

RESEARCH ARTICLE



## *Lycium barbarum* polysaccharides protect mice from hyperuricaemia through promoting kidney excretion of uric acid and inhibiting liver xanthine oxidase

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### ABSTRACT

**Context:** *Lycium barbarum* L. (Solanaceae) polysaccharides (LBPs) are important active constituents that have demonstrated kidney protection.

**Objective:** This study investigated the effect of LBPs on hyperuricaemia and explored the underlying mechanism in mice.

**Materials and methods:** Thirty-six C57BL/6 mice were randomly divided into the control group, hyperuricaemia group, allopurinol group (5 mg/kg) and three LBP groups (n = 6). The LBP groups were treated orally with LBPs at 50, 100 and 200 mg/kg body weight for 7 days. We examined the levels of serum uric acid ( $S_{UA}$ ) and urinary uric acid ( $U_{UA}$ ), as well as xanthine oxidase (XOD) activities. mRNA and protein were quantified by quantitative real-time PCR and Western blotting, respectively.

**Results:** LBPs treatment (100 and 200 mg/kg) significantly reduced the  $S_{UA}$  levels to 4.83 and 4.48 mg/dL, and markedly elevated the  $U_{UA}$  levels to 4.68 and 5.18 mg/dL ( $p < 0.05$ ), respectively, while significantly increased the mRNA and protein expression levels of renal organic anti-transporter 1 (OAT1) and organic anti-transporter 3 (OAT3), and markedly decreased the levels of glucose transporter 9 (GLUT9) ( $p < 0.05$ ). Additionally, the serum XOD activities were reduced to 31.5 and 31.1 mU/mL, and the liver XOD activities were reduced to 80.6 and 75.6 mU/mL after treatment with 100 and 200 mg/kg LBPs ( $p < 0.01$ ), respectively.

**Discussion and conclusions:** This study demonstrated the potential role of LBPs in reducing the uric acid level in hyperuricemic mice. A border study population should be evaluated. These results are valuable for the development of new anti-hyperuricaemia agents from LBPs.

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### Introduction

Hyperuricaemia, characterized by high blood uric acid levels, is a metabolic disease. Under the condition of chronic hyperuricaemia, uric acid is crystallized and deposited as monosodium urate in the joint, being closely correlated with gout. Xanthine oxidase (XOD), the key enzyme in purine metabolism in the liver, is responsible for catalysing purine to uric acid (Choi et al. 2005; George and Struthers 2009). Thus, reducing the activity of XOD could be used to treat hyperuricaemia. In addition to the synthesis of uric acid, molecules responsible for the secretion and reabsorption of uric acid in the kidney also contribute to abnormal uric acid levels in the blood, such as renal organic anti-transporter 1 (OAT1), organic anti-transporter 3 (OAT3) and glucose transporter 9 (GLUT9). Presently, two types of agents have been used in the treatment of hyperuricaemia: one mainly accelerates uric-acid metabolism, and the other is used primarily to inhibit the activity of XOD, such as allopurinol. However, these agents have many adverse reactions, such as allergic reactions, kidney dysfunction, gastrointestinal symptoms and fever

(Chung et al. 2015; Yang et al. 2015). Therefore, a safer and more effective agent with no side effects is needed for the treatment of hyperuricaemia.

*Lycium barbarum* L. (Solanaceae), a traditional Chinese medicine, has the effects of nourishing the liver and kidney and replenishing vital essence to improve eyesight. *Lycium barbarum* polysaccharides (LBPs) are the main effective ingredient of *Lycium barbarum*, comprised of glucose, arabinose, galactose, mannose, xylose, rhamnose and fucose (Zeng et al. 2019; Liu et al. 2020). Studies have demonstrated that LBPs have multiple pharmacological functions, including antioxidant, anti-inflammatory, antitumor, immune regulation, neuroprotective, hypoglycaemic and hypolipidemic effects (Li XM et al. 2007; Miao et al. 2010; Cheng et al. 2015; Xing et al. 2016; Po et al. 2017; Tang ZY et al. 2017; Pop et al. 2020). Moreover, Li J et al. (2017) demonstrated the protective effects of LBPs in renal damage. Thus far, the use of LBPs to treat hyperuricaemia has not been reported. In this study, we examined the efficacy of LBPs in reducing the serum uric acid levels in a mouse model of

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hyperuricaemia induced by potassium oxonate. We also investigated the possible mechanisms of this effect.

