Studies on the Anti-Gouty Arthritis and Anti-hyperuricemia Properties of Astilbin in Animal Models

Abstract: The purpose of this study was to investigate potential anti-gouty effect of astilbin (AS) and its possible mechanisms. In mice with hyperuricemia induced by potassium oxonate (OXO) and yeast extract powder (YEP), AS and febuxostat (FB) reduced the serum uric acid (UA) and xanthine oxidase (XO). Moreover, AS and FB reduced the levels of reactive oxygen species and increased the content of superoxide dismutase (SOD), glutathione peroxidase and catalase present in the serum. In acute gouty arthritis rats induced by intraarticular monosodium urate crystal injection, AS and Colchicine (COL) alleviated the ankle joints swelling, and reduced the inflammatory cell infiltration. AS also reduced the levels of interleukin 1β, interleukin 6, tumor necrosis factor alpha and monocyte chemoattractant protein 1 in liver. The present study first confirmed the anti-gouty effect of AS in mice with hyperuricemia and rats with acute gouty arthritis, which provides the experimental evidence for further evaluation of AS as a candidate for gout treatment.

Keywords: Astilbin, Hyperuricemia, Acute gouty arthritis, Interleukin 1β, Xanthine oxidase

1 Introduction

As a common arthritis, gout seriously affects the people’s normal life by causing pain, fatigue and high fever [1]. According to epidemiological studies, the morbidity of gout in Asia has increased, especially in China [2]. Monosodium urate (MSU) engulfing by macrophages promotes the release of interleukin 1β (IL-1β) responsible for the occurrence and amplification of an inflammatory response which is considered to be the pathogenesis of gouty arthritis [3, 4].

Hyperuricemia, defined as a level of serum uric acid (UA) higher than 6.8 mg/dL, is the basis of gouty arthritis [5, 6]. Excessive production of UA and obstruction of UA excretion can lead to hyperuricemia [7] which promotes the production of IL-1β. With the catalysis of xanthine oxidase (XO), purines were metabolized to UA which produces a lot of active oxygen molecules [8].

Based on the pathogenesis of gout there are two treatment strategies, 1) inhibiting inflammation, and 2) lowering serum UA level. The clinical agents for gouty arthritis treatment include colchicine (COL), corticosteroids and non-steroidal anti-inflammatory drugs [9, 10]. Allopurinol and febuxostat (FB) are the main clinical agents for treatment of hyperuricemia [11, 12], but show little effect on gouty arthritis [13]. However, the side-effects including liver damage, nephrotoxicity and bone marrow inhibition have been observed in clinics [11, 12, 14].

As a flavonoid compound, astilbin (AS) is widely distributed in Rhizoma Smilacis Glabrae [15] and its chemical structure is shown in Figure 1. AS was confirmed to show multifarious biological effects containing anti-inflammation [16], anti-oxidation [17] and immune regulation [18]. AS inhibited the expression of nitric oxide and inducible nitric oxide synthase by regulating phosphor C-Jun N-terminal kinase in lipopolysaccharide-treated RAW 264.7 cells [19] suggesting its anti-oxidative effects. AS improved liver injury by inhibiting the
production of TNF-α [20], and stimulated IL-10 to show the effect of alleviating contact hypersensitivity [21]. However, the anti-gouty arthritis and anti-hyperuricemia effects of AS have not been reported yet.

In this study, the potassium oxonate (OXO) and yeast extract powder (YEP) treated hyperuricemia mice, and MSU injected rats with gouty arthritis were carried out to inspect the anti-gouty effects of AS and the possible mechanisms.

2 Materials and methods

2.1 Animal care

The animal protocol was approved by the Animal Ethics Committee of Jilin University (Reference NO. 2018SY0602). Forty-eight male BALB/c mice (8-weeks old) and forty-eight male wistar rats (8-weeks old) were used for the study which was performed by Liaoning Changsheng Biotechnology Co., Ltd., Liaoning, China (SCXK (Liao) 2015-0001). The animals were housed in plastic cages and maintained under standard laboratory conditions of 22°C ± 2°C, relative humidity of 55% and 12-hour light/dark cycle (lights on 07:00-19:00) during the study. All mice and rats were fed with double distilled water and adequate food was available throughout the study.

