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Antihyperuricemic and nephroprotective effects of extracts from *Orthosiphon stamineus* in hyperuricemic mice

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Keywords
adenosine deaminase; hyperuricemia; *Orthosiphon stamineus*; urate transporters; xanthine oxidase

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Abstract

Objectives To investigate the antihyperuricemia and nephroprotective effects of *Orthosiphon stamineus* extracts on hyperuricemia (HUA) mice and explore the potential mechanisms.

Methods *Orthosiphon stamineus* extracts were extracted using 50% ethanol and enriched using ethyl acetate, and characterised utilising UPLC/ESI-MS. A potassium oxonate (PO) induced hyperuricemic mouse model was used to evaluate antihyperuricemia and nephroprotective effects of *O. stamineus* ethyl acetate extracts (OSE).

Key findings Eight constituents from OSE were identified and OSE treatment ameliorated HUA by regulating key indicators of kidney dysfunction and xanthine oxidase, adenosine deaminase activity and urate transporters in hyperuricemic mice. Moreover, in renal histopathology analysis, OSE significantly alleviated kidney injury.

Conclusions These findings demonstrate that OSE has antihyperuricemic and nephroprotective effects on PO-induced HUA mice and those results indicate that OSE could be a safe and effective agent or functional ingredient for treating HUA.

Introduction

As a universal metabolic disease, hyperuricemia (HUA) is an important cause of gout, hypertension, atherosclerosis and cardiovascular disease.[1] HUA is mainly caused by UA overproduction or renal urate underexcretion, and is characterised by urate concentration higher than 6.8 mg/dl.[2] Xanthine oxidase (XOD), which plays an important role in the purine metabolic pathway, can catalyse the last two steps of urate biosynthesis.[3] Adenosine deaminase (ADA) is extensively spread in human tissues, and can irreversibly deaminate adenosine, converting it to the related nucleoside inosine.[4,5] In the current treatment of gout, it is effective in restraining UA generation and maintaining normal UA levels by suppressing XOD and ADA activity.

In humans, approximately 30% of UA excretion occurs in the intestine routes by mechanisms that have not yet been investigated in detail, and urate transporters account for the other 70%. URAT1 (SLC22A12) has been identified and demonstrated to be responsible for renal urate handling and the regulation of circulating urate levels.[6] GLUT9 (SLC2A9) is known as a modulator of UA levels and is strongly associated with the reabsorption of UA.[7] In contrast, OAT1 (SLC22A6) and OAT3 (SLC22A8) participate in the urate excretion process via mediating pericellular cell uptake of urate and transporting organic anions across the basolateral membrane of human proximal renal tubules epithelium.[8] So that these renal urate transporters can be considered as credible drug targets for treating HUA.

Allopurinol (AP) is recognised as a first-line drug for lowering uric acid (UA) levels and has been used clinically for more than 40 years, but it has caused drug allergies, hepatotoxicity and nephrotoxicity.[9] Previous studies have validated that extracts of natural products, such as the ethyl acetate fraction of *Chaenomeles sinensis* (Thouin) Koehne fruit extract, anthocyanins from Purple Sweet Potato

(*Ipomoea batatas* L.), ethanolic extract of *Lychnophora pinaster* and *Rhizoma Alpiniae officinarum* ethanolic extract,[10–13] are effective antihyperuricemic and nephroprotective agents. Hence, it is necessary to explore supplementary natural products for treating HUA.

The *Orthosiphon stamineus* Benth. (*O. stamineus*) from the family of Labiatae is widely distributed in southeast Asia and has been used as a traditional material medica for many centuries for treating various disorders disease such as chronic nephritis, cystitis, urinary calculi and rheumatoid arthritis.[14] In southern China, the aerial part of this plant is used as a diuretic agent to treat patients infected with urinary inflammatory and lithiasis.[15] Previous study has reported the diuretic and hypouricemic effects of *O. stamineus* methanol extracts in rats, but key indicators of kidney dysfunction and the mechanism of hypouricemic effects were not evaluated. Therefore, based on our previous research on biological activity of natural products, the aim of this study was to explore the antihyperuricemic and nephroprotective effects of *O. stamineus* extracts and the underlying mechanism.[16–18]

Materials and Methods

Reagents and materials

HPLC grade acetonitrile and methanol were acquired from Merck (Darmstadt, Germany). AP, potassium oxonate (PO), caffeic acid and rosmarinic acid were obtained from Aladdin Chemistry (Shanghai, China). XOD and ADA assay kits were produced by Nanjing Jiancheng Biotech (Nanjing, China). Uric acid (UA), creatinine (Cr), blood urea nitrogen (BUN) and adenosine deaminase (ADA) assay kits were purchased from Meikang Medical System Biotechnology (Ningbo, China). The antibody against mURAT1 and mGLUT9 were purchased from ProteinTech Group (Wuhan, China). Antibodies against mOAT1 and mOAT3 and RNA TriQuick Reagent were from Solarbio (Beijing, China). Secondary antibodies and RIPA buffer were purchased from Beyotime Biotechnology (Shanghai, China).

