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The inhibitory effect of lotus leaf extract on hyperuricemia and its potential mechanism

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Abstract

Objective: Lotus leaf is a traditional Chinese herb that has been used successfully for centuries for relieving edema by inducing diuresis. Based on its good clinical evidence and anti-hypertensive effectiveness, this study aimed to investigate the potential mechanism of the hyperuricemic inhibitory effects of lotus leaf crude extract (LL) and lotus leaf total alkaloids fraction (LA).

Methods: The xanthine oxidase (XOD) inhibitory effect of LL and LA was analyzed *in vitro* by determining mRNA expression and protein expression levels of hepatic XOD. The hyperuricemic inhibitory effect of the lotus leaf was analyzed *in vivo* in a potassium oxonate (PO)-induced rat model by determining mRNA expression for renal urate transporters.

Results: At a concentration of 40 μg/mL, LL and LA suppressed XOD enzymatic activity by 37.35% ± 9.50% and 47.73% ± 8.32%, respectively. Both LL and LA administration significantly reduced the concentration of uric acid in the serum and liver of PO-induced hyperuricemic rats. Both LL and LA administration could inhibit XOD mRNA and protein expression, activate renal organic anion transporter 1/3 mRNA expression, and inhibit renal urate reabsorption by decreasing renal GLUT9 and renal urate transporter 1.

Conclusions: Insight was gained into the mechanism behind the hyperuricemic inhibitory effects of LL and LA. Our results suggest that they act on two targets: decreasing the production of uric acid by inhibiting mRNA and protein expression of XOD in the liver, and regulating the mRNA expression of renal urate transporters in the kidneys.

Keywords: Hyperuricemia, Lotus leaf, Renal urate transporters, Uric acid, Xanthine oxidase

1 Introduction

Uric acid is a product of purine metabolism that is freely excreted in the urine. An excess of uric acid in the body caused by increased production and/or decreased excretion causes hyperuricemia, which is an independent risk factor for the development of gout, renal dysfunction, hypertension, hyperlipidemia, diabetes, and obesity[1–3].

Inhibitors of the enzyme xanthine oxidase (XOD) are widely used as a treatment for hyperuricemia. XOD catalyzes the conversion of xanthine to urate[4] and is created by conversion of the enzymatic protein xanthine dehydrogenase, which is involved in the oxidative metabolism of purines. One of these inhibitors, which plays a significant role in urate reduction, is allopurinol[5], a purine analog that is an isomer of hypoxanthine.

Folium nelumbinis (lotus leaf) is the dried leaf of *Nelumbo nucifera* Gaertn., a perennial aquatic plant that grows widely across China. The dried leaves of the lotus have been widely used as a foodstuff for thousands of years in China, and according to the Compendium of Materia Medica, the tropism of taste of lotus leaf is bitter and mild. As a medicine, it is associated with the hepatic, spleen, and stomach meridians, which can be used in the treatment of a variety of symptoms, including generalized edema, carbuncles, fever, and dehydration. Lotus leaf has also been demonstrated to have an inhibitory effect on hyperlipidemia, which makes it suitable for treating related conditions such as gout and hypertension.

The major medicinal components of lotus leaves are tannins, alkaloids (e.g. nuciferine, roemerine, nornuciferine, N-nornuciferine, and pronuciferine), and flavonoids (e.g. quercetin, isoquercetin, and leucocyanidin)[6] .In particular, the alkaloids found in lotus leaves have been reported to show remarkable biological activities, including antioxidant[7-8], anti-obesity[9], and hypotensive[8] effects. These properties suggest that lotus leaves could be a potential XOD inhibitor and could be used to decrease serum uric acid in hyperuricemic rats.

2 Materials and methods

2.1 Plant materials and reference standard

Lotus leaf used in this study was obtained from Yonggang Chinese Medicine Co. Ltd., Bozhou, China, and identified and authenticated by Dr. Hong Liu at School of Traditional Chinese Materia Medica, Tianjin University of traditional Chinese Medicine. Voucher specimens were deposited at the School of Traditional Chinese Materia Medica , Tianjin University of traditional Chinese Medicine. Nuciferine was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, China.

2.2 Lotus leaf crude extract and total alkaloids fraction extraction and quality analysis

The methods we used for obtaining lotus leaf crude extract (LL), extracting the total alkaloids fraction, and

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performing quality analysis have been reported previously[10]. Briefly, lotus leaves were extracted with 70% EtOH containing 1% HCl at room temperature for 24 h, which was then repeated a second time. The combined 70% EtOH solution was concentrated in a rotatory evaporator in vacuo at 40°C for 2 h to obtain the LL. Then, the crude extract was isolated with ethyl acetate using a Soxhlet extractor for 3 h. The ethyl acetate extract was used as the lotus leaf total alkaloids fraction (LA) (0.23%). The nuciferine content was determined by high-performance liquid chromatography (HPLC) analysis under the following conditions: HPLC column, COSMOSIL C_{18} ($4.6 \times 150\,mm^2$, 5 μm); detection, UV detector at 270 nm; column temperature, 25°C; mobile phase, CH_3CN–H_2O–triethylamine–HAc (33:64.8:1.5:0.7, v/v); flow rate, 1.0 mL/min. In comparison with the standard sample, the nuciferine content of LA was 20.6%.

