Inhibitory Effects of *Apium graveolens* on Xanthine Oxidase Activity and Serum Uric Acid Levels in Hyperuricemic Mice

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ABSTRACT: Celery (*Apium graveolens*) is traditionally used to treat rheumatism and cardiovascular disorders. Hyperuricemia is considered as a predisposing factor for gout and is also suggested to be associated with coronary artery disease. In the present study, the effect of hydroalcoholic extracts from *A. graveolens* (AGE) against potassium oxonate (PO)-induced hyperuricemia was investigated in mice. AGE (250, 500, and 1,000 mg/kg) or allopurinol (5 mg/kg, as positive control) were orally administrated 1 h after PO injection (250 mg/kg, ip) for two weeks. After that, the serum uric acid level and hepatic xanthine dehydrogenase (XDH) and xanthine oxidase (XO) activities were measured. In addition, the antioxidant activity of AGE was determined by assessment of hepatic lipid peroxidation, *in vivo* and the ferric reducing/antioxidant power assay, *in vitro*. The extract exhibited good capacity to reduce ferric ion to ferrous ion with mean value of 63.8±8.5 μmol/g. The data also showed that oxonate treatment produced a significant increase in serum uric acid level (4.6 vs. 2.3 mg/dL, *P* <0.001), liver XO/XDH activities (*P*<0.01 and *P*<0.001, respectively), and hepatic lipid peroxides levels (about two fold, *P*<0.01), compared to the healthy mice. AGE significantly decreased the serum uric acid level, hepatic XO/XDH activities, and lipid peroxidation, in a dose-dependent manner. Oral administration of 1,000 mg/kg AGE for two weeks reversed the elevated serum uric acid level (2.7 vs. 4.6 mg/dL, *P*<0.001) and significantly inhibited liver XO/XDH activities (*P*<0.001) and diminished hepatic lipid peroxidation (0.45 vs. 0.82 nmol/mg protein, *P*<0.05), compared with hyperuricemic mice. AGE (1,000 mg/kg) per se did not significantly modify these parameters. Our results demonstrated that AGE could reduce the serum uric acid level via inhibition of hepatic XDH/XO and indicated its potential utility as an effective hypouricemic bioactive agent or functional food.

Keywords: *Apium graveolens*, hyperuricemia, xanthine dehydrogenase (XDH), xanthine oxidase (XO), hypouricemic agent

INTRODUCTION

Excessive production of uric acid leads to deposition of monosodium uric acid crystals in soft tissues and joints which are associated with gout (1-3). These crystals cause the pathology of gout by activating the inflammatory responses, resulting in bone and cartilage erosions (4). Oxidative damage is also closely related to the pathogenesis of gout disease (2). Therefore, inhibition of uric acid overproduction, inflammation, and oxidative stress have importance in gout management (2). The most therapeutic strategies to treat hyperuricemia is the development of xanthine oxidase (XO) inhibitors which could effectively reduce the uric acid contents of plasma and urine and decrease tophaceous uric acid deposition (1). Allopurinol, a XO inhibitor, is widely used as a uric acid-lowering agent (5). However, the clinical use of allopurinol is limited because of severe adverse effects such as hepatotoxicity, nephrotoxicity or hypersensitivity reactions. Therefore, searching the new XO inhibitors, in particular from natural sources, is a promising approach (5-8).

*Apium graveolens* Linn. (Apiaceae), commonly known as celery, has a long history of usage in Ayurveda and Unani medicine. *A. graveolens* is widely cultivated in Central Europe, North Africa, North-Western Himalayas, and Western India. The whole plant or its seeds have been used as food or medicine (9,10). Celery has been effective in the prevention of cardiovascular disease (11) and lowering blood pressure (12). It also has antifungal (13), anti-inflammatory (14,15), antioxidant (16), anti-gastric ulcer...
(17), and anticoagulant activities (11). An antirheumatic formulation of the seeds has shown to induce significant arthritic pain relief (9,10).

In the current study, the effect of hydroalcoholic extract of A. graveolens (AGE) on hyperuricemia induced by potassium oxonate (PO) was investigated in mice. Moreover, the activities of XO and xanthine dehydrogenase (XDH) in hepatic tissue were also measured.