Plant material

Orthosiphon stamineus leaves and stems were collected in Xishuangbanna in China, in April 2016, and were identified by Lecturer. Ai-cun Zhou from Zhejiang Agriculture & Forestry University. A voucher specimen (No. zlc 007) was deposited in the Zhejiang University of Technology (Hangzhou, China). The dried leaves and stems of *O. stamineus* were ground in an electric grinder until a coarse powder was obtained, and the powder was subsequently weighed. The dried powder material (100 g) was extracted using 50% ethanol at 80°C (reflux, 40 min × 3), and the solvent was evaporated using a rotary vacuum evaporator (Heidolph, Schwabach, Germany) to obtain a crude ethanol extract. The crude extracts were suspended in H_2O and then partitioned with petroleum ether. The aqueous solution was evaporated, suspended in H_2O and partitioned with ethyl acetate. The ethyl acetate fraction was evaporated and freeze-dried and stored at −20°C before use, respectively.

Characterisation of *Orthosiphon stamineus* ethyl acetate extracts by UPLC/ESI-MS

The components of OSE were analysed by an Acquity UPLC system (Waters, Milford, MA, USA) equipped with an automatic injector, a column oven and a TUV detector. An ACQUIT UPLC HSS T3 column (2.1 × 100 mm, 1.7 μm; Waters) was used. The mobile phase consisted of 0.1% (*v*/*v*) formic acid waster (A) and acetonitrile (B). A gradient programme was performed: 0 min, 15% B; 4 min, 17% B; 10 min, 21% B; 20 min, 30%; 23 min, 40% B; 24 min, 40% B; 27 min, 50% B; 32 min, 50%; 37 min, 90% B; and 40 min, 90%. The column temperature was maintained at 30°C. The flow rate used was 0.2 ml/min, and detection performed at 280 nm. The crude extract and OSE fraction were filtered through a 0.20 μm PTFE membrane filter, and 1 μl of sample was injected for UPLC analysis. The typical base peak ion chromatograms of OSE presented in Figure 1, and the qualitative analysis of the main compounds of OSE was shown in Table 1.[19]

A Thermo Scientific Ultimate 3000 and LCQ Fleet was used for UPLC/ESI-MS analysis, and ESI-MS was carried out in negative ion mode. The full data were scanned the *m*/*z* range of 50–1000. The ESI parameters were set at 6.0 l/min and 200°C (dry gas N_2), 0.8 bar (nebuliser gas N_2 pressure), 3500 V (capillary voltage), (see Data S1).

Animals, hyperuricemia induction and experimental design

Male Kunming mice (20 ± 2 g) were supplied by Beijing Vital River Laboratory Animal Technology (Permit Number: CNAS LA0004). The mice were housed at a standard 12-h light–12-h dark cycle and within a stable temperature (22–24°C) with normal chow and water ad libitum. Experiments were started after at least 1 week of environmental adaptation. Hyperuricemia in the mice was induced by PO (280 mg/kg) as previously described.[20,21] The mice were divided into 6 groups randomly (n = 6 each): the normal control group (NC), model control group (MC), 10 mg/kg AP group, OSE high dose (2000 mg/kg) group (OSH), OSE medium dose (1000 mg/kg) group (OSM) and OSE low dose (500 mg/kg) group (OSL), and were orally administered with PO 0.5% CMC-Na (vehicle), AP