2.3 XOD inhibition in vitro assay

The assay method described by Morikawa et al.[11] was used with a slight modification. Briefly, the reaction mixture for the XOD inhibition assay, comprising 920 μL of 50 mM sodium carbonate buffer (pH 10.2), 120 μL of 3 mM xanthine, 40 μL of 3 mM ethylenediaminetetraacetic acid, 40 μL of sample solution dissolved in distilled water or 1% dimethyl sulfoxide, and 40 μL XOD (58 mU/mL), was incubated for 20 min at 37°C. After incubation, the solution was mixed with 0.1 mL of 2 M HCl to stop the reaction. XOD inhibition assays were performed on LL, LA, and allopurinol (obtained from Chongqing Kerui Pharmaceutical Co., Ltd., China) at a concentration of 40 μg/mL. Measurements were performed in duplicate, and the inhibition ratio was determined.

2.4 PO-induced hyperuricemia in rats

A total of 62 male Sprague-Dawley rats (5 weeks old and weighing 220–260 g), were purchased from River Laboratory Animal Technology Co. Ltd. (Beijing, China). All animals were raised under specific pathogen free conditions, randomly assigned three rats per cage, with bedding material and air filtration. All animals were allowed to eat a standard diet and drink ad libitum and were adapted to the experimental conditions at (22 ± 2)°C and 60% ± 5% humidity with a fixed 12 h artificial light period for 1 week. The experimental rats were overseen and approved by the Science and Technological Committee and the Animal Use and Care Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2018017). After 1 week of adaptation, the rats were randomized into eight groups ($n = 6$–8): (1) normal group [5% acacia water solution); (2) control group (5% acacia water solution, potassium oxonate (PO)-induced hyperuricemia][12]; (3) positive drug group (5% acacia water solution, PO-induced hyperuricemia + allopurinol: 10 mg/kg/d), and drug groups; (4) 5% acacia water solution, PO-induced hyperuricemia + LL-high: 400 mg/kg/d; (5) 5% acacia water solution, PO-induced hyperuricemia + LL-medium: 200 mg/kg/d; (6) 5% acacia water solution, PO-induced hyperuricemia + LL-low: 100 mg/kg/d; (7) 5% acacia water solution, PO-induced hyperuricemia + LA-high: 200 mg/kg/d; and (8) 5% acacia water solution, PO-induced hyperuricemia + LA-Low: 100 mg/kg/d. Due to previous experiments found that the effect of LA high-dose group (400 mg/kg/d) is often not as good as other dose groups, we considered whether the dose of 400 mg/kg is too high for this model, so only two doses of total alkaloids (200 and 100 mg/kg/d) were showed in this paper, and the high-dose was set at 200 mg/kg. The details of animal grouping and administration are given in Table 1. Other than the normal group, each group of rats had acute hyperuricemia induced using PO (Sigma Chemical Co., Santa Cruz, USA), a urate oxidase inhibitor[13–14]. These rats were intragastrically given to PO (300 mg/kg body weight), 1 h before the administration of treatment doses. The treatment doses for the allopurinol group and the five LL/LA groups were 10 mL/kg bodyweight, suspended in 5% acacia solution, while the control group received only 5% acacia water solution. After intragastric administration of PO, blood samples (ca. 0.6 mL) were collected from the infraorbital venous plexus under ether anesthesia at 1.5 h, 2 h, and 3 h and were then centrifuged at 3,500 × *g* for 10 min. Supernatant serum was collected and stored at −20°C until assayed. Serum uric acid (SUA) levels of the PO-induced rats were determined using commercial kits (BioSino Bio-technology and Science Inc., China), and the protocol was performed without modification. In addition, hepatic and renal samples were collected and immediately frozen in liquid N_2 and stored at −70°C until used for real-time reverse transcription polymerase chain reaction (RT-PCR) and Western Blot (WB) analysis.

2.5 Hepatic uric acid assay

A 2 g of the sampled hepatic tissue was grinding with a homogenizer in 10 mL ice-cold saline, then centrifuged at 8,000 × *g* for 10 min at 4°C. The supernatant was collected for hepatic uric acid detection, which was performed using

Table 1

Animal grouping and administration.

Number	Groups		Details
1	Normal group		5% acacia water solution
2	Control group		5% acacia water solution, PO-induced hyperuricemia
3	Positive drug group	Allopurinol	5% acacia water solution, PO-induced hyperuricemia + allopurinol: 10 mg/kg/d
4	LL groups	LL-high	5% acacia water solution, PO-induced hyperuricemia + LL-high: 400 mg/kg/d
5		LL-medium	5% acacia water solution, PO-induced hyperuricemia + LL-medium: 200 mg/kg/d
6		LL-low	5% acacia water solution, PO-induced hyperuricemia + LL-low: 100 mg/kg/d
7	LA groups	LA-high	5% acacia water solution, PO-induced hyperuricemia + LA-high: 200 mg/kg/d
8		LA-low	5% acacia water solution, PO-induced hyperuricemia + LA-low: 100 mg/kg/d

LA: Lotus leaf total alkaloids fraction; LL: Lotus leaf crude extract; PO: Potassium oxonate.

commercial kits (BioSino Bio-technology and Science Inc., China).

