y=ax+b$)	$Y=8452.83x+177959.6$	$Y=971.99x+1809520$
Slope	8452.83	971.99
Intercept	177959.6	1809520
Coefficient of correlation	0.9964	0.9849
Limit of detection	2.34 $\mu\text{g/mL}$	0.97 $\mu\text{g/mL}$
Limit of quantification	7.09 $\mu\text{g/mL}$	0.32 $\mu\text{g/mL}$
Accuracy (% RSD)	100.3-102.7, % RSD 1.29%	98.6-101.7, % RSD 1.54%
Precision (% RSD)	Inter-day=0.40 Intra-day=0.78	Inter-day=0.052 Intra-day=0.083
Mean, mg/mL	0.002 ± 0.004	0.56 ± 0.005

% RSD-Relative Standard Deviation

Table 2. The results of Antioxidant activity

Sample	DPPH ($\text{IC}_{50}=\mu\text{g/ml}$)	ABTS ($\text{IC}_{50}=\mu\text{g/ml}$)
Methanol extract	91.04 ± 0.23	387.2 ± 0.96
Ethanol extract	93.14 ± 0.2	436.3 ± 0.1
Gallic acid standard	34.8 ± 0.12	67.1 ± 0.2
% RSD	0.95	1.53

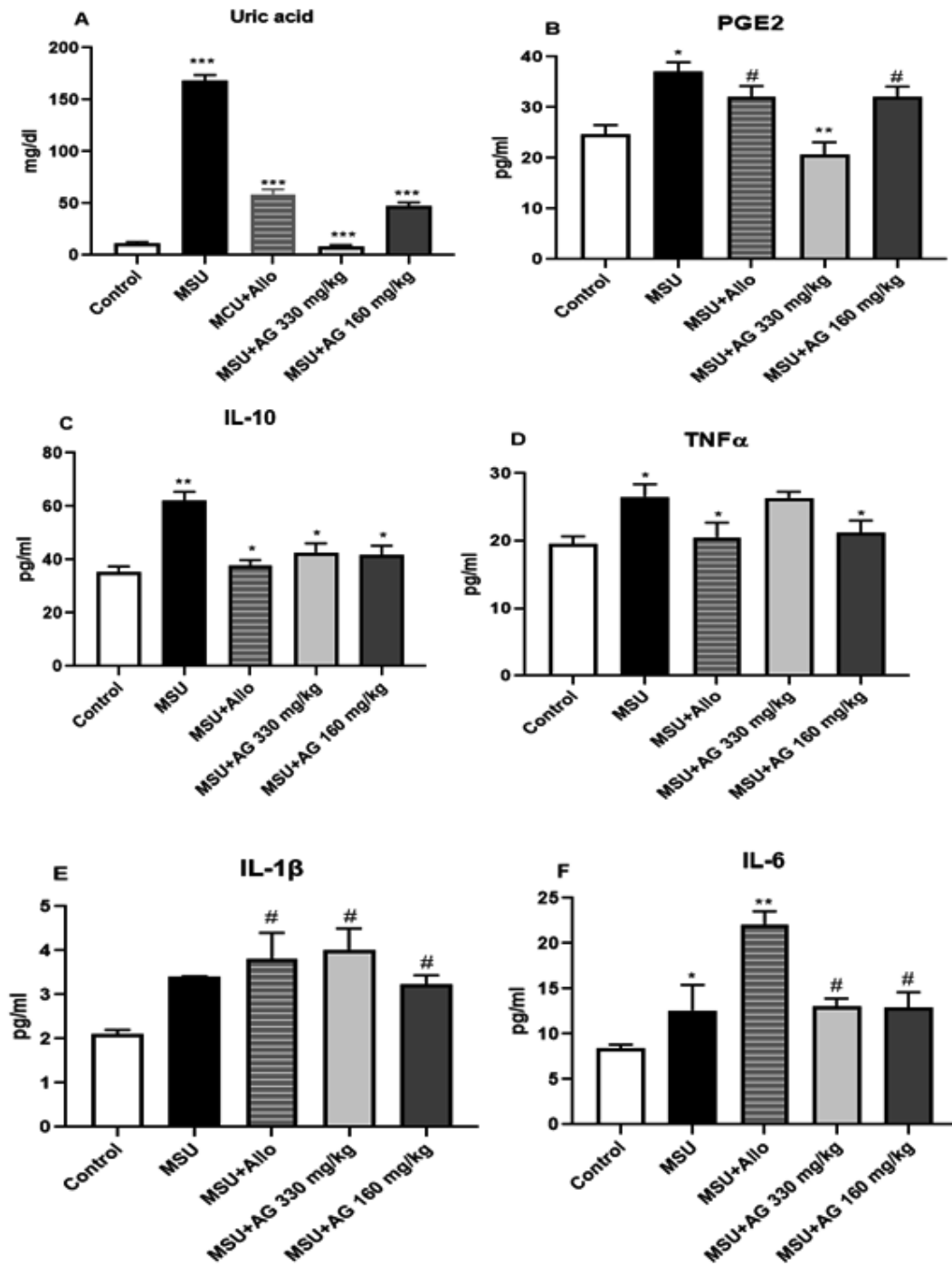


Fig. 6. The results of pro-inflammatory cytokines in rats (at 24 h)
 Each group: n=5, means \pm SD, * $p < 0.05$ and ** $p < 0.00$, # $p > 0.05$ (one-way ANOVA)



Fig. 7. Representative photographs of the hind paw. The affected side (arrows).
 1-Control group, 2-MSU group, 3-MSU+Allo group, 4-MSU+AG160 mg/kg group, 5-MSU+AG330 mg/kg group