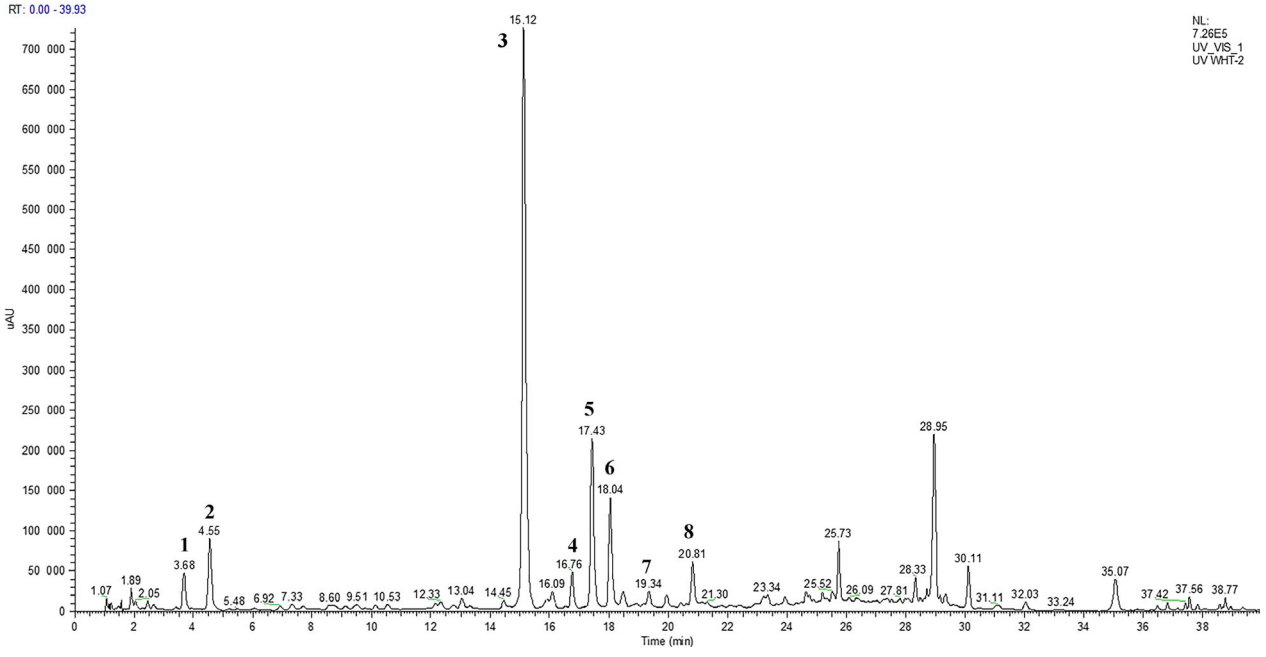

Figure 1 UPLC chromatogram of *Orthosiphon stamineus* ethyl acetate extracts (OSE) at 280 nm (1: Protocatechualdehyde, 2: Caffeic acid, 3: Rosmarinic acid, 4: Caffeic acid tetramer, 5: Lithospermic acid, 6: Salvianolic acid B, 7: Isorhamnetin-3-O-hexoside and 8: Caffeic acid derivative).

(positive control drug) and OSE (500, 1000 and 2000 mg/kg) for seven consecutive days (at 8:00–9:00 a.m.). AP and OSE were dispersed in 0.5% CMC-Na and were intragastrically administered to the mice 1 h after PO administration. After the seventh day of treatment, food was withdrawn from the cages 12 h before the mice were sacrificed. All studies were performed in strict accordance with the guidelines for the Care and Use of Laboratory Animals and approved by the Ethics Committee of Zhejiang University of Technology (Permit NO. 20190308040).

One hour after the last drug administration, all mice were sacrificed. Whole blood samples were collected and coagulated in room temperature approximately 1 h, and the serum was obtained by centrifugation (10 min, 3700*g*). Serum protein was removed with acetonitrile. Liver and kidney were excised and weighed, placed on ice and stored at −78°C until use.

Assays for the levels of uric acid, creatinine, blood urea nitrogen and hepatic xanthine oxidase and adenosine deaminase activity

Serum levels of UA, Cr, BUN, as well as hepatic XOD and ADA activity were determined using commercially available assay kits following the manufacturer's protocols.

Renal histological analyses

Kidney samples were washed in phosphate buffer and fixed in 10% formalin. Tissues were dehydrated in graded ethanol solutions and embedded in paraffin. Tissue sections of 4 μm thick were cut and stained with haematoxylin and eosin (H&E) according to a standard protocol. Morphological evaluation was observed under light microscope.

Table 1 UHPLC and mass spectral characteristics of *Orthosiphon stamineus* ethyl acetate extracts

Peak	RT (min)	$[M-1]^-$ (*m/z*)	Fragments	Identification
1	3.83	136.97	136	Protocatechualdehyde
2	4.72	179.06	135	Caffeic acid
3	15.32	359.34	197, 161	Rosmarinic acid
4	16.88	719.53	359, 197, 161,	Caffeic acid tetramer
5	17.56	537.34	493, 295, 135	Lithospermic acid
6	18.21	717.47	519, 321, 295, 185	Salvianolic acid B
7	19.49	477.39	343, 295, 161	Isorhamnetin-3-O-hexoside
8	20.95	701.4	503, 321, 185	Caffeic acid derivative

Quantitative real-time PCR

RNA TriQuick Reagent was used to extract the total RNA from mouse kidney cortex tissue. Total RNA was mixed with HiScript III RT SuperMix for qPCR (+gDNA wiper), according to the cDNA synthesis instructions and followed by quantitative qPCR analysis (SYBR Green; Applied Biosystems, Waltham, MA, USA). GAPDH used as an endogenous control and was analysed by the $2^{-\Delta\Delta Ct}$ method.[22] The primers were synthesised by Tsing Ke Biological Technology (Hangzhou, China) and sequences were shown in Table 2.