## Materials and methods

### Chemicals and reagents

LBPs ( $\geq 90\%$ ) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Potassium oxonate (97%) and allopurinol ( $\geq 99\%$ ) was purchased from Sigma-Aldrich Co. LLC (Shanghai, China). Uric acid (UA), serum creatinine ( $S_{CR}$ ), blood urine nitrogen (BUN) and xanthine oxidase activity assay kit were purchased from Abcam Inc. (Cambridge, UK). The RNA isolation kit was obtained from Promega Biotechnology Co., Ltd. (Beijing, China). PrimeScript<sup>TM</sup> RT reagent kit with gDNA Eraser and TB Green<sup>®</sup> Premix Ex Taq<sup>TM</sup> II (Tli RNaseH Plus) was purchased from Takara Biomedical Technology Co., Ltd. (Dalian, China). Protease and phosphatase inhibitors were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Anti-OAT1, anti-OAT3 and  $\beta$ -actin antibody were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Anti-GLUT9 was purchased from Abcam Inc. (Cambridge, UK). The Amersham ECL System was purchased from GE Healthcare (Pittsburgh, PA, USA). Other biochemical reagents and chemicals were of analytical grade.

### Animals

Eight-week-old C57BL/6 male mice (Vital River Laboratory Animal Technology Co., Ltd., Beijing, China), weighing 20–25 g, were housed at room temperature ( $23 \pm 1^\circ\text{C}$ ) under standard conditions: humidity of  $50 \pm 5\%$ , allowing free access to water and food under 12h light/dark cycle (light at 8:00 am). The study was approved by the Ethics Committee of Central Hospital of Fushun for Animal Experiments and was performed according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication 86-23, 1985 revision).

Thirty-six mice were randomly divided into the control group, hyperuricaemia group, allopurinol (5 mg/kg) group and LBP groups (50, 100 and 200 mg/kg) ( $n = 6$ ). The concentrations of LBPs were determined according to the literature (Wu et al. 2010). Potassium oxonate, a urate oxidase inhibitor, was used to induce a hyperuricaemia mouse model (Stavric et al. 1975). In addition to the control group, the remaining five groups were intraperitoneally injected with potassium oxonate (250 mg/kg) at 8:00 am for seven consecutive days. Allopurinol or LBPs (50, 100 and 200 mg/kg) were orally administered at 9:00 am for seven consecutive days in the corresponding group.

Finally, whole blood was collected 1 h after the last administration on the 7<sup>th</sup> day. After coagulation for 1 h at room temperature, the blood was centrifuged at 4000 g for 10 min and the serum was collected. The urine was collected using a mouse metabolic cage. The serum and urine samples were stored at  $-20^\circ\text{C}$  until analysis. The liver and kidney tissues of the mice were quickly separated on ice and stored at  $-80^\circ\text{C}$  for testing.

### Blood and urine sample analyses

The collected serum and urine samples of the mice were used to detect the levels of serum uric acid ( $S_{UA}$ ),  $S_{CR}$ , BUN and urinary uric acid ( $U_{UA}$ ) according to the manufacturer's instructions using appropriate kits.

### Serum and hepatic XOD activity analysis

Briefly, the liver tissue of the mice was thoroughly homogenized in an ice bath by placing it in sodium phosphate buffer at pH 7.4. The homogenate was centrifuged at 4000 g for 20 min at  $4^\circ\text{C}$  to extract the supernatant for subsequent use. The serum and hepatic XOD activities were measured using the xanthine oxidase activity assay kit according to the manufacturer's instructions. The absorbance of each well was measured at 570 nm using an Infinite M Plex Fully Loaded Multimode Plate Reader (Tecan, Männedorf, Switzerland). Each assay was performed in duplicate. XOD activity (mU/mL) was calculated using the formula provided by the manufacturer's instructions.

