

Anti-gouty arthritis and anti-hyperuricemia properties of celery seed extracts in rodent models

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Abstract. Gout is a type of serious arthritis that is caused by hyperuricemia. Celery is an umbelliferous plant that was shown to exhibit anti-inflammatory activity in rodent. The present study aimed to investigate the effects and potential preliminary mechanisms of celery seed aqueous extract (CSAE) and celery seed oil extract (CSOL) for gout treatment. The components of CSAE and CSOL were systematically analyzed. In mice with hyperuricemia induced by potassium oxonate and yeast extract, CSAE and CSOL treatment reduced the serum levels of uric acid and xanthine oxidase. In addition, CSAE and CSOL reduced the levels of reactive oxygen species and increased the serum levels of superoxide dismutase and glutathione peroxidase in mouse serum. In rats with acute gouty arthritis induced by intra-articular injection of monosodium urate crystals, CSAE and CSOL treatment alleviated the swelling of the ankle joints and reduced inflammatory cell infiltration around the ankle joints. In addition, CSAE and CSOL reduced the levels of interleukin (IL)-1 β and tumor necrosis factor α and increased the levels of IL-10. The results of the present study suggested that celery seed extracts may have anti-gout properties, partially through anti-inflammatory and antioxidative effects.

Introduction

Gout is a common form of arthritis associated with pain, fatigue and high fever (1). According to epidemiological studies, the

incidence of gout increased from 1.42% in 1997 to 2.49% in 2012 in Britain (2), which is partly influenced by dietary changes and age (3). Hyperuricemia, defined as a level of serum uric acid (UA) >6.8 mg/dl, is caused by the overactivation of xanthine oxidase (XO) following excessive purine intake (4,5). High levels of UA contribute to the deposition of monosodium urate (MSU) in joints and other tissues (6). The deposition of MSU in the joint cavity activates inflammatory cytokines, inducing the accumulation of macrophages and neutrophils, which leads to gouty arthritis (7,8). Oxidative stress serves an important role in the pathogenesis of gout (9) and is responsible for a series of inflammatory events (10), such as the production of interleukin (IL)-1 β (11).

Based on the pathogenesis of gout, inhibiting inflammation and lowering the serum UA level are considered to be effective treatment strategies. Colchicine (COL), corticosteroids and non-steroidal anti-inflammatory drugs are commonly used in the treatment of gouty arthritis (12,13). Allopurinol and febuxostat (FBX) are the main clinical agents for treating hyperuricemia (14,15). However, a number of adverse effects have been reported, including liver damage, nephrotoxicity, bone marrow suppression and hypersensitive bodily reactions (14-16). It is therefore particularly important to find alternative treatment agents for hyperuricemia and gouty arthritis.

Natural products have received increasing attention in clinical applications owing to their diverse efficacies and low adverse effects (17). Celery is an annual or perennial umbelliferous plant widely distributed in subtropical and tropical regions of Europe, Africa and Asia (18). Celery has exhibited antifungal, anti-inflammatory and anti-gastric ulcer effects in rodents, and may lower blood pressure in patients with hypertension (19-23). n-Butanol extracts from celery seeds have been reported to improve lipid peroxidation through antioxidation in diabetic rats (24). Methanol and petroleum extracts from celery seeds reduced blood UA levels in rats (25). However, the antioxidant and anti-inflammatory activities of celery seeds, especially their aqueous extracts and volatile oil, have not been fully reported in gout.

Rats with acute gouty arthritis, which was induced by MSU to simulate acute gout in humans, have been used to investigate the effects of various agents on joint swelling and inflammation (26). Mice with hyperuricemia, which was

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induced by yeast extract powder and potassium oxonate (OXO) to simulate hyperuricemia in humans, have been used to investigate compounds that lower UA level (27). Based on our previous study, the two animal models can be used together to investigate the anti-gout activity of various compounds (28). The aim of the present study was to systematically analyze the main components of the aqueous extract and volatile oil of celery seeds and to determine their anti-gout effects in mice with hyperuricemia and rats with acute gouty arthritis. The specific roles of the antioxidant and anti-inflammatory activities of the aqueous extract and volatile oil of celery seeds were also investigated.

Materials and methods

Preparation of celery seed aqueous and oil extracts using supercritical carbon dioxide. To prepare celery seed aqueous extract (CSAE), 30 g celery seed powder (Changchun Yonglong Food Co., Ltd.) was added to 300 ml distilled water and heated at 80°C for 2.5 h twice. The supernatants from different samples were collected by centrifugation at 7,100 x g, at 20°C for 10 min and pooled together. CSAE powder was prepared using a R206 rotary evaporator spray drier (Shanghai Senco Technology Co., Ltd.). The extraction rate of CSAE was 10.0±0.5% (w/w).

To prepare celery seed oil extract (CSOL), 300 g celery seed powder was placed in a HA221-50-06 supercritical carbon dioxide extraction system (Nantong Wenaoh Import And Export Co., Ltd.) under the following conditions: 35°C and 25 MPa in the extraction tank; 40°C and 8 MPa in the first separation; and 30°C and 6 MPa in the second separation. The extraction period was 2 h, and the oil was collected every 15 min. The extraction rate of CSOL using supercritical carbon dioxide was 7.8±0.3% (w/w).

Composition analysis of CSAE and CSOL

Main components analysis. The main components of CSAE powder, including total sugar, mannitol, reducing sugar, protein, crude fat, total flavonoids and total triterpenes were determined, as previously described, by the phenol sulfuric acid method (29), high-performance liquid chromatography (30), 3,5-dinitrosalicylic acid reducing sugar assay (31), Kjeldahl method (32), Soxhlet extractor method (33), aluminum trichloride colorimetric method (34) and vanillin-glacial acetic acid-perchloric acid colorimetric method (35), respectively.

Fatty acid analysis. A 5% KOH-methanol solution was added to the CSAE powder or CSOL, incubated in a 60°C water bath for 30 min and mixed with 14% BF₃-methanol solution at 60°C for 3 min. The samples were mixed with hexane and the levels of fatty acids were analyzed using a QP2010 gas chromatography-mass spectrometer (Shimadzu Corporation). GC was performed using high-pressure-55% phenyl methyl siloxane chromatographic column (30x0.32 mm²; diameter, 0.25 μm). The sample inlet temperature was set at 270°C, and the column temperature at 100°C. The heating rate was set at 10°C/min to 170°C, and at 3°C/min to 250°C. The carrier gas used was helium, with a flow rate of 2.4 ml/min at 0.4 MPa. For mass spectrometry, electron positive ionization was used as ion source, and the transition was m/z 380.0→20.0, the

temperature of ion source was 200°C, the interface temperature was 250°C, the solvent removal time was 1.5 min, and the detector voltage was 1.14 kV.

