Reduction of the Plasma Uric Acid Level in Potassium Oxoate-Induced Hyperuricemic Rats by Heat-Concentrated *Prunus mume* Fruit Extract Containing Three Chlorogenic Acid Isomers

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Abstract: Gout is a common rheumatic disease, resulting from hyperuricemia. *Prunus mume* fruit extract, after being heat-concentrated named mei extract, was empirically found to reduce the risk of gout. While neochlorogenic acid was found as the predominant phenolic compound in the fresh juice of *Prunus mume*, neochlorogenic acid, chlorogenic acid, and cryptogenic acid were detected as the major phenolic compounds in the mei extract. In vitro testing showed that all the three chlorogenic acid isomers exhibited comparable inhibitory activities on xanthine oxidase. The hypouricemic effects of the mei extract were evaluated in potassium oxonate-induced hyperuricemic rats. Oral administrations of the mei extract significantly reduced the plasma uric acid level in hyperuricemic rats, but did not elevate the urinary uric acid level. The results provide in vivo evidence for the anti-hyperuricemic effects of mei extract for the first time, rationalize its therapeutic usage for the treatment of hyperuricemia and gout, and propose chlorogenic acid isomers as the active ingredients. Mei extract seems to be a potential natural functional food product.

Keywords: chlorogenic acid isomer; hyperuricemia; mei extract; *Prunus mume*; xanthine oxidase

1. Introduction

Hyperuricemia, characterized by high blood uric acid, results from uric acid overproduction in the liver and its ineffectual excretion from the kidney. It is defined as when serum urate reaches saturation point at 6.8 mg/dL, and is the key risk factor of gout. Gout is a common rheumatic disease associated with monosodium urate crystallization in joints and connective tissues, leading to acute inflammatory arthritis [1]. Epidemiology studies have indicated that the consumption of purine-rich foods, such as meat and seafood, significantly increases the risk of hyperuricemia, whereas the intake of fruit, coffee, and red wine substantially decreases the risk [2].

Three main classes of urate-lowering drugs are available: xanthine oxidase (XO) inhibitors, that inhibit urate production; uricosurics, that normalize urate excretion; and recombinant uricases, that catalyze urate to more hydrophilic, readily excretable allantoin [3]. XO, the key enzyme for uric acid formation, converts hypoxanthine into xanthine, and xanthine into uric acid at the end of purine metabolism [4]. In most mammals, uric acid is converted into allantoin by urate oxidase and excreted via urea. In contrast, humans lost urate oxidase at an early stage of their evolution, and the sparingly soluble uric acid is the final product of purine catabolism [5]. Being an isostere of hypoxanthine and xanthine, allopurinol is a suitable inhibitor of XO, and used as a drug to treat hyperuricemia and gout. However, adverse effects, such as gastrointestinal distress, worsening renal functions,

and hypersensitivity were reported for allopurinol, when used as an anti-hyperuricemia drug [6]. Therefore, there remains a need to search for superior anti-hyperuricemia compounds from natural sources, particularly those from food and fruit, with no or fewer side effects.

Prunus mume, is a Rosaceous family member widely cultivated in Mainland China, Taiwan, Japan, and Korea. The fruit is designated 'mei' in Mandarin, 'ume' in Japanese, or 'maesil' in Korean. The chemical constituents of mei fruit comprise phenylpropanoid sucrose esters, flavonoids, organic acids, terpenes, sterols, lignans, furfurals, benzyl glycosides, cyanogenic glycosides, and alkaloids [7]. Due to the presence of cyanogenic glucosides, mei fruit is not readily edible without post-harvest treatment. Derived products, such as vinegar, liquor, pickled fruit, and heat-concentrated juice of the fruit (named mei extract), are available for culinary usages and medicinal applications, to alleviate hyperuricemia, fever, chronic cough, fatigue, diarrhea, and abdominal pain caused by infection [8,9]. Therefore, mei extract products have been consumed as folk medicines in East Asia.

The methanol extract of *Prunus mume* fruit has been demonstrated to exhibit an anti-hyperuricemic effect in an animal model [9]. The results showed that the anti-hyperuricemic activity resulted from the inhibition of XO activities in the liver; the authors further speculated that triterpenoids might be the active ingredients, as the methanol extract of *Prunus mume* fruit was used in the animal study. However, no convincing data were provided to support this speculation. Focusing on the anti-hyperuricemia effect, this study aimed to compare the chemical constituents of fresh *Prunus mume* juice and mei extract, to investigate the XO inhibitory activities of the major phenolic compounds in mei extract, and to evaluate the anti-hyperuricemic effects of mei extract in an animal model.