MATERIALS AND METHODS

Materials
PO, 2,4,6-tri(2-pyridyl)-s-triazine, ferrous sulfate, ferric chloride, hydrogen peroxide (H$_2$O$_2$), xanthine, nicotinamide adenine dinucleotide (NAD), allopurinol, hydrochloric acid (HCl), and bicinchoninic acid protein assay kit were purchased from Sigma (St. Louis, MO, USA).

Animals
Adult male mice (20–30 g) were provided by the Institutional Animal House and kept under 12/12-h light/dark cycle at 21±2°C with access to water and food ad libitum. The animal experiments were approved by the Institutional Animal Ethics Committee and were in accordance with the Guidelines for the Care and Use of Laboratory Animals of National Institutes of Health (ethics number: IR900613).

Preparation of hydroalcoholic extract of A. graveolens (AGE)
The fresh herb of celery (A. graveolens) of a commercial cultivar was harvested and authenticated by the University’s Herbarium Unit (voucher specimen No. AG-3278-21). The whole plant was washed, dried, and grounded to a fine powder with a blender. Then, the powder (100 g) was soaked in 1,000 mL of 70% ethanol, for 48 h at 4°C with access to water and food ad libitum. The resultant was filtered, and the solvent was removed using rotary evaporator. The residue (yield 11% w/w) was kept at −20°C until use (18,19).

Antioxidant capacity of A. graveolens extract in vitro
The total antioxidant (reducing) power of AGE was determined using the ferric reducing/antioxidant power (FRAP) assay, as described previously. Briefly, 50 μL of the extract was added to 2 mL of freshly prepared and prewarmed (37°C) FRAP reagent (300 mM acetate buffer, 10 mM tripyridyltriazine in 40 mM HCl, and 20 mM ferric chloride in the ratio of 10:1:1) in a test tube and incubated at 37°C for 10 min. The absorbance of the blue colored (ferrous tripyridyltriazine) complex was measured against the reagent blank at 593 nm. The standard curve was constructed using ferrous sulfate solution (100–1,000 μM), and the data were expressed as μmol ferric ions reduced to ferrous form per g dry extract mass (FRAP value). The measurements were performed in triplicate (20,21).

Experimental design
To explore the anti-gout activity of AGE, hyperuricemia was induced in mice using PO, as an uricase inhibitor (22). The mice were randomly divided into seven groups each comprising eight animals and fasted 2 h prior to drug administration. The control mice received saline solution as a vehicle. In hyperuricemic mice, PO (250 mg/kg) was injected intraperitoneally. In treatment groups, AGE (250, 500, and 1,000 mg/kg) or allopurinol (5 mg/kg, as positive control) were orally administrated 1 h before PO (250 mg/kg) injection and then once daily for 14 days. Another group of mice was orally administrated by AGE (1,000 mg/kg), alone for 2 weeks.

Oral gavage was performed using a 1 mL insulin syringe equipped with a ball tipped plastic (flexible) disposable feeding needle to deliver the solution to the back of the throat. A habituation to oral gavage was performed using water. Fresh solutions of AGE at concentrations of 25, 50, and 100 mg/mL in saline solution were prepared and administered at 10 mL/kg (19).

Blood and liver sampling
Mice were euthanized under deep anesthesia 1 h after the final drug administration, and blood samples were drawn from the heart. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 3,000 g for 15 min to obtain the serum. The serum was then stored at −20°C until analyzed. Serum uric acid was determined using alkaline phosphotungstate assay (22).

The livers were quickly removed and chopped on the ice. After freezing in liquid nitrogen, the samples were stored at −70°C until analysis. For determination of activities of XO and XDH, 0.5 g of livers was homogenized on ice in 2.5 mL phosphate buffer (50 mM, pH 7.4). The homogenates were centrifuged at 3,000 g for 10 min at 4°C, the supernatants were then carefully removed and underwent further centrifugation at 15,000 g for 60 min at 4°C. The supernatants were then used for further analyses (22,23).

Measurement of XO and XDH activities
The activities of XO and XDH were measured based on oxidation of xanthine to uric acid using NAD$^+$ (in the case of XDH) or H$_2$O$_2$ (for XO) as the electron acceptors. Briefly, 100 μL enzyme solution in 50 μM phosphate buffer (pH 7.4) was preincubated with 200 μM NAD$^+$ or H$_2$O$_2$ at 37°C for 15 min. Then, 50 μM xanthine was added to the mixture and further incubated for 30 min
at 37°C. After that, the reaction was stopped by adding 0.6 M HCl and the absorbance was monitored at 290 nm (22).