Amino acid analysis. The CSAE powder and CSOL were hydrolyzed with 6 mol/l HCl at 110±1°C for 22 h. Following vacuum drying, the samples were dissolved in 1 ml pH 2.2 buffer (19.6 g sodium citrate and 16.5 ml hydrochloric acid dissolved in 1 l of deionized water; pH 2.2). The amino acid content was quantified by an L-8900 automatic amino acid analyzer (Hitachi High-Technologies Corporation).

Mineral analysis. The CSAE powder was pretreated with hydrogen nitrate 110°C and 3 MPa for 30 min. The levels of mercury, lead, selenium, arsenic, cadmium, zinc, iron, manganese, chromium, calcium, copper, sodium and potassium were detected by inductively coupled plasma optical emission spectrometry as previously described (36-38).

Flavonoid analysis. The flavonoid content of CSOL was analyzed using the Agilent 1260 LC system with a diode array detector-fluorescence detector and a C-18 column (150x4.6 mm; particle size, 5 μm) (Agilent Technologies, Inc.) with the injection volume of 10 μl and the flow rate of 1 ml/min by elution containing 98% methanol and 2% ultrapure water at 20°C. The standards were obtained from Shanghai Yuanye Bio-Technology Co. Ltd.

Animal experiments. The working concentrations of FBX and COL were selected according to previous studies (39,40). Celery seed treatment doses were selected following preliminary experiments (data not shown).

Establishment of mouse hyperuricemia model and drug treatment. A total of 120 male BALB/c mice (age, 8 weeks; weight, 18-20 g) were provided by Liaoning Changsheng Biotechnology Co., Ltd. All mice were housed in plastic cages at 23±1°C with 55% relative humidity, a 12-h light/dark cycle (7:00-19:00) and standard food and sterile mineral water *ad libitum*.

Mice were divided into two main groups: i) To investigate CSAE (AE); and ii) to investigate CSOL (OL). Mice used as control to examine CSAE were defined as 'CTRL (AE)' mice; mice used as control to examine CSOL were defined as 'CTRL (OL)' mice. A total of 60 mice were randomly divided into 5 groups (n=12 mice/group): i) Control [CTRL (AE)] mice group, which received 10 ml/kg saline by gavage; ii) hyperuricemia model mice [MC (AE)] group, which received 10 ml/kg saline by gavage; iii) positive control [FBX (AE)] group, which were MC mice that received 0.6 mg/ml FBX (Jiangsu Wabang Biochemical Pharmaceutical Group Co., Ltd.) at 10 ml/kg by gavage; iv) low-dose CSAE-treated model (CSAE-low) group, which received 75 mg/kg CSAE (equal to 0.75 g celery seed) dissolved in 10 ml saline by gavage; and v) high-dose CSAE-treated model (CSAE-high) group, which received 300 mg/kg (equal to 3 g celery seed) dissolved in 10 ml saline by gavage. CTRL (AE), MC (AE) and FBX (AE) were used as untreated control, model control and positive control groups, respectively, in the experiments analyzing the effects of CSAE in hyperuricemia model mice.

Table I. Composition of celery seed aqueous extract.

Component	Compound	Content (%)
Main component	Total sugar	90.3
	Total triterpenes	0.038
	Mannitol	0.515
	Reducing sugar	2.73
	Crude fat	0.7
	Protein	4.66
	Total flavonoids	0.014
Fatty acid	Capric acid (C10:0)	2.36x10 ⁻⁴
	Undecanoic acid (C11:0)	0.01x10 ⁻⁴
	Lauric acid (C12:0)	3.16x10 ⁻⁴
	Tridecanoic acid (C13:0)	ND
	Myristic acid (C14:0)	16.48x10 ⁻⁴
	Myristoleic acid (C14:1n5)	0.41x10 ⁻⁴
	Pentadecanoic acid (C15:0)	3.04x10 ⁻⁴
	Pentadecenoic acid (C15:1n5)	0.11x10 ⁻⁴
	Hexadecanoic acid (C16:0)	1761.24x10 ⁻⁴
	Palmitoleic acid (C16:1n7)	17.08x10 ⁻⁴
	Heptadecanoic acid (C17:0)	6.36x10 ⁻⁴
	Heptadecenoic acid (C17:1n7)	3.43x10 ⁻⁴
	Stearic acid (C18:0)	289.50x10 ⁻⁴
	Oleic acid (C18:1n9)	1 85.10x10 ⁻⁴
	Elaidic acid (C18:1n9t)	ND
	Linoleic acid (C18:2n6c)	6087.74x10 ⁻⁴
	Trans-linoleic acid (C18:2n6t)	ND
	α-linolenic acid (C18:3n3)	568.85x10 ⁻⁴
	γ-Linolenic acid (C18:3n6)	103.81x10 ⁻⁴
	Arachidic acid (C20:0)	54.82x10 ⁻⁴
	Paullinic acid (C20:1)	61.99x10 ⁻⁴
	Eicosadienoic acid (C20:2)	ND
	Eicosatrienoic acid (C20:3n3)	ND
	Dihomo-γ-linolenic acid (C20:3n6)	98.90x10 ⁻⁴
	Arachidonic acid (C20:4n6)	115.07x10 ⁻⁴
	Eicosapentaenoic acid (C20:5n3)	120.20x10 ⁻⁴
	Heneicosanoic acid (C21:0)	71.64x10 ⁻⁴
	Docosanoic acid (C22:0)	88.04x10 ⁻⁴
	Erucic acid (C22:1n9)	107.84x10 ⁻⁴
	cis-13,16-Docosadienoic acid methyl ester (C22:2)	119.11x10 ⁻⁴
	Docosahexaenoic acid (C22:6n3)	ND
	Tricosanoic acid (C23:0)	106.60x10 ⁻⁴
Tetracosanoic acid (C24:0)	ND	
Nervonic acid (C24:1n9)	ND	
Octanoic acid (C8:0)	0.02x10 ⁻⁴	
Amino acid	Aspartic acid	2.38x10 ⁻¹
	L-Threonine	1.68x10 ⁻¹
	Serine	0.22x10 ⁻¹
	Glutamic acid	2.55x10 ⁻¹
	Glycine	1.08x10 ⁻¹

Table I. Continued.

