Original Article
Hypouricemic and nephroprotective effects of Jianpi Huashi decoction in a rat model of hyperuricemia

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Abstract: Hyperuricemia, indicated by elevated serum level of uric acid, is a risk factor for developing gout, hypertension, renal disease and cardiovascular disease. In the present study, we evaluated the therapeutic efficacy of a novel hypouricemic agent, Jianpi Huashi Decoction (JPHSD), derived from Chinese herbal medicines *Rhizoma Smilacis Glabrae, Plantago asiatica* L.*, kudzu* root*, Cichorium intybus* L.*,* seeds of *Coix lacryma-jobi* L.*,* and *Alismatis Rhizoma*, and tuna extract in a rat model of hyperuricemia induced by potassium oxonate. The results showed that JPHSD effectively reduced the serum level of uric acid in hyperuricemic rats. The hypouricemic effect of JPHSD was achieved in two aspects: reducing the production of uric acid by inhibition of hepatic xanthine oxidase activity, and promoting renal excretion of uric acid through regulation of major urate transporters. Consequently, the oxidative stress induced by hyperuricemia was attenuated, and the renal damage was ameliorated by JPHSD treatment. Our study demonstrated that the novel agent JPHSD had both hypouricemic and nephroprotective effects in hyperuricemic rats.

Keywords: JPHSD, hyperuricemia, uric acid, urate transporter, ion transporter, oxidative stress

Introduction

Hyperuricemia is the underlying cause of gout, a metabolic disorder characterized by increased level of uric acid in the blood. The excessive serum uric acid crystallizes and deposits in the joints and leads to recurrent inflammatory arthritis [1]. The incidence rates of hyperuricemia and gout have increased rapidly over the past decade, which is believed to be associated with the change in life styles and diet structure, and influence from environmental factors [2-4]. Gout is also frequently accompanied by other medical conditions, including diabetes, hypertension, hyperlipemia, renal disease, atherosclerosis, and cardiovascular disease [1, 5, 6]. Therefore, it will be of great significance to design effective therapeutics to treat and prevent hyperuricemia, and to improve the quality of patients with the disease.

Uric acid is the metabolic product of purine metabolism, which involves breakdown of hypo-xanthine into xanthine and subsequently uric acid by the enzyme called xanthine dehydrogenase (XDH) and its converted form xanthine oxidase (XOD) [7, 8]. When the uric acid metabolic pathway is disrupted, either by over-production of uric acid due to increased purine intake or decreased excretion of uric acid from the kidney due to impaired renal function, hyperuricemia develops and the risk of having gout increases substantially [9, 10]. Inhibition of XOD activity has been proved to effectively lower serum level of uric acid and prevent recurrent gout attacks. The XOD inhibitor allopurinol is currently in clinical use to treat hyperuricemia and chronic gout [11, 12]. Allopurinolhas long been the first-line therapy for gout, but the use of suboptimal dose in patients with impaired renal function is ineffective. In addition, severe allergic reaction and hypersensitivity syndromes are the most common side effects of allopurinol which limit its use [13]. Febuxostat is a new XOD inhibitor currently tested in clinical trials. Available data from comparative studies

showed that febuxostat had a similar hypouricemic effect as allopurinol but higher risk of causing liver damage [14, 15]. When XOD inhibitors are ineffective or the patients are unresponsive to the treatment, other therapeutic agents are introduced to those patients, such as benzbromarone and probenecid. Benzbromarone is a uricosuric drug with strong inhibitory activity on the uric acid transporter 1 (URAT1) mediated urate reabsorption, and thus highly effective in treating hyperuricemia [16]. However, its safety has been doubted due to the irreversible adverse effects and serious hepatotoxicity [17]. It has been withdrawn from the US market since 2003. Probenecid targets the organic anion transporters (OAT) in the kidney, thereby blocks the transport of uric acid across the cell membrane into plasma and eventually reduces the serum concentration of uric acid [18]. However, severe and potentially life-threatening adverse reaction and liver damage have been reported in several cases [17, 19]. A new therapeutic alternative with high efficacy and low risk is in urgent need.

In this study, we evaluated the efficacy of a novel agent developed in our laboratory, designated as Jianpi Huashi Decoction (JPHSD), in reducing serum uric acid levels in a rat model of hyperuricemia. JPHSD contains extracts from Chinese herbal medicines *Rhizoma Smilacis Glabrae, Plantago asiatica* L.*, kudzu* root*, Cichorium intybus* L.*,* seeds of *Coix lacryma-jobi* L.*,* and *Alismatis Rhizoma*, all of which have long been used in traditional Chinese medicine to treat gout orhave documented anti-gout and anti-inflammatory effects [20, 21]. In addition, recent studies have suggested that peptides might have anti-hyperuricemic effect. In a study using fractions of the proteolytic digest of shark cartilage, peptides have been shown to function as the bioactive component to modulate hyperuricemia in rats [22]. Further investigation showed that the dipeptides derived from milk protein could non-competitively inhibit the XOD activity [23, 24]. To develop a novel agent with high anti-hyperuricemic efficacy, we combined the Chinese herbal medicines with tuna extract which is a rich source of peptides, and studied the combined effect in rats. Humans lack uricase, a uric acid-oxidizing enzyme, so the end product of purine metabolism, uric acid, gets excreted in urine. While in rats, uric acid is further oxidized to allantoin by uricase [25]. Therefore, inhibition