Western blot assay

The kidney tissues were weighed and washed with 4°C saline solution, crushed and homogenised on ice in 10 μl/1 mg of RIPA buffer (containing 1 mmol PMSF), then centrifuged at 3000*g* for 10 min at 4°C. The supernatants were centrifuged again (12 000*g*, 5 min, 4°C) and obtained for Western blot analysis. The protein concentrations were measured by a BCA protein assay, and equal amounts of proteins were then loaded for SDS-PAGE polyacrylamide gel electrophoresis, and transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were then blocked with PBST containing 5% fat-free milk and incubated with specific primary antibodies against URAT1, GLUT9, OAT1 and OAT3 at 4°C overnight, followed by the horseradish peroxidase-conjugated secondary antibodies incubation at room temperature for 1 h and visualised with Clarity Western ECL Substrate (Bio-Rad Laboratories).

In vitro inhibitory activity on xanthine oxidase

As previously reported, with minor modifications, the inhibition of XOD activity was quantified by the change of xanthine concentration in the enzyme-catalysed reaction.[23] All samples were dissolved in phosphate buffer (pH = 7.4, containing 75 mmol/l phosphate ion and 200 μmol/l EDTA). The assay mixture: sample solution 200 μl, XOD solution 100 μl (39.2 μ/l in the same buffer), all solutions were prepared immediately before use. After preincubation at 25°C for 15 min, 400 μl of substrate solution was added to the reaction (1.0 mmol/l xanthine in the same buffer). The enzyme reaction was incubated at 25°C for 30 min, and absorbance of xanthine was determined by HPLC at 254 nm. HPLC analyses were performed using the Shimadzu LC – 20AT (Shimadzu), and an Inertsil ODS-3 column (4.6 × 250 mm, 5 μm, Shimadzu) was used.

Statistical analysis

All data are presented as the mean ± SEM using SPSS 18.0 software. Statistical comparison was evaluated by one-way analysis of variance (ANOVA). $P < 0.05$ was considered as significant.

Results

Effects of *Orthosiphon stamineus* ethyl acetate extracts on serum uric acid, creatinine and blood urea nitrogen levels

To explore the antihyperuricemic activity of OSE on HUA mice, serum UC levels were determined and data were shown in Figure 2a. Serum Cr and BUN levels were evaluated because they were considered as the signals of glomerular injury (Figure 2b and 2c). Compared with NC mice, after PO administration, the serum levels of UA, Cr and BUN in MC group were significantly increased by 53.6, 15.8 and 37.9%, respectively ($P < 0.001$, $P < 0.01$), suggested that the HUA mice model was successfully established. After 10 mg/kg AP treatment, serum UA, Cr and BUN levels were significantly decreased compared with that of NC group. Furthermore, 500, 1000 and 2000 mg/kg doses of OSE significantly declined serum UC levels by 8, 26 and 73 ($P < 0.05$, $P < 0.01$ and $P < 0.001$), respectively, indicating its antihyperuricemic effect. In addition, all serum Cr and BUN levels of OSE treated hyperuricemic mice were lower than those of AP group, suggesting a nephroprotective effect.

Table 2 Primer sequences

Genes	Forward primer (5′–>3′)	Reverse primer (5′–>3′)
URAT1	GTGTTAAGTGGCCTAGCTG	CTTCACTGACTGTTTCTGGA
GLUT9	GTGGACTCAATGCGATCTG	TGATGTATGGGATCTTGTCCT
OAT1	TGCATGACACTAAACATGGA	CCAAGCTGTAGACATAGCC
OAT3	GGCTTATCTGACTTGTTCCG	AAAGCCAGTAGCAAACCAG
GAPDH	AGAAGGCTGGGGCTCATTTG	AGGGGCCATCCACAGTCTTC

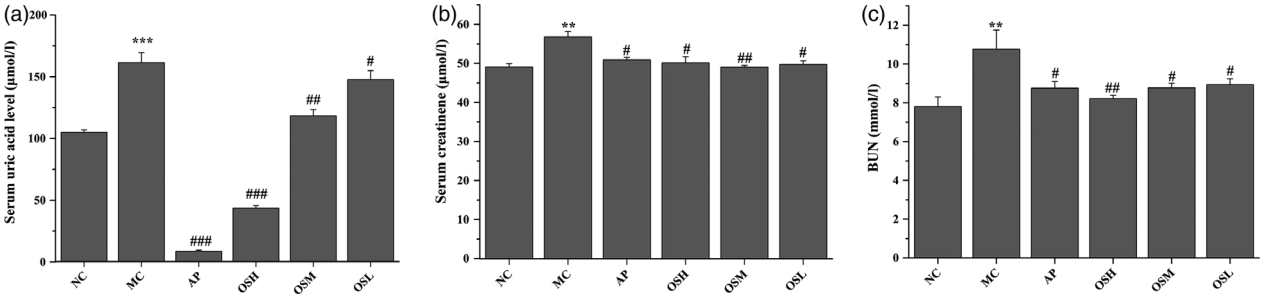

Figure 2 Effects of *Orthosiphon stamineus* ethyl acetate extracts (OSE) on the serum UC (a), creatinine (Cr; b) and blood urea nitrogen (BUN; c) levels in hyperuricemic mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs the MC group ($n = 6$). MC, model control group; NC, normal control group.