2.6 RT-PCR for relative gene expression in rats: hepatic and renal

Briefly, total RNA was extracted from tissue with TRIzol reagent (Invitrogen, USA). One microgram of RNA was reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) to obtain cDNA according to the protocols provided by the manufacturer. The total reaction volume was 20 μL with the reaction incubated as follows in C1,000 Touch™ Thermal Cycler (BIO-RAD, USA): 10 min at 25°C, 120 min at 37°C, 5 min at 85°C, hold at 4°C.

RT-PCR was performed with an Applied Biosystems 7,500 RT-PCR PCR System (Applied Biosystems, USA) using Power SYBR® Green PCR master mix (Applied Biosystems, USA) according to the protocols provided by the manufacturer. Briefly, PCR was performed in a final volume of 20 μL including 10 ng sample cDNA, 5 μM specific forward and reverse primers, and 10 μL Power SYBR® green PCR Master Mix. PCR reactions consisted of an initial denaturation cycle at 95°C for 10 min, followed by 40 amplification cycles: 15 s at 95°C and 1 min at 60°C. The primers used are given in Table 2. Results are presented as levels of expression relative to those of controls after normalization to GAPDH using the $2^{-\Delta\Delta Ct}$ method. Analysis was carried out in triplicates.

2.7 WB for hepatic XOD

Each frozen tissue was homogenized while ice-cold using a RIPA protein extraction kit (Sangon Biotech Co. Ltd., China), and the protein concentration of the supernatant was measured by using a BCA protein assay kit (Thermo Fisher Scientific Inc., USA), both following the manufacturer's instructions. Equal amounts of hepatic proteins (40 μg) were resolved by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to 0.45 μm polyvinylidene difluoride membranes (Millipore, MA). The XOD blot was blocked with 5% non-fat dry milk-TBST buffer [Tris-buffered saline (TBS) containing 0.1% Tween-20] for 1 h at room temperature. The membranes were incubated overnight at 4°C with 1:2,000 dilutions of antibodies for XOD (Santa Cruz Biotechnology, Inc., USA). Equal lane loading was assessed using β-actin (Sigma Chemical Co., USA). The blots were rinsed seven times with TBST buffer for 3 min each. Washed blots were incubated with 1:10,000 dilution of the rabbit peroxidase conjugated-secondary antibody (Beijing Zhongshan Golden Bridge Biotechnology Ltd. China) for 2 h and washed five times with TBST buffer. The transferred proteins were visualized with an enhanced chemiluminescence detection kit (Millipore Co. Ltd. USA).

Table 2

Gene-specific primers used for quantitative RT-PCR.

Gene	Sequence
GAPDH	Forward: 5′-TGAGGCCGGTGCTGAGTATGT-3′
	Reverse: 5′-CAGTCTTCTGGGTGGCAGTGAT-3′
XOD	Forward: 5′-CTTTGCGAAGGATGAGGTT-3′
	Reverse: 5′-CACTCGGACTACGATTCTGTT-3′
OAT1	Forward: 5′-TCATCTACTCTTGGTTCTTCATTG-3′
	Reverse: 5′-CGGAGCACCTCTATACTTAGC-3′
OAT3	Forward: 5′-CCGCTGAAGACTGGTGGGAT-3′
	Reverse: 5′-TAGGCTATGGTGGAGGTGATG-3′
URAT1	Forward: 5′-CACTGCCTGACACCATCCA-3′
	Reverse: 5′-CTCCTTCTCCTCCTTCCATTGA-3′
GLUT9	Forward: 5′-AGTCCTACTGCTTCCTCGTCTTTG-3′
	Reverse: 5′-CCTTGTTCCTCTTGGCGAATGC-3′

OAT: Organic anion transporter; RT-PCR: Real-time reverse transcription-polymerase chain reaction; URAT: Renal urate transporter 1; XOD: Xanthine oxidase.

2.8 Statistical analysis

All the grouped data were statistically analyzed using the software SPSS 26.0. Values are expressed as mean ± standard error of the mean (mean ± SEM). The significance of differences between mean values was evaluated using a one-way analysis of variance (ANOVA). $P < 0.05$ was considered to indicate statistical significance.

3 Results

3.1 XOD inhibition in vitro assay

The XOD inhibitory activity of LL and LA were evaluated by HPLC (Figure 1). Both LL and LA inhibited XOD activity. At a concentration of 40 μg/mL, the inhibition ratio of LL was 37.35% ± 9.50% and for LA it was 47.73% ± 8.32% (Table 3).