Component	Compound	Content (%)
Minerals	Alanine	1.32x10 ⁻¹
	Cysteine	3.52x10 ⁻¹
	Valine	0.77x10 ⁻¹
	Methionine	2.00x10 ⁻¹
	Isoleucine	1.17x10 ⁻¹
	Leucine	1.88x10 ⁻¹
	Tyrosine	0.69x10 ⁻¹
	Phenylalanine	15.35x10 ⁻¹
	Lysine	0.89x10 ⁻¹
	Histidine	4.77x10 ⁻¹
	Arginine	0.77x10 ⁻¹
	Proline	3.95x10 ⁻¹
	Mercury	ND
	Lead	0.18x10 ⁻⁴
	Selenium	ND
	Arsenic	0.19x10 ⁻⁴
	Cadmium	ND
Zinc	29.94x10 ⁻⁴	
Iron	36.16x10 ⁻⁴	
Manganese	21.50x10 ⁻⁴	
Chromium	1.92x10 ⁻⁴	
Calcium	2,426x10 ⁻⁴	
Copper	2.05x10 ⁻⁴	
Sodium	1,258x10 ⁻⁴	
Potassium	8,079x10 ⁻⁴	

ND, not detected.

The remaining 60 mice were randomly divided into 5 groups (n=12 mice/group): i) CTRL (OL) group, which received 5 ml/kg of olive oil by gavage; ii) MC (OL) group, which received 5 ml/kg of olive oil by gavage; iii) FBX (OL) group, which comprised MC mice that received 6 mg/kg FBX dissolved in 5 ml olive oil by gavage; iv) low-dose CSOL-treated (CSOL-low) model group, which received 0.058 ml/kg CSOL (equal to 0.75 g celery seed) in 5 ml olive oil by gavage; and v) high-dose CSOL-treated (CSOL-high) model group, which received 0.233 ml/kg (equal to 3 g celery seed) in 5 ml olive oil by gavage. CTRL (OL), MC (OL) and FBX (OL) were used as untreated control, model control and positive control groups, respectively, in the experiments analyzing the effects of CSOL in hyperuricemia model mice.

With the exception of the CTRL mice, 12 h prior to the oral administration of the aforementioned agents (saline, olive oil, FBX, CSAE or CSOL), 20 g/kg yeast extract powder was administered by gavage to the mice once a day for 8 days. On day 6, 7 and 8, 1 h prior to the oral administration of the aforementioned agents, mice were intraperitoneally injected with 300 mg/kg OXO (Sigma-Aldrich; Merck KGaA) to induce hyperuricemia (28); the CTRL mice were injected with 0.9% saline following the same schedule. During the 8 days, the aforementioned agents (saline, olive oil, FBX, CSAE, CSOL

Table II. Composition of celery seed oil extract.

Component	Compound	Content (%)
Fatty acid	Capric acid (C10:0)	0.118x10 ⁻²
	Undecanoic acid (C11:0)	0.029x10 ⁻²
	Lauric acid (C12:0)	1.315x10 ⁻²
	Tridecanoic acid (C13:0)	0.059x10 ⁻²
	Myristic acid (C14:0)	9.120x10 ⁻²
	Myristoleic acid (C14:1n5)	0.013x10 ⁻²
	Pentadecanoic acid (C15:0)	1.033x10 ⁻²
	Pentadecenoic acid (C15:1n5)	0.049x10 ⁻²
	Hexadecanoic acid (C16:0)	983.766x10 ⁻²
	Palmitoleic acid (C16:1n7)	8.086x10 ⁻²
	Heptadecanoic acid (C17:0)	4.521x10 ⁻²
	Heptadecenoic acid (C17:1n7)	3.506x10 ⁻²
	Stearic acid (C18:0)	242.853x10 ⁻²
	Oleic acid (C18:1n9)	115.324x10 ⁻²
	Elaidic acid (C18:1n9t)	ND
	Linoleic acid (C18:2n6c)	5,174.071x10 ⁻²
	Trans-linoleic acid (C18:2n6t)	ND
	α-linolenic acid (C18:3n3)	320.313x10 ⁻²
	γ-Linolenic acid (C18:3n6)	0.436x10 ⁻²
	Arachidic acid (C20:0)	21.331x10 ⁻²
	Paullinic acid (C20:1)	6.764x10 ⁻²
	Eicosadienoic acid (C20:2)	1.579x10 ⁻²
	Eicosatrienoic acid (C20:3n3)	0.435x10 ⁻²
	Dihomo-γ-linolenic acid (C20:3n6)	0.114x10 ⁻²
	Arachidonic acid (C20:4n6)	1.543x10 ⁻²
	Eicosapentaenoic acid (C20:5n3)	0.035x10 ⁻²
	Heneicosanoic acid (C21:0)	1.082x10 ⁻²
	Docosanoic acid (C22:0)	21.793x10 ⁻²
	Erucic acid (C22:1n9)	0.257x10 ⁻²
	cis-13,16-Docosadienoic acid methyl ester (C22:2)	0.211x10 ⁻²
	Docosahexaenoic acid (C22:6n3)	0.084x10 ⁻²
	Tricosanoic acid (C23:0)	2.165x10 ⁻²
	Tetracosanoic acid (C24:0)	9.214x10 ⁻²
Nervonic acid (C24:1n9)	0.053x10 ⁻²	
Octanoic acid (C8:0)	0.251x10 ⁻²	
Amino acid	Aspartic acid	0.007
	L-Threonine	ND
	Serine	0.003
	Glutamic acid	ND
	Glycine	0.001
	Alanine	ND
	Cysteine	ND
	Valine	ND
	Methionine	0.003
	Isoleucine	ND
	Leucine	ND
	Tyrosine	ND
Proline	0.112	

Table II. Continued.

Component	Compound	Content (%)
Flavonoids	Lysine	0.003
	Arginine	0.007
	Histidine	0.004
	Phenylalanine	ND
	Dihydromyricetin	ND
	Myricetin	ND
	Naringenin	0.18x10 ⁻²
	Apigenin	0.012x10 ⁻²
	Taxifolin	0.52x10 ⁻²
	Eriodictyol	0.032x10 ⁻²
	Luteolin	0.024x10 ⁻²
	Aromadendrin	0.095x10 ⁻²
Quercetin	2.3x10 ⁻²	
Kaempferol	ND	

ND, not detected.

or COL) were administered every day. On day 8, after the last administration of the agents, over 150 μl of blood were collected from the caudal vein of all mice, and then the mice were sacrificed. Liver samples were collected from all mice post mortem. In summary, yeast extract powder was administered by gavage once a day.