Effects of *Orthosiphon stamineus* ethyl acetate extracts on hepatic xanthine oxidase and adenosine deaminase activity

Figure 3 shows that hepatic XOD and ADA activities in hyperuricemic mice were significantly improved by 61.5 and 69.6% (both $P < 0.01$), compared with those in NC group. Meanwhile, in the MC group, AP markedly lowered XOD and ADA activity in the liver by approximately 37 and 34% (both $P < 0.01$). About 2000 and 1000 mg/kg OSE administration significantly lowered hepatic XOD activity by 30% ($P < 0.01$) and 16% ($P < 0.05$), respectively, and hepatic ADA activity by 29% ($P < 0.01$) and 20% ($P < 0.05$), respectively. Unfortunately, low dose of OSE did not have a considerable impact on XOD and ADA activity in the liver.

Renal histopathology analysis

Histopathological staining depicts that the glomeruli of the kidneys in hyperuricemic mice were prominently damaged (Figure 4b), showing degeneration in the cortex and outer stripe of the outer medulla, compared with those in the NC group (Figure 4a). However, AP did not improve renal injury significantly, and the AP group exhibited congestion in the renal interstitium (Figure 4c). In contrast, the mice in the other three groups retained nearly normal kidney morphology (Figure 4d–4f). These results indicated that OSE could ameliorate the kidney damage and have renal nephroprotective effects on PO-induced hyperuricemic mice.

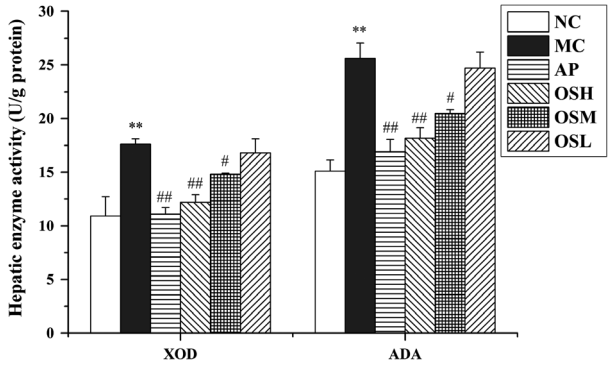

Figure 3 Effects of *Orthosiphon stamineus* ethyl acetate extracts (OSE) on hepatic xanthine oxidase (XOD) and adenosine deaminase (ADA) activity. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs MC group ($n = 6$). MC, model control group; NC, normal control group.

Effects of *Orthosiphon stamineus* ethyl acetate extracts on renal mRNA expression levels

As presented in Figure 5, compared with the NC group, the mice that received PO significantly upregulated mRNA expression levels of URAT1 and GLUT9 ($P < 0.05$ and $P < 0.01$). Both AP and OSM treatment clearly downregulated both UTAT1 and GLUT9 mRNA expression levels compared with those in the MC mice ($P < 0.01$ and $P < 0.05$). The mRNA expressions of renal OAT1 and OAT3 were apparently downregulated after PO administration in MC mice (both $P < 0.01$). However, at the mRNA level, the expression in the AP group was lower than those in the NC group. OSE at high and medium doses was significantly upregulated mRNA expression levels of OAT1 and OAT3 ($P < 0.001$).

Effects of *Orthosiphon stamineus* ethyl acetate extracts on renal protein expression levels

Assays were carried out to explore the effects of OSE on renal relative protein expression levels and investigate the underlying antihyperuricemia mechanisms. As observed in Figure 6, compared with NC mice, the improved renal protein levels of URAT1 and GLUT9 and diminished renal OAT1 and OAT3 levels were investigated in hyperuricemic mice. AP treatment downregulated URAT1 and GLUT9 protein expression levels, and upregulated protein