2.2 The establishment of mice with hyperuricemia and agent treatment process

All mice were randomly divided into four groups. The control mice (CTRL) (n=12) and the model mice (n=12) (YEP and OXO only treated mice) were administrated orally with normal saline for 8 days; meanwhile, the febuxostat (FB) (Jiangsu Wanbang Biochemical Pharmaceutical Group Co., Ltd., Jiangsu, China) treated mice (n=12) and astilbin (AS) (Shanghai Yuanye Biotechnology Co. Ltd., Shanghai, China) treated mice (n=12) were administrated orally with 6 mg/kg of FB and 15 mg/kg of AS for 8 days [22]. One hour before the normal saline, AS and FB gavage, except for CTRL mice, other mice were intragastrically (i.g.) treated with 20 g/kg of YEP for 8 days, and the intraperitoneally (i.p.) injected with OXO (300 mg/kg) (Sigma-Aldrich, USA) during the last 3 days. Control mice received oral administration of normal saline for 8 days, intraperitoneal injection of normal saline in the last 3 days. The last day, 6 hours after treatment, blood samples were collected from each mouse. After euthanizing by injection with 200 mg/kg of 1.5% pentobarbital, liver tissues were collected.

2.3 The establishment of rats with acute gouty arthritis and agent treatment process

All rats were randomly divided into four groups. The control rats (CTRL) (n=12) and the model rats (n=12) (MSU only treated rats) were administrated orally with normal saline for 7 days; meanwhile, the colchicine (COL) (Yunnan Phytopharmaceutical Co. Ltd, Yunnan, China) treated rats (n=12) and AS treated rats (n=12) were administrated orally with 0.3 mg/kg of COL and 10 mg/kg of AS for 7 days respectively. On the sixth day, rats were intraarticularly injected (i.i.) with 100 μg of MSU on the right ankle joint except for CTRL rats, which were injected with the same volume of 0.9% saline (i.i.). The right ankle circumference was measured with vernier caliper at 0, 4, 12, 24 and 48 hours after treatment. The circumference was computed based on the mean of long and short diameters of ankle joint multiplied by 3.14. The swelling ratio (%) is calculated following the formula:

$$\text{Swelling ratio (\%)} = \frac{C_t - C_0}{C_0} \times 100$$

Where $C_t$ represents the circumference at different hours and $C_0$ represents the circumference at 0 hours. The percentages of swelling ratio of other groups were calculated compared to the CTRL rats, which was considered to be 100%.

Blood was sampled from each rat after the measurement of ankle circumference at 48 hours. After euthanizing by injection with 200 mg/kg of 1.5% pentobarbital, liver tissues and right ankle joint were collected.

Figure 1: Chemical structure of astilbin.
2.4 Biochemical assay

In hyperuricemia mice, the levels of serum UA (#MAK077; Sigma-Aldrich, USA), blood urine nitrogen (BUN) (#C013-2; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and hepatic XO (#MAK078; Sigma-Aldrich, USA) were determined by related commercialized kits base on manufacturer’s instructions. The serum levels of reactive oxygen species (ROS) (#43124), catalase (CAT) (#43356), superoxide dismutase (SOD) (#43125), glutathione peroxidase (GSH-Px) (#43390) and IL-1β (#42776) were detected by ELISA assay kit (Yuanye Bio-Technology Co. Ltd., Shanghai, China) based on instructions.

In the rats with acute gouty arthritis, the level of IL-1β (#43360), interleukin 6 (IL-6) (#41731), tumor necrosis factor alpha (TNF-α) (#41721) and monocyte chemoattractant protein 1 (MCP-1) (#41640) in serum and liver were detected with ELISA assay Kits (Yuanye Bio-Technology Co. Ltd, Shanghai, China) based on instructions.

2.5 Pathological examine of the ankle joint

The right ankle of each rat was collected. After decalcification and dehydration with 4% paraformaldehyde and 10% ethylenediaminetetraacetic acid, the samples were embedded in paraffin, sliced into 5 μm thickness sections and stained with hematoxylin and eosin (H&E). Histopathological slices of rat ankle joints were analyzed by microscope (200×), and histopathological slices of rat joint capsules analyzed by microscope (100×) under an inverted microscope CKX41 (Olympus, Japan).