2. Materials and Methods

2.1. Chemicals and Materials

The acetic acid and acetonitrile used in the high-performance liquid chromatography (HPLC) were purchased from ECHO Chemical Co. (Miaoli, Taiwan). Chlorogenic acid, dimethyl sulfoxide (DMSO), xanthine oxidase from bovine milk, allopurinol, and xanthine were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Neochlorogenic acid and cryptochlorogenic acid were purchased from Wuhan ChemFaces Biochemical Co. (Wuhan, Hubei, China). Potassium oxonate was purchased from Combi-Blocks Inc. (Dan Diego, CA, USA). Fruits of *Prunus mume* was collected from the Qing San Orchard, in Shuili Township, Nantou County, Taiwan in March 2020.

2.2. Mei Extract Production and Crude Extraction

Fresh fruits of *Prunus mume* (10 kg) were cored, juiced, and kept in a 100 °C water bath with constant stirring until being concentrated approximately 10-fold. Subsequently, the mei extract (10 g) was redissolved in water (100 mL) and centrifuged. The supernatant was filtered through a 0.45 μm polypropylene membrane filter (Pall Corporation, Glen Cove, NY, USA) for the following analyses.

2.3. HPLC Analysis

The chemical compounds were analyzed with a C18 column (Syncronis, Thermo Scientific, Waltham, MA, USA) in the HPLC system coupled to a Model 600E photodiode array detector (Waters Corporation, Milford, MA, USA) according to the protocol described in a previous study [10]. The ingredients were eluted with mixture solutions of (A) water with 0.5% acetic acid and (B) acetonitrile. The flow rate was set at 1 mL/min. The gradient was as follows: 0–20 min, linearly gradient from 5% to 10% B; 20–40 min, linearly gradient from 10% to 15% B; 40–60 min, linearly gradient from 15% to 25% B; 60–70 min, linearly gradient from 25% to 5% B. The absorbance was detected at a wavelength of 320 nm.

2.4. Mass Spectrometric Analysis

The chromatographic condition was the same as that of the HPLC analysis. An LTQ linear ion trap tandem mass spectrometer (Thermo Electron, San Jose, CA, USA) was equipped with an electrospray ionization interface and connected to a Surveyor LC system (Thermo Electron, San Jose, CA, USA). Mass spectrometric analysis was conducted using the methods described previously with slight modifications [11]. The mass spectra were obtained with negative electrospray ionization (ESI) mode. The flow rates of the sheath gas, auxiliary gas, and sweep gas were set at 50, 13, and 3 arbitrary units, respectively. The heated capillary temperature was set at 300 °C, and the collision energy was 25 eV. The first scan was operated in full-scan mode ranging from m/z 200 to 800. The second scans were data-dependent MSn scans with relative collision energy of 27%. The highest intensity ion of the previous scan was chosen as the precursor ion for the successive MS/MS scans.

2.5. Xanthine Oxidase Inhibitory Assay

The XO inhibitory activity was assayed spectrophotometrically with 96-well plates (Corning Incorporated, Corning, NY, USA), according to the procedure reported previously [4]. The assay mixture consisted of test solution (50 µL), phosphate buffer (70 mM, pH 7.5, 35 µL), and enzyme solution (0.01 units/mL in phosphate buffer, 30 µl). The mixture was pre-incubated at 25 °C for 15 min. Subsequently, substrate solution (150 µM xanthine in phosphate buffer, 60 µL) was added to initiate the reaction. The assay mixture was incubated at 25 °C for 30 min. HCl (1 N, 25 µL) was added to quench the reaction, and the absorbance at 290 nm was measured with a Tecan infinite 200 PRO spectrophotometer (Tecan, Männedorf, Switzerland). A blank was prepared in a similar way, but the enzyme solution was the last added. One unit of XO was defined as the amount of enzyme required to produce 1 µmol of uric acid/min at 25 °C. The XO inhibitory activity was calculated as $(1 - B/A) \times 100\%$, where B and A were the enzyme activities with and without the test materials.

2.6. Animals

Thirty-five Sprague–Dawley rats (12 weeks old) were purchased from BioLASCO, Taiwan Co., Ltd. (Taipei, Taiwan), and acclimated for 1 week before the experiment. Following the environmental conditions described previously [12], three to four animals were housed per cage and maintained at 23 ± 2 °C, $60 \pm 10\%$ humidity, with a 12 h light/dark cycle. The rats had free access to regular rat diet (LabDiet 5001) and purified water. The animal experiment was approved by the Institutional Animal Care and Use Committee of the National Chung-Hsing University (IACUC Approval Number: 108–109).

2.7. Hyperuricemia Model and Drug Administration

A hyperuricemia animal model induced by potassium oxonate was adopted from previous studies [13,14], with slight modifications. The rats were randomly divided into five groups, (1) control group: distilled water; (2) hyperuricemia model group: potassium oxonate (250 mg/kg) dissolved in distilled water; (3) positive control group: hyperuricemic rats treated with allopurinol (5 mg/kg); (4) low dosage of mei extract group: hyperuricemic rats treated with 263 mg/kg of mei extract; (5) high dosage of mei extract group: hyperuricemic rats treated with 526 mg/kg of mei extract. Rats were treated with allopurinol or mei extract 1 h after potassium oxonate induction. All agents were given by oral administration once daily for seven consecutive days. Samples of blood and urine were collected 1 h after the last administration on the fifteenth day.