### RNA isolation and quantitative real-time PCR

Total RNA was extracted from mouse renal tissue using the RNA isolation kit, and quantitative real-time PCR using PrimeScript<sup>TM</sup> RT reagent kit with gDNA Eraser and TB Green<sup>®</sup> Premix Ex Taq<sup>TM</sup> II (Tli RNaseH Plus) was performed according to our previous report (He et al. 2019) with the following primers: Slc22a6 (OAT1)-F: 5'-TCTGCCTCTCTATGCTGTGG-3'; Slc22a6 (OAT1)-R: 5'-GATTGTATGGCCCTCGGTA-3'; Slc22a8 (OAT3)-F: 5'-GCCAACCACAACCTTGCTACA-3'; Slc22a8 (OAT3)-R: 5'-GATCCAGCCATCCAAGCATG-3'; Slc2a9 (GLUT9)-F: 5'-GGCTCCTTTTCCCTTTCATC-3'; Slc2a9 (GLUT9)-R: 5'-CTGAGCCTTGTTCCTCTTGG-3'; GAPDH-F: 5'-AGTGTTTCCTCGTCCCGTAG-3'; GAPDH-R: 5'-GCCGTGAGTGGAGTCATACT-3'.

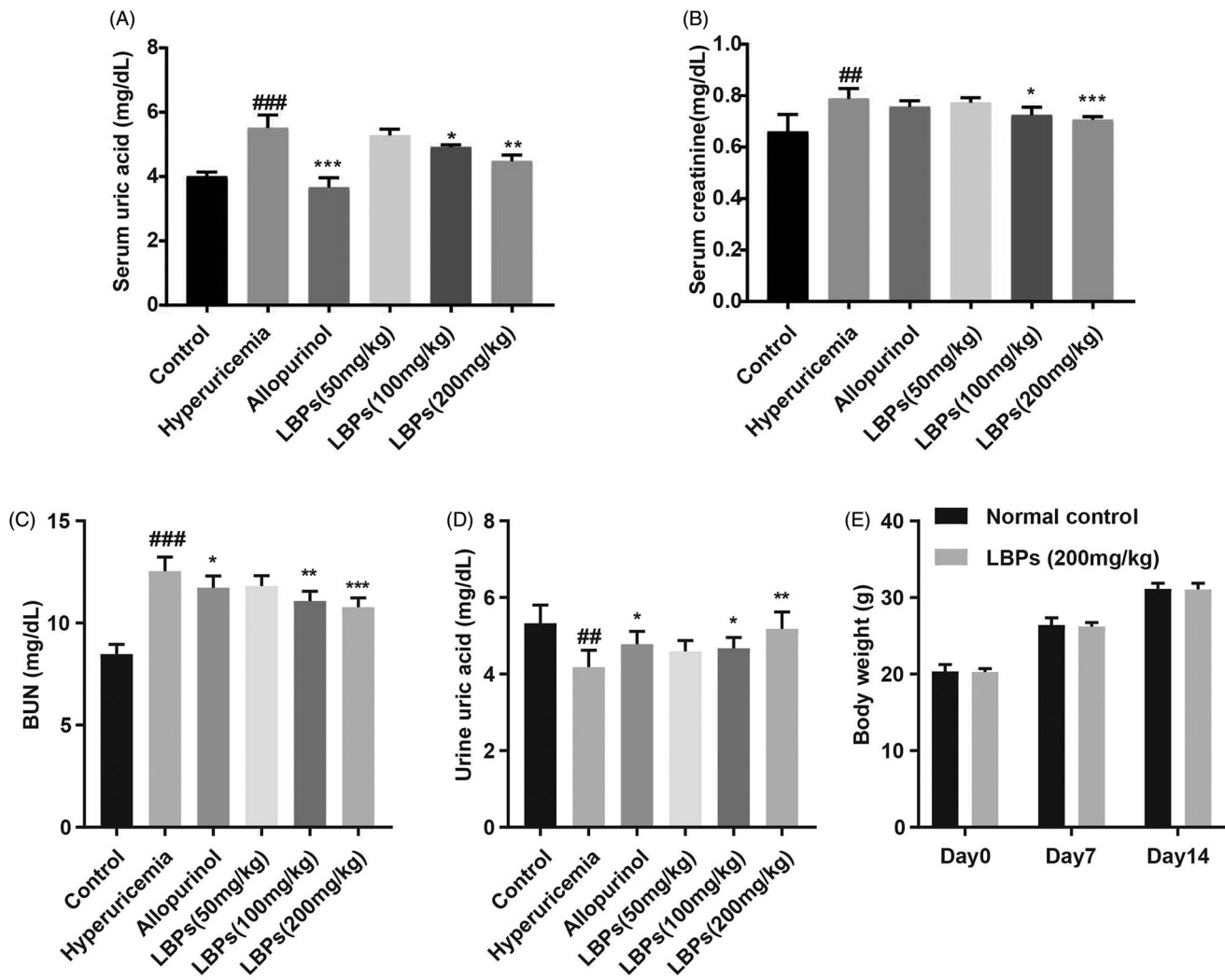
The relative mRNA expression levels of Slc22a6, Slc22a8 and Slc2a9 were normalized to that of GAPDH.