2.6 Western Blot

Liver tissues collected from mice with hyperuricemia were extracted with RIPA buffer (Sigma-Aldrich, USA) containing a 1% protease inhibitor cocktail (Sigma-Aldrich, USA) and 2% phenylmethanesulfonyl fluoride (Sigma-Aldrich, USA). A BCA assay kit was used to detect the protein concentration of samples. 30 μg protein of each group sample was separated by the 10% SDS-PAGE and transferred onto PVDF membranes (0.45 μm, Merck Millipore, Germany). After incubated with 5% bovine serum albumin for 4 hours at 4°C, the membranes were incubated with XO (#bs-8552R, Bios, Beijing, China) and GAPDH (E-ab-20059, Elabscience, Wuhan, China) antibodies (Diluting rate of 1:1000) for 12 hours at 4°C. After washing with tris-buffered saline (contains 0.1% Tween-20), the membranes were incubated with HRP-conjugated secondary antibodies (diluting rate of 1:2000) for 2 hours at room temperature. The enhanced chemiluminescent detection kit (Merck Millipore, Germany) was used to develop the protein bands, and the imaging system (BioSpectrum600, USA) was used to visualize the bands. The ImageJ software (National Institute of Health, USA) was used as the pixel density quantified tool.

2.7 Statistical analysis

Mean ± standard deviation (S.D.) was used for the representation of all data, and One-way analysis of variance (ANOVA) was used to perform the statistical analysis followed by post-hoc Dunn’s multiple comparisons test with SPSS 16.0 (Version 16.0) (IBM corporation, Armonk, USA). P<0.05 was considered to be statistically significant.

3 Results

3.1 The therapeutic effects of AS and FB on mice with hyperuricemia

The accumulation of serum UA promotes the production of sodium urate in the joint leading to severe and painful arthritis [23]. YEP and XO administration caused a 33.44% enhancement on serum UA levels (P<0.05, Figure 2A), which were reduced 82.9% by FB (P<0.001, Figure 2A) and 70.38% by AS (P<0.001, Figure 2A). Compared with hyperuricemia mice, only AS, but not FB, strongly reduced the levels of IL-1β (P<0.05, Figure 2B) and BUN (P<0.001, Figure 2C).

XO, the critical enzyme, plays an important role responsible for the purine disintegration and development of UA [24]. In hyperuricemia mice, the hyper-levels of XO were reduced by both FB and AS after an 8-day treatment as shown by ELISA (P<0.01, Figure 2A) and western blot (P<0.01, Figure 2E).

Since the production of UA is accompanied by the over-accumulation of ROS, and anti-oxidation is considered as a useful therapeutic regimen for hyperuricemia [25, 26]. In mice with hyperuricemia, the high amounts of XO were reduced by both FB and AS after an 8-day treatment as shown by ELISA (P<0.01, Figure 2D) and western blot (P<0.01, Figure 2E).

3.2 The therapeutic effects of AS and FB on mice with hyperuricemia

The accumulation of serum UA promotes the production of sodium urate in the joint leading to severe and painful arthritis [23]. YEP and XO administration caused a 33.44% enhancement on serum UA levels (P<0.05, Figure 2A), which were reduced 82.9% by FB (P<0.001, Figure 2A) and 70.38% by AS (P<0.001, Figure 2A). Compared with hyperuricemia mice, only AS, but not FB, strongly reduced the levels of IL-1β (P<0.05, Figure 2B) and BUN (P<0.001, Figure 2C).

XO, the critical enzyme, plays an important role responsible for the purine disintegration and development of UA [24]. In hyperuricemia mice, the hyper-levels of XO were reduced by both FB and AS after an 8-day treatment as shown by ELISA (P<0.01, Figure 2A) and western blot (P<0.01, Figure 2E).