2.8. Blood and Urine Collection and Determination of Uric Acid Content

Before sacrifice, rats were housed in metabolic cages for 8 h to collect fasting urine, which was measured with a graduated cylinder (Sigma-Aldrich, St. Louis, MO, USA). After sacrifice, blood samples were collected by cardiac puncture and allowed to clot for at least 1 h at room temperature. Serum was collected after centrifugation ($3000 \times g$, 4 °C) for 10 min.

The serum and urine samples were delivered to the Union Clinical Laboratory (Taichung, Taiwan) for biochemical examinations. The content of uric acid in the serum or urine was determined by using the Siemens ADVIA Chemistry XPT System (Siemens Healthineers, Erlangen, Germany). The fractional excretion of uric acid (FEUA) was calculated using the following equations:

$$FEUA\ (\%) = (UUA \times SCr)/(SUA \times UCr) \times 100$$

2.9. Statistical Analysis

The data were presented as mean values $\pm$ standard error of the mean (SEM). Statistical calculations were performed by SPSS. The significance of differences was determined by analysis of variance (ANOVA) and unpaired t-test. A level of $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Chlorogenic Acid Isomers in Fresh Prunus mume Juice and Mei Extract

Three chlorogenic acid isomers, neochlorogenic acid, chlorogenic acid and cryptogenic acid, were comparably detected in heat-concentrated mei extract, while neochlorogenic acid was found as the only major constituent in the fresh juice of the *Prunus mume* fruit by comparison with authentic compounds in the HPLC analysis (Figure 1). The contents of the three chlorogenic acid isomers in the juice of the *Prunus mume* fruit and mei extract are listed in Table 1. Overall, the total content of chlorogenic isomers increased from 0.62 ± 0.02 to 2.22 ± 0.07 mg per gram when the *Prunus mume* juice was heat-concentrated to mei extract. This result indicates that the 10-fold heat concentration elevated the content of chlorogenic acid isomers by 3.6-fold (2.22 mg/g $\div$ 0.62 mg/g = 3.58), which also implies that 36% (2.22 mg/g $\div$ (0.62 mg/g $\times$ 10)) of the chlorogenic acid isomers from the fresh juice remained, while the rest might be degraded during the heating process.

Table 1. Quantification of chlorogenic acid isomers in fresh juice of *Prunus mume* fruit (cored), and heat-concentrated mei extract. Data are presented as mean $\pm$ SEM (n = 3) milligram per gram of juice or mei extract.

	Neo-CGA	**CGA**	**Crypto-CGA**	**Total**
Fresh juice of cored fruit	0.46 ± 0.01	0.16 ± 0.01	ND	0.62 ± 0.02
Heat-concentrated mei extract	0.90 ± 0.03	0.62 ± 0.01	0.70 ± 0.03	2.22 ± 0.07

ND: not detectable.

The chemical structures of the three chlorogenic acid isomers were further verified by comparison with mass spectrometry data reported formerly (Figure 2) [15,16]. Their UV absorption spectra showed a maximum wavelength at 323.2 nm, and ESI-MS in negative ion mode yielded $[M - 1]^-$ at m/z 353. The product ions of $[M - 1]^-$ gave diagnostic fragmentation patterns to distinguish the chlorogenic acid isomers. The peak at 22 min was assigned as neochlorogenic acid by fragmentation m/z 353 in MS1 and m/z 173.0, 179.0, and 191.0 in MS2, where m/z 191.0 was predominant, and m/z 179.0 had a relative intensity above 75% of the base peak. The peak at 33 min was assigned as chlorogenic acid by m/z 353 in MS1 and m/z 191 in MS2. The peak at 35 min was assigned as cryptochlorogenic acid by m/z 353 in MS1 and m/z 173.0, 179.0 and 191.0 in MS2, where the fragment ion at m/z 173.0 was in the highest abundance, and m/z 179.0 showed a relative intensity above 70% of the base peak.

3.2. In Vitro XO Assay

The XO inhibitory activities of the three chlorogenic acid isomers at 1 mM were measured in vitro, and are shown in Figure 3. The results show that all the three chlorogenic acid isomers exhibited XO inhibitory activities. The inhibitory activity of chlorogenic acid was found to be slightly lower than that of neochlorogenic acid or cryptochlorogenic acid.