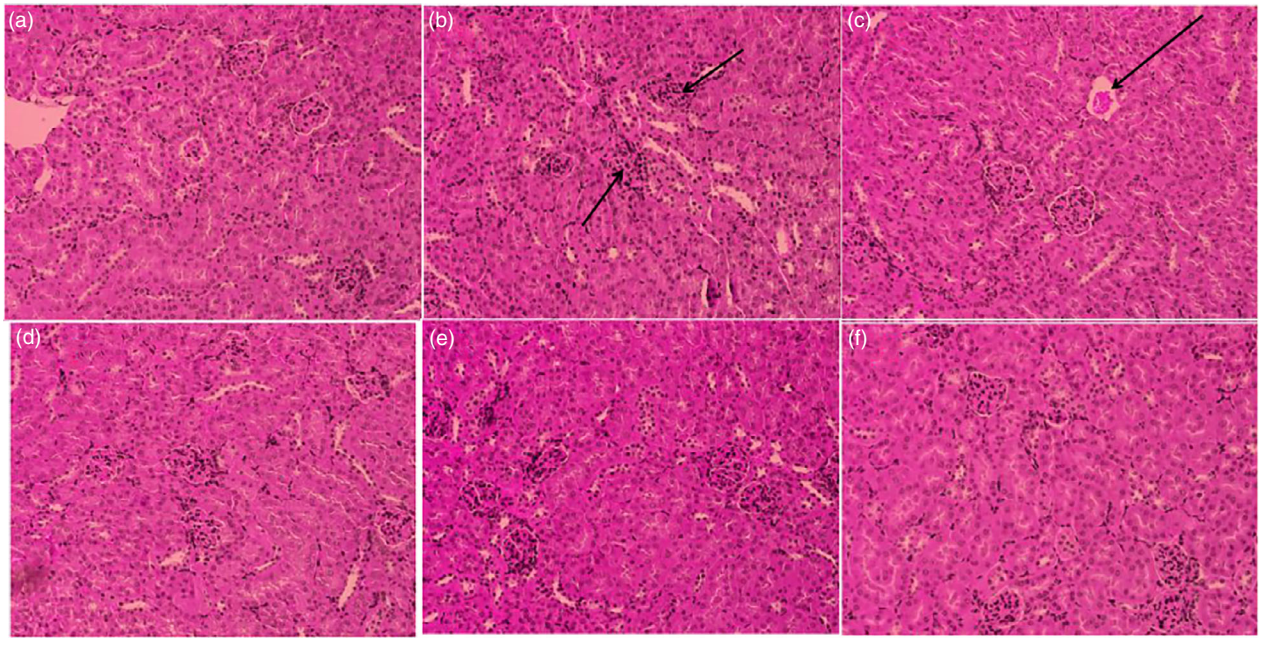

Figure 4 Micrograph of renal histopathology (images, original magnification ×200). a: NC group; b: MC group; c: AP group; d: OSH group; e: OSM group; and f: OSL group. AP, allopurinol; MC, model control group; NC, normal control group; OSE, *Orthosiphon stamineus* ethyl acetate extracts; OSH, OSE high dose; OSL, OSE low dose; OSM, OSE medium dose.

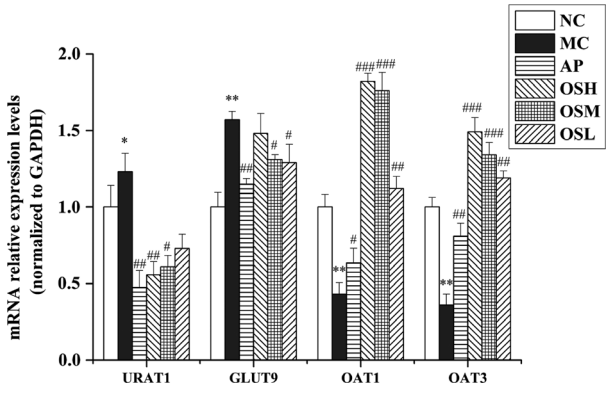

Figure 5 Effects of *Orthosiphon stamineus* ethyl acetate extracts (OSE) on renal mRNA expression levels. GAPDH was used as an endogenous control. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs MC group ($n = 6$). MC, model control group; NC, normal control group.

expression levels of OAT1 and OAT3. OSH and OSM significantly downregulated renal URAT1 and GLUT9 expression levels and upregulated OAT3 expression levels, but did not have much impact on OAT1 protein expression levels.

Effect of in vitro inhibitory activity on xanthine oxidase

To confirm the inhibition effects caffeic acid and rosmarinic acid on XOD, the inhibitory activity was analysed in enzymatic reactions. AP was used as a positive control. The results showed that caffeic acid and rosmarinic acid inhibited XOD activity in a dose-dependent manner and that the IC_{50} was 3.94 and 8.76 mg/ml, respectively. As a XOD inhibitor, AP presented a significant inhibitory activity on XOD (Figure 7).