of uricase activity is essential to establish a rat model of hyperuricemia. In this study, we used potassium oxonate, a well documented effective uricase inhibitor, to induce a hyperuricemic condition in rats and evaluated the efficacy of JPHSD in reducing level of serum uric acid.

Materials and methods

Animals

Male Sprague-Dawley (SD) rats (age: 7-8 weeks; weight: 180-220 g) were purchased from the Experimental Animal Center in Guangzhou University of Chinese Medicine (Guangzhou, Guangdong, China) with the batch number of SCXK-2013-0020. Rats were allowed to acclimatize for 1 week before the experiment. All rats were maintained under specific pathogen free (SPF) laboratory conditions with temperature at $22 \pm 1°C$, relative humidity $55 \pm 5\%$, and automatic 12-h light/12-h dark cycle. They were allowed free access to food and water. All the cages, food, and water were processed by 121°C steam sterilization. All protocols for animal experiments were approved by the Animal Care Committee of Guangzhou University of Chinese Medicine.

Reagents and preparation of JPHSD

JPHSD was developed and prepared in our laboratory. Briefly, mixture of Chinese herbal medicines *Rhizoma Smilacis Glabrae* (from Guangxi, China), *Plantago asiatica* L. (from Jiangxi, China), *kudzu* root (from Anhui, China), *Cichorium intybus* L. (from Heilongjiang, China), seed of *Coix lacryma-jobi* L. (from Hebei, China), and *Alismatis Rhizoma* (from Sichuan, China) (mixed according to 10:5:5:4:10:4 by weight) were subjected to extraction by refluxing with distilled water (1:12, w/v) for 1.5 h. The extract process was repeated with distilled water (1:8, w/v) for 1 h. The filtrates were combined and evaporated, and the concentrated herbal extract was obtained [yield 20% (w/w)]. Wild tunas were caught and stored at -20°C until use. After rehydration, heads and internal organs were removed, and the remaining parts were grounded followed by crude proteolytic digestion with food-grade protease including Papain (Nanning pangbo, China) and Neutrase (Novozymes, China) at 50-60°C for 4 hours. The digested samples were heated at 95°C for 20 minutes to stop protease activity, and then centrifuged at

8000 rpm for 20 minutes at 4°C. Next, the supernatants were collected, clarified by filtration, and spray dried. The resulting powder was resolved in the concentrated herbal extract to make JPHSD. After sterilization, JPHSD was aliquoted and ready for use.

Concentrated JPHSD was diluted to the required concentration using 0.1% carboxymethylcellulose sodium (CMC-Na, purchased from Matrix Laboratories Ltd, Xiamen, Fujian, China). Three different doses of JPHSD - 1.67,3.33, and 6.67 mL/kg per day- were used in the experiments. Allopurinol and potassium oxonate (Oxo) were purchased from Matrix Laboratories Ltd. Benzbromarone was purchased from Kunshan-Rotam Reddy Pharmaceutical Co. (Kunshan, Jiangsu, China). The assay kit for measurement of serum uric acid was purchased from Shanghai Kehua Bioengineering Institute (Shanghai, China), and those for measurement of XOD, SOD and MDA activities were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Results of the assays were determined using the AU5421 automatic biochemistry analyzer (Olympus, Tokyo, Japan). Primary antibodies against GLUT9, OCT1, OCT2, OCTN1, OCTN2 and OAT1 were purchased from Santa Cruz (Dallas, Texas, USA), anti-URAT1 was purchased from Abbiotec (San Diego, CA, USA), and anti-GAPDH was purchased from Abcam (Cambridge, MA, USA). Secondary antibodies (goat anti-rabbit and goat anti-mouse) were purchased from Boster Bio (Pleasanton, CA, USA).