No significant difference was detected between the inhibitory activity of neochlorogenic acid and that of cryptochlorogenic acid.
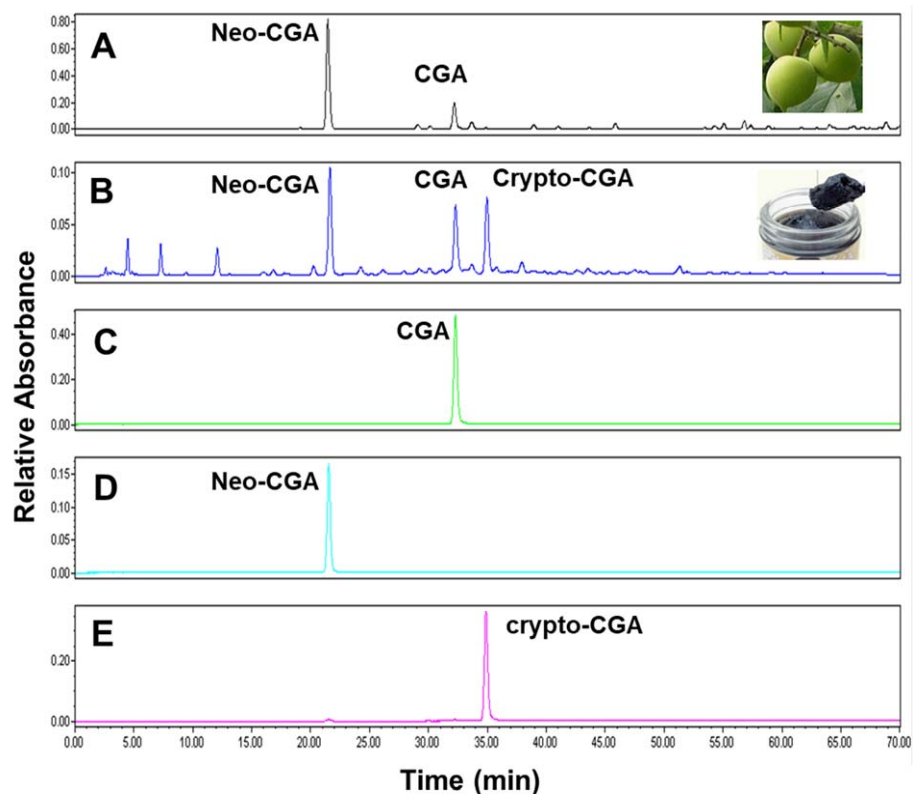
Figure 1. HPLC profiles (detected at 320 nm) of the chemical constituents in fresh juice of *Prunus mume* fruit (**A**) and mei extract (**B**). Three major peaks, neochlorogenic acid (neo-CGA), chlorogenic acid (CGA), and cryptochlorogenic acid (crypto-CGA) are labeled. Authentic standard compounds of CGA, neo-CGA, and crypto-CGA are shown in (**C–E**).
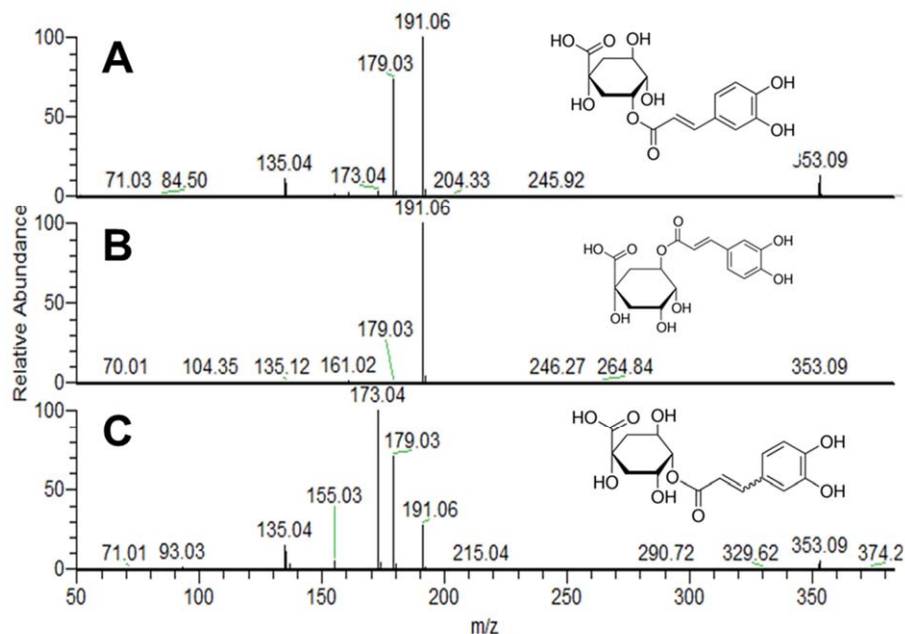
Figure 2. MS^2 of $[M-H]^-$ ions and chemical structures of neochlorogenic acid (**A**), chlorogenic acid (**B**) and cryptochlorogenic acid (**C**).
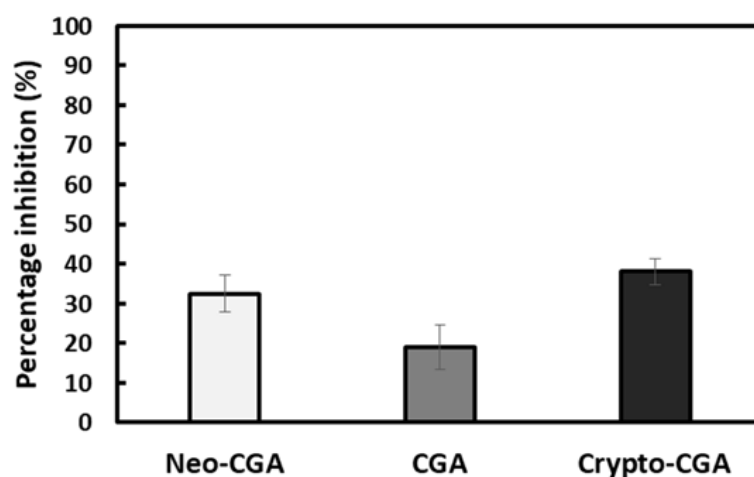
Figure 3. XO inhibitory activities of neochlorogenic acid, chlorogenic acid and crypto-chlorogenic acid. Data are presented as mean $\pm$ SEM (n = 6).