Since the production of UA is accompanied by the over-accumulation of ROS, and anti-oxidation is considered as a useful therapeutic regimen for hyperuricemia [25, 26]. In mice with hyperuricemia, the high amounts of XO were reduced by both FB and AS after an 8-day treatment as shown by ELISA (P<0.01, Figure 2A) and western blot (P<0.01, Figure 2E).

Since the production of UA is accompanied by the over-accumulation of ROS, and anti-oxidation is considered as a useful therapeutic regimen for hyperuricemia [25, 26]. In mice with hyperuricemia, the high amounts of XO were reduced by both FB and AS after an 8-day treatment as shown by ELISA (P<0.01, Figure 2A) and western blot (P<0.01, Figure 2E).
Figure 2: The therapeutic effects of AS on mice with hyperuricemia. In mice with hyperuricemia established via intragastrical administration with 20 g/kg of YEP for 8 days, and the intraperitoneal injection with 300 mg/kg of OXO, AS strongly reduced the serum levels of (A) UA, (B) IL-1β, (C) BUN and (D) XO level of hepatic. (E) AS reduced the XO expression in liver. The data of quantified protein expressions are normalized by GAPDH, analyzed using a one-way ANOVA and expressed as mean ± S.D. (n = 12). *P<0.05 and **P<0.01 vs. CTRL mice. *P<0.05, **P<0.01 and ***P<0.001 vs. vehicle treated mice with hyperuricemia. YEP, yeast extract powder; OXO, potassium oxonate; FB, febuxostat tablets; AS, astilbin; UA, uric acid; XO, xanthine oxidase; BUN, blood urine nitrogen.
3.2 The anti-inflammation effects of AS on rats with acute gouty arthritis

Compared with CTRL rats, MSU caused significant swelling on the right ankle joints of rats ($P<0.01$, Figure 3A), which were suppressed by AS, especially at 4, 24 and 48 hours ($P<0.05$, Figure 3A). COL showed significant suppressing effect on the swelling at 4, 12 and 24 hours after MSU injection ($P<0.01$, Figure 3A). According to the histopathological examination, MSU injection caused an inflammatory cell infiltration in the ankle joints (Figure 3B) and joint capsules (Figure 3C), which were strongly prevented by AS and COL.

In gouty arthritis rats, the high levels of IL-1β, IL-6, TNF-α and MCP-1 in the liver and serum were noted ($P<0.05$) (Table 2), which were all strongly reduced by COL ($P<0.05$) (Table 2). Similar to COL, AS reduced 29.41% ($P<0.001$), 23.53% ($P<0.01$), 34.17% ($P<0.001$) and 47.52% ($P<0.001$) levels of IL-1β, IL-6, TNF-α and MCP-1 in the liver, respectively (Table 2). In the serum, AS reduced 14.74% ($P<0.05$) and 21.1% ($P<0.001$) levels of IL-1β and MCP-1, respectively (Table 2).

**Table 1:** The effects of FB and AS on anti- and pro-oxidation cytokines in mice with hyperuricemia.

|               | CTRL       | YEP+OXO    | YEP+OXO+ FB (6 mg/kg) | YEP+OXO+ AS (15 mg/kg) |
|---------------|------------|------------|-----------------------|------------------------|
| SOD (U/mL)    | 24.77±2.09 | 22.58±1.47 | 23.24±1.93            | 25.73±2.64             |
| GSH-Px (U/mL) | 39.41±3.69 | 35.2±2.03  | 36.2±4.15             | 38.1±3.17              |
| CAT (U/mL)    | 5.26±0.33  | 4.89±0.28  | 5.27±0.25             | 5.49±0.51              |
| ROS (U/mL)    | 13.03±0.8  | 14.42±1.62 | 13.11±1.64            | 12.1±1.65              |

Data are represented as mean±S.D. ($n=12$). *$P<0.05$ vs. CTRL mice, **$P<0.05$ and ***$P<0.01$ vs. vehicle treated mice with hyperuricemia. YE, yeast extract powder; OXO, potassium oxonate; FB, febuxostat tablets; AS, astilbin; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; ROS, reactive oxygen species.