Thiobarbituric acid reactive species (TBARS) measurement
The extent of hepatic oxidative damage in different treatment groups was assessed using thiobarbituric acid (TBA) reagent that forms the pink colored complex with the end products of lipid peroxidation with peak absorbance at 532 nm. In brief, 1 mL of 10% homogenate sample (in phosphate buffered saline) was mixed with 2 mL of trichloroacetic acid (TCA)-TBA-HCl reagent (15% TCA, 0.67% TBA, and 0.25 N HCl) and heated for 45 min in a boiling water bath. After cooling, the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was collected, and the absorbance was read against blank, at 532 nm. The amount of malondialdehyde produced was calculated, using a molar absorption coefficient of \(1.56 \times 10^5 \text{ M}^{-1} \text{cm}^{-1}\) and expressed as nmol/mg protein (21). The protein content of the samples was determined using bicinchoninic acid protein assay kit.

Statistical analysis
Data were presented as mean±standard error of mean (SEM). For data analysis, we used GraphPad Prism 6.01 software (GraphPad Software, La Jolla, CA, USA) and the values were compared using the one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test for multiple comparisons. The \(P<0.05\) was considered statistically significant.

RESULTS

In vitro total antioxidant capacity of AGE by the FRAP assay
The extract exhibited good capacity in reducing ferric ion (Fe\(^{3+}\)) to ferrous ion (Fe\(^{2+}\)) with mean value of 63.8 ±8.5 μmol/g.

AGE significantly and dose-dependently decreased serum uric acid level in hyperuricemic mice
As shown in Fig. 1, serum uric acid level was significantly \((P<0.001)\) increased in the PO animals compared to the control mice. Treatment with AGE markedly decreased the uric acid level from 4.6 mg/dL (PO group) to 2.7 mg/dL (500 mg/kg AGE group) \((P<0.001)\), which is similar to the values of control mice (2.3 mg/dL). Also, in the positive control group, allopurinol caused a significant decrease in the serum uric acid level (0.8 mg/dL). As shown in Fig. 1, AGE by itself did not significantly change the serum uric acid level (2.0 mg/dL).

AGE significantly inhibited hepatic XO and XDH activities in hyperuricemic mice
Fig. 2 and 3 show the effects of AGE and allopurinol on the XO and XDH activities in mice liver. Following PO administration, the XO activity was significantly increased, reached to 134.2%, as compared to the control mice \((P<0.001)\). The increased XO activity was probably responsible for the elevated serum uric acid level. In contrast, AGE significantly and dose-dependently decreased the elevated XO activity to 83.2%, 78.2% \((P<0.001)\), and 49.2% \((P<0.001)\) of control value (for 250, 500, and 1,000 mg/kg AGE, respectively). Treatment with allopurinol (as positive control) also significantly decreased XO activity to 33.2% of control value \((P<0.001)\). On the other hand, AGE (1,000 mg/kg) per se did not significantly modify XO activity.

Fig. 3 presents the liver XDH activity of hyperuricemic mice treated with AGE or allopurinol. Compared with the control group, PO-treated animals had increased levels \((P<0.001)\) of XDH activity, indicating uric acid overproduction. In the same way, AGE significantly decreased XDH activity. For the high-, medium-, and low-dose AGE
groups, XDH activity inhibited to about 80.1%, 97.5%, and 123.5% of control. Allopurinol also caused about a five-fold decrease in the enzyme activity, as compared to PO group (*P<0.001, Fig. 3).

AGE dose-dependently attenuated liver lipid peroxidation in hyperuricemic mice
As illustrated in Fig. 4, TBARS levels were significantly increased (about two fold) in hyperuricemic mice compared to control animals (P<0.01). On the other hand, hyperuricemic mice treated with relatively high doses of the extract showed significantly lower lipid peroxides levels. Hepatic TBARS levels significantly decreased to 0.63±0.08, 0.52±0.06 and 0.45±0.09 nmol/mg protein for hyperuricemic mice treated with 250, 500, and 1,000 mg/kg AGE, respectively (both *P<0.05 compared with the PO group; Fig. 4).