Rat model of hyperuricemia and drug administration

All rats were randomly divided into 7 groups (n = 10 per group): Normal (no treatment), Oxo (treated with potassium oxonate and water), Oxo + AP (treated with potassium oxonate and allopurinol), Oxo + BB (treated with potassium oxonate and benzbromarone), Oxo + JPHSD_L (treated with potassium oxonate and low dose of JPHSD), Oxo + JPHSD_M (treated with potassium oxonate and medium dose of JPHSD), and Oxo + JPHSD_H (treated with potassium oxonate and high dose of JPHSD). The rat model of hyperuricemia was established by administration of potassium oxonate (1.50 g/kg·d) through oral gavage once daily at 9:00 a.m. for 30 consecutive days. 0.1% CMC-Na solution

was used as vehicle control in the normal group. Allopurinol (27.0 mg/kg·d), benzbromarone (4.5 mg/kg·d), and JPHSD (1.67, 3.33, and 6.67 mL/kg·d) were given to the groups Oxo + AP, Oxo + BB, Oxo + JPHSD_L, Oxo + JPHSD_M, and Oxo + JPHSD_H, respectively, by oral gavage once daily at 1:00 p.m. for 30 consecutive days. 0.1% CMC-Na solution was given to the normal group and Oxo group as control. All doses of drug administered were determined based on the body weight measured immediately prior to each treatment. The volume of all the gavages was 10 mL/kg·d. The activity level, health status, and the amount of food intake for all rats were monitored daily.

Sample collection and measurements

Whole blood samples were collected at 4:00 p.m. on days 30. The blood was allowed to clot at room temperature and the serum was isolated by centrifugation at 2500 x g for 10 min. Simultaneously, liver and kidney were removed promptly and examined for morphology and pathologic changes. Portions of liver and kidney (including renal cortex and medulla) were fixed in formaldehyde solution (10% formalin, purchased from Zhongnan Laboratory Chemicals, Guangzhou, Guangdong, China) and processed for histological analysis. Another portion of liver or renal cortex was stored in RNAlater tissue storage reagent (Invitrogen, Grand Island, NY, USA) at -80°C for total RNA isolation. A third portion of renal cortex was frozen by liquid nitrogen flash freezing and stored at -80°C for total protein isolation. The rest of liver and kidney tissues were homogenized in 5 mM Tris-HCl buffer (pH 7.4). The homogenates were centrifuged at 15,000 x g for 10 min at 4°C, and the supernatant was collected for measurement of XOD, SOD, and MDA using the assay kits according to the manufacturer's instructions.

RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from tissue samples stored in RNAlater reagent following the manufacturer's instructions. cDNA was prepared from 1 μg RNA using the PrimeScript II 1st Strand cDNA Synthesis Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed using

Table 1. Primer sequences used in qRT-PCR analysis to measure the expression level of major transporters

Gene	Genebank	Primer	Primer Sequence
OAT1	NM_017224.2	Forward	GTACCCCACAGTGATTCGGC
		Reverse	GAGGCATGGAGGGGTAGAAC
OCT1	NM_012697.1	Forward	GTCACATCTGTGTCCGGTGT
		Reverse	CCACTAGCCCCACTGTGAAG
OCT2	NM_031584.2	Forward	TGAGGACGCTGGCAAGAAAT
		Reverse	AAGGCCCATGTGCATGATGA
OCTN1	NM_022270.1	Forward	CATCCGAGACTGGAGGATGC
		Reverse	ATGCCATTCATCTTTGCGGC
OCTN2	NM_019269.1	Forward	GTATCCCCAGATCCCCGGAC
		Reverse	AGCCATTGGGGATGATGCTG
GLUT9	NM_001191551.1	Forward	GAGGTGGACCATCAGAGCAC
		Reverse	GCTCTGCTCTACCCCTTGTC
URAT1	NM_001034943.1	Forward	GCCGCCACTGATTTGTGATT
		Reverse	GTGAGGGCTGGTGCAAAAAG
GAPDH	NM_017008.3	Forward	TGCCACTCAGAAGACTGTGG
		Reverse	TTCAGCTCTGGGATGACCTT

SYBR Green PCR Master mix in the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Expression levels of target genes were normalized to *GAPDH* internal control. All samples were processed in triplicate. The sequences of PCR primers are listed in **Table 1**.

Protein isolation and Western blotting

Total protein was extracted from frozen renal cortex samples. Briefly, every 10 mg of samples were homogenized in 200 μl of lysis buffer supplemented with phosphatase inhibitors, protease inhibitors and PMSF (Keygentec Inc., Nanjing, China) on ice. The homogenate was centrifuged at 12000 rpm, 4°C for 15 min, and the supernatant was collected and stored at -80°C until further use. Protein concentration was determined using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Equal amount (40-60 μg) of total proteins was separated on 10% SDS-PAGE, followed by transferring to a polyvinylidene difluoride (PVDF) membrane (Merck Millipore, Billerica, MA, USA). Membrane was blocked in TBS-T containing 5% skim milk for 1 h at room temperature with gentle agitation, and incubated at 4°C overnight with antibodies against GLUT9, OCT1, OCT2, OCTN1 and OCTN2 (1:1000), and URAT1, OAT1 and GAPDH (1:500). The membrane was then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2500) after washing in TBS-T for 15 min and repeating 3 times. The immunoreactive bands were visualized after addition of BeyoECL Plus reagents (Beyotime Inc., Haimen, Jiangsu, China) during exposure to X-ray film (Kodak, New Haven, CT, USA).