Establishment of rat acute gouty arthritis model and drug treatment. A total of 120 male Wistar rats (age, 8 weeks; weight, 180–220 g) were obtained from Liaoning Changsheng Biotechnology Co., Ltd. All rats were housed in plastic cages at 23±1°C with 55% relative humidity, a 12-h light/dark cycle (7:00–19:00) and *ad libitum* access to standard food and mineral water.

A total of 60 rats were randomly divided into 5 groups (n=12 rats/group): i) CTRL (AE) group, which received 5 ml/kg saline by gavage for 8 days; ii) rat gouty arthritis MCr (AE), which receive 5 ml/kg saline by gavage for 8 days; iii) positive control [COL (AE)] model group, which received 0.4 mg/kg COL (Yunnan Phytopharmaceutical Co., Ltd.) dissolved in 5 ml saline by gavage for 8 days; iv) SAE-low group, which received 50 mg/kg CSAE (equal to 0.5 g celery seed) dissolved in 5 ml saline by gavage for 8 days; and v) CSAE-high group, which received 200 mg/kg (equal to 2 g celery seed) dissolved in 5 ml saline by gavage for 8 days. CTRL (AE), MCr (AE) and COL (AE) were used as the untreated control, model control and positive control groups, respectively, in the experiments analyzing the effects of CSAE in gouty arthritis model rats.

The remaining 60 rats were randomly divided into 5 groups (n=12 mice/group): i) CTRL (OL) group, which received 1 ml/kg olive oil by gavage for 8 days; ii) MCr (OL) group, which received 1 ml/kg olive oil by gavage for 8 days; iii) COL (OL) group, which was model rats that received 0.4 mg COL dissolved in 1 ml/kg olive oil by gavage for 8 days; iv) CSOL-low group, which received 0.039 ml CSOL (0.5 g celery seed) dissolved in 0.961 ml/kg olive oil by gavage

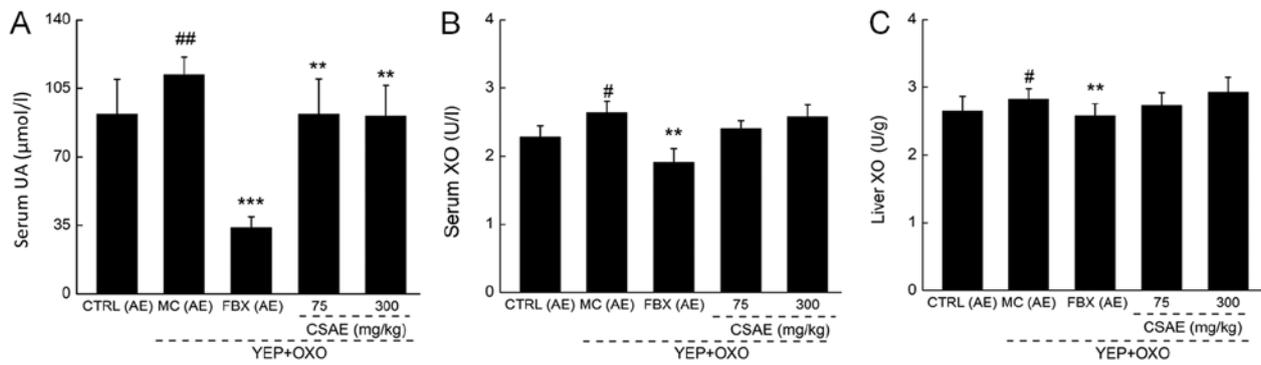


Figure 1. Effects of CSAE on mice with hyperuricemia. (A) CSAE reduced the serum levels of UA in model mice. (B and C) CSAE slightly affected XO activity in (B) serum and (C) liver of model mice. Data are expressed as the mean ± SD; n=12; [#]P<0.05 and ^{##}P<0.01 vs. CTRL (AE); ^{**}P<0.01 and ^{***}P<0.001 vs. MC (AE). CSAE, celery seed aqueous extract; CTRL, control; FBX, MC mice treated with febuxostat; MC, hyperuricemia model mice; OXO, potassium oxonate; UA, uric acid; XO, xanthine oxidase; YEP, yeast extract powder.

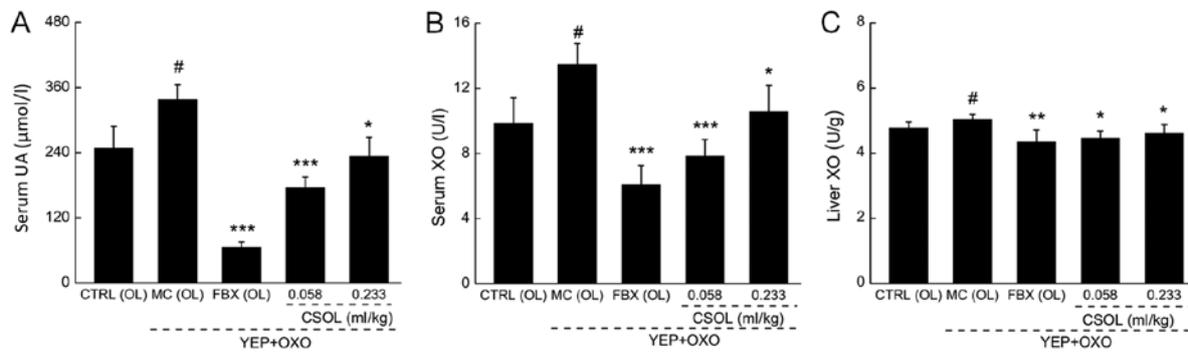


Figure 2. Effects of CSOL on mice with hyperuricemia. (A) CSOL treatment reduced the serum levels of UA in model mice. (B and C) CSOL suppressed XO activities in (B) serum and (C) liver in treated model mice. Data are expressed as the mean ± SD; n=12; [#]P<0.05 vs. CTRL (OL); ^{*}P<0.05, ^{**}P<0.01 and ^{***}P<0.001 vs. MC (OL). CSOL, celery seed oil extract; CTRL, control; FBX, MC mice treated with febuxostat; MC, model mice; OXO, potassium oxonate; UA, uric acid; XO, xanthine oxidase; YEP, yeast extract powder.

for 8 days; and v) CSOL-high group, which received 0.155 ml CSOL (2 g celery seed) dissolved in 0.845 ml/kg olive oil by gavage for 8 days. CTRL (OL), MCr (OL) and FBX (OL) were used as the untreated control, model control and positive control groups, respectively, in the experiments analyzing the effects of CSOL in gouty arthritis model rats.