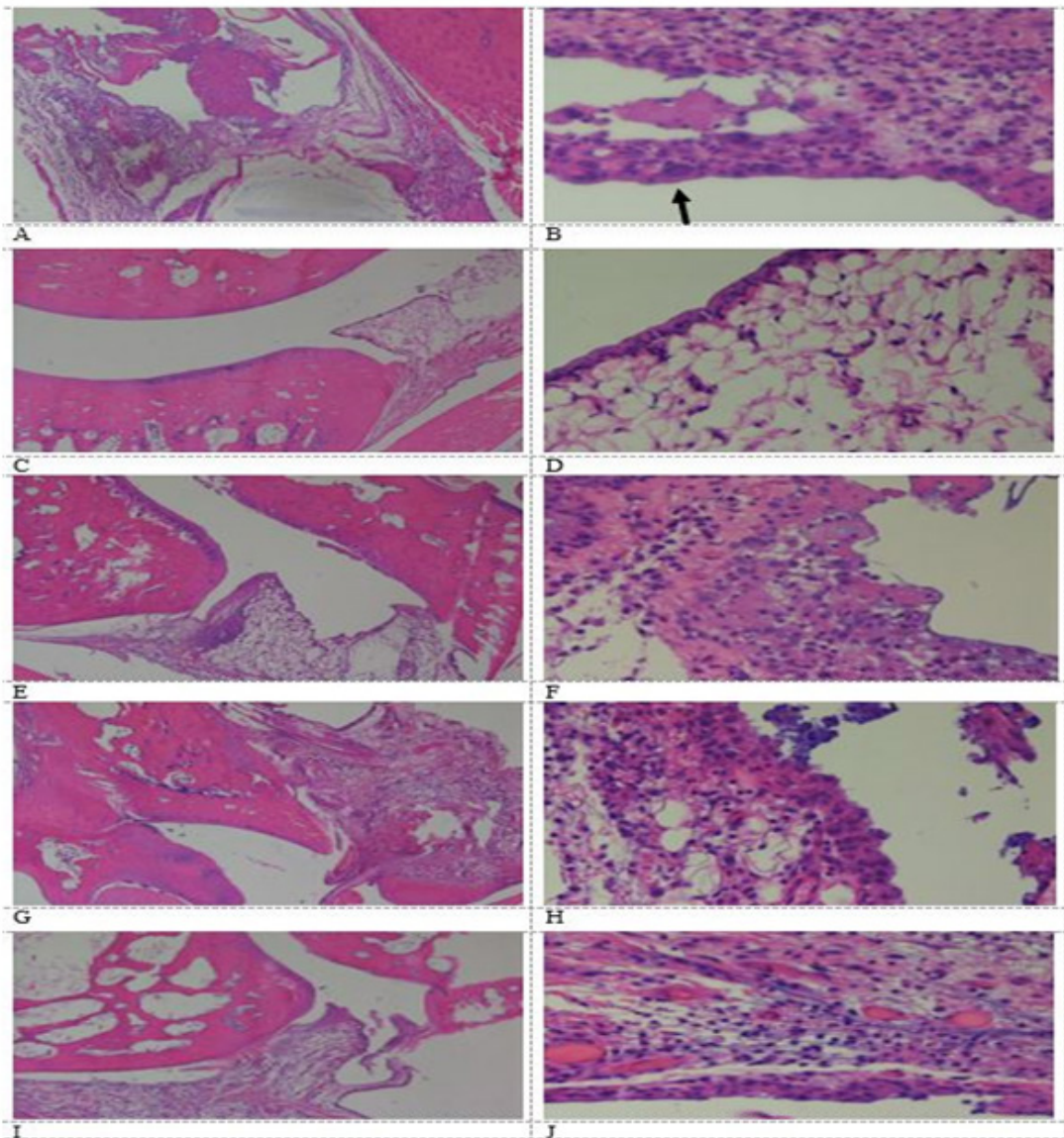


Fig. 8. Photomicrograph of anti-inflammatory effect of AG160 mg/kg, AG330 mg/kg on the synovial membrane. A, B- Control groups showed dense inflammatory cell infiltration (multinucleated giant cell-arrow). C, D- MSU groups. E, F- Allopurinol treated groups. G, H- AG160 mg/kg treated groups. I, J- AG330 mg/kg treated groups. Magnification: 40 \times , 400 \times .

For instance, Xin-Yu Liu *et al* discovered that when *Astragalus membranaceus* Bunge was isolated, the entire flavonoid had pronounced anti-arthritic effects against rats that had been given FCA-induced arthritis. Signs of FCA-induced rats include increased body weight, reduced paw swelling, inflammatory cell infiltration and

synovial hyperplasia, a decreased immune tissues index, and inhibited production of inflammatory mediators¹⁰.

We determined that the main biologically active compounds are astragaloside IV and formononetin in *Astragalus galactites* (Pall.) through qualitative HPLC methods. Formononetin,