3.3. Effects of Mei Extract on Serum and Urinary Uric Acid Levels in Hyperuricemic Rats

The effects of mei extract and allopurinol on the serum uric acid level of the potassium oxonate-induced hyperuricemic rats were detected (Figure 4). The administration of potassium oxonate (PO) significantly elevated the serum uric acid content compared to the normal control (NC) group. The administration of mei extract or allopurinol successfully reduced the serum uric acid level elevated by potassium oxonate treatment in the rats. The high dosage of mei extract showed a better anti-hyperuricemia effect than the low dosage of mei extract, and its reduction of the serum uric acid level was found to be statistically significant when compared with the PO group. However, the difference between the reduction of the serum uric acid level in the rats treated with the high dosage of mei extract, and that in the rats treated with the low dosage of mei extract, was not statistically significant at $p < 0.05$. Possibly, the uptake of chlorogenic acid isomers into the circulation system had already reached a plateau when the rats were fed with the low dosage of mei extract.
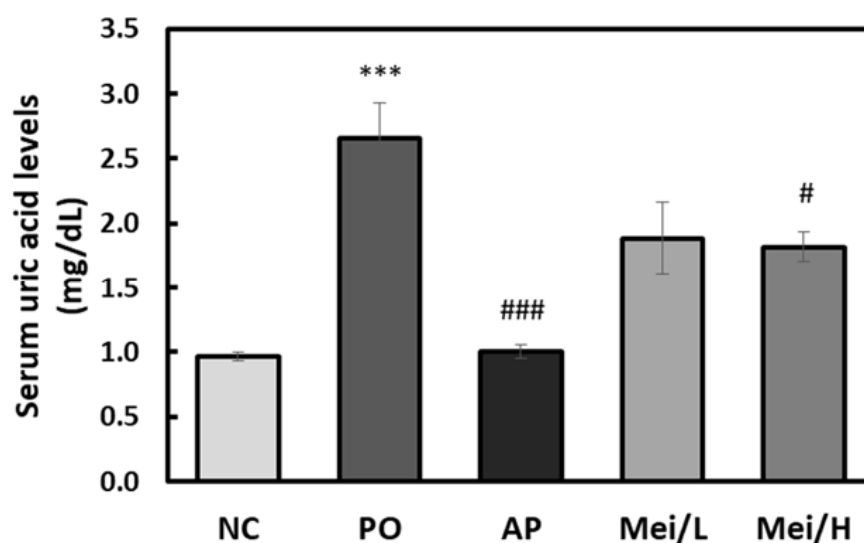
Figure 4. Effect of mei extract on serum uric acid levels of potassium oxonate (PO)-induced hyperuricemic rats. AP represents treatment with allopurinol. Mei/L and Mei/H represents treatment with low and high dosage of mei extract, respectively. Data are presented as mean $\pm$ SEM (n = 5–6). *** $p < 0.001$ compared with normal control (NC) and # $p < 0.05$, ### $p < 0.001$ compared with PO group.