### Western blotting

Protein extracts were obtained following the collection of kidney tissue in RIPA lysis buffer supplemented with protease and phosphatase inhibitors. Western blotting was performed as previously described (He et al. 2019). The PVDF membrane was incubated with anti-OAT1, anti-OAT3 or anti-GLUT9 at a dilution of 1:200–1:1000 overnight at  $4^\circ\text{C}$ . An antibody against  $\beta$ -actin (1:1000) was used as a loading control. A horseradish peroxidase-labeled secondary antibody was added for 1 h at room temperature, and the enzymatic substrate was added. The Amersham ECL System was used to develop the blot, and the films were scanned for quantification by densitometry using ImageJ software (National Institutes of Health, Bethesda, MD, <http://imagej.nih.gov/ij/>, 1997e2012).

### Statistical analysis

All the data in this study were analysed using SPSS 17.0 software for Windows (IBM Corp., Armonk, NY, USA). The data were expressed as the means  $\pm$  standard deviation (SD). Statistical comparisons between the two groups were performed by Student's *t*-test. A *p* value  $< 0.05$  was considered statistically significant.



**Figure 1.** Effect of LBPs on the (A)  $S_{UA}$  levels, (B)  $S_{CR}$  levels, (C) BUN levels, (D)  $U_{UA}$  levels of potassium oxonate-induced hyperuricemic mice and (E) body weight (g) of wild-type C57BL/6 mice. The data are presented as means  $\pm$  SD,  $n=6$  per group.  $##p < 0.01$  and  $###p < 0.001$  compared with the control group.  $*p < 0.05$ ,  $**p < 0.01$  and  $***p < 0.001$  compared with the hyperuricaemia group.

## Results

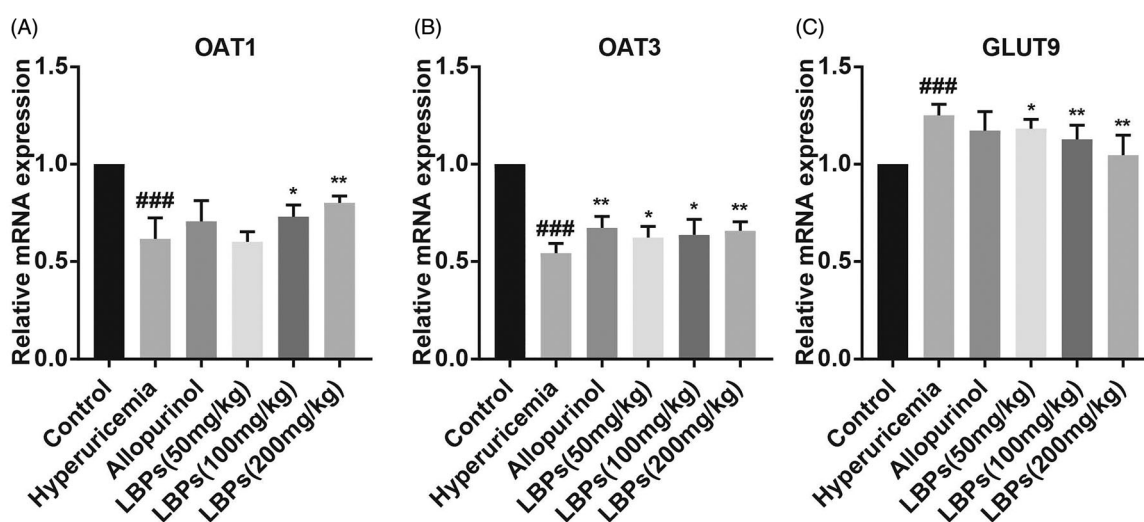
### Effect of LBPs on the $S_{UA}$ , $S_{CR}$ , BUN and $U_{UA}$ levels in mice with hyperuricaemia

