Research Article

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

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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

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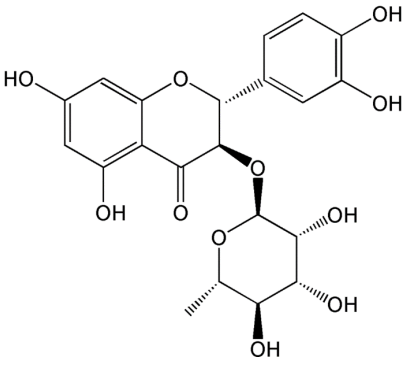

Figure 1: Chemical structure of astilbin.

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 Ct represents the circumference at different hours and C0 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.

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, Bioss, 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 chemilumenescent 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 OXO 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 2D) 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 ROS, and low amounts of SOD, GSH-Px and CAT in serum were noted ($P<0.05$, Table 1). AS resulted in 13.95% ($P<0.01$), 8.35% ($P<0.05$) and 12.27% ($P<0.05$) increase on SOD, GSH-Px and CAT, and 11.1% ($P<0.05$) reduction on ROS of serum levels of mice (Table 1). Comparatively, FB only enhanced the levels of SOD and CAT ($P<0.05$) in serum of mice with hyperuricemia (Table 1).

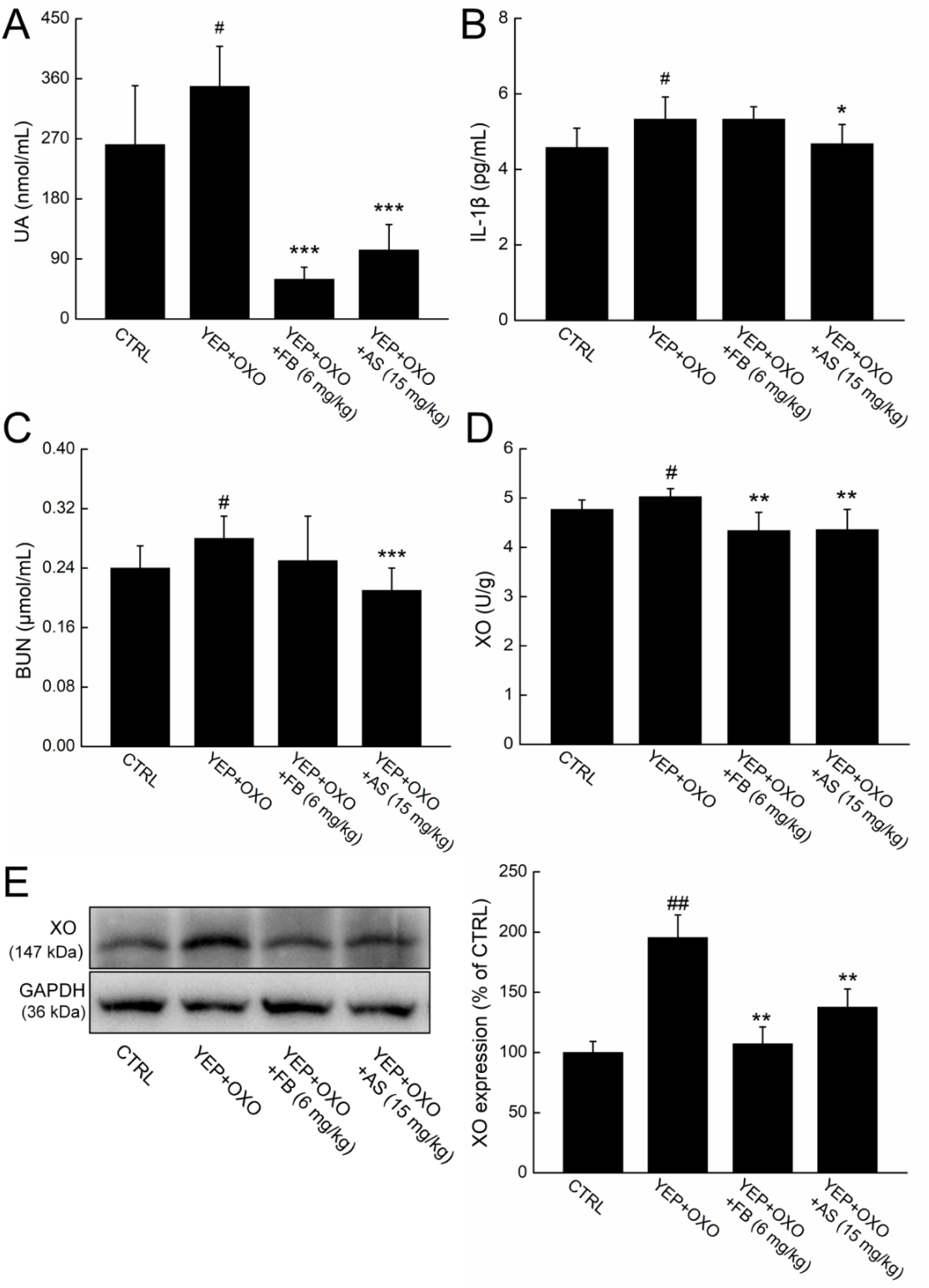

Figure 2: The therapeutic effects of AS on mice with hyperuricemia. In mice with hyperuricemia established via intragastrical administration with 20 g/kg of YEP for 8 days, and the intraperitoneal injection with 300 mg/kg of OXO, AS strongly reduced the serum levels of (**A**) UA, (**B**) IL-1β, (**C**) 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.