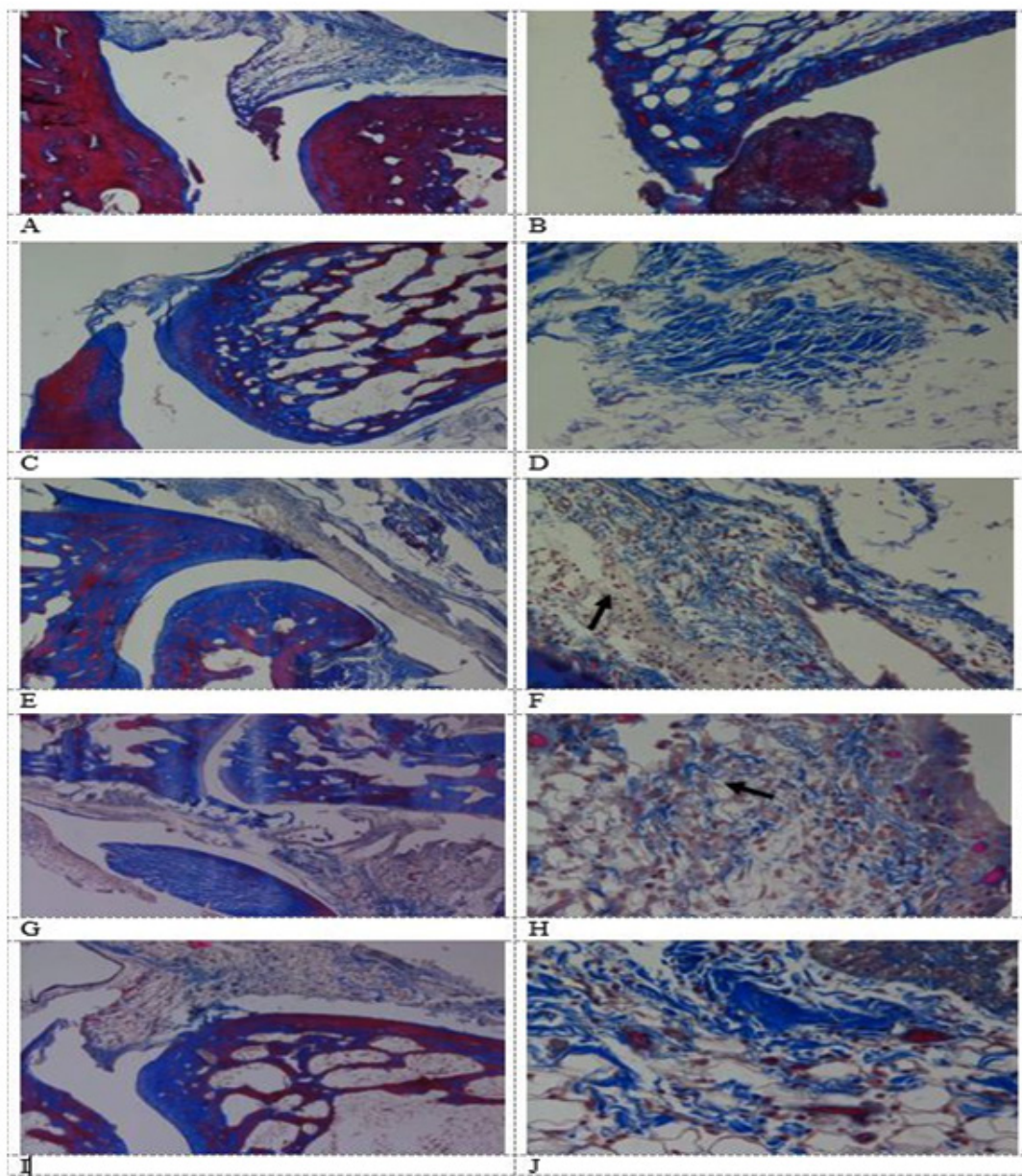


Fig. 9. Photomicrograph of anti-inflammatory effect of AG160 mg/kg, AG330 mg/kg on the synovial membrane. A, B- Control groups. C, D- MSU groups. E, F- Allopurinol treated groups. G, H- AG160 mg/kg treated groups. I, J- AG330 mg/kg treated groups. Masson, 40x, 400x

Table 3. Semi-quantitative analysis of AG

Groups	Inflammatory cell infiltration	Edema	MSU crystal like structure	Synovial lining cell proliferation	New vascularization
Control	-	-	-	-	-
MSU	+++	++	+	+	++
MSU+Allo	++	++	+	+	+++
MSU+AG160 mg/kg	++	++	+	++	+++
MSU+AG330 mg/kg	+	++	-	++	+++

(0-) none, (1+) mild, (2++) moderate, (3+++) severe

an active ingredient in *Astragalus membranaceus*, inhibits the expression of the inflammatory proteins COX-2 and cyclin D1, which can stop the pathogenesis and progression of esophageal cancer²². In addition, AS-IV was reported to study the molecular mechanisms, including the pathways and target genes, through which AS-IV inhibits the growth of tumors²³. According to a study by Xiaodong Ding et al, the cp genome has 110 complete genes, including 75 protein-coding genes (75 PCGs), 4 ribosomal RNA genes (4 rRNAs), and 30 tRNA genes (30 tRNAs), total plastome length of AG is 126,117 bp. and phylogenetic tree demonstrates that AG and *A. laxmannii* are the most closely related species².

As a result of our research, the amount of Astragaloside IV and formononetin constituents was determined 0.56 mg/mL and 0.002 mg/mL by using HPLC in the AG, respectively. The developed methods show high reliability and validity, as evidenced by the % RSD values in all validation experiments being less than 2%.