After intraperitoneal injection of potassium oxonate, the  $S_{UA}$  level was significantly increased in the hyperuricaemia group compared with that in the control group ( $p < 0.001$ , Figure 1(A)), suggesting that the mouse model of hyperuricaemia was successfully established. The  $S_{UA}$  levels in the allopurinol group were reduced compared with those in the hyperuricaemia group ( $p < 0.001$ , Figure 1(A)), which was used as a positive control. In the LBP (50, 100 and 200 mg/kg) groups, the  $S_{UA}$  level was also decreased ( $p < 0.05$  or  $p < 0.01$ ) and correlated with the concentration of LBPs, although no significant difference was found between the 50 mg/kg LBP group and hyperuricaemia group, indicating that LBPs reduce the  $S_{UA}$  level in the hyperuricemic mice in a dose-dependent manner (Figure 1(A)). At the same time, we examined the levels of  $S_{CR}$  and BUN. Consistently, decreases in both the  $S_{CR}$  levels and BUN levels were found in the LBP groups ( $p < 0.05$ ,  $p < 0.01$  or  $p < 0.001$ ; Figure 1(B,C)). We also examined the  $U_{UA}$  levels in all groups. As shown in Figure 1(D), the levels of  $U_{UA}$  in the LBP-administered hyperuricemic mice were increased compared with hyperuricemic mice

without LBP treatment ( $p < 0.05$  or  $p < 0.01$ ), suggesting that LBPs affect the renal excretion of uric acid. What's more, the acute toxicity study showed no significant changes in body weight (Figure 1(E)), respiration, behaviour changes or death, indicating the safety of the maximal tolerable dose of LBPs. Therefore, LBPs reduced the  $S_{UA}$  levels in hyperuricemic mice likely by increasing the excretion of uric acid from the blood into urine.

### Effect of LBPs on the protein and mRNA expression levels of OAT1, OAT3 and GLUT9 in kidney tissue of hyperuricemic mice

Many studies have demonstrated that certain molecules are responsible for the excretion and absorption of UA in the kidney, such as OAT1, OAT3 and GLUT9, which are encoded by *Slc22a6*, *Slc22a8* and *Slc2a9*, respectively. To further investigate the mechanism, we performed quantitative real-time PCR to examine the mRNA levels of OAT1, OAT3 and GLUT9 in mouse kidney tissue in different groups. Compared with the control group, the mRNA levels of OAT1 and OAT3 were downregulated, and that of GLUT9 was upregulated, in the hyperuricaemia group ( $p < 0.001$ , Figure 2(A-C)), indicating that



**Figure 2.** Effect of LBPs on the relative mRNA expression levels of OAT1, OAT3 and GLUT9 in the kidney tissue of potassium oxonate-induced hyperuricemic mice. The data are presented as means  $\pm$  SD,  $n=6$  per group. ### $p < 0.001$  compared with the control group. \* $p < 0.05$  and \*\* $p < 0.01$  compared with the hyperuricaemia group.

the transport of UA from the blood into urine was inhibited in hyperuricemic mice. However, LBPs increased the mRNA levels of OAT1 and OAT3 but decreased the mRNA level of GLUT9 in a dose-dependent manner compared with the hyperuricaemia group ( $p < 0.05$  or  $p < 0.01$ , Figure 2(A–C)).