DISCUSSION
Hyperuricemia is considered as a predisposing factor for gout and is also thought to be associated with a number of clinical disorders, including endothelial dysfunction and atherosclerosis, hypertension, coronary artery disease, diabetes, dyslipidemia, and chronic kidney disease. It has been reported that hyperuricemia may increase production of oxygen free radicals, promote lipid peroxidation, and also upregulate proinflammatory mediators (1,2,24-28). It can be caused by uric acid overproduction, decreased renal excretion of uric acid, or both (3). Hyperuricemia can be effectively controlled by inhibiting XO, which is generated from XDH during oxidative stress and inflammatory processes. The enzyme oxidizes hypoxanthine to xanthine and then to uric acid. Therefore, inhibition of uric acid overproduction, inflammation and oxidative stress has considerable values in the management of gout, cardiovascular and metabolic disorders (1,2).

As mentioned earlier, it was shown that the methanol and petroleum ether extracts of celery leaves and seeds could significantly decrease serum uric acid level in rats (7). However, the mechanism underlying the hypouricemic effects including XO and XDH activities were not explored. Therefore, we explored the effects of AGE on hepatic XO and XDH activities and serum uric acid level as well as hepatic lipid peroxidation. We found that the AGE dose-dependently and significantly inhibited hepatic XO and XDH activities, lowered serum uric acid level, and decreased liver lipid peroxidation in PO-induced hyperuricemic mice. Moreover, the extract exhibited good antioxidant capacity in vitro, as measured by FRAP assay, which is consistent with other results (29).

A. graveolens contains furocoumarins (apigravin, celerin, and umbelliferone), flavonoids (apigenin, apiin, kaempferol, and luteolin), phenolic compounds (caffeic acid, p-coumaric acid, and ferulic acid), and tannins (30,31). Celery essential oil has also been shown to have a series of phthalide derivatives (32). Anti-gout activity has been described for phthalide derivatives, phenolic compounds, and tannins (7,33). The hyperuricemic and XO inhibitory activity of apigenin were investigated in several studies. Lin et al. (34) reported that apigenin could interact with the XO active site and had the comparable inhibitory effect as allopurinol in vitro. Flemmig et al. (35) also showed that apigenin could moderately inhibit XO activity in vitro. Apigenin, at dose of 25 mg/kg, also significantly decreased serum uric acid and inhibited liver XO activity by 38.4% in hyperuricemic mice (36). In contrast, Huang et al. (37) indicated that apigenin at doses as high as 700 mg/kg failed to decrease serum uric acid level and liver XO activity of hyperuricemic mice. Luteolin, another major component of celery, also reversibly inhibited XO in a competitive manner, by interacting with the primary amino acid residues located within the active site pocket of XO (38). It was also reported that kaempferol,
another flavonoid found in celery, reversibly inhibited XO by occupying the catalytic center of the enzyme. In addition, luteolin exhibited a strong synergistic effect with kaempferol (39).