**Figure 3:** The anti-inflammation effects of AS on rats with acute gouty arthritis established via intraarticularly injection with 100 μg of MSU at the right ankle joint. (A) AS reduced the swelling ratio of ankle joints in rats. The percentage of swelling ratio of other groups were calculated compared with CTRL rats, which was considered to be 100%. Data are analyzed using a one-way ANOVA and expressed as mean ± S.D. ($n=12$). **$P<0.01$ and ***$P<0.001$ vs. CTRL rats. *$P<0.05$ and **$P<0.01$ vs. vehicle treated rats with acute gouty arthritis. AS prevented the inflammation of (B) ankle joints (200×) (Scale bar: 50 mm) and (C) joint capsules (100×) (Scale bar: 100 mm) detected via H&E staining. MSU, monosodium urate; COL, colchicine; AS, astilbin.
Table 2: The effect of COL and AS on the inflammation cytokines in rats with acute gouty arthritis.

|                | CTRL           | MSU            | MSU+ COL (0.3 mg/kg) | MSU+AS (10 mg/kg) |
|----------------|----------------|----------------|----------------------|-------------------|
| Serum          |                |                |                      |                   |
| IL-1β (pg/ml)  | 3.32±0.18      | 3.8±0.48*      | 2.85±0.68*           | 3.24±0.32*        |
| IL-6 (pg/ml)   | 15.83±1.85     | 17.52±0.82*    | 15.12±1.24*          | 16.32±0.67        |
| TNF-α (pg/ml)  | 26.77±1.12     | 28.77±0.84**   | 27.02±1.46*          | 27.21±0.74        |
| MCP-1 (pg/ml)  | 59.79±2.56     | 64.84±3.52**   | 59.28±4.5**          | 51.16±1.53**      |
| Liver          |                |                |                      |                   |
| IL-1β (pg/mg)  | 0.51±0.07      | 0.68±0.08**    | 0.49±0.06***         | 0.48±0.04***      |
| IL-6 (pg/mg)   | 1.7±0.2        | 2.38±0.37**    | 1.96±0.14            | 1.82±0.33**       |
| TNF-α (pg/mg)  | 5.81±1.32      | 7.58±1.09**    | 5.93±0.12            | 4.99±0.8**        |
| MCP-1 (pg/mg)  | 8.71±1.36      | 11.47±1.38***  | 8.95±0.7***          | 6.02±1.06***      |

Data are represented as mean±S.D. (n = 12). *P<0.05, **P<0.01 and ***P<0.001 vs. CTRL rats, #P<0.05, ##P<0.01 and ###P<0.001 vs. vehicle treated rats with acute gouty arthritis. MSU, monosodium urate; COL, colchicine; AS, astilbin; IL-1β, interleukin 1β; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; MCP-1, monocyte chemoattractant protein 1.

4 Discussion

By employing the mice model with hyperuricemia and the rats model with gouty arthritis, the anti-gout activity of AS was for the first time confirmed in this study. AS both reduced the high serum UA in hyperuricemia mice, and also suppressed the swelling rates of ankle joints and regulated the inflammatory cytokines in rats with acute gouty arthritis.

The development of hyperuricemia accompanied by the increase of oxygen free radical production, promoted lipid peroxidation and even increased the level of pro-inflammatory cytokines [27, 28]. Natural flavonoids exhibit antioxidant activities via collecting free radicals [29, 30]. As a typical flavonoid, AS has been found to inhibit XO activity and enhance the anti-oxidative function in mice with hyperuricemia. AS enhanced the levels of anti-oxidative cytokines such as SOD, GSH-Px and CAT, and suppressed the pro-oxidative cytokines, especially ROS in serum of mice with hyperuricemia. As effective antioxidant enzymes, O₂⁻ and H₂O₂ could be transformed to H₂O by SOD and GSH-Px catalysis [31, 32]. SOD and GSH-Px protect tissues from oxidative damage via scavenging ROS [33]. As reported, the over-accumulation of ROS helps the production of UA [25]. In the acute pararquet poisoning patients, the negative correlation between the levels of GSH-Px, SOD and XO activities has been confirmed [34]. As the key enzyme during the process in the catalytic reaction of xanthine and hypoxanthine to UA [35, 36], XO can regulate the production of ROS [25]. In brains of female rats treated with gamma-irradiation and carbon tetrachloride, the enhanced levels of SOD and GSH-Px by flaxseed oil can inhibit XO gene expression [37]. XO is the main source of the ROS productions in vivo [38]. AS suppressed the hyper-levels of UA in mice with hyperuricemia, at least partially, related to the inhibition on XO activity via adjusting the anti- and pro-oxidative cytokines.