3.2 LL and LA decreases serum uric acid levels in hyperuricemic rats

The administration of 300 mg/kg PO to rats control group for 1 week resulted in a significant elevation of serum uric acid levels compared with normal rats. The serum uric acid levels of the control group increased over time: the SUA level of the control group at 1.5 h was nearly two times that of the normal group, and at 2 and 3 h the control group SUA level was almost three times that of the normal group. At 1.5 h, the SUA levels of the 400 mg/kg and 200 mg/kg LL dosage groups had significantly declined, but the 100 mg/kg dosage group had only slightly declined compared with the control group. This pattern was repeated for the groups administered LA. For the 400 mg/kg of LL group and the 200 mg/kg and 100 mg/kg LA groups, SUA levels gradually decreased to normal by the 3 h mark (Table 4).

3.3 LL and LA decreases hepatic uric acid levels in hyperuricemic rats

The effects of LL and LA on hepatic uric acid levels in hyperuricemic rats were also observed. Compared to the normal group, the level of hepatic uric acid was elevated from (0.44 ± 0.01) μmol/L to (0.58 ± 0.02) μmol/L ($P < 0.01$) in the PO-induced control group. The hepatic uric acid levels of each dosage group of LL and LA were significantly lower compared to the control group ($P < 0.01$) (Table 5).

3.4 Hepatic XOD mRNA and protein expression assay

Compared with the normal group, the PO-induced control group up-regulated hepatic XOD mRNA expression almost 2-fold ($P < 0.01$). All three dosages of LL and

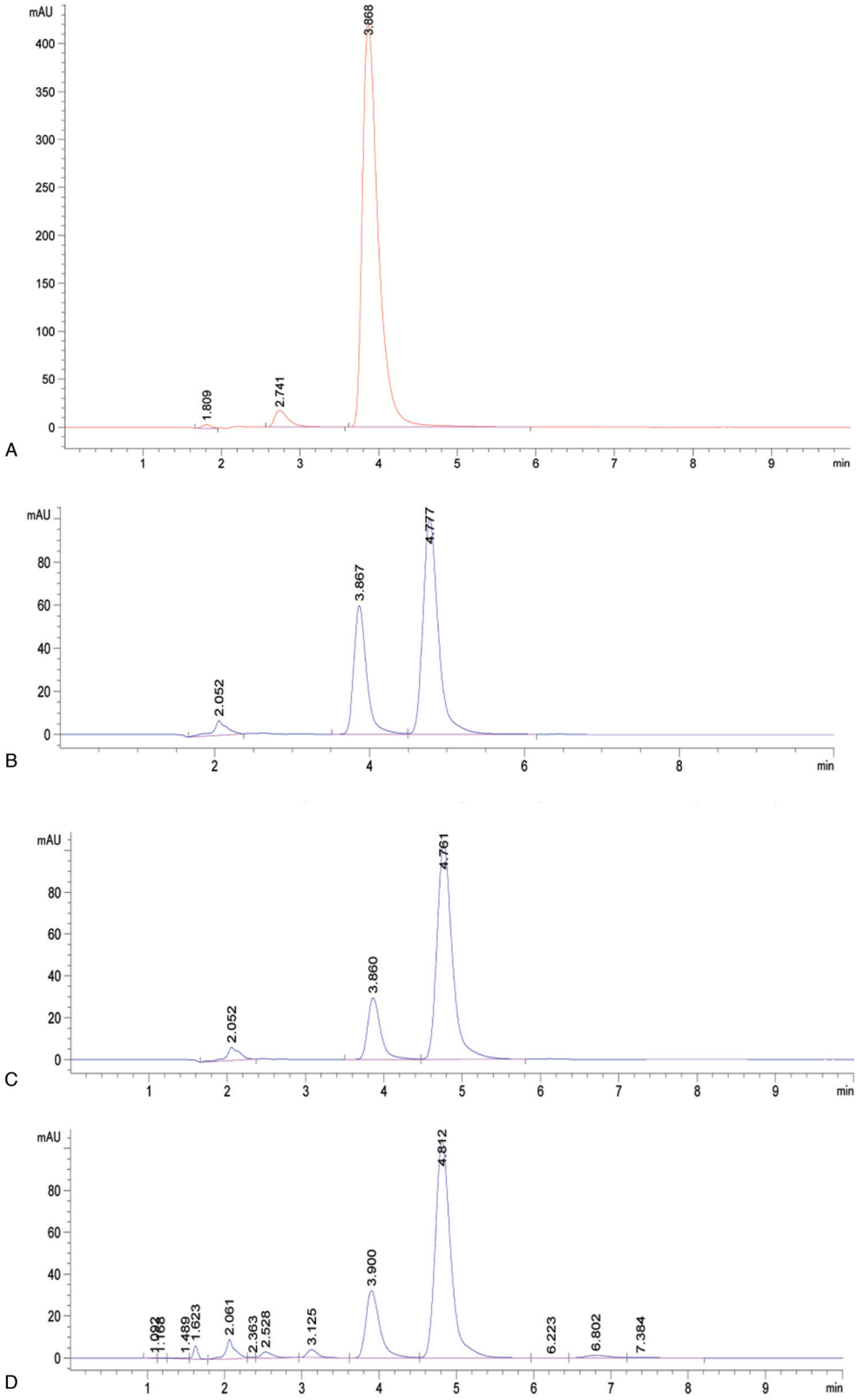

Figure 1. The formation of uric acid was determined by HPLC analysis under the following conditions: HPLC column, COSMOSIL C_{18} (4.6 × 150 mm^2, 5 μm); detection, UV detector at 284 nm; column temperature, 25°C; mobile phase, CH_3OH – 74 mM phosphate (98:2, v/v); flow rate, 1.0 mL/min. All HPLC runs were performed in duplicate. (A) Uric acid standard. (B) XOD inhibition of LL. (C) XOD inhibition of LA. (D) XOD inhibition of allopurinol. HPLC: High-performance liquid chromatography; LA: Lotus leaf total alkaloids fraction; LL: Lotus leaf crude extract; XOD: Xanthine oxidase.