MSU (Sigma-Aldrich; Merck KGaA) suspension was prepared with sterile water in biological safety cabinets. The endotoxins in the MSU samples were detected by a commercial kit (Tachypleus Amebocyte Lysate for Endotoxin Detection Kit; cat. no. RG025006; Xiamen Bioendo Technology Co., Ltd.) to exclude a potential effect induced by Endotoxin. On day 6, the rats were intra-articularly injected with 30 mg/ml MSU to the right ankle (0.1 ml) at 4:00 PM (28), with the exception of the CTRL rats, which were injected with 0.9% saline at the same time.

Following the 8-day treatment, the right ankle circumferences of all rats were measured using Vernier calipers at 24 and 48 h, and the swelling ratio (%) was calculated as follows: Swelling ratio (%)=(C_t-C₀)/C₀, where C_t is the circumference at time t, and C₀ is the circumference at 0 h. Prior to euthanasia, blood samples were collected from the caudal vein of the rats.

Biochemical assay. In the hyperuricemia MC mouse model, the serum levels of UA, and XO, and the liver levels

of XO were determined using an XO Activity Assay kit (cat. no. MAK078; Sigma-Aldrich; Merck KGaA) and a UA Assay kit (cat. no. MAK077; Sigma-Aldrich; Merck KGaA) according to the manufacturer's instructions. The serum levels of reactive oxygen species (ROS; cat. no. 43124), superoxide dismutase (SOD; cat. no. 43125) and glutathione peroxidase (GSH-Px; cat. no. 43390) were determined using ELISA kits from Shanghai Yuanye Bio-Technology Co., Ltd. according to the manufacturer's instructions.

In the acute gout rat model, the serum levels of IL-1β (cat. no. 43360), IL-10 (cat. no. 41736), IL-6 (cat. no. 41731), IL-17 (cat. no. 41736), monocyte chemoattractant protein 1 (MCP-1; cat. no. 41640) and tumor necrosis factor α (TNF-α; cat. no. 41721) were determined by ELISA kits from Shanghai Yuanye Bio-Technology Co. Ltd.

Pathological section of the ankle joint. The right ankle of each rat was collected, fixed in 4% paraformaldehyde and decalcified with 10% ethylenediaminetetraacetic acid. Following decalcification and dehydration via increasing ethanol series, followed by incubation in 50% of ethanol + 50% dimethylbenzene for 1 h at room temperature, samples were incubated twice with dimethylbenzene for 20 min at room temperature, the samples were embedded in paraffin, sliced into 5-µm sections and stained with hematoxylin and eosin (H&E). The slides were stained with hematoxylin for 5 min at room temperature

Table III. Effects of CSAE on the oxidative stress-related factors in mice with hyperuricemia.

Group	SOD (U/ml)	GSH-Px (U/ml)	ROS (U/ml)
CTRL (AE)	41.2±3.6	61.3±7.3	59.5±6.5
MC (AE)	36.8±3.8 ^a	53.6±6.1 ^a	64.0±3.9 ^a
FBX (AE) (6 mg/kg)	43.0±4.6 ^b	57.2±5.9	54.5±59.0 ^c
CSAE (75 mg/kg)	45.1±4.7 ^c	61.2±4.2 ^b	55.3±6.1 ^c
CSAE (300 mg/kg)	47.8±3.5 ^d	50.2±1.9	57.6±4.2 ^b

^aP<0.05 vs. CTRL (AE); ^bP<0.05, ^cP<0.01 and ^dP<0.001 vs. MC (AE). Results are presented as the mean ± SD, n=12. CSAE, celery seed aqueous extract; CTRL, control; FBX, MC mice treated with febuxostat; GSH-Px, glutathione peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; MC, model mice.

Table IV. Effects of CSOL on the oxidative stress-related factors in mice with hyperuricemia.

Group	SOD (U/ml)	GSH-Px (U/ml)	ROS (U/ml)
CTRL (OL)	24.8±2.1	39.4±3.7	21.1±0.7
MC (OL)	21.4±2.1 ^b	35.2±2.0 ^a	22.6±1.3 ^a
FBX (OL) (6 mg/kg)	23.2±1.9 ^c	36.3±4.1	20.0±1.3 ^d
CSOL (0.058 ml/kg)	23.0±1.6	37.8±1.2 ^c	20.0±1.0 ^d
CSOL (0.233 ml/kg)	25.2±1.6 ^d	35.6±3.7	22.3±2.0

^aP<0.05 and ^bP<0.01 vs. CTRL (OL); ^cP<0.05 and ^dP<0.01 vs. MC (OL). Results are presented as the mean ± SD; n=12. CSOL, celery seed oil extract; CTRL, control; FBX, MC mice treated with febuxostat; GSH-Px, glutathione peroxidase; MC, model mice; ROS, reactive oxygen species; SOD, superoxide dismutase.

and eosin for 3 min at room temperature and observed under a light microscope (magnification, x200), and three fields of view were examined per section.

Statistical analysis. All data are expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance followed by Dunn's multiple comparison post-hoc test using SPSS software (version 16.0; SPSS, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Composition of CSAE and CSOL. The CSAE contained 90.3% total sugar, 2.73% reducing sugar, 0.515% mannitol, 4.66% protein, 0.7% crude fat and 0.014% total flavonoids (Table I). Among the 35 types of the detected fatty acids, the contents of C16:0 (0.17%) and C18:2n6c (0.61%) were the highest (Table I). A total of 17 different amino acids were detected in CSAE (Table I). The overall mineral content was low, and the solution contained, among other salts, 0.24% calcium and 0.0022% manganese (Table I).