Discussion

Hyperuricemia is strongly associated with numerous metabolic disorders, such as hyperlipidemia, hypertension, gout and cardiovascular disease. Allopurinol is recognised as the first-line drug for the treatment of HUA, but it has caused serious side effects during clinical use, including drug allergies, hepatotoxicity and nephrotoxicity. As it has been previously reported that herbal medicine, such as Pu-erh ripened tea, Konjac glucomannan, *Corylopsis coreana* Uyeki and *Smilax china* L.,[24–26] are more effective and safer for treating HUA and gout. In herbal extract, caffeic acid and rosmarinic acid were verified to be potent antihyperuricemic agents.[27–29] However, most active ingredients are a mixture of herbal extracts. *Orthosiphon stamineus* is known for its diuretic effect and antioxidant activity,[30,31] but its antihyperuricemia effect has not been reported yet. Therefore, this study aimed to explore the effects of OSE on HUA because of its increasing popularity. In this study, treatment with OSE could effectively normalise serum levels of UC, Cr and BUN in hyperuricemic mice and

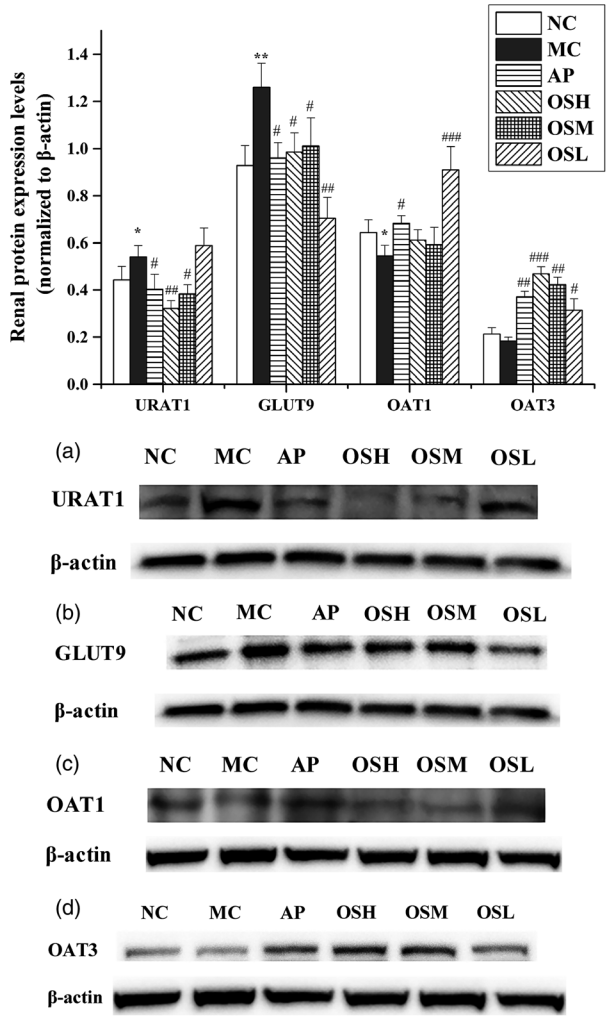

Figure 6 Effects of *Orthosiphon stamineus* ethyl acetate extracts (OSE) on renal protein expression levels. The renal protein levels were normalised to those of mβ-actin. (a: URAT1; b: GLUT9; c: OAT1; and d: OAT3) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs MC group ($n = 6$). MC, model control group; NC, normal control group.

downregulate the hepatic XOD and ADA activity. Renal histological analyses demonstrated that OSE could ameliorate renal inflammation damages in PO-induced hyperuricemic mice. In addition, the expressions levels of urate related transporters were regulated. Those results indicated that OSE has antihyperuricemic and nephroprotective effects, suggesting that OSE could be a promising agent for HUA treatment and have a potential value as a new candidate for a healthy dietary source.

The catabolism of purine nucleotides to UA is extremely complicated. Previous studies have emphasised the significance of XOD and ADA in the UA synthesis pathway systematically.[32] Therefore, we explored the inhibitory effects of OSE on XOD and ADA activity in liver. After PO treatment, hepatic XOD and ADA activity clearly proliferated, leading to an improvement in UA production further. We found OSE treatment could significantly inhibit hepatic XOD and ADA activity and reduce serum UC levels in hyperuricemic mice. Those results indicated that the hypouricemic mechanism of OSE may through influencing catalytic activity of XOD and ADA and relative urate transporter protein expression level.

Potassium oxonate is widely used as an inducing agent leading to HUA in rodents because of its inhibitory effect on uricase, which degrades UC into allantoin acid. Similar to previous reports,[33,34] we found that the serum UA levels of the mice treated with a 280 mg/kg PO for consecutively 7 days generated an approximately 53.6% increment compared with those of NC mice. At the same time, OSH, OSM and OSL clearly lessened serum levels of UA, Cr and BUN in the hyperuricemic mice.