the 200 mg/kg dosage of LA had significantly inhibited hepatic XOD mRNA expression compared with the control group ($P < 0.01$ or $P < 0.05$). As for protein expression, the PO-induced control group had significantly greater hepatic XOD expression than the normal group, and all dosages of LL and the LA had inhibited hepatic XOD protein expression compared to the control group (Figure 2).

Table 3

XOD inhibitory activity of LL and LA.

Group	Concentration (μg/mL)	XOD inhibition ratio (%)
Lotus leaf crude extract	40	37.35 ± 9.50
Lotus leaf total alkaloids fraction	40	47.73 ± 8.32
Allopurinol	40	81.64 ± 2.62

LA: Lotus leaf total alkaloids fraction; LL: Lotus leaf crude extract; XOD: Xanthine oxidase.

3.5 Renal uric acid transporter gene expression assay

According to the reaction mechanism of the LL, the LA decreased uric acid effects and the renal uric acid excretion genes, the organic anion transporter (OAT) 1/3, renal urate transporter 1 (URAT1), and SLC2A9 (GLUT9) mRNA expression has been determined. As shown in Figure 3, 200 mg/kg and 100 mg/kg dosages of LL and LA administration stimulated the OAT1 mRNA expression compared to the control group ($P < 0.01$), and each dosage of LL and LA administration stimulated the OAT3 mRNA expression compared to the control group ($P < 0.01$ or $P < 0.05$), while merely 100 mg/kg dosages of LA administration inhibited renal URAT1 gene expression compared to the control group ($P < 0.05$). Moreover, 400 and 200 mg/kg dosages of LL administration and 100 mg/kg dosages of LA administration significantly inhibited renal GLUT9 mRNA expression compared to the control group ($P < 0.01$ or $P < 0.05$) (Figure 3).

4 Discussion

The increased production or decreased excretion of urate (or both) plays a critical role in various types of hyperuricemia. Urate is produced only in tissues that contain XOD, primarily in the liver, and three-quarters of the urate produced is excreted by the kidneys[15]. Therefore, hepatic XOD activity (which regulates the production of urate) and renal urate transporter activity (which regulates the excretion of urate in the kidneys) both play important roles in urate homeostasis[16]. So, the LL and LA inhibitory effects on XOD activity suggest that they could reduce uric acid in the body. We found that LL has a better inhibitory effect on XOD activity than LA, but that allopurinol was superior to both (Table 3).

To clarify whether LL and LA can decrease the serum uric acid in hyperuricemia rats by inhibiting hepatic XOD activity, we induced mild hyperuricemia in rats by administering the urate oxidase inhibitor, PO. The inducement of hyperuricemia in rats was not associated with any acute renal injury[17]. In this study, the serum uric acid levels of PO-induced hyperuricemic rats were significantly higher than we expected. Nevertheless, both LL and LA inhibited serum uric acid increase for the 3 h were measured (Table 4). However, it should be noted that rats possess Rasburicase, a uricase that degrades uric acid to allantoin, and which humans do not possess. As a consequence, humans have less ability to regulate serum uric acid levels, and high levels overall. As well as serum uric acid levels, we also measured uric acid levels in the liver, the main site of uric acid production by XOD, besides the intestine. We demonstrated that hepatic uric acid was significantly decreased both in LL and LA groups (Table 5), and then demonstrated that both LL and LA can inhibit hepatic mRNA and protein expression of XOD (Figure 2). This suggests that the inhibition of hepatic XOD activity could be the mechanism by which LL and LA decrease serum uric acid levels.

Besides the effects of a lotus leaf in the production of uric acid in the liver, we also determined the effects of a lotus leaf in the excretion of uric acid in the kidneys. Ordinarily, approximately 70% of the daily urate production is excreted through the kidneys, where organic anion transporters of the renal proximal tubule play an important role[18-19]. Basolateral uptake *via* OAT1/3 and bidirectional transport of urate may involve apical absorption via the URAT1[20]. The function of OAT3 operates similarly to OAT1 and may mediate renal urate secretion. OAT1 and OAT3, in response to an increase in uric acid intake, are upregulated and stimulate urinary uric acid excretion, which likely contributes to protection from hyperuricemia[19,21-22]. We demonstrated that both LL and LA can stimulate the mRNA expression of OAT1 and OAT3, and that this stimulation increases uric acid excretion (Figure 3A and B). We also examined another member of the OAT family, SLC22A12 (URAT1), which is a urate–anion exchanger and could also affect the excretion of uric acid[23]. We demonstrated that the groups treated with LL and LA show similar mRNA expression of URAT1 to the non-hyperuricemic normal group, whereas the untreated hyperuricemic control group showed more abnormal URAT1 mRNA expression. This suggests that LL and LA can affect uric acid excretion by affecting URAT1 mRNA expression (Figure 3C).