We also detected the protein expression levels of OAT1, OAT3 and GLUT9 by western blot analysis. As shown in Figure 3, although the protein level of OAT3 was elevated in hyperuricemic mice treated with allopurinol ( $p < 0.01$ ), no significant difference was found between the two groups above in the protein expression of OAT1 and GLUT9. However, the protein levels of OAT1 and OAT3 were significantly increased, and that of GLUT9 was decreased, in the LBP groups compared with those in the hyperuricaemia group ( $p < 0.05$ ,  $p < 0.01$  or  $p < 0.001$ ; Figure 3). In summary, LBPs affect OAT1, OAT3 and GLUT9 expression, likely by regulating the excretion and absorption of  $S_{UA}$  in the hyperuricemic mouse kidney.

#### Effect of LBPs on XOD activities in the serum and liver of mice with hyperuricaemia

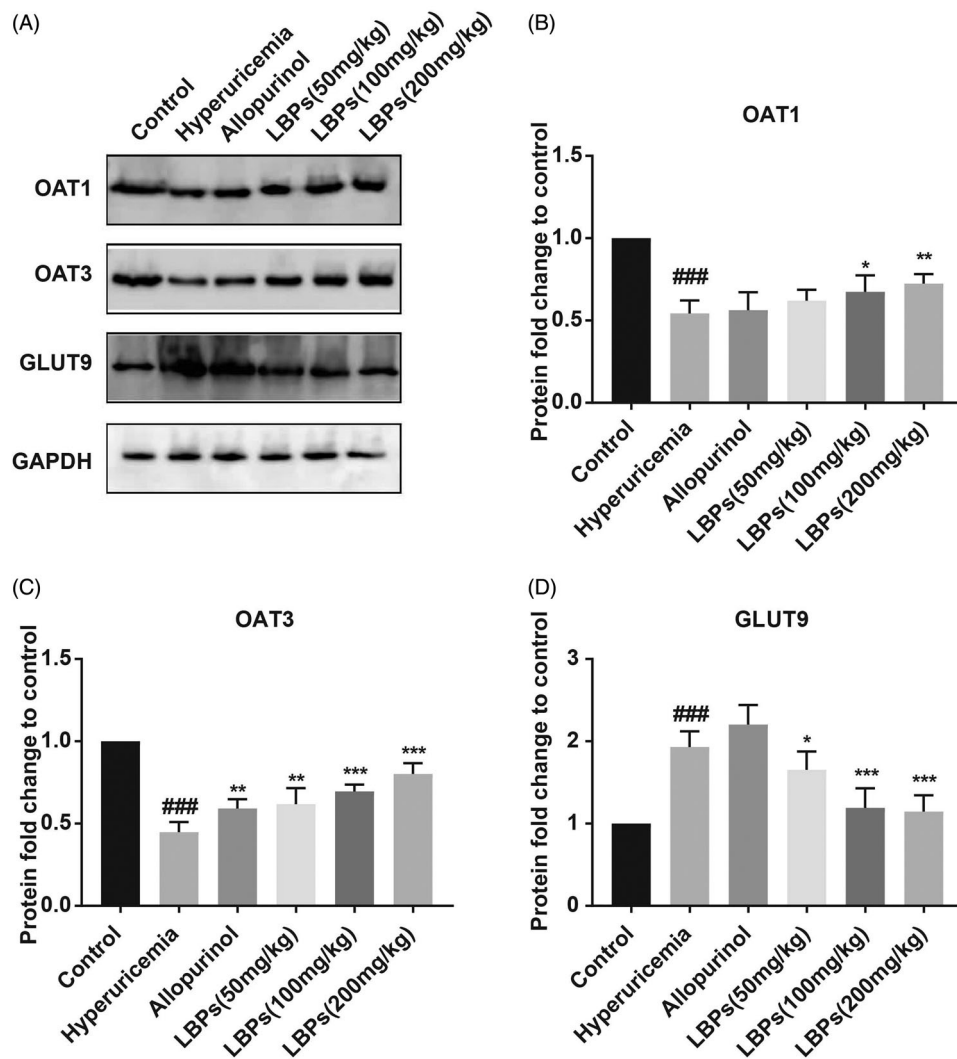
We further determined whether LBPs decrease the  $S_{UA}$  level in hyperuricemic mice by reducing its synthesis. To test this hypothesis, we tested the serum and hepatic XOD activity in mice. As expected, the serum and hepatic XOD activities were significantly increased in the hyperuricaemia group compared with those in the control group ( $p < 0.001$ , Figure 4(A,B)); by contrast, the serum XOD and hepatic XOD activities were significantly inhibited in the allopurinol group compared with those in the hyperuricaemia group ( $p < 0.01$ , Figure 4(A,B)). In the LBP (100 and 200 mg/kg) groups, the serum XOD and hepatic XOD activities were also inhibited compared with those in the hyperuricaemia group ( $p < 0.01$ , Figure 4(A,B)), indicating the potential mechanism of the decreased  $S_{UA}$  levels in hyperuricemic mice treated with LBPs occurs by suppressing XOD activity.