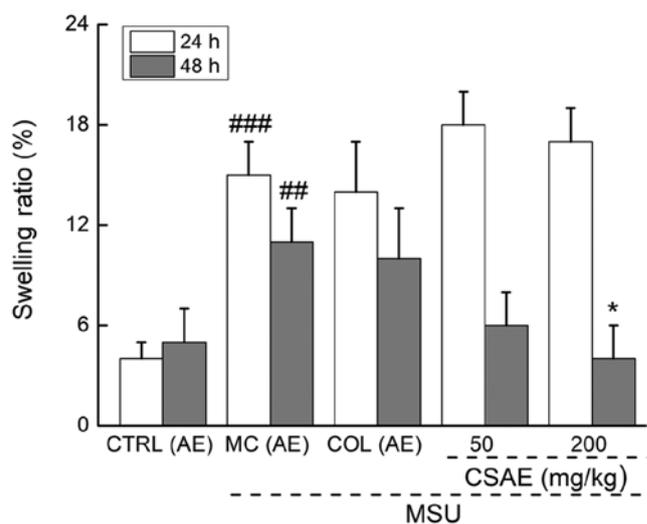


Figure 3. CSAE decreases the ankle joint swelling rate of rats with acute gouty arthritis. Data are expressed as the mean ± SD; n=12; ^{##}P<0.01 and ^{###}P<0.001 vs. CTRL (AE); ^{*}P<0.05 vs. MCr (AE). COL, MCr rats treated with colchicine; CSAE, celery seed aqueous extract; CTRL, control; MCr, acute gouty arthritis model rats; MSU, monosodium urate.

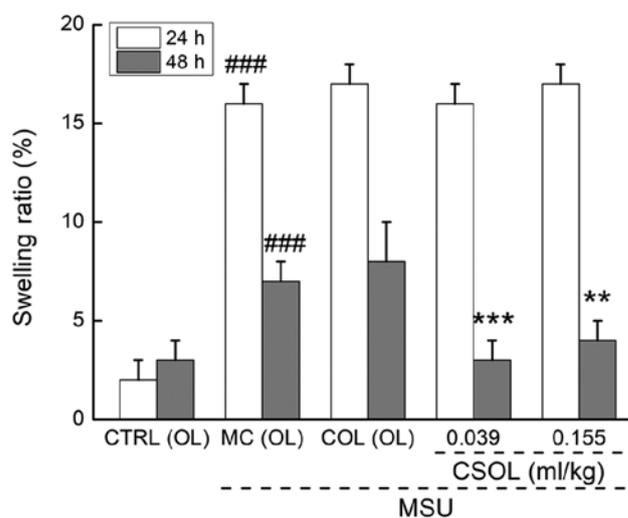


Figure 4. CSOL decreases the ankle joint swelling rate of rats with acute gouty arthritis. Data are expressed as means ± SD; n=12; ^{###}P<0.001 vs. CTRL (OL); ^{**}P<0.05 and ^{***}P<0.01 vs. MCr (OL). COL, MCr rats treated with colchicine; CSOL, celery seed oil extract; CTRL, control; MCr, model rats; MSU, monosodium urate.

In the CSOL, among the 35 types of the detected fatty acids, the contents of C18:2n6c (51.74%), C16:0 (9.8%), C18:3n3 (3.2%), C18:0 (2.4%) and C18:1n9 (1.15%) were the highest. Of the 17 amino acids identified in CSAE, only nine were detected in CSOL. The total flavonoid content in CSOL was 0.03%, which included seven distinct flavonoids, including naringenin, quercetin and taxifolin (Table II).

Effects of CSAE and CSOL in hyperuricemia MC mice
Reductive effects of CSAE and CSOL on the levels of UA and XO. The accumulation of UA in the body induces sodium urate precipitation in the joint cavity, causing severe painful arthritis (41). In the two separate experiments, a significant

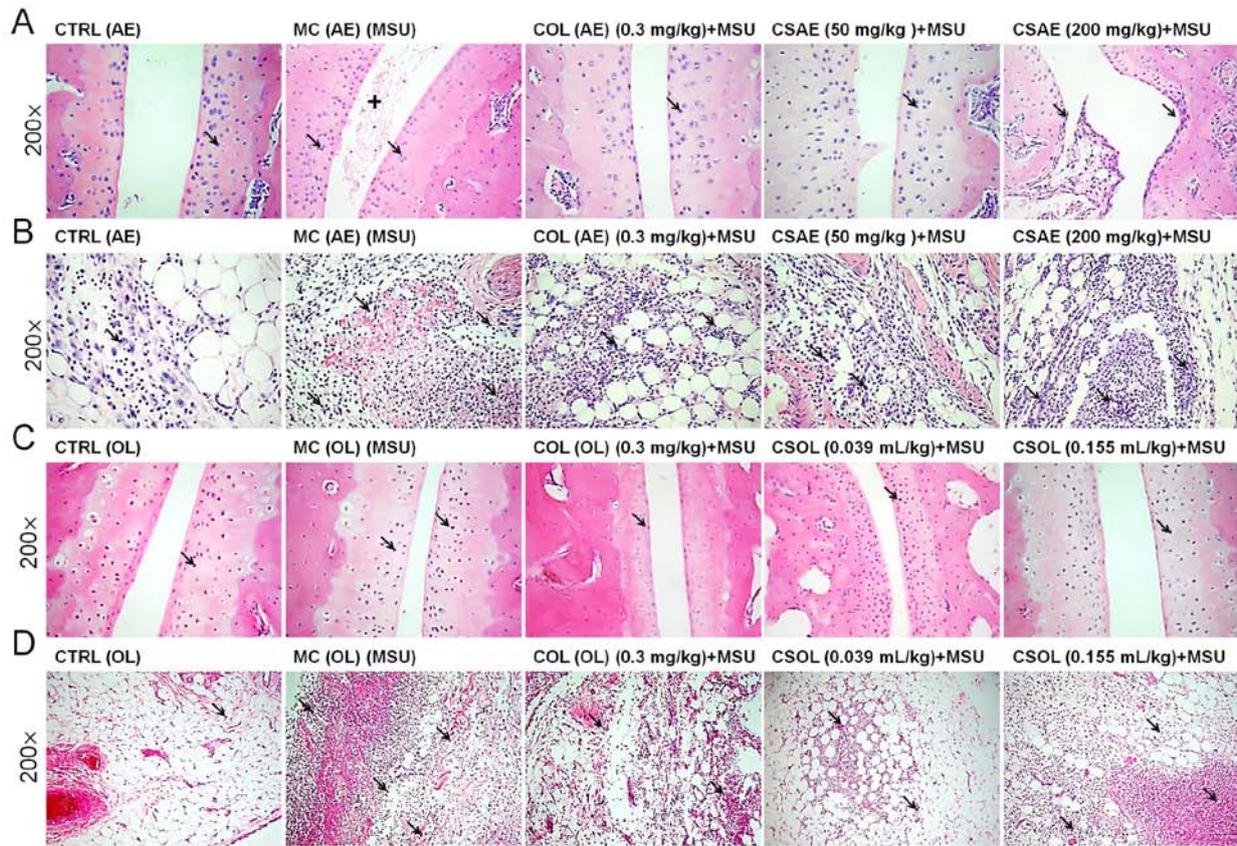


Figure 5. CSAE and CSOL reduce the pathological alterations of ankle joints in rats with acute gouty arthritis. (A and B) H&E stained histopathological slices of (A) ankle joints and (B) joint capsules in CSAE-treated model rats. (C and D) H&E stained histopathological slices of (C) ankle joints and (D) joint capsules of CSOL-treated model rats. Magnification, x200; n=6. The arrows indicate inflammatory cells. '+' indicates cell debris. COL, MCr rats treated with colchicine; CSAE, celery seed aqueous extract; CSOL, celery seed oil extract; CTRL, control; MCr, model rats; MSU, monosodium urate.