Similar to COL, AS strongly suppressed the swelling of ankle joints and the cytokines including IL-1β, IL-6, TNF-α and MCP-1 of acute gouty arthritis rats. Through endocytosis, MSU can enter the cells and stimulates the production of IL-1β from synovial cells, monocyte macrophages and neutrophils [23, 39]. In gouty patients, extremely high levels of IL-1β can be found [40]. Due to the important roles of IL-1β on gout attack, researchers focus their study on IL-1β [41]. IL-1β is responsible for the release of keys cytokines including IL-6, TNF-α and MCP-1, which lead to the spread of gouty inflammation [42]. TNF-α and MCP-1 may prime and enhance maturing of monocytes to macrophages in inflammatory ocular diseases [43], and MCP-1 promotes the priming and trafficking of monocytes in gout [44]. Flavonoids from lotus plumule showed the significant anti-inflammatory activities by inhibiting the production of IL-1β and IL-6 [45]. Based on our present study, the suppression of the swelling of gouty arthritis rats ankle joints of AS may be related to its modulation on inflammatory cytokines, especially IL-1β.

There is still limitations in the present research. The detail mechanisms during AS-mediated anti-gouty activity still need systematical investigation via applying genomics or proteomics and the relationship between the anti-oxidation and anti-inflammation of AS will be studied in our group.
In conclusion, AS can reduce the UA levels of serum in hyperuricemia mice, and decreased the swelling of ankle joints in rats with acute gouty arthritis, which may be related to its regulation on XO activity and the levels of inflammatory cytokines via the anti-oxidative property. Our data provides the experimental evidence for further evaluation of AS as a candidate for gout treatment.

Acknowledgements: This work was supported by the National Key Research & Development Program of China (No. 2018YFE0107800), the Special Projects of Cooperation between Jilin University and Jilin Province in China (SXGJSF2017-1) and the “13th Five-year” Science and Technology Projects from Education Department in Jilin Province (JJKH20190108K).

Availability of data and materials: All data generated and analyzed during the present study are included in this published article.

Conflict of Interest: The authors have declared that there is no conflict of interest.