#### Discussion

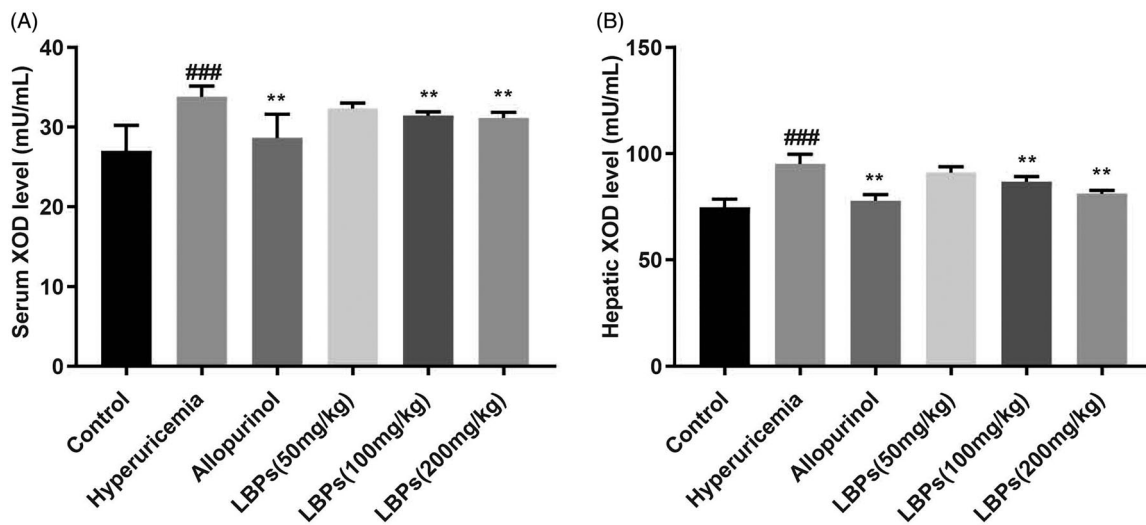
Hyperuricaemia is a metabolic disease caused by disorders of uric acid metabolism, which is characterized by elevated blood uric acid levels. Hyperuricaemia can cause gouty arthritis, renal