increase in serum UA levels was observed in mice with hyperuricemia compared with the respective untreated CTRL mice ($P<0.05$; Figs. 1A and 2A). FBX treatments resulted in a >60% reduction of the UA enhancement compared with the respective MC group ($P<0.001$; Figs. 1A and 2A). Low- and high-dose CSAE and CSOL administration strongly reduced serum UA levels in mice with hyperuricemia compared with those in the respective MC group ($P<0.05$; Figs. 1A and 2A).

XO is a regulator of purine metabolism, which regulates the levels of the final product of purine metabolism, UA (42). Significantly higher levels of XO were observed in the serum ($P<0.05$, Figs. 1B and 2B) and liver ($P<0.05$, Figs. 1C and 2C) of MC mice compared with the CTRL group. Low- and high-dose CSAE and CSOL slightly decreased the pathologically elevated XO levels; CSOL-low treatment reduced the XO activity by 41.9% in the serum ($P<0.001$; Fig. 2B) and by 11.3% in the liver ($P<0.05$; Fig. 2C).

Regulatory effect of CSAE and CSOL on oxidative stress.

The production of UA is accompanied by a large amount of ROS, and hyperuricemia is associated with the occurrence of oxidative stress (43,44). Low SOD and GSH-Px levels and high ROS levels were observed in hyperuricemia MC mice compared with the CTRL group ($P<0.05$; Tables III and IV). Low-dose CSAE treatment resulted in 14.2 and 22.6% increase of GSH-Px ($P<0.05$) and SOD ($P<0.05$) levels, respectively, and a 13.6% reduction of ROS levels ($P<0.01$) in the serum

compared with untreated MC mice. Similar regulatory effects on the anti- and pro-oxidative factors were observed in the CSOL-treated compared with untreated MC mice ($P<0.05$; Tables III and IV). However, FBX treatment only enhanced the levels of SOD and reduced the levels of ROS in the serum compared with untreated MC mice ($P<0.05$; Tables III and IV).

Effects of CSAE and CSOL on MSU-induced acute gouty arthritis model rats

CSAE and CSOL regulate the swelling and pathological changes of ankle joints. Compared with the CTRL rats of the two experiments, the swelling rates of the right ankle joint in the MCr rats with MSU-induced gouty arthritis increased by >100% at 24 and 48 h ($P<0.001$; Figs. 3 and 4). This effect was suppressed by CSAE-high and low- and high-dose CSOL administration at 48 h ($P<0.05$; Figs. 3 and 4). Compared with MCr rats with MSU-induced gouty arthritis, COL treatment did not reduce the swelling ($P<0.05$; Figs. 3 and 4).

In the MCr rats, there was a notable presence of foreign substances, such as cell debris, in the ankle joint cavity, a narrow joint space and enhanced numbers of inflammatory cells around the joint cavity were observed compared with the untreated CTRL rats of the two experiments (Fig. 5). These effects were detected in both ankle joints and joint capsules. These pathological changes of the ankle joints of rats with acute gouty arthritis appeared to be relieved by COL, CSAE-low and CSOL-low treatments (Fig. 5). However, CSAE-high and

Table V. Effects of CSAE on inflammatory factors in MSU-induced rats with acute gout.

Group	IL-1 β (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	MCP-1 (pg/ml)	TNF- α (pg/ml)
CTRL (AE)	5.3 \pm 0.6	28.1 \pm 3.2	16.0 \pm 3.2	116.8 \pm 11.1	44.0 \pm 2.8
MCr (AE)	6.7 \pm 1.5 ^a	35.3 \pm 7.9 ^a	13.6 \pm 1.2 ^a	127.1 \pm 23.9	51.3 \pm 9.6
COL (AE) (0.3 mg/kg)	5.1 \pm 0.8 ^b	27.4 \pm 3.7 ^b	14.2 \pm 1.1	92.0 \pm 12.2 ^c	37.1 \pm 4.9 ^c
CSAE (50 mg/kg)	5.2 \pm 0.4 ^b	31.1 \pm 1.9	16.6 \pm 1.7 ^c	106.9 \pm 23.7	40.8 \pm 4.4 ^b
CSAE (200 mg/kg)	5.2 \pm 0.4 ^b	28.1 \pm 2.7 ^b	14.8 \pm 0.7 ^b	107.6 \pm 7.9	42.5 \pm 3.8 ^b

^aP<0.05 vs. CTRL (AE); ^bP<0.05 and ^cP<0.01 vs. MCr (AE). Results are presented as the mean \pm SD; n=12. COL, MCr rats treated with colchicine; CSAE, celery seed aqueous extract; CTRL, control; IL, interleukin; MCr, model rats; MCP-1, monocyte chemoattractant protein 1; TNF- α , tumor necrosis factor α .

Table VI. Effects of CSOL on the inflammatory factors in MSU-induced acute gouty rats.

Group	IL-1 β (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	MCP-1 (pg/ml)	TNF- α (pg/ml)
CTRL (OL)	2.7 \pm 0.1	17.7 \pm 2.9	4.7 \pm 0.2	73.1 \pm 3.1	22.7 \pm 0.5
MCr (OL)	3.1 \pm 0.5 ^a	20.4 \pm 2.1 ^a	4.2 \pm 0.5 ^a	76.5 \pm 1.1	23.3 \pm 0.7
COL (OL) (0.3 mg/kg)	2.6 \pm 0.5 ^b	17.1 \pm 0.9 ^b	4.4 \pm 0.3	71.3 \pm 4.1	22.2 \pm 0.8
CSOL (0.039 ml/kg)	2.5 \pm 0.1 ^b	19.4 \pm 1.7	5.2 \pm 0.8 ^b	74.0 \pm 10.2	20.0 \pm 1.2 ^b
CSOL (0.155 ml/kg)	2.9 \pm 0.2	18.8 \pm 1.1	4.8 \pm 0.2 ^b	72.7 \pm 2.6	21.5 \pm 1.0

^aP<0.05 vs. CTRL (OL); ^bP<0.05 vs. MCr (OL). Results are presented as the mean \pm SD; n=12. COL, MCr rats treated with colchicine; CSOL, celery seed oil extract; CTRL, control; IL, interleukin; MCr, model rats; MCP-1, monocyte chemoattractant protein 1; TNF- α , tumor necrosis factor α .