Reference

[1] Lan X, Chen, H Ralph S. Gout: An evidence-based review. J Clin Rheumatol. 2008;14(6 Suppl):55-62.
[2] Chaiamnuay P, Darmawan J, Muirden KD, Assawatanabodee P. Epidemiology of rheumatic disease in rural Thailand: A who-ilar coprdor study. Community oriented programme for the control of rheumatic disease. J Rheumatol. 1998;25(7):1382.
[3] Cronstein BN, Terkeltaub R. The inflammatory process of gout and its treatment. Arthritis Res Ther. 2006;8 Suppl 1:53.
[4] Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006;440(7081):237-241.
[5] Loeb JN. The influence of temperature on the solubility of monosodium urate. Arthritis Rheum. 1972;15(2):189-192.
[6] Kapoor N, Saxena S. Potential xanthine oxidase inhibitory activity of endophytic lasiodiplodia pseudotheobromae. Appl. Biochem. Biotechnol. 2014;173(6):1360-1374.
[7] Ichida K, Matsuo H, Takada T, Nakayama A, Murakami K, Shimizu T, et al. Decreased extra-renal urate excretion is a common cause of hyperuricemia. Nat. Commun. 2012;3:7.
[8] Chambers DE, Parks DA, Patterson G, Roy R, McCord JM, Yoshida S, et al. Xanthine oxidase as a source of free radical damage in myocardial ischemia. Journal of molecular and cellular cardiology. 1985;17(2):145-152.
[9] Terkeltaub RA. Colchicine update: 2008. Seminars in arthritis and rheumatism. 2009;38(6):411-419.
[10] Fam AG. Strategies and controversies in the treatment of gout and hyperuricaemia. Bailliere's clinical rheumatology. 1990;4(2):177-192.
[11] Terkeltaub R, Bushinsky DA, Becker MA. Recent developments in our understanding of the renal basis of hyperuricemia and the development of novel antihyperuricemic therapeutics. Arthritis Research & Therapy. 2006;8(Suppl 1):S4-S4.
[12] Fam AG. Difficult gout and new approaches for control of hyperuricemia in the allopurinol-allergic patient. Current rheumatology reports. 2001;3(2):29-35.
[13] Shen MN, Zhang JY, Qian K, Li CM, Xu WY, Gu BJ, et al. Febuxostat in the treatment of gout patients with low serum uric acid level: 1-year finding of efficacy and safety study. Clin. Rheumatol. 2018;37(11):3107-3113.
[14] Fam AG. Treating acute gouty arthritis with selective COX 2 inhibitors. BMJ (Clinical research ed.). 2002;325(7371):980-981.
[15] Yuan M, Yan Z, Liu Y, Chen D, Yang Z, He L, et al. Chemical profiles, antioxidant activity and acute toxicity of raw and sulfur-fumigated smilacis glabrae rhizoma. J Ethnopharmacol. 2019;234:76-84.
[16] Chen F, Zhu X, Sun Z, Ma Y. Astilbin inhibits high glucose-induced inflammation and extracellular matrix accumulation by suppressing the Tlr4/myd88/nf-kappab pathway in rat glomerular mesangial cells. Front Pharmacol. 2018;9:1187.
[17] Zhang Q-F, Zhang Z-R, Cheung H-Y. Antioxidant activity of rhizoma smilacis glabrae extracts and its key constituent-astilbin. Food Chem. 2009;115(1):297-303.
[18] Meng QF, Zhang Z, Wang YJ, Chen W, Li FF, Yue LT, et al. Astilbin ameliorates experimental autoimmune myasthenia gravis by decreased th17 cytokines and up-regulated t regulatory cells. J Neuroimmunol. 2016;298:138-145.
[19] Lu Cl, Zhu YF, Hu MM, Wang DM, Xu XJ, Lu CJ, et al. Optimization of astilbin extraction from the rhizome of smilax glabra, and evaluation of its anti-inflammatory effect and probable underlying mechanism in lipopolysaccharide-induced raw264.7 macrophages. Molecules. 2015;20(1):625-644.
[20] Wang J, Zhao Y, Xu Q, Astilbin prevents concanavalin a-induced liver injury by reducing tnf-alpha production and t lymphocyte activation. J. Pharm. Pharmacol. 2004;56(4):495-502.
[21] Fei MJ, Wu XF, Xu Q, Astilbin inhibits contact hypersensitivity through negative cytokine regulation distinct from cyclosporin a. J. Allergy Clin. Immunol. 2005;116(6):1350-1356.
[22] Wang M, Zhao J, Zhang N, Chen JH. Astilbin improves potassium oxonate-induced hyperuricemia and kidney injury through regulating oxidative stress and inflammation response in mice. Biomed. Pharmacother. 2016;83:975-988.
[23] Sabina EP, Rasool M. An in vivo and in vitro potential of Indian ayurvedic herbal formulation triphala on experimental gouty arthritis in mice. Vascul Pharmacol. 2008;48(1):14-20.
[24] Merriman TR. An update on the genetic architecture of hyperuricemia and gout. Arthritis Res Ther. 2015;17:98.
[25] Oguz N, Kirca M, Cetin A, Yesilkaya A. Effect of uric acid on muscle cells. Journal of receptor and signal transduction research. 2017;37(5):500-505.
[26] Tang L, Xu Y, Wei Y, He X. Uric acid induces the expression of tnfalpha via the rosiglitazone-induced signaling pathway in rat vascular smooth muscle cells. Molecular medicine reports. 2017;16(5):6928-6933.
[27] Singh M, Kalia AN, Sharma R, Balakumar P. Hyperuricemia: Is it a risk factor for vascular endothelial dysfunction and
associated cardiovascular disorders? Current Hypertension Reviews. 2009;5(1):-

[28] Billiet L, Doaty S, Katz JD, Velasquez MT. Review of hyperuricemia as new marker for metabolic syndrome. ISRN rheumatology. 2014;2014:852954.