Table 4

Effects of LL and LA on serum uric acid of hyperuricemic rats (mean ± SEM, $n = 6$–8).

Group	Dosage (mg/kg)	Serum uric acid (μmol/L)		
		1.5 h	2 h	3 h
Normal	–	43.05 ± 5.02**	37.31 ± 5.65**	31.64 ± 5.77**
Control	–	85.49 ± 6.76	97.28 ± 9.79	103.15 ± 8.94
Allopurinol	10	37.19 ± 5.60**	36.23 ± 7.18**	43.10 ± 12.07**
Lotus leaf crude extract	400	69.42 ± 6.83	57.16 ± 7.08**	48.94 ± 3.25**
	200	69.30 ± 6.63	62.81 ± 4.62**	58.58 ± 6.46**
	100	62.75 ± 2.70**	65.91 ± 7.06*	53.17 ± 6.22**
Lotus leaf total alkaloids fraction	200	60.80 ± 10.46*	59.55 ± 9.02*	47.92 ± 7.74**
	100	59.46 ± 12.31	57.79 ± 8.60**	48.44 ± 7.58**

LA: Lotus leaf total alkaloids fraction; LL: Lotus leaf crude extract; SEM: Standard error of the mean.
* $P < 0.05$, ** $P < 0.01$ versus the control group.

Table 5

Effects of LL and LA on hepatic uric acid of hyperuricemic rats (mean ± SEM, $n = 6–8$).

Group	Dosage (mg/kg)	Hepatic uric acid (μmol/L)
Normal	–	$0.44 \pm 0.01^{**}$
Control	–	0.58 ± 0.02
Allopurinol	10	$0.50 \pm 0.02^{**}$
LL	400	$0.49 \pm 0.01^{**}$
	200	$0.52 \pm 0.01^{**}$
	100	$0.50 \pm 0.03^{**}$
LA	200	$0.53 \pm 0.01^{**}$
	100	$0.47 \pm 0.00^{**}$

LA: Lotus leaf total alkaloids fraction; LL: Lotus leaf crude extract; SEM: Standard error of the mean. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the control group.

Another influence on uric acid levels is SLC2A9 (GLUT9), which influences renal excretion and is expressed in more distal, relatively anaerobic, nephron segments. GLUT9 may alter the levels of lactate and other anions, which affect the reabsorption of filtered urate from the urine in exchange for cytosolic organic anions[24]. In this study, with the exception of the 200 mg/kg LA dosage group, the other LL and LA groups were shown to have inhibited GLUT9 mRNA expression compared to the untreated control group. This suggests that LL and LA could affect uric acid excretion by affecting GLUT9 mRNA expression (Figure 3D).

Protein expression of OAT1/3, SLC2A9 (GLUT9), and SLC22A12 (URAT1), as well as expression of other proteins, such as the multidrug resistance protein 4[25] and Na+-phosphate cotransporter[26], which are also highly influential on urate excretion, will be examined in future studies.

5 Conclusions

This study shows that LL and LA inhibited uric acid production in hyperuricemic rats by decreasing mRNA and protein expression of hepatic XOD. It also shows that LL and LA inhibited renal urate reabsorption by down-regulating renal GLUT9 and URAT1, and activated urate secretion by upregulating renal OAT1/3. The suppression of XOD activity and modulation of urate transporters to promote uric acid excretion by lotus leaf was shown to relieve hyperuricemia in rats. This suggests that the lotus leaf could be a potential target for the development of novel treatments for hyperuricemia in humans (Figure 4).

Conflict of interest statement

The authors declare no conflict of interest.