Histological analysis of liver and kidney tissues

Liver and kidney tissues fixed in formaldehyde solution were dehydrated in alcohol and embedded in paraffin. A series of tissue sections (4 μm) were made, and hematoxylin and eosin (H&E) staining was performed for histological evaluation using an OLYMPUS BX41 microscope (Olympus, Center Valley, PA, USA).

Statistical analysis

All the assays were performed at least three times. All data were expressed as the mean ± standard error of themean (S.E.M.) from 10 rats per group, and comparison between groups was made by one-way analysis of variance (ANOVA). *P* values were further adjusted by Bonferroni's multiple comparison tests. A value of $P<0.05$ was considered statistically significant.
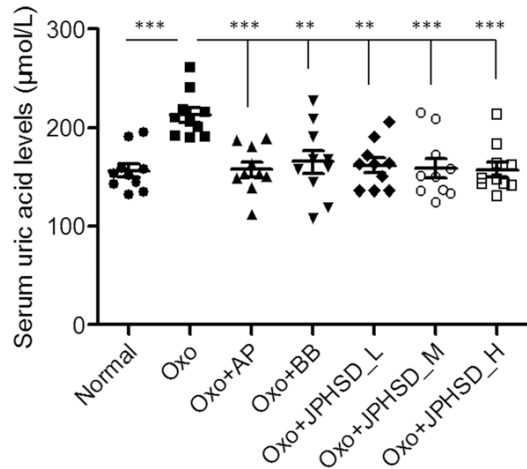
Results

*Hypouricemic effect of JPHSD in potassium oxonate-induced hyperuricemia in rats*

The model of potassium oxonate-induced hyperuricemia was successfully established in rats, as indicated by drastic increasing of serum uric acid levels in rats after treatment of potassium for 30 days when compared with the normal group ($P<0.001$) (**Figure 1**). After treatment for 30 days, both allopurinol and benzbromarone could significantly reduce the serum uric acid levels in the hyperuricemic rats (both $P<0.01$) (**Figure 1**). The same effect could be observed in the hyperuricemic rats ($P<0.01$) with treatment of JPHSD at different doses (**Figure 1**), suggesting high sensitivity of JPHSD in lowering the serum uric acid levels.

Figure 1. Hypouricemic effect of different doses of JPHSD in potassium oxonate-induced hyperuricemic rats. The levels of serum uric acids in 7 different groups of rats were measured on day 30 after treatments (n=10 per group). The results were expressed as mean ± S.E.M for each group. **represents *P*<0.01 and ***represents *P*<0.001 when compared with the Oxo group.
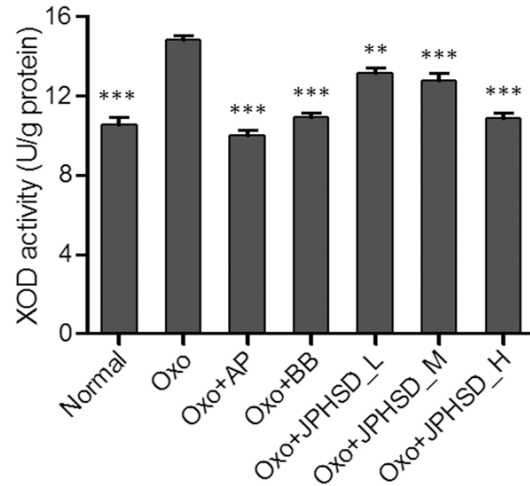
Figure 2. Effect of JPHSD treatment on enzymatic activities of hepatic XOD in hyperuricemic rats. The activities of XOD in liver of hyperuricemic rats were measured using the standard assay kits. The results were expressed as mean ± S.E.M. of ten rats for each group. **represents *P*<0.01 and ***represents *P*<0.001 when compared with the Oxo group.

Inhibitory effects of JPHSD on hepatic XOD activities

Potassium oxonate-induced hyperuricemia was further validated by the increased hepatic activity of XOD, which is akey mediator in purine metabolism and uric acid production and a well-known therapeutic target for many hypouricemic agents. As shown in **Figure 2**, allopurinol and benzbromarone significantly suppressed the activities of XOD to the normal levels (both *P*<0.001). All of the three doses of JPHSD could also potently inhibit the hepatic XOD activity to different levels (*P*<0.01). Higher dose of JPHSD is more effective in suppressing the hepatic XOD activity. These results suggested that some components of JPHSD exerted the hypouricemic function through inhibition of serum uric acid production by modulating the XOD activity.

JPHSD regulated expression of major urate and organic ion transporters

Previous studies have indicated that approximately 70% of uric acids produced daily are removed from human bodies through urinary excretion [26]. A variety of molecular transporters play important roles in maintaining the balance between urine uric acid and serum uric acid. Therefore, we set out to investigate the

potential regulatory role of JPHSD on those major transporters in renal tissues. Quantitative real-time PCR (qRT-PCR) (**Figure 3A-F**) and Western blotting (**Figure 3G**) were performed to measure the levels of transcripts and proteins of these transporters, respectively. GLUT9 belongs to the family of glucose transporters, but has been shown to possess urate transport activity and help maintain the urate homeostasis [27]. Expression of GLUT9 was greatly up-regulated in potassium oxonate-induced hyperuricemic rats, which was consistent with its role in urate uptaking. In allopurinol and benzbromarone treated rats, with the reduction of serum uric acid to the normal value, both of the mRNA and protein levels of renal GLUT9 were decreased. In addition to the similar inhibitory effect with allopurinol and benzbromarone, a dose-dependent reduction of GLUT9 expression was observed in JPHSD treated rats. URAT1 is a major urate transporter and mediates urate reabsorption in the kidney [28]. Its central role in maintaining urate homeostasis has made it a direct target of many therapeutic agents. As shown in **Figure 3**, renal URAT1 mRNA and protein levels were highly elevated in hyperuricemic rats, while inhibited with the treatment of allopurinol and benzbromarone. A similar dose-dependent inhibition of URAT1 was shown in JPHSD treated rats. However, JPHSD was not as potent as allopurinol and
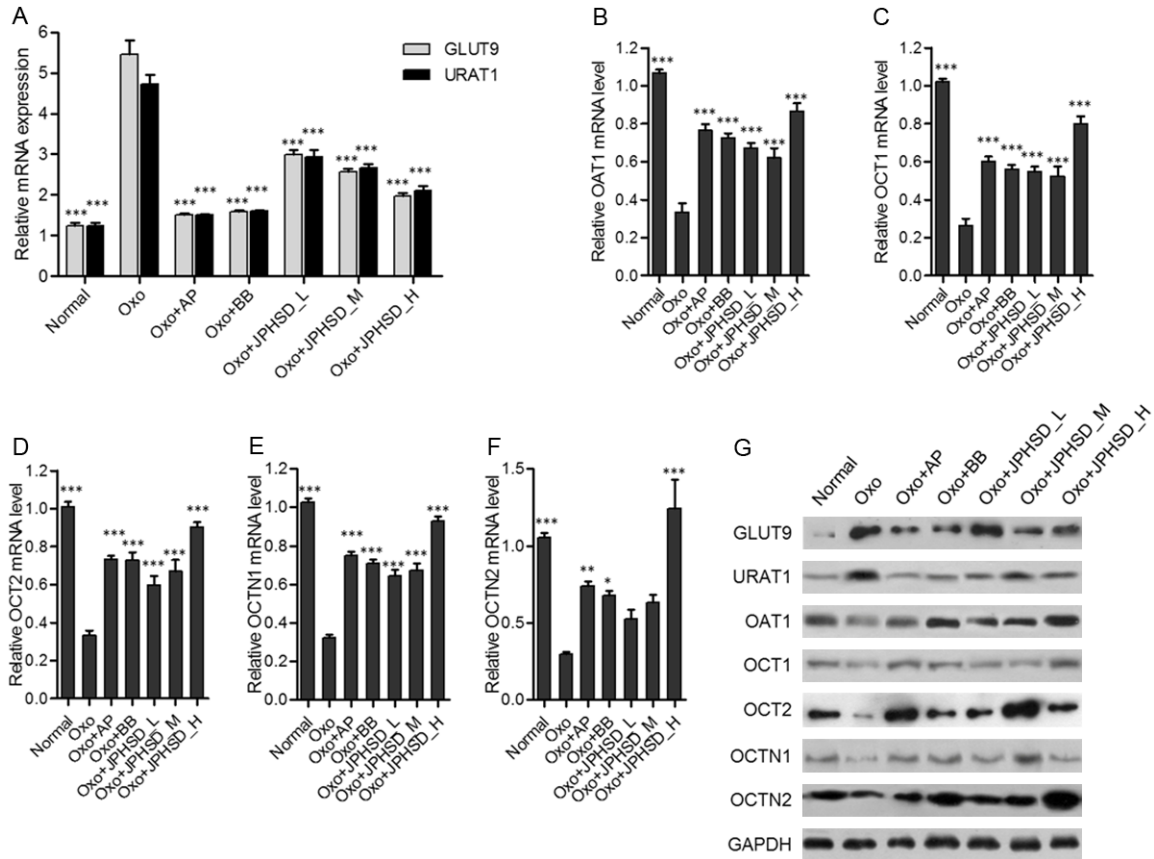
Figure 3. Effect of JPHSD treatment on expression of urate and ion transporters in kidney of hyperuricemic rats. Renal cortex samples were isolated from rats in 7 different groups (n = 10 per group). Expression of mRNA (A to F) and protein (G) was analyzed for various urate and ion transporters. GAPDH was used as internal controls in both qRT-PCR and Western blotting. (A to F) The results were expressed as mean ± S.E.M of ten rats for each group. *represents $P<0.05$, **represents $P<0.01$, ***represents $P<0.001$ when compared with the Oxo group. (G) Protein lysates from all the rats in each group were analyzed and representative blots are shown.

benzbromarone in modulating URAT1 expression. OAT1, an organic anion transporter, plays a major role in urate excretion [29]. As expected, down-regulation of OAT1 was observed in hyperuricemic rats, and the expression was partially restored in allopurinol and benzbromaroneeated rats. Low and medium doses of JPHSD exerted similar regulatory effects on OAT1 expression, and high dose of JPHSD was slightly more potent than allopurinol and benzbromarone as shown in **Figure 3**. Organic cation transporters, also play important roles in urate handling and the body's process of detoxification [30]. Expressions of organic cation transporters, including OCT1, OCT2, OCTN1 and OCTN2, were all down-regulated in hyperuricemic rats induced by potassium oxonate, and the hypouricemic agent such as allopurinol and benzbromarone was capable of partially restoring their expressions. Treatment of JPHSD

similarly enhanced the mRNA and protein levels of all four transporters, with the high dose of JPHSD being the most potent.

JPHSD attenuated hyperuricemia-associated oxidative stress

Hyperuricemia is frequently accompanied by oxidative stress and elevation of serum uric acid level has been considered a risk factor for development of multiple metabolic disorders and cardiovascular diseases [31]. Liver and kidney are the two major organs prone to be affected by oxidative stress-induced tissue damage. Therefore, we investigated the activity of an antioxidant enzyme, superoxide dismutase (SOD), and accumulation of an oxidative stress marker, malondialdehyde (MDA), in hepatic tissues and renal cortex. As shown in **Figure 4**, the SOD activities declined dramati-
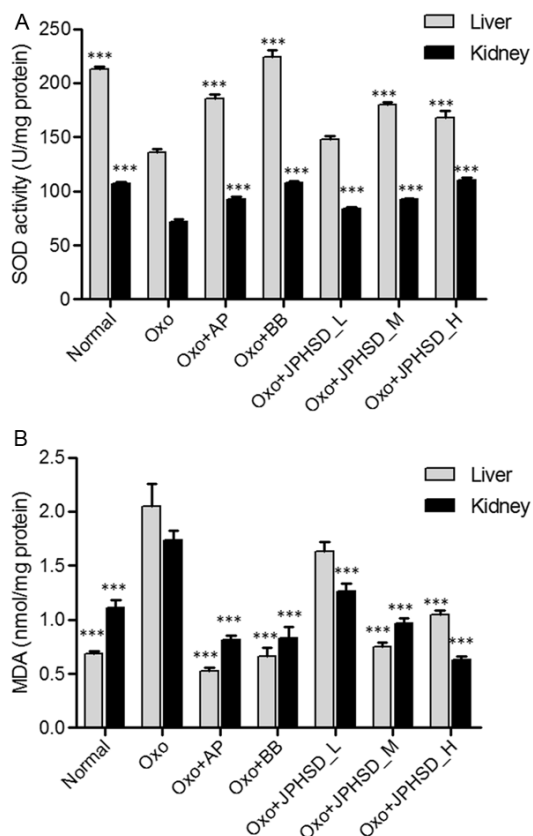
Figure 4. Effect of JPHSD on hyperuricemia-induced oxidative stress. SOD enzymatic activity and accumulation of MDA were measured in liver and kidney tissues of rats in 7 different groups (n = 10 per group). The results were expressed as mean ± S.E.M. of ten rats for each group. ***represents *P*<0.001 when compared with the Oxo group.

cally in hepatic and renal tissues of hyperuricemic rats induced by potassium oxonate. Accordingly, increased accumulation of MDA was observed in both tissues from the hyperuricemic rats. Treatment of allopurinol and benzbromarone relieved the oxidative stress by restoring the SOD activities and reducing the production of MDA. JPHSD, especially at medium and high doses, exhibited similar effects as AP and BB, indicating that JPHSD was capable of attenuating the hyperuricemia-associated oxidative stress.

*JPHSD ameliorated renal damage induced by hyperuricemia*

Histopathology of liver and kidney tissues following treatment was examined in each group. The morphology of liver tissues appeared normal in all groups: the structure of liver lobules

and portal area was unchanged, the arrangement of hepatocytes cords was maintained, and no proliferation of endothelial cells, hepatic stellate cells, Kupffer cells and the fibrous connective tissues was observed (**Figure 5**). Compared with the renal tissues from the Normal group, those from the Oxo group showed profound morphological changes, which were characterized by tubular atrophy, interstitial cell infiltration and deposition of big urate crystalsin the tubules and collecting ducts (**Figure 5**). Treatment of allopurinol and benzbromarone greatly alleviated the renal damages caused by potassium oxonate, with normal kidney structured restored (**Figure 5**). Medium and high doses of JPHSD displayed similar nephroprotective effects as allopurinol and benzbromarone. As shown in **Figure 5**, tubular atrophy was relieved, interstitial cell infiltration disappeared, and the number and size of urate crystals were greatly reduced. The results suggested that JPHSD could protect the kidney from hyperuricemia-induced tissue damage.

Discussion

Hyperuricemia is a major risk factor for gout and is caused by the imbalance between production and excretion of uric acid. The current therapeutic agents for treating hyperuricemia are commonly associated with undesirable adverse reaction and hepatotoxicity [3]. Many investigators have directed their attention to natural products and herbal medicines, making efforts to identify the active components within them and develop novel hypouricemic agents with higher clinical efficacy and safety. Herbal medicines *Rhizoma Smilacis Glabrae, Plantago asiatica* L.*, kudzu* root*, Cichorium intybus* L.*,* seeds of *Coix lacryma-jobi* L.*,* and *Alismatis Rhizoma* have long been used in traditional Chinese medicine to treat hyperuricemia, gout, and other associated medical conditions. Simiao pill, a commonly prescribed medication in Chinese medicine, is composed of some ingredient mentioned above [32]. We improved the medication by adding peptide-containing tuna extract to the formula and developed the JPHSD. In the present study, we demonstrated the efficacy of JPHSD in lowering serum uric acid level and the potential protective effects on liver and renal function by ameliorating the oxidative stress.
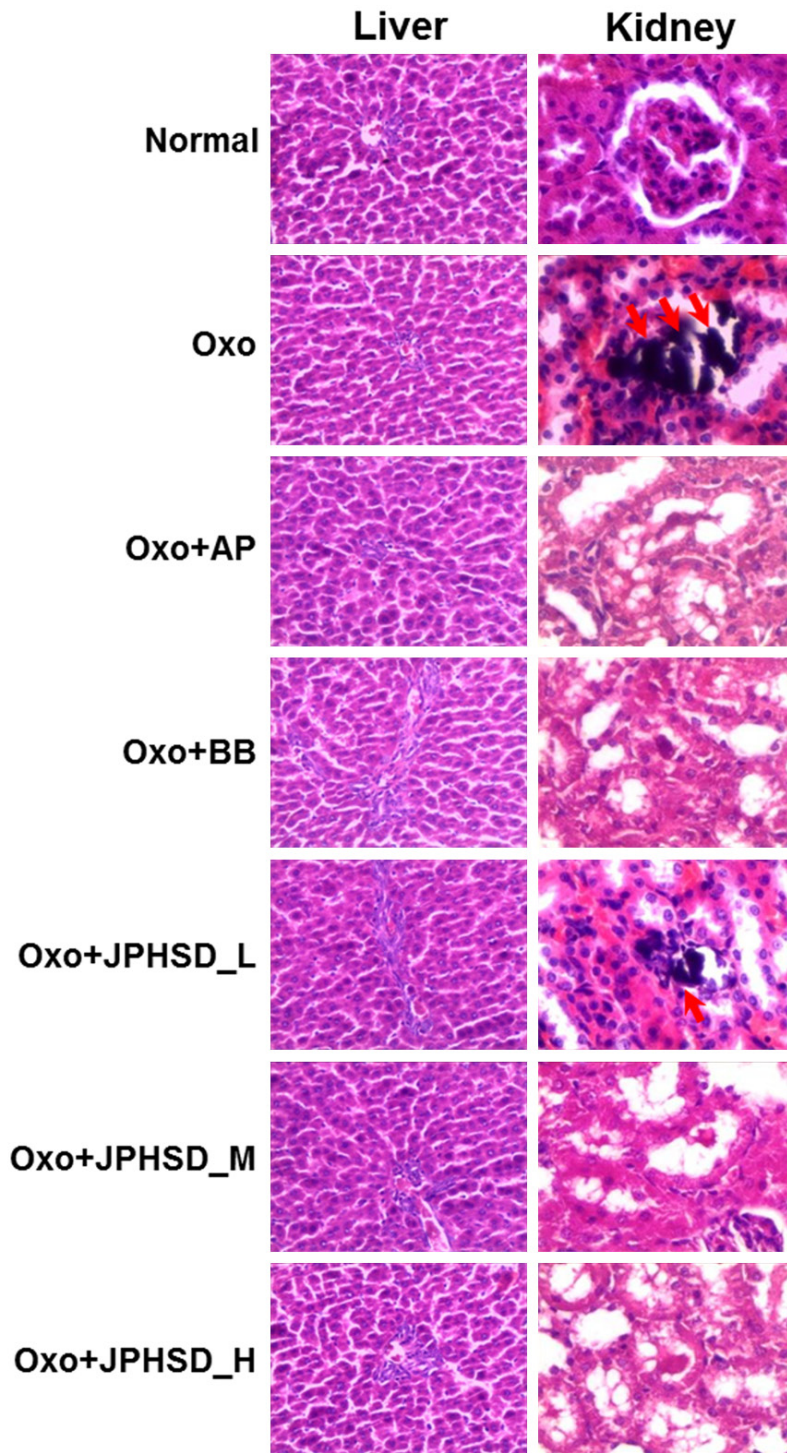
Figure 5. Histological analysis of liver and kidney tissues from normal and hyperuricemic rats treated with AP, BB, and different doses of JPHSD. Liver and kidney tissue sections were stained with H&E for morphological examinations. Samples from all the rats in each group were examined and representative images are shown. Note the altered morphology and dark purple urate crystals accumulating in the tubules of renal tissues from the Oxo and Oxo + JPHSD_L groups (indicated by red arrows). Original magnification, ×200 (liver); ×400 (kidney).

Our results showed that the medium dose of JPHSD was capable of reducing the serum uric acid level to the normal value in the hyperuricemic rats, but it only partially inhibited the hepatic XOD activity. These findings indicated that some component(s) in JPHSD might utilize a different mechanism to exert the hypouricemic function and regulate the serum level of uric acid. Since majority of the uric acid reabsorption and excretion occurs in the kidney, we hypothesized that the expression of urate and organic ion transporters could be regulated by JPHSD in the kidney. By measuring both the transcript and protein levels of those transporters, we demonstrated the uricosuric effect of JPHSD via modulation of urate transport. The urate transporters and ion transporters cooperate with one another in the proximal tubule of kidney to maintain the serum uric acid level in the normal range. For example, URAT1 and GLUT9 are essential transporters for urate reabsorption which results in an increase of uric acid in the serum; while OAT1 and other ion transporters function to promote urine excretion of uric acid thus leading to reduced uric acid level in the serum [33]. Previous studies have demonstrated that some of these transporters, including URAT1 and GLUT9, are the therapeutic targets of uricosuric drugs [32, 34]. The results from our study showed similar regulatory effects of JPHSD in affecting the expression of a variety of

transporters. However, the underpinning mechanism of this regulation awaits further investigation.

Besides functioning as ion transporters to help maintain the uric acid balance, OAT1, OCT1, OCT2, OCTN1 and OCTN2 are also responsible for renal excretion of metabolic wastes, xenobiotics and toxins [35]. Previous studies have suggested that their expression is positively associated with the enhancement of kidney transport capacity and improvement of renal function; oppositely, down-regulation of expression is usually an indicator of renal dysfunction or tissue damage [36]. In this study, JPHSD up-regulated expression of all five ion transporters, which implied an improved renal function in rats treated with medium/high doses of JPHSD. Indeed, results from histopathological studies demonstrated the nephroprotective effects of JPHSD, treatment of which restored the normal morphology and structure of renal tissues, greatly reduced the accumulation of urate crystals in tubules, and prevented infiltration of interstitial cells. Further functional studies of the kidney should be carried out to provide additional evidence.

The XOD mediated metabolic pathway is associated with generation of reactive oxygen species (ROS) and free radicals, which may result in cellular damages when produced in a large amount [6]. SOD is one of the major antioxidant enzymes that remove the excessive free radicals from body to attenuate their damaging effects and promote tissue repair. MDA is a product of lipid peroxidation and its accumulation in the body reflects the status of tissue damage [37]. Through simultaneous measurements of SOD enzymatic activity and amount of MDA present in the same tissue, the antioxidant capacity can be determined. In our model of hyperuricemia induced by potassium oxonate, the antioxidant capacities in liver and renal tissues were greatly compromised. JPHSD functioned to attenuate the potassium oxonate-induced oxidative stress in rats by enhancing the antioxidant activity of SOD and reducing lipid peroxidation.

There are two major mechanisms that contribute to pathogenesis of hyperuricemia: overproduction of uric acid and underexcretion of uric acid in urine. Our results showed that JPHSD was capable of reducing the production of uric acid by suppressing the XOD-mediated metabolic pathway. In addition, JPHSD exhibited uricosuric effect through modulation of urate and organic ion transporters. Since JPHSD is a mixture of extracts from multiple herbal medicines and tuna, future investigation should focus on identifying the active component(s) responsible for the XOD inhibitory effect, the uricosuric effect, or both.

JPHSD did not appear to have severe toxicity or cause undesired side-effect. Body weight of all treated animals was carefully monitored daily through the course of the study, and none dropped out of the study due to severe weight loss and other life-threatening conditions. Histopathological studies revealed no dramatic changes in morphology and structure of hepatic and renal tissues. Before going any further, the clinical efficacy and safety of JPHSD in patients with hyperuricemia and gout should be determined in controlled clinical trials. Only with confirmative results obtained from those studies, JPHSD could then be used as an alternative or in combination with AP, BB, or otherhypouricemic agents to treat gout and other related disorder and minimize the side effects, particularlyin long-term treatment.

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Disclosure of conflict of interest

None.

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