Celery, or its constituents, has been shown to have antioxidant and anti-inflammatory effects (30,40), which may be involved in the anti-gout, cardiovascular, hepatic or renal protective effects of the medicinal herb (31). Several studies showed that celery contains high levels of phenolic and flavonoid compounds and exhibits significant antioxidant and free radical scavenging activities in vitro. An extremely significant positive correlation between the antioxidant activity and the contents of total flavonoids or total phenolics was observed (41,42). Popović et al. (43) suggested that the diethyl ether, chloroform, ethyl acetate, n-butanol, and water extracts of celery leaves and roots are good scavengers of hydroxyl (OH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and decrease liposomal peroxidation, which may be due to the presence of flavonoids. Moreover, in vivo experiments revealed antioxidant properties of the extracts by diminishing lipid peroxidation and promoting the antioxidant defense systems in the liver and blood of mice treated with carbon tetrachloride. In the same way, celery seeds showed antioxidant and cyclooxygenase inhibitory effects reflecting the anti-inflammatory action via the presence of phenolic compounds; sedanolide and senkyunolide-N (16). In another study, the methanolic extract of A. graveolens ameliorated liver oxidative stress in adjuvant-induced arthritic rats by reducing the generation of liver free radicals and increasing the liver antioxidant enzyme activity (44). Another study found that celery roots and leaves juices improved the biochemical parameters in rats’ liver homogenate and blood hemolysate samples and revealed protective effects against doxorubicin toxicity (45). In a study by Al-Sa’aidi et al. (46), it was found that the n-butanol extract of celery seed modified the glyceric and insulin levels, ameliorated hepatic lipid peroxidation, and improved intracellular glutathione and antioxidant enzymes (mainly superoxide dismutase, catalase, glutathione transferase, and reductase) in streptozotocin-induced diabetic rats. Antioxidant and cyclooxygenase (COX-I and COX-II) inhibitory activities have also been described for several constituents in the celery plant (16). For example, apigenin, an antioxidant, inhibits the production of hydrogen peroxide and IgE, which are responsible for inflammation and allergic responses (47,48). Apigenin also had inhibitory effects on cyclooxygenase and lipoxygenase (49). Li et al. (50) showed that apiin, the flavonoid isolated from celery leaf, possess remarkable antioxidant activities in vitro and in vivo. Apiin effectively scavenged DPPH, OH, and superoxide (O_2-) free radicals in vitro. In vivo, apiin also significantly decreased TBARS levels and improved the total antioxidant capacity, and enhanced activities of the enzymes superoxide dismutase, glutathione peroxidase and catalase in serum, brain, heart, liver and kidney of male mice.

A. graveolens extracts were also reported to have anti-inflammatory activity via suppression of carrageenan-induced paw edema in rats (51). Ramezani et al. (52) also showed that aqueous and hexane extracts from A. graveolens seeds have remarkable anti-inflammatory and antinociceptive activities in formalin and xylene-induced ear edema tests in mice. The croton-oil ear test in mice showed that the hydroalcoholic extract of celery leaves exerted anti-inflammatory activity with a potency seven times lower than that of indomethacin. The anti-inflammatory activity was attributed to the inhibitory effect of the extract and its major constituent, apiin, on nitric oxide (NO) production and inducible NO synthase expression (14). In addition, two aglycones of a phthalide glycoside and a megastigmane glycoside, isolated from 70% ethanolic extract of A. graveolens, showed potent inhibitory activity against lipopolysaccharide-induced NO production in RAW 264.7 macrophages, in vitro (53). Apiuman, a pectic polysaccharide isolated from fresh celery stalks, also exerted anti-endotoxemic effect in LPS-intoxicated mice, and this effect was shown to be mediated by diminished neutrophils migration, decreased interleukin (IL)-1β and increased IL-10 production (54).

A. graveolens and the flavonoids content, mainly apiin and apigenin, also exhibit healing properties (30). Recently Kooti et al. (19) showed that hydroalcoholic extract of celery leaf could significantly increase the number of sperms, sertoli cells, and primary spermatocyte in the rat. In one study, L-3-n-butylphthalide from celery seeds, significantly reversed memory impairment in APP/PS1 transgenic mice model of Alzheimer’s disease via brain-derived neurotrophic factor/tropomyosin receptor kinase B/phosphoinositide-3-kinase/Akt signaling pathway (55).

In conclusion, the present study demonstrated that hydroalcoholic extract of A. graveolens possesses hypouremic activity in PO-induced hyperuricemia in mice by inhibiting XDH and XO activities. These findings suggest the use of A. graveolens as a dietary supplement or nutraceutical for the treatment of hyperuricemia, but the molecular mechanisms underlying these effects require further investigation.