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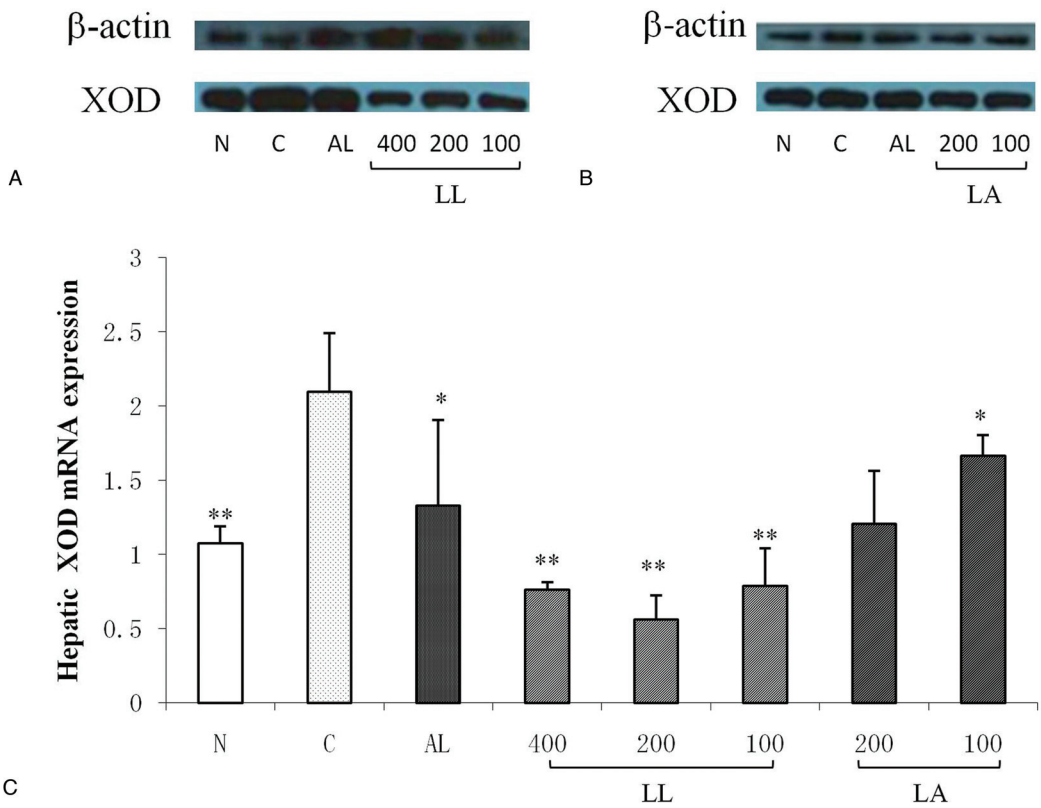

Figure 2. Effects of LL and LA on hepatic XOD mRNA and protein expression in hyperuricemic rats. Values represent the mean ± SEM of six determinations. $^{*}P < 0.05$; $^{**}P < 0.01$ versus the control group. (A) LL suppressed hepatic XOD protein expression. (B) LA suppressed hepatic XOD protein expression. (C) LL and LA suppressed hepatic XOD mRNA expression. N: normal group; C: control group; AL: positive drug group; LL (400 mg/kg/d, 200 mg/kg/d, 100 mg/kg/d) and LA (200 mg/kg/d, 100 mg/kg/d). AL: Allopurinol; LA: Lotus leaf total alkaloids fraction; LL: Lotus leaf crude extract; SEM: Standard error of the mean; XOD: Xanthine oxidase.