In daily life, many people probably regard high purine food or drinks as the main cause of HUA, while urate excretion is usually not seriously concerned. However, insufficient excretion of renal urate is the cause of approximately 90% of gout patients in clinical practice, and damaged urate excretion pathway was the major cause of HUA.[35] In humans, nearly 90% of filtered

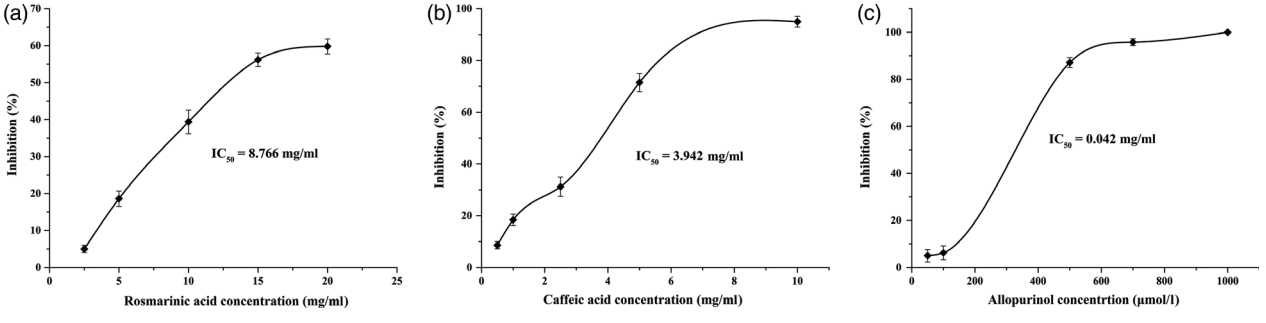

Figure 7 Inhibitory effect of Rosmarinic acid (a), Caffeic acid (b) and Allopurinol (c) on xanthine oxidase (XOD). Data are expressed as the mean ± SEM ($n = 3$)

urate is reabsorbed in renal proximal tubules.[36] Renal urate transporters, such as URAT1 and GLUT9, have been reported to be involved in promoting urate excretion. In this study, PO treatment significantly upregulated URAT1 and GLUT9 expression at mRNA and renal protein levels, while OSE treatment induced an apparently reduction in the mRNA and protein expression levels of both in hyperuricemic mice. Furthermore, OAT1 and OAT3 have been verified to be involved in the secretion of urate.[37,38] PO administration downregulated OAT1 and OAT3 expressions at mRNA and protein levels, which was consistent with previous reports. These results suggested that PO could not only promoted urate reabsorption but also attenuated urate secretion. OSE treatment significantly downregulated URAT1 and GLUT9 expression levels and upregulated OAT1 and OAT3 expression, which demonstrated that OSE may reduce urate reabsorption and enhance UA excretion. In addition, renal histopathology analysis demonstrated that OSE administration could repair PO-induced kidney damages and have renal nephroprotective effects on PO-induced hyperuricemic mice.

Taken together with these findings and our results, it is presented that OSE treatment ameliorated PO-induced HUA and kidney damages, suggesting that OSE could be a potential antihyperuricemia agent. It is essential to mention that the doses which OSE exhibited its effects in this study were excessive, and achieving antihyperuricemic effects through the consumption of such a massive amount of *O. stamineus* is practically impossible for normal people. Instead, *O. stamineus* consumption should occur in the long-term. With regard to this, longer clinical trials and studies on lower OSE doses are necessary to further demonstrate the antihyperuricemic potency of *O. stamineus*. Furthermore, more effective components of OSE will be identified.

Conclusion

In this paper, antihyperuricemic and nephroprotective effect mechanisms of OSE on hyperuricemic mice were investigated for the first time. Eight phenolic acid constituents from OSE were identified, and OSE treatment decreased serum UA level by decreasing hepatic XOD and ADA activity in hyperuricemic mice. In addition, OSE significantly downregulated renal mURAT1 and mGLUT9 levels and upregulated renal mOAT1 and mOAT3 levels. All these results validated hypouricemic effect of OSE and indicated that OSE might be a potential dietary supplement for HUA treatment.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1.

Figure 1. Mass spectral of protocatechualdehyde.

Figure 2. Mass spectral of caffeic acid.

Figure 3. Mass spectral of rosmarinic acid.

Figure 4. Mass spectral of caffeic acid tetramer.

Figure 5. Mass spectral of lithospermic acid.

Figure 6. Mass spectral of salvianolic acid B.

Figure 7. Mass spectral of isorhamnetin-3-O-hexoside.

Figure 8. Mass spectral of caffeic acid derivative.

Figure 9. Chemical structures of OSE (1: Protocatechualdehyde, 2: Caffeic acid, 3: Rosmarinic acid, 4: Caffeic acid tetramer, 5: Lithospermic acid, 6: Salvianolic acid B, 7: Isorhamnetin-3-O-hexoside, 8: Caffeic acid derivative).