**ACKNOWLEDGEMENTS**

This work was supported by a grant (900613) from the Vice-Chancellor for Research and Technology, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran.
AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Nuki G, Simkin PA. 2006. A concise history of gout and hyperuricemia and their treatment. Arthritis Res Ther 8: S1.
2. Terkeltaub R. 2010. Update on gout: new therapeutic strategies and options. Nat Rev Rheumatol 6: 30-38.
3. Terkeltaub RA. 2003. Gout. N Engl J Med 349: 1647-1655.
4. Dalbeth N, Haskard D. 2005. Mechanisms of inflammation in gout. Rheumatology 44: 1090-1096.
5. Dalbeth N, Kumar S, Stamp L, Gow P. 2006. Dose adjustment of allopurinol according to creatinine clearance does not provide adequate control of hyperuricemia in patients with gout. J Rheumatol 33: 1646-1650.
6. Chao J, Terkeltaub R. 2009. A critical reappraisal of allopurinol dosing, safety, and efficacy for hyperuricemia in gout. Curr Rheumatol Rep 11: 135-140.
7. Mohamed DA, Al-Okbi SY. 2008. Evaluation of anti-gout activity of some plant food extracts. Pol J Food Nutr Sci 58: 389-395.
8. Mo SF, Zhou F, Lv YZ, Hu QH, Zhang DM, Kong LD. 2007. Hypouricemic action of selected flavonoids in mice: structure-activity relationships. Biol Pharm Bull 30: 1551-1556.
9. Hassanen NHM, Eissa AMF, Hafez SAM, Mosa EAM. 2015. Antioxidant and antimicrobial activity of celery (Apium graveolens) and coriander (Coriandrum sativum) herb and seed essential oils. Int JCurr Microbiol Antimicrob Agents 4: 284-296.
10. Fazal SS, Singla RK. 2012. Review on the pharmacognostical & pharmacological characterization of Apium graveolens Linn. Indo Global J Pharm Sci 2: 36-42.
11. Sowbhagya HB, Srinivas P, Krishnamurthy N. 2010. Effect of enzymes on extraction of volatiles from celery seeds. Food Chem 120: 230-234.
12. Gharouni M, Sarkarati A. 2000. Application of Apium graveolens in treatment of hypertension. Tehran Univ Med J 58: 67-69.
13. Momin RA, Nair MG. 2001. Mosquitocidal, nematocidal, and antifungal compounds from Apium graveolens L. seeds. J Agric Food Chem 49: 142-145.
14. Mencherini T, Cau A, Bianco G, Della Loggia R, Aquino RP, Autore G. 2007. An extract of Apium graveolens var. dulce leaves: structure of the major constituent, apiin, and its anti-inflamatory properties. J Pharm Pharmacol 59: 891-897.
15. Lewis DA, Tharib SM, Veitch GBA. 1985. The Anti-inflammatory activity of celery Apium graveolens L. (Fam. Umbelliferae). Int J Crude Drug Res 23: 27-32.
16. Momin RA, Nair MG. 2002. Antioxidant, cyclooxygenase and topoisomerase inhibitory compounds from Apium graveolens Linn. seeds. Phytomedicine 9: 312-318.
17. Al-Howiriny T, Alsheikh A, Alqasoumi S, Al-Yahya M, ElTahir K, Rafatullah S. 2010. Gastric antiulcer, antisecretory and cytoprotective properties of celery (Apium graveolens) in rats. Pharm Biol 48: 786-793.
18. Zhou K, Zhao F, Liu Z, Zhuang Y, Chen L, Qiu F. 2009. Triterpenoids and flavonoids from celery (Apium graveolens). J Nat Prod 72: 1563-1567.
19. Kooti W, Mansouri E, Ghasemiboroon M, Harizi M, Ashtary-Larky D, Afrisham R. 2014. The effects of hydroalcoholic extract of Apium graveolens leaf on the number of sexual cells and testicular structure in rat. Jundishapur J Nat Pharm Prod 9: e17532.
20. Nićetin M, Lončar B, Filipović V, Knežević V, Kuljanin T, Pezo L, Gorjanović S. 2015. Changes in antioxidant activity and phenolic content of celery leaves and root during osmotic treatment. J Hyg Eng Des 13: 75-81.
21. Sadeghnia HR, Shaterzadeh H, Forouzanfar H, Hosseinzadeh H. 2017. Neuroprotective effect of safranal, an active ingredient of Crocus sativus, in a rat model of transient cerebral ischaemia. Folia Neuropathol 55: 206-213.