The effects of mei extract and allopurinol on the urine uric acid level of potassium oxonate-induced hyperuricemic rats were examined (Figure 5). Both the urine uric acid level and the hour urine output value showed no significant differences among the examined groups. The 8-h urine uric acid excretion level of the PO group was not significantly different from the NC group. Treatment with allopurinol or mei extract did not alter the 8-h urine uric acid level significantly in hyperuricemic rats. The results indicate that the anti-hyperuricemic effect of mei extract was likely due to XO inhibition but not uricosuric effects. In the PO-induced hyperuricemic model, urine samples were commonly collected 1 h after the last administration [9], or for 5–24 h in metabolic cages [13,17]. Thus, rat urine samples were collected for 8 h to analyze the uric acid concentration and urine volume in this study. It is expected that similar results, showing no statistical significance in the uric acid excretion level, would be detected if the urine collection period was extended to 24 h.
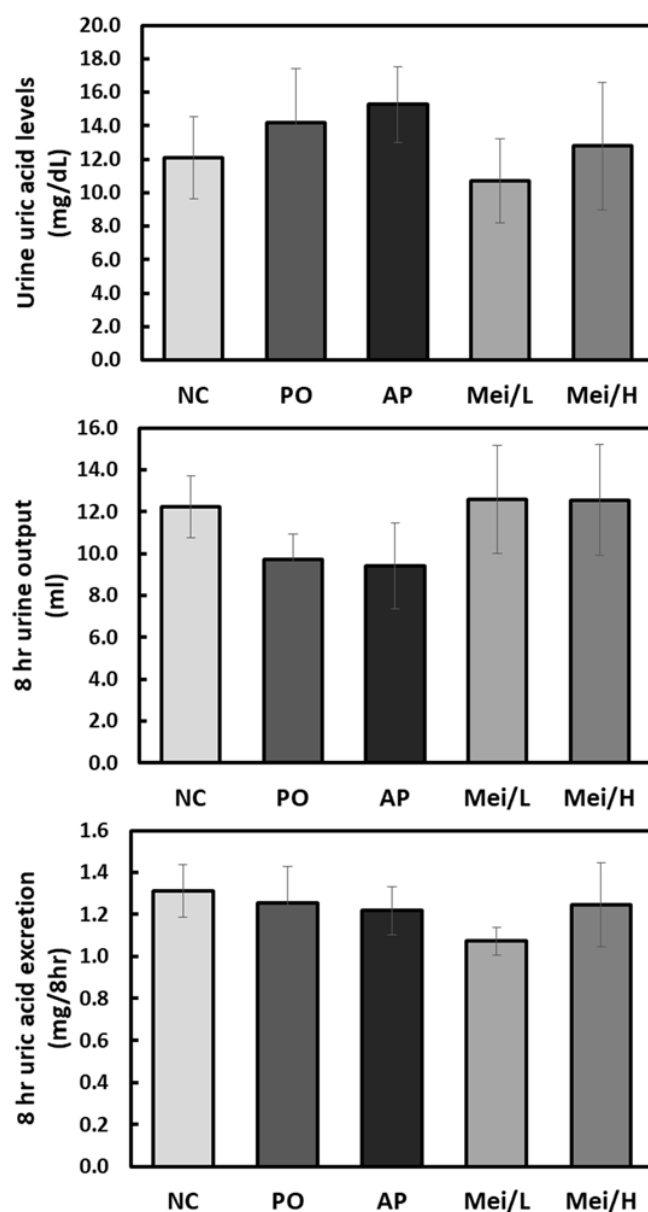
Figure 5. Effects of mei extract on urine uric acid, urine output, and 8-h uric acid excretion levels of potassium oxonate (PO)-induced hyperuricemic rats. NC designates the normal control group, and AP represents treatment with allopurinol. Mei/L and Mei/H represents treatment with low and high dosage of mei extract, respectively. Data are presented as mean $\pm$ SEM (*n* = 5–6). No statistically significant difference is observed between any two groups.