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$^{\#}$	36.26±4.15	38.14±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. YEP, yeast extract powder; OXO, potassium oxonate; FB, febuxostat tablets; AS, astilbin; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; ROS, reactive oxygen species.

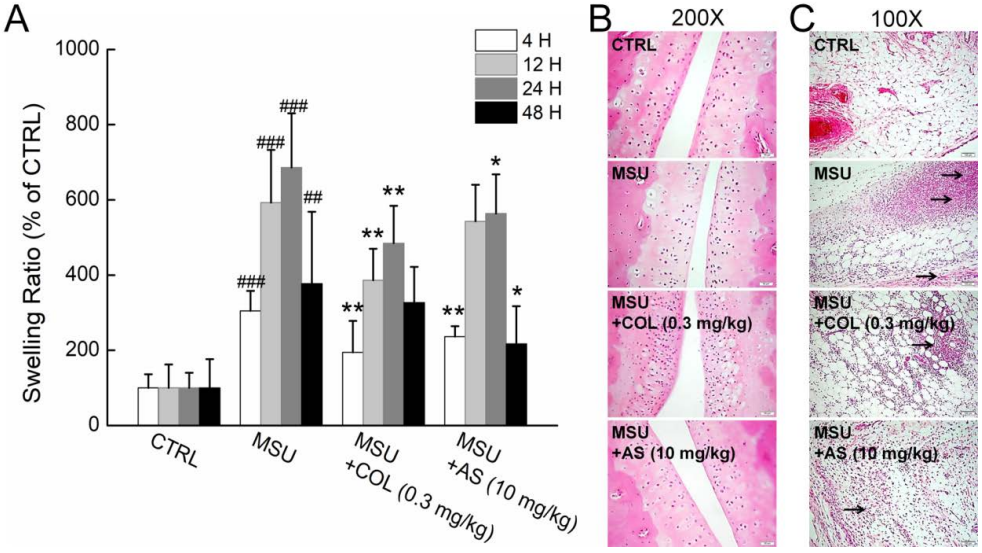

Figure 3: The anti-inflammation effects of AS on rats with acute gouty arthritis established via intraarticularly injection with 100 μg of MSU at the right ankle joint. (**A**) AS reduced the swelling ratio of ankle joints in rats. The percentage of swelling ratio of other groups were calculated compared 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.

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 2: The effect of COL and AS on the 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 found 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, O^{2-} and H_2O_2 could be transformed to H_2O 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 paraquat 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.

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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.

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