Acupuncture and Herbal Medicine

Penthorum chinense Pursh. attenuates hyperuricemia by regulation of uric acid excretion and gut microbiota

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Abstract

Objective: Hyperuricemia (HUA) is a metabolic disease that threatens human health. The role of *Penthorum chinense* Pursh. (PCP) in the treatment of HUA has begun to receive attention in recent years. This study aimed to investigate the effects and potential mechanisms of PCP in HUA treatment.

Methods: A HUA murine model was induced in C57/BL6 mice using potassium oxonate (PO) and adenine (AD). Serum uric acid (SUA) was measured using ultra-performance liquid chromatography (UPLC). Serum creatinine (Scr) was detected using a creatine oxidase assay kit, and serum blood urea nitrogen (BUN) was detected using a urease indophenol blue assay kit. Protein expression levels were detected using western blotting, and gut microbiota were detected using 16S rRNA.

Results: PCP substantially improved the serum contents of SUA, Scr, and BUN and alleviated kidney injury. PCP promotes renal uric acid excretion by downregulating GLUT9 and URAT1 expression and upregulating ABCG2 and OAT1 expression PCP also regulated the NOD-like receptor family, pyrin domain-containing protein 3 (NLRP3) pathway and reduced the expression of inflammatory factors, thus attenuating kidney injury in HUA mice. PCP regulated the structure of the gut microbiota, including the relative abundance of beneficial bacteria, such as *Lactobacillus* and *Alistipes,* which promoted uric acid metabolism and anti-inflammatory effects.

Conclusions: PCP can reduce uric acid levels by promoting renal uric acid excretion and regulating the gut microbiota. PCP improves kidney injury by inhibiting the activation of the NLRP3 signaling pathway and reducing the levels of inflammatory factors.

Keywords: Gut microbiota, Hyperuricemia, NLRP3, *Penthorum chinense* Pursh.

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Introduction

Hyperuricemia (HUA) is characterized as a metabolic disorder resulting from dysregulation of uric acid (UA) homeostasis, manifested by serum UA exceeding 420 μmol/L[1]. High serum UA concentrations may result in numerous diseases, including chronic kidney disease (CKD), cardiovascular disease, type 2 diabetes mellitus, gout, and metabolic syndrome[2]. Approximately 30% of UA in the diet comes from purine catabolism, with the remainder being endogenous[3]. HUA can be caused by excessive production of UA in the liver and insufficient excretion through the kidneys and is typically treated using two mechanisms[4]. High concentrations of UA that exceed the kidney's excretory capacity precipitate and crystallize in the kidney, causing damage, activating the NOD-like receptor family, pyrin domain-containing protein 3 (NLRP3) inflammasome, altering the gut microbiota, and affecting UA transporters[5].

Gut microbiota refers to the entire population of bacteria that play a role in maintaining intestinal homeostasis[6]. There is growing evidence that the gut microbiota regulate immune and hormonal systems, and host metabolism[7]. The gut microbiota has been implicated in

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various diseases, including diabetes, obesity, and ulcerative colitis[8]. Studies have supported the hypothesis that gut microbiota play an important role in HUA[9]. The gut microbiota is involved in purine and UA metabolism[10]. Patients with gout have an altered gut microbiome with substantially less gut microbiota expressing a gene called *uricase*[11].

HUA affects the composition and metabolites of the gut microbiota, particularly short-chain fatty acids (SCFA)[12]. SCFA in hyperuricemic mice are reduced but few studies have elucidated the mechanism between gut microbiota and HUA[13]. A recent report suggested that gut microbiota could be a candidate intervention target for alleviating the pathogenesis of HUA[14]. Therefore, kidney health and gut microbiota integrity are critical for the prevention of HUA.

In recent years, low-UA drugs have increasingly been used in the medical arena for the treatment of HUA, a condition characterized by high levels of UA in the blood, and other UA-related diseases[12]. This therapeutic arsenal includes xanthine oxidase (XOD) inhibitors and renal UA reabsorption inhibitors, two distinct classes of medications that lower the UA concentration in the body[13]. However, it is important to note that like all medications, these low-UA drugs, which include popular options such as febuxostat and benzbromarone, have potential risks and side effects[14]. The prolonged use of these drugs is known to inhibit UA production or promote UA excretion, with some patients experiencing severe hypersensitivity reactions, gastrointestinal and renal toxicity, and in severe cases, even hepatotoxicity[13]. These risks emphasize the urgent need for the medical community to develop safer and more effective treatments for UA-related ailments, and many are looking for Chinese herbal medicines as a potential source of such treatments.

Penthorum chinense Pursh. (PCP) is primarily distributed in the Wumeng Mountain region, which represents the geographical junction of the Yunnan, Guizhou, and Sichuan provinces[14]. The Miao people, indigenous to the region, have traditionally used this plant for gastrointestinal health and endearingly called it "fairy grass." Modern pharmacology has corroborated the Miao people's empirical knowledge, which imparts diverse biological functions of PCP. These include confirmed antioxidant properties, countering of oxidative stress, demonstrated anti-inflammatory properties, established hepatoprotective properties, and properties useful for treating nephrotic syndrome[15–16]. Recent academic efforts have increasingly focused on elucidating PCP's efficacy in protecting the liver. These include experimental models of alcoholic fatty liver, characterized by fat deposition in liver cells from excessive alcohol consumption, and non-alcoholic fatty liver, in which fatty liver develops in alcohol-light or non-drinkers. These studies have primarily used mice as experimental models[17].