The urine uric acid to creatinine ratio (UUA/UCr) of the PO group was higher in comparison with the NC group (Figure 6). Nevertheless, their difference was not statistically significant at $p < 0.05$. It was noticed that the allopurinol and mei extract groups had comparable UUA/UCr values, which were lower than that of the PO group although the difference was not significant. The values of fractional excretion of uric acid (FEUA) in all the groups were below 10%. Noticeably, the FEUA level in the PO group was significantly lower than that in the NC group. It seemed that the administration of allopurinol significantly elevated the FEUA level, whereas the effects of the administration of mei extract were not as obvious.
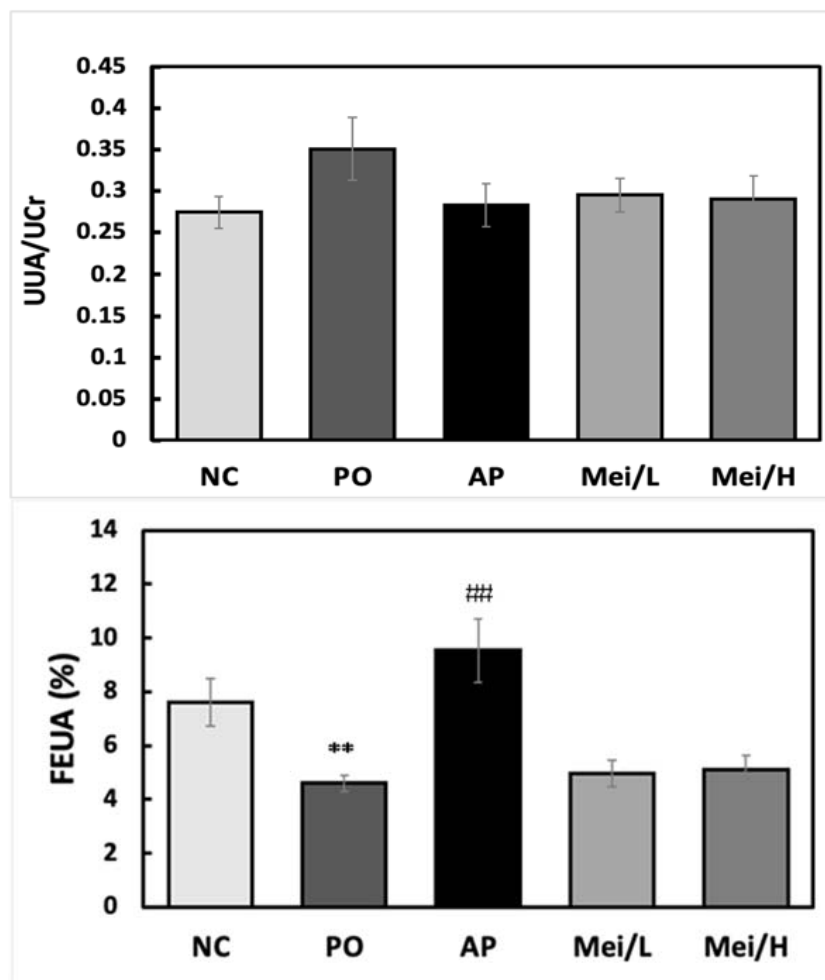
Figure 6. Effects of mei extract on urine uric acid to creatinine ratio (UUA/UCr) and fractional excretion of uric acid (FEUA) of potassium oxonate (PO)-induced hyperuricemic rats. NC designates the normal control group, and AP represents treatment with allopurinol. Mei/L and Mei/H represents treatment with low and high dosage of mei extract, respectively. Data are presented as mean ± SEM (n = 5–6). ** $p < 0.01$ compared with the NC group and ## $p < 0.01$ compared with PO group.

4. Discussion

In this study, mei extract was produced from fresh fruit juice by 10-fold concentration under heat. Three abundant phenolic compounds, neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid, were identified in the mei extract (Figure 1). As neochlorogenic acid was present as the only major phenolic compound in the fresh juice, the presence of abundant chlorogenic acid and cryptogenic acid in the mei extract was presumably derived from isomerization of neochlorogenic acid during the heating concentration process. The relative contents of the three isomers in the heat-concentrated mei extract were found in the order of neochlorogenic acid > cryptogenic acid > chlorogenic acid. This result was

in accordance with a previous computational thermodynamic investigation, suggesting that the order of structural stability was a consequence of varied O H···O chains with hydrogen bonds [18].

The three identified chlorogenic acid isomers, neochlorogenic acid, chlorogenic acid and cryptogenic acid, are phenolics with reputed antioxidant, anti-inflammatory, and anti-hyperuricemic activities [19]. Chlorogenic acid and its isomers have been reported to be present in several natural sources, such as *Coffea arabica* [20]. Fortunately, the conversions of chlorogenic acid and its isomers in the mei extract were not so complex as those found in coffee beans. Among the commonly observed chemical transformations in *Coffea arabica*, including acyl migration, trans-cis isomerization, dehydration, and epimerization, only acyl migration was detected in mei extract in the present study. The major constituent Neo-CGA in the fresh juice of the *Prunus mume* fruits was gradually transformed to Crypto-CGA and CGA through the acyl migration mechanism under the heating process for the preparation of the mei extract.

It has been demonstrated that chlorogenic acid could alleviate PO-induced hyperuricemia in an animal model [21]. Chlorogenic acid markedly reduced the serum uric acid level by inhibiting XO, but not elevating the urinary uric acid level. The authors concluded that the main anti-hyperuricemic mechanism of chlorogenic acid could result from its inhibition of XO activity, which is consistent with our results. Furthermore, the authors illustrated that chlorogenic acid could ameliorate monosodium urate (MSU)-induced gouty inflammation in rats. MSU crystals might stimulate macrophages and neutrophils, leading to a rapid rise in pro-inflammatory cytokines and acute inflammatory responses. Their experimental data suggested that chlorogenic acid might suppress symptoms of inflammation (paw swelling in rats), by reducing inflammatory cell infiltration in the connective tissues and down-regulating the production of IL-1β, IL-6, and TNF-α.