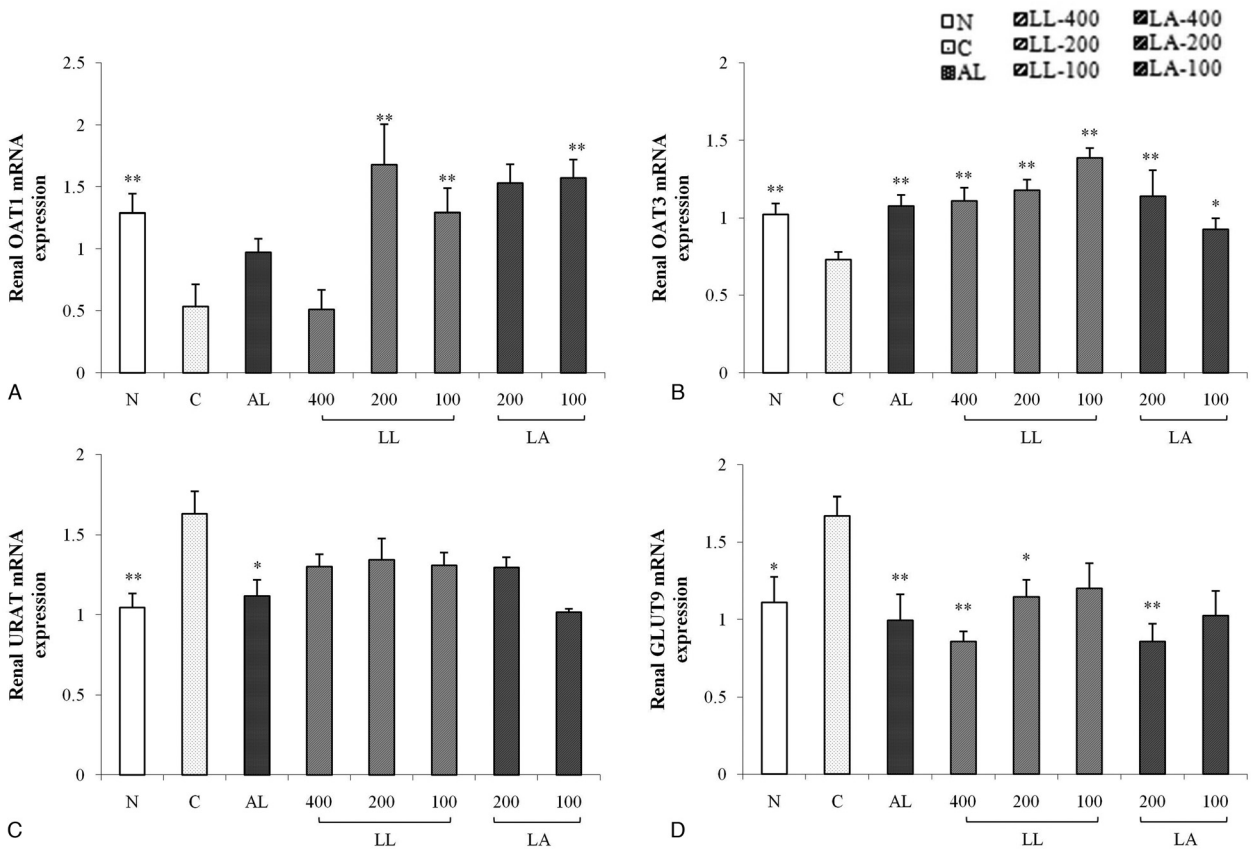

Figure 3. Effects of LL and LA on renal uric acid excretion genes expression in hyperuricemic rats. Values represent the mean ± SEM of six determinations. $^{*}P < 0.05$; $^{**}P < 0.01$ versus the control group. (A) Renal OAT1 mRNA expression. (B) Renal OAT3 mRNA expression. (C) Renal URAT1 mRNA expression. (D) Renal GLUT9 mRNA expression. N: normal group; C: control group; AL: positive drug group; LL (400 mg/kg/d, 200 mg/kg/d, and 100 mg/kg/d); and LA (200 mg/kg/d and 100 mg/kg/d). AL: Allopurinol; LA: Lotus leaf total alkaloids fraction; LL: Lotus leaf crude extract; OAT: Organic anion transporter; SEM: Standard error of the mean; URAT: Renal urate transporter 1; XOD: Xanthine oxidase.

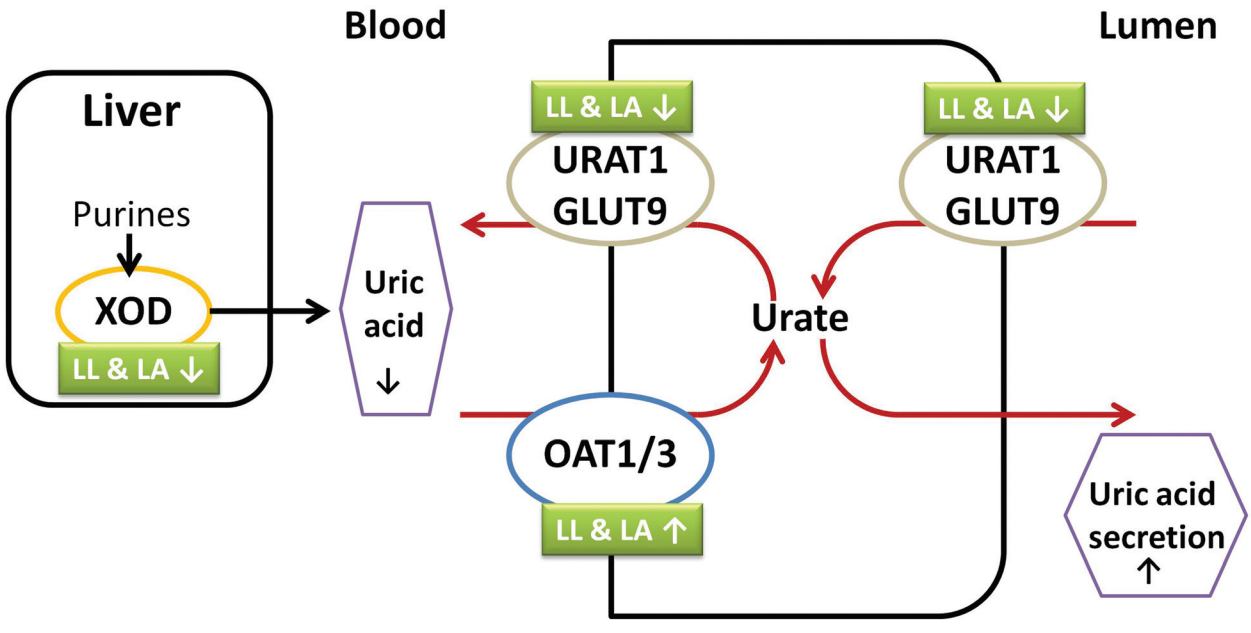

Figure 4. The schematic illustration of the mechanism of LL and LA, which inhibits uric acid generation in hyperuricemic rats. LA: Lotus leaf total alkaloids fraction; LL: Lotus leaf crude extract.

Author contributions

Yating An conducted the research and investigation process, specifically performed the experiments, and data/evidence collection and wrote the main part of the manuscript. Jia Hao took part in the planning and execution of the extraction experiments, also wrote the main part of the manuscript. Jian Li performed the HPLC experiments and analyzed the results. Lei Wang took part in the planning and execution of the experiments. Yi Zhang is responsible for supervising and leading the planning and execution of the research activity. All authors read and approved the final manuscript.

Ethical approval of studies and informed consent

The experimental rats were overseen and approved by the Science and Technological Committee and the Animal Use and Care Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2018017).

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None.

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