CSOL-high failed to relieve the inflammation of the joints (Fig. 5), possibly due to a negative feedback regulation.

Effects of CSAE and CSOL on inflammatory factors. A previous study has demonstrated that the pathogenesis of gout is associated with inflammation (45). Compared with the CTRL rats of the two experiments, enhanced levels of the pro-inflammatory cytokines IL-1 β and IL-6 and reduced levels of the anti-inflammatory cytokine IL-10 were observed in the respective hyperuricemia or gouty arthritis MCr rats (P<0.05; Tables V and VI). CSAE-high reduced the levels of IL-1 β by 22.4% (P<0.05), IL-6 by 20.4% (P<0.05) and TNF- α by 17.2% (P<0.05), and enhanced the IL-10 levels by 8.8% (P<0.05) compared with untreated MCr rats; however, it did not affect the levels of MCP-1 in the serum of rats with acute gouty arthritis (Table V). CSOL-low resulted in 14.2 and 19.4% reductions in the serum levels of TNF- α (P<0.05) and IL-1 β (P<0.05), respectively, and a >14.3% increase in the serum levels of IL-10 (P<0.05) compared with untreated acute gouty arthritis MCr rats (Table VI). CSOL did not affect the levels of IL-6 or MCP-1 in rats with acute gouty arthritis (Table VI).

Discussion

The results of the present study suggested that CSAE and CSOL exerted slightly suppressive effects on the serum UA levels and XO activity in mice with hyperuricemia induced by OXO and yeast extract, and reduced the ankle joint

swelling rates in rats with acute gouty arthritis induced by an intra-articular injection of MSU.

The occurrence of hyperuricemia increases the production of oxygen free radicals, promotes lipid peroxidation and upregulates pro-inflammatory factor expression (6,46-48). Celery juice and celery root can increase the antioxidant content in rats (49). One of the major functions of the flavonoids in plants is to scavenge free radicals and exert anti-oxidant effects (50,51). In the present study, CSAE and CSOL, which contain various types of flavonoids, such as quercetin and taxifolin, inhibited XO activity, promoted oxidative stress factors SOD and GSH-Px and reduced levels of ROS in mice with hyperuricemia mice. As an effective antioxidant enzyme, SOD catalyzes the rapid conversion of O₂ and •O₂⁻ to H₂O₂, following which H₂O₂ can be converted to H₂O by GSH-Px catalysis inside cells (52). A negative correlation between the levels of XO activity and SOD and GSH-Px has been reported in patients with acute paraquat poisoning (53). Flaxseed oil has been demonstrated to inhibit the gene expression levels of XO by increasing the activity of SOD and GSH-Px in the brains of female rats treated with g-irradiation and carbon tetrachloride (54). XO is a key enzyme in the catalytic conversion of xanthine and hypoxanthine to UA (55,56), which is responsible for the generation of ROS (43). As a feedback response, a large amount of ROS is generated alongside the production of UA (43). Therefore, the suppressive effects of CSEA and CSOL on UA in mice with hyperuricemia may be, at least partially, associated with oxidative stress inhibition.

During the development of gouty arthritis, MSU enters cells through endocytosis and induces inflammation (57). MSU stimulates synovial cells, monocyte macrophages and neutrophils to produce IL-1 β , which promotes the release of a series of inflammatory cytokines, such as IL-6, TNF- α and MCP-1 (41), leading to the spread of inflammation (58). In clinical trials, high levels of pro-inflammatory factors, especially IL-1 β , have been detected in patients with gout (59). IL-1 β has been investigated for its important roles in gout, and piperine has been shown to exhibit anti-gouty arthritis effect by inhibiting IL-1 β (60). IL-1 inhibitors, such as anakinra and canakinumab, which are drugs approved by the U.S. Food and Drug Administration and the European Medicines Agency (61), are reportedly effective against gouty arthritis (62,63). In addition, sustained oxidative stress can lead to chronic inflammation (64). The overproduction of ROS is a pathogenetic factor of acute gouty arthritis (65,66). Excessive ROS production activates the inflammasome, specifically NACHT, LRR and PYD domains-containing protein 3, and promotes the production of IL-1 β in gouty arthritis (67). In the present study, CSAE and CSOL reduced the pro-inflammatory factors and enhanced the anti-inflammatory factor in the serum, and mitigated the pathological changes of the ankle joints of rats with MSU-induced acute gouty arthritis. The results of the present study suggested that the anti-inflammatory properties of CSAE and CSOL, as well as their modulatory effect on inflammatory cytokines, especially IL-1 β , may be central to their anti-gout effects, possibly through the modulation of oxidative stress.

There were certain limitations to the present study. Although the anti-gout effects of two celery seed extracts, CSAE and CSOL, were demonstrated in two rodent models, the results did not clearly determine which extract exhibited stronger effects. The contents of CSAE and CSOL were systematically detected; however, which component exhibited the anti-gouty arthritis and anti-hyperuricemia properties remains to be determined. Based on the current data, it is difficult to establish quality standards for CSAE and CSOL. Additionally, although the anti-gout effects of CSAE and CSOL were demonstrated to be related to antioxidation and anti-inflammation, the detailed mechanisms require further systematic investigation.

In conclusion, the present study demonstrated that CSAE and CSOL exhibited the effect of suppressing serum UA levels in mice with hyperuricemia and the swelling rates of ankle joints in rats with gouty arthritis, which may be associated with the modulation of XO activity and inflammation response by oxidative stress regulation, providing experimental evidence to support the further evaluation of CSAE and CSOL as agents for gout treatment.

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Availability of data and materials

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

Authors' contributions

DW and NH designed the experiments; SL, LL, HY, XJ and WH performed the experiments; SL, LL and HY processed data; DW, SL and LL wrote the paper; DW and NH revised the paper.

Ethics approval and consent to participate

The experimental animal protocol was approved by the Animal Ethics Committee of Jilin University (Changchun, China; approval no. 20171124).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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