insufficiency, systemic inflammation, cardiovascular disease, and other metabolic abnormalities (Abeles 2015; Cabău et al. 2020). Hyperuricaemia is mainly caused by insufficient excretion of uric acid by the kidneys or excessive uric acid production by the liver. Uric acid excretion disorders are caused primarily by abnormal urate transporters in the proximal tubules, such as OAT1 (Slc22a6), OAT3 (Slc22a8) and GLUT9 (Slc2a9) (Yong et al. 2017; Fang et al. 2019). Among them, OAT1 and OAT3 are used to excrete urate from the kidney into the urine, which is then excreted from the body, while GLUT9 regulates the reabsorption of urate (Yong et al. 2017; Fang et al. 2019). The excessive production of uric acid is mainly due to the enhanced activity of the key enzyme XOD during uric acid production or the large intake of purines in the diet (Tang et al. 2018). Therefore, regulation of these transporters in the kidney and XOD activity in the liver are key targets for the treatment of hyperuricaemia. Studies have shown that LBPs have protective effects on liver and kidney tissue (Li et al. 2017; Rjeibi et al. 2018; Liao et al. 2019). However, the effect of LBPs on hyperuricaemia remains unclear. In the present study, we observed that LBPs decreased the  $S_{UA}$  levels in hyperuricemic mice in a dose-dependent manner. Moreover, the BUN and  $S_{CR}$  levels were decreased in hyperuricemic mice treated with LBPs, indicating the protective effect of LBPs on the kidney and corroborating the findings of previous reports (Li et al. 2017; Rjeibi et al. 2017). Conversely, the  $U_{UA}$  level was increased in hyperuricemic mice treated with LBPs. These data demonstrated that LBPs reduced the  $S_{UA}$  levels, and the underlying mechanism is via increased UA excretion.

To test the hypothesis above, we assessed the mRNA expression levels of Slc22a6, Slc22a8 and Slc2a9 in the kidneys of each mouse group. The results showed that LBPs upregulated the expression levels of Slc22a6 and Slc22a8 but downregulated those of Slc2a9 in the hyperuricemic mouse kidney. At the protein level, LBPs increased the expression levels of OAT1 and OAT3 and decreased the expression level of GLUT9 in the hyperuricemic mouse kidney. These results indicated that LBPs reduce the level of uric acid in the blood by increasing the excretion of uric acid and reducing the reabsorption of uric acid by the kidney. The clinical first-line anti-hyperuricaemia drug benzbromarone works by strengthening the excretion of uric acid in the kidney's



**Figure 3.** Effect of LBPs on the protein levels of OAT1, OAT3 and GLUT9 in the kidney tissue of potassium oxonate-induced hyperuricemic mice. (A) The protein levels were measured by western blotting, and (B–D) densitometry was calculated. The data are presented as means  $\pm$  SD,  $n = 6$  per group. ### $p < 0.001$  compared with the control group. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  compared with the hyperuricaemia group.



**Figure 4.** Effect of LBPs on the (A) serum and (B) liver XOD activities of potassium oxonate-induced hyperuricemic mice. The data are presented as means  $\pm$  SD,  $n = 6$  per group. ### $p < 0.001$  compared with the control group. \*\* $p < 0.01$  compared with the hyperuricaemia group.

proximal tubule, thereby maintaining blood uric acid at a suitable level. However, many studies have demonstrated the hepatotoxicity of benzbromarone (Wang et al. 2016, 2017), which restricted its usage in some countries, such as the United States.

Allopurinol is another clinical treatment for gout, mainly by inhibiting XOD activity, thereby reducing liver uric acid production. However, studies have reported that allopurinol may cause serious side effects such as rash, liver toxicity or gastrointestinal toxicity (Umamaheswari et al. 2009). Therefore, it is necessary to develop new anti-hyperuricaemia drugs. In the present study, we found that LBPs could significantly reduce XOD activity in the blood and liver of hyperuricemic mice. According to reports in the literature, the pharmacological effects of LBPs are related to their ability to scavenge oxygen free radicals (Li et al. 2007), and active oxygen species, including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide anion (O<sup>2-</sup>), are produced during the metabolism of purine (Berry and Hare 2004). These findings may explain why LBPs inhibit XOD activity, but further verification is needed.

## Conclusions

Our study demonstrated that LBPs exert an anti-hyperuricaemia effect by increasing the expression of renal OAT1 and OAT3, decreasing the expression of renal GLUT9, and inhibiting XOD activity. The results obtained under our experimental conditions are valuable for the development of new anti-hyperuricaemia agents. Therefore, the anti-hyperuricaemia effect of LBPs on other animals should be further investigated.

## Disclosure statement

The authors declare that there are no conflicts of interest.

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