22. Haidari F, Keshavarz SA, Mohammad Shahi M, Mahboob SA, Rashidi MR. 2011. Effects of parsley (Petroselinum crispum) and its flavonol constituents, kaempferol and quercetin, on serum uric acid levels, biomarkers of oxidative stress and liver xanthine oxidoreductase activity in oxoxante-induced hyperuricemic rats. Iran J Pharm Res 10: 811-819.
23. Haidari F, Keshavarz SA, Rashidi MR, Shahi MM. 2009. Orange juice and hesperetin supplementation to hyperuricemic rats alter oxidative stress markers and xanthine oxidoreductase activity. J Clin Biochem Nutr 45: 285-291.
24. Hussain S, Iqbal T. 2015. Association of serum uric acid with family history of coronary artery disease in a Pakistani population. Balkan Med J 32: 235-236.
25. Balakumar P, Sharma R, Kalia AN, Singh M. 2009. Hyperuricemia: is it a risk factor for vascular endothelial dysfunction and associated cardiovascular disorders?. Curr Hypertens Rev 5: 1-6.
26. Billiet L, Doaty S, Katz JD, Velasquez MT. 2014. Review of hyperuricemia as new marker for metabolic syndrome. ISRN Rheumatology 2014: 852954.
27. Kuvabara M. 2016. Hyperuricemia, cardiovascular disease, and hypertension. Pulse 3: 242-252.
28. Puddu P, Puddu GM, Cravero E, Vizioli L, Muscar A. 2012. Relationships among hyperuricemia, endothelial dysfunction and cardiovascular disease: molecular mechanisms and clinical implications. J Cardiol 59: 235-242.
29. Fernandes RP, Trindade MA, Tonin FG, Lima CG, Pugine SM, Munekata PE, Lorenzo JM, de Melo MP. 2016. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. J Food Sci Technol 53: 451-460.
30. Kooti W, Ali-Akbari S, Asadi-Samani M, Ghadery H, Ashtary-Larky D. 2015. A review on medicinal plant of Apium graveolens. Adv Herb Med 1: 48-59.
31. Kooti W, Daraei N. 2017. A review of the antioxidant activity of celery (Apium graveolens L.). J Evid Based Complementary Altern Med 22: 1029-1034.
32. Bjeldanes LF, Kim IS. 1977. Phthalide components of celery essential oil. J Org Chem 42: 2333-2335.
33. Owen PL, John T. 1999. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. J Ethnopharmacol 64: 149-160.
34. Lin CM, Chen CS, Chen CT, Liang YC, Lin JK. 2002. Molecular modeling of flavonoids that inhibits xanthine oxidase. Biochem Biophys Res Commun 294: 167-172.
35. Flemming J, Kuchta K, Arnhold J, Rauwald HW. 2011. Olea europaea leaf (Ph.Eur.) extract as well as several of its isolated phenolics inhibit the gout-related enzyme xanthine oxidase. Phytotherapy 18: 561-566.
36. de Souza MR, de Paula CA, Pereira de Resende ML, Grabe-Guimarães A, de Souza Filho JD, Saúde-Guimarães DA. 2012. Pharmacological basis for use of Lychnophora trichoacantha in gouty arthritis: anti-hyperuricemic and anti-inflammatory effects of its extract, fraction and constituents. J Ethnopharmacol 142: 845-850.
37. Huang J, Wang S, Zhu M, Chen J, Zhu X. 2011. Effects of genistein, apigenin, quercetin, rutin and astilbin on serum uric acid levels and xanthine oxidase activities in normal and
37. Woods JA, Jewell C, O'Brien NM. 2001. Sedanolide, a natural phthalide from celery seed oil: effect on hydrogen peroxide and \textit{tert}-butyl hydroperoxide-induced toxicity in HepG2 and CaCo-2 human cell lines. \textit{In Vitro Mol Toxicol} 14: 233-240.

38. Al-Hindawi MK, Al-Deen IH, Nabi MH, Ismail MA. 1989. Anti-inflammatory activity of some Iraqi plants using intact rats. \textit{J Ethnopharmacol} 26: 163-168.

39. Ramezani M, Nasri S, Yassa N. 2009. Antinociceptive and anti-inflammatory effects of isolated fractions from \textit{Apium graveolens} seeds in mice. \textit{Pharm Biol} 47: 740-743.

40. Xiang J, Pan J, Chen F, Zheng L, Chen Y, Zhang S, Feng W. 2014. L-3-\textit{n}-butylphthalide improves cognitive impairment of APP/PS1 mice by BDNF/TrkB/PI3K/AKT pathway. \textit{Int J Clin Exp Med} 7: 1706-1713.