[29] Nickavar B, Kamalinejad M, Izadpanah H. In vitro free radical scavenging activity of five salvia species. Pakistan journal of pharmaceutical sciences. 2007;20(4):291-294.

[30] Yao Y, Sang W, Zhou M, Ren G. Phenolic composition and antioxidant activities of 11 celery cultivars. Journal of food science. 2010;75(1):C9-13.

[31] Song Y, Driessens N, Costa M, De Deken X, Detours V, Corvilain B, et al. Roles of hydrogen peroxide in thyroid physiology and disease. The Journal of clinical endocrinology and metabolism. 2007;92(10):3764-3773.

[32] Poobathy R, Sinniah UR, Xavier R, Subramaniam S. Catalase and superoxide dismutase activities and the total protein content of protocorm-like bodies of dendrobium sonia-28 subjected to vitrification. Appl. Biochem. Biotechnol. 2013;170(5):1066-1079.

[33] Taysi S, Tascan AS, Ugur MG, Demir M. Radicals, oxidative/nitrosative stress and preeclampsia. Mini-Rev. Med. Chem. 2019;19(3):178-193.

[34] Zhang J, Lv G, Zhao Y. The significance of serum xanthine oxidase and oxidation markers in acute paraxquat poisoning in humans. Clin Biochem. 2011;44(2):221-225.

[35] Ramallo IA, Zacchino SA, Furlan RL. A rapid tlc autographic method for the detection of xanthine oxidase inhibitors and superoxide scavengers. Phytochemical analysis : PCA. 2006;17(1):15-19.

[36] Unno T, Sugimoto A, Kakuda T. Xanthine oxidase inhibitors from the leaves of lagerstroemia speciosa (L.) pers. Journal of ethnopharmacology. 2004;93(2-3):391-395.

[37] Ismail AFM, Salem AAM, Eassawy MMT. Modulation of gamma-irradiation and carbon tetrachloride induced oxidative stress in the brain of female rats by flaxseed oil. J Photochem Photobiol B. 2016;161:91-99.

[38] Medow MS, Bamji N, Clarke D, Ocon AJ, Stewart JM. Reactive oxygen species (ros) from nadph and xanthine oxidase modulate the cutaneous local heating response in healthy humans. J. Appl. Physiol. 2011;111(1):20-26.

[39] Rock KL, Hiroshi K, Jiann-Jyh L. Uric acid as a danger signal in gout and its comorbidities. Nat Rev Rheumatol. 2012;9(1):13-23.

[40] Zeng M, Dang W, Chen B, Qing Y, Xie W, Zhao M, et al. Il-37 inhibits the production of pro-inflammatory cytokines in mus crystal-induced inflammatory response. Clin Rheumatol. 2016;35(9):2251-2258.

[41] Sabina EP, Nagar S, Rasool M. A role of piperine on monosodium urate crystal-induced inflammation--an experimental model of gouty arthritis. Inflammation. 2011;34(3):184-192.

[42] Jeong JH, Hong S, Kwon OC, Ghang B, Hwang I, Kim YG, et al. Cd14(+) cells with the phenotype of infiltrated monocytes consist of distinct populations characterized by anti-inflammatory as well as pro-inflammatory activity in gouty arthritis. Front Immunol. 2017;8:1260.

[43] Sharon Y, Schlesinger N. Beyond joints: A review of ocular abnormalities in gout and hyperuricemia. Curr. Rheumatol. Rep. 2016;18(6):8.

[44] Grainger R, McLaughlin R, Harrison AA, Harper JL. Hyperuricaemia elevates circulating ccl2 levels and primes monocyte trafficking in subjects with inter-critical gout. Rheumatology. 2013;52(6):1018-1021.

[45] Chen GL, Fan MX, Wu JL, Li N, Guo MQ. Antioxidant and anti-inflammatory properties of flavonoids from lotus plumule. Food Chem. 2019;277:706-712.