The total flavonoid extract, a chemical compound found in PCP, can inhibit alcoholic liver disease. The mechanism by which this effect is achieved may involve the regulation of bile acid excretion and expression of genes related to exogenous chemical efflux[18]. Given the earlier findings, investigating PCP's potential to prevent HUA and renal injury is important. HUA refers to elevated UA levels in the blood, potentially leading to kidney stones, gout, and kidney disease if left untreated. In the current study, we investigated the preventative effects of a water extract of PCP against HUA and renal injury. Additionally, we investigated the potential renal protective effects of PCP water extract in hyperuricemic mice. We also investigated the mechanism by which the water extract of PCP exerts its renoprotective effects. Our findings serve as valuable theoretical references for elucidating the biological functions of PCP and its potential application in the treatment of HUA.

Methods

Preparation of PCP aqueous extract

One kilogram of PCP was extracted (purchased from Lichang Planting Cooperative of Jiuhe Township Yulong County, Yunnan Province) in the laboratory of Chinese Medicine Chemistry, Academy of Chinese Medicine, Tianjin University of Chinese Medicine. After adding 10 times the amount of water for the first time, soaking it for 12 h, boiling it at high heat, boiling it at low heat for 2 h, and filtering the residue. Eight times the amount of water was added for the second and third time, for 2 h each time. The filtrate was merged three times, concentrated to 1 g/mL, and stored in the refrigerator at 4°C.

Reagents and drugs

Potassium oxonate (PO) and adenine (AD) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Commercial kits used to determine the creatinine and blood urea nitrogen (BUN) were obtained from the Jiancheng Institute of Biotechnology (Nanjing, China). Acetonitrile, acetic acid, and methanol were purchased from Fisher (USA). Antibodies against URATl, GLUT9, OAT1, ABCG2, NLRP3, ASC, caspase-1, and β-actin were purchased from Abcam Inc. (Cambridge, MA, USA). Polyvinylidene fluoride (PVDF) membranes were purchased from Merck Millipore (USA). A bicinchoninic acid assay (BCA) protein assay kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Animals

Fifty specific pathogen-free, male, C57BL/6J mice (8 weeks, 18–22 g) were purchased from Beijing Huafukang Biotechnology Company. They were provided with free access to food and water. Animal experiments were approved by the Science and Technological Committee and the Animal Use and Care Committee of TJUTCM (TCM-LAEC2022060). The environment temperature was (20–26)°C, the humidity was maintained at (40–70)%, the ventilation condition was 10 to 15 times/h of fresh air, and the lighting was alternated for 12 h every day.

Murine model of HUA

PO and AD have been used to induce HUA in murine models[19]. Fifty male C57BL/6J mice were divided into five groups: normal, control, Ben (50 mg/kg/day), and

PCP extract (300 and 600 mg/kg/day). The model group was comprised of a control group. The low- and high-dose groups were administered PCP extracts at 300 and 600 mg/kg, respectively. PCP extracts were freshly prepared in ultrapure water and administered orally to experimental mice. Mice in the AD-induced HUA model were administered AD (75 mg/kg/day) or PO (200 mg/kg/day) intragastrically. PCP extract was administered to the low- and high-dose groups *via* oral gavage at doses of 300 and 600 mg/kg, respectively. The positive control group was administered benzbromarone (50 mg/kg) *via* oral gavage. Both normal and control groups were administered an equivalent volume of ultrapure water *via* oral gavage. All groups, except the normal group, were administered AD (75 mg/kg) and PO (200 mg/kg) intragastrically, 1-hour post-administration; the normal group received an equivalent volume of ultrapure water. The experimental protocol was maintained for 21 days. On the 21st day, 1 h after administration, livers and kidneys were collected.

Blood and tissue

Blood samples were collected 1 h after the final administration on the 21st day. Samples were kept for 2 h at room temperature and centrifuged at 3,500 rpm for 10 min to obtain the serum. Serum was used for the determination of UA and stored at –20°C until biochemical assays were performed. The kidney was cut into tubes for histological analysis and the rest was stored at –80°C for the western blotting analysis.

Measurement of UA concentration

Serum and plasma UA levels were determined using ultra-performance liquid chromatography (UPLC)[20]. A total of 270 µL $HCLO_4$ (0.3M) was added to 30 µL of the plasma sample, mixed using the vortex, and placed on ice for 30 min. The sample was then centrifuged at 10,000×*g* for 10 min at 4°C. An amount of 50 µL Na_2HPO_4 solution (0.8M) was added to the 200 µL supernatant, and centrifuged again at 10,000×*g* for 10 min at 4°C. The UA content of the 10-µL supernatant was detected using UPLC.

The UPLC conditions were Waters Acquity UPLC system H-class (Waters Co., Ltd., Milford, Massachusetts, USA); detector, photo-diode array; column, Acquity UPLC BEