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Research on the pharmacodynamics and mechanism of Fraxini Cortex on hyperuricemia based on the regulation of URAT1 and GLUT9

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ABSTRACT

Fraxini Cortex (also known as Qinpi, QP) has been used for the treatment of hyperuricemia with a significant difference on efficacy of QP from different regions. However, it`s still unknown whether proportion of components is the key and why same kind of herbs have different therapeutic effects. In this study, different sources of QP were collected from Shaanxi Qinpi extracts (SQPE), Henan Qinpi extracts (HQPE), Hebei Qinpi extracts (GQPE) provinces in China. Rat model of hyperuricemia with hypoxanthine combined with potassium oxonate were established to determine the levels of blood urea nitrogen (BUN), serum uric acid (SUA), urine uric acid (UUA) and creatinine (Cr). Hematoxylin-eosin staining (H&E) and Periodic Acid-Schiff staining (PAS) were performed for renal pathology while Western blot analysis and real-time PCR analysis for proteins and mRNA expression levels. High-performance liquid chromatograph (HPLC) was used for components and composition analysis. Our results demonstrated that QPE from different regions could alleviate hyperuricemia via increasing significantly the SCr and BUN levels whereas decreasing markedly UCr, SUA and UUA levels. Additionally, QPE could also improve the pathological changes of the kidneys. The protein and mRNA levels of urate reabsorption transporter 1 (URAT1) and glucose transporter 9 (GLUT9) were down-regulated by QPE treatment. SQPE hold a better activity on improving hyperuricemia and regulating URAT1 and GLUT9. HPLC analysis showed that the proportion of four components aesculin, aesculetin, fraxin, fraxetin were 9.002: 0.350: 8.980: 0.154 (SQPE); 0.526: 0.164: 7.938: 0.102 (HQPE); 12.022: 1.65: 0.878: 1.064 (GQPE). These data indicate that this proportion of effective components may be an important factor for efficacy of QP and had implications for the treatment of hyperuricemia.

1. Introduction

Hyperuricemia has been regarded as one of leading cause of mortality and morbidity for people due to a disorder of purine metabolism or the excretion of uric acid in a timely manner [1]. Many factors contribute to the pathogenesis of hyperuricemia, such as bad habit of eating. It can be led to a net out-of-balance of reabsorption and secretion of serum uric acid and the formation of uric acid crystals in the joints and kidneys, resulting in serious gout [2]. Many mechanisms have been proposed to explain the possible pathogenesis. Researches have shown that the urate reabsorption transporter 1 (URAT1) and glucose transporter 9 (GLUT9) are two important mediators in the process of hyperuricemia [3,4]. The evidence showed that the level of uric acid can be inhibited through regulating urate transport mediated

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by the major reabsorptive urate transporters URAT1 and GLUT9 [5]. Therefore, it is very important to target these two transporters for improving hyperuricemia.

Interestingly, many Chinese Medical Materia have been found to be beneficial for the treatment of hyperuricemia [6–8]. However, a significant difference on the efficacy of Chinese Medical Materia from different sources was found in the experimental study or clinical application. For example, Zhu found that Scutellaria in Hebei origin had superior effects in anti-chemical liver injury, antiseptic effect, Antiviral effect and immunomodulatory effect compared with these from other areas [9]. This difference in efficacy may result from differences in the proportion of components [10]. Wang studied the protective effects of aconitine and glycyrrhetinic acid on the protection of H9c2 cells under the oxygen-glucose deprivation condition. Finally, the best efficacy was found when the ratio of two effective components was 1:1 [11].

Fraxini Cortex (also known as Qinpi in China), the dry bark of Fraxinus rhynchophylla Hance and Fraxinus stylosa Lingelsh, has been used as an important traditional Chinese herbal medicine for the treatment of gout and hyperuricemia [12]. A large number of studies have shown those components of Fraxinus general coumarins are the main active components of Qinpi in the treatment of hyperuricemia [13,14]. The optimized combination of components of Qinpi could achieve the best efficacy on treating hyperuricemia. However, the regulation of components of Qinpi on URAT1 and GLUT9, and the relationship between its regulation and the combination of these components, remain unknown.

Therefore, the aim of this study was to: (1) evaluate the improvement of Qinpi on hyperuricemia; (2) know whether this improvement is related to GLUT9 and URAT1; (3) know also whether the differences of proportions of the same components will lead to the different efficacy. This research will provide useful information for the treatment of Qinpi on hyperuricemia.

2. Materials and methods

2.1. Chemicals and reagents

Hypoxanthine (HX) (Lot No., P329005), Potassium oxonate (OXO) (Lot No., BDYQSJ-25) were purchased from Sigma Aldrich Co., (Sigma, St. Louis, MO, USA). Blood urea nitrogen (BUN), serum uric acid (SUA), urine uric acid (UUA) and creatinine (Cr) quantification kits were provided by the Jiancheng Bioengineering Institute (Nanjing, China). Trizol reagent (Lot No., 135404) was purchased from Invitrogen (Carlsbad, CA, USA). Acetonitrile and formic acid used for HPLC analysis was purchased from Burdick & Jackson (Muskegon, MI, USA). Water used for analysis HPLC was obtained from a water purification system (Milli-Q Reagent Water System, MA, USA). Standard material of aesculin, aesculetin, fraxin and fraxetin were obtained from National Institutes for Food and Drug Control (Beijing, China). Anti-URAT1 (Lot No., 14937-1-AP) were purchased from Proteintech Group, Inc. (Wuhan, China), anti-GLUT9 (Lot No., PA5-966) were purchased from Invitrogen (Carlsbad, CA, USA), anti-β-actin (Lot No., sc-8432) were purchased from Santa Cruz technology Co., Ltd. (Shanghai, China). And other reagents were obtained from Nanjing Reagent Co., Ltd. (Nanjing, China).

2.2. Preparation of Qinpi extracts

Different sources of Fraxini Cortex (Qinpi) were obtained from Shangluo, Shaanxi Province (batch number 20160507), Pingdingshan, Henan Province (batch number 20160412), Anguo, Hebei Province (batch number 20160502), they are stored at room temperature, and the relative humidity was 65% at *Shaanxi University of Chinese Medicine*. All the medicinal materials were identified by Dr. G. Zhang from Shaanxi University of Chinese Medicine. Qinpi of 300 g was extracted with 2400 mL 95% ethanol at the ratio of 1/8, (g/mL) under 80 °C for 2 h. This operation is repeated for two times. The collected extracts were centrifuged under 3000 rpm for 15 min at 4 °C with a 5430R centrifuge (Eppendorf, Riesae, Germany; rotor:16 × 5.0 mL), and then concentrated at 65 °C under reduced pressure for 1.5 h. Finally, the extract was dried at 65 °C for 8 h to obtain powder. These powders were konwn as: SPQE (the origin is Shangluo, Shaanxi Province), HQPE (the origin is Pingdingshan, Henan Province) and GQPE (the origin is Anguo, Hebei Province), the yield of QPE was as the follows: SQPE: 8.05%, HQPE: 6.03%, GQPE: 7.57%; and the contents of four components (Aesculin, aesculetin, fraxin and fraxetin, calculated by the standard curves) of QPE was as the follows: SQPE: 58.05%, HQPE: 46.03%, GQPE: 47.57%.

2.3. HPLC analysis of QPE

Agilent 1200 series high-performance liquid chromatograph which was equipped with a diode array detector (HPLC-DAD) (Agilent Technologies, Santa Clara, CA, USA) and was applied for the components analysis of QPE. Chromatographic analysis was performed according to the following conditions: Agilent TC - C18 column (4.6 * 250 mm, 5 m) was used as chromatographic column; acetonitrile was used as mobile phase A while 0.1% formic acid water as mobile phase B. Elution program was set at flow rate of 0.8 mL/min: 0–20 min, 5%–10%A, 20–30 min, 10%-10%A; 30–80 min, 10%–18%A. The samples were detected at 254 nm wavelength. The column temperature was maintained at 25 °C while the injection volume of 5 μL. The standard curve was made by a mixed solution including Aesculin, Aesculetin,Fraxin and Fraxetin with external standard method.

2.4. Experimental animals

Male Sprague-Dawley (SD) rats, weight 200 ± 20 g (Mean ± SD) and aged 2-month-old, were obtained from *Xi`an Jiaotong University* (SCXK 2016-003) and fed freely for two weeks, animal indoor illumination 12 h day/night cycle, room temperature was maintained at 22–25 °C. All the animals were fed in the Animal Experiment Center at Pharmacology Laboratory of Traditional Chinese Medicine in Shaanxi University of Chinese Medicine. The 120 rats were divided randomly into the following groups: control group, model group (OXO 250 mg/kg/d. i. g. and H X 10 mg/kg/d. s. c.) for 2 weeks, positive control allopurinol group (APL 50 mg/kg/d), QPE high dose group (200 mg/kg/d, i. g.), QPE medium dose group (100 mg/kg/d. i. g.), QPE low dose group (50 mg/kg/d. i. g.) for three origins. This animal experiment was approved by the ethics committee of *Shaanxi University of Chinese Medicine,* approval number: 21060306. In the experiment, the number of animals was strictly controlled and the degree of pain was reduced to a minimum. All the experimental samples were collected in one day.

2.5. Serum and urine samples

After 2 weeks, the rats were fasted for 12 h and then 24 h urine samples were collected with metabolic cages. After been anesthetized with 10% Chloral hydrate at dose of 0.3 mL per 100 g body weight, the abdominal aorta blood samples of rats were collected at room temperature. Both the urine and serum samples were centrifuged at 3000 rpm for 10 min. The supernatant was taken and then stored at −20 °C for the measurements of biochemical indexes.

2.6. Kidney tissue samples

At the end of administration, the animals were anesthetized with 10% chloral hydrate and then aseptic removal of both kidneys. The double kidney weight of rat kidney were measured and recorded. The single kidney tissue was cut into about 0.4 cm * 0.4 cm * 0.2 cm sizes, then the blocks were removed into the capsule. Next, the blocks were washed with PBS, then blotted with filter paper, after that the blocks