As illustrated in the XO inhibitory assay (Figure 3), all the three chlorogenic acid isomers exhibited inhibitory activities. The results were also in agreement with data published previously [22]. According to the current investigation, it seems that the anti-hyperuricemic effect of the mei extract can be attributed to inhibition of XO in the liver by chlorogenic acid isomers, but not the facilitation of uric acid excretion. The contents of the three chlorogenic acid isomers in the mei extract were compromised by heat degradation at 100 °C; the 10-fold concentration by heating led to an only 3.6 times, instead of 10 times, increase in the total content of chlorogenic acid isomers. Obviously, the therapeutic effect of the mei extract as an anti-hyperuricemic food supplement was substantially reduced using the current production conditions. An improved concentration process, with a higher yield of chlorogenic acid isomers, should be designed economically.

In the 14-day animal experiment, the elevation of serum uric acid induced by potassium oxonate was significantly suppressed by the treatment with mei extract (Figure 4). This result is in agreement with a previous study evaluating the effects of a methanolic extract of the *Prunus mume* fruit on hyperuricemic mice in a 7-day assessment, though the authors proposed that triterpenoids might be the active ingredients [9]. As chlorogenic acid isomers in the *Prunus mume* fruit should be largely extracted out by methanol, the putative active ingredients in the methanolic extract for the anti-hyperuricemic effects in mice were assumed to be chlorogenic acid isomers. Whether any unidentified triterpenoids in the *Prunus mume* fruit might also be active ingredients for its anti-hyperuricemic effects remains to be further verified. Moreover, it has been reported that chlorogenic acid exhibited anti-hyperuricemic activity, inhibited liver XO activity, and promoted reduction of inflammation at a dose of 10 mg/kg in Swiss mice in a 3-day experiment [19]. The dosage used in the mice was roughly equivalent to 5 mg/kg of chlorogenic acid for rats, which was 4–5 times higher than that used in our animal study. However, the longer experimental period in our study might compensate for the dosage effect.

Potassium citrate is clinically used as a urinary alkalinizing agent and can be prescribed to alleviate renal uric acid stones by increasing the solubility of urate crystals [23]. It has been demonstrated that water extracts of the lemon fruit lowered the serum uric acid level

in an 11-day mice experiment, independent of XO inhibition [24]. Potassium citrate at a dose of 5 mg/kg mice was found to exhibit significant anti-hyperuricemic effects; in contrast, citric acid was found to be rather ineffective [25]. This dosage in mice was equivalent to potassium 0.96 mg/kg rat (5 mg × (3 × 39.1 g/mole ÷ 306.39 g/mole) × 0.5). In the heat-concentrated mei extract, the potassium content was approximately 29.27 mg/g, while the citric acid content was 456.02 mg/g on average [26]. The effective dose of the mei extract, at 526 mg/kg, used in this study would theoretically supply citric acid of 240 mg/kg rat (456.02 mg/g × 0.526 g/kg), and potassium 15.4 mg/kg rat (29.27 mg/g × 0.526 g/kg). Whether the potassium content (15.4 mg/kg) in the company of excess citric acid (240 mg/kg) in the mei extract is able to exhibit anti-hyperuricemic effects by urinary alkalinization remains to be investigated.

A previous study has compared the hypouricemic effects of allopurinol (an XO inhibitor) and benzbromarone (a uricosuric agent) in PO-pretreated rats in a 14-day animal experiment [27]. Compared with the PO group, the serum uric acid levels of the allopurinol and benzbromarone groups were significantly reduced. In contrast, the urine uric acid level of the benzbromarone group was significantly increased compared with the PO group, whereas the change in the allopurinol group was not significant. The FEUA of the allopurinol group was not significantly different from the PO group, whereas that of benzbromarone was increased by almost 4-fold. In our study, the serum uric acid lowering effects of allopurinol and mei extract were demonstrated. Nonetheless, the underperformance in urine parameters and FEUA indicates that mei extract could not possibly be used as a uricosuric agent.

5. Conclusions

Isomerization led to the presence of three abundant chlorogenic acid isomers in heat-concentrated mei extract. The hypouricemic effects of the mei extract were observed in potassium oxonate-induced hyperuricemic rats. All the three chlorogenic acid isomers exhibited XO inhibitory activities, and were assumed to be the active ingredients responsible for the anti-hyperuricemic effects of the mei extract. The results provided an empirical association between the consumption of mei extract and the reduction of the risk of gout. Mei extract seems to be a suitable natural product for the development of functional food supplements. However, plentiful loss of chlorogenic acid isomers in the preparation of the mei extract was detected in the current process. The economic production of mei extract may be optimally developed by tracking the contents of chlorogenic acid isomers during its manufacturing processes. The contents of chlorogenic acid isomers are suggested to be quantified batch by batch for the quality control of mei extract products.

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