We found that the DPPH and ABTS radical scavenging activity in AG may be the cause of the high concentration of phenolic compounds established from our preliminary investigation. The results of the research showed that AG's methanolic extract has more antioxidant activity than the ethanolic extract. The compounds ononin and formononetin have been discovered to have extensively reported anti-inflammatory, anticancer, and antioxidant capacities according to a study by Ong SKL et al and Machado Dutra J et al^{24,25}. The compounds astragaloside IV and astragaloside activity for liver protection, as reported by Zhang J et al., have been identified as their anti-inflammatory, anti-apoptotic, and antioxidant

capacity and also their significance in improving immunity²⁶. Therefore, the AG medicinal plant we studied has antioxidant and anti-inflammatory properties, we are focused on researching and developing of new herbal medicines for gouty arthritis in the future.

The results of this study indicated that AG prevented gouty arthritis caused by MSU, a protective effect that seemed to be mediated by preventing the release of pro-inflammatory cytokines. Because little is known about the mechanisms underlying crystal formation and growth, gout therapy centered on regulating the size and shape of MSU crystals has not yet been developed²⁷. However, it may have a pharmacological effect of monosodium urate (MSU) in regulating the NLRP3 pathways in the pathogenesis and treatment of MSU-induced gouty arthritis in mice and rats²⁸. Tumor necrosis factor (TNF)- α , interleukin (IL)-1 α , IL-6, and PGE2 are among the inflammatory mediators created by blood cell infiltration after MSU injection²⁹. By giving AG160 mg/kg and AG330 mg/kg doses to rats that had gouty arthritis created experimentally, we were able to assess their effectiveness against the disease. Comparing the AG330 mg/kg group to other groups, our research suggested that it is comparatively useful for treating gouty arthritis. A report by Feng Lin et al discovered that fermented astragalus significantly lowered plasma concentrations of creatinine and urea nitrogen, preventing kidney disease in hyperuricemic mice. They also found the levels of key inflammatory cytokines that further confirm that fermented astragalus reduced inflammation by inhibiting the process. The higher of astragaloside IV, formononetin, total flavonoids, and other active

components may be related to the mentioned previously outcomes through fermentation³⁰. Astragaloside IV was identified to reduce the activation of macrophages while on RA, thereby preventing inflammatory-mediated cartilage and bone erosion by Wang B *et al*⁹. According to research by Lima Cavendish R *et al* and Yang Q *et al*, astragalosides and formononetin have been shown to be antinociceptive substances for inflammatory diseases^{31,32}. Alkaloids, terpenoids, saponins, and flavonoids—particularly flavones and flavanols—have all been shown to have uric acid-lowering properties³³.

We found that noted total flavonoid, formononetin, and astragaloside IV have been proven to have anti-gouty effects in inflammatory pain in AG compared with studies of another astragalus. These biologically active compounds (flavonoids, saponins, and phenolic acids) are explained by the identified UPC2-QTOF-MS analysis in the chloroform fraction and phenolic acids are explained by the identified HPLC analysis of AG that we have previously investigated.

On the other hand, the previously mentioned researcher's investigations confirmed the anti-gout and anti-inflammatory properties of chemical compounds like formononetin, astragaloside IV, and total flavonoids, which is consistent with what we have discovered. The advantage of the study of Mongolian plants is that the chemical compounds of subject plants are determined by using HPLC and evaluated the effect against MSU-induced gouty arthritis for the first time.

CONCLUSION

In conclusion, the main compounds of the AG medicine plant are flavonoids and saponin it is an acute anti-gouty due to the result revealed that AG significantly decreased PGE2 and UA decreased amount while experimenting. Therefore, this study contributes to preventing and treating hyperuricemia in AG. We first time generated a hyperuricemia rat model using an MSU-induced in Mongolia. In the future, we are researching and developing new herbal medicines for the treatment of gouty arthritis.

ACKNOWLEDGMENT

The authors would like to thank the team from the Research Center at the Institute of Traditional Medicine and Technology for their help during this study.

Conflict of Interest

The authors have declared no conflicts of interest.

Funding Source:

There is no organization that funds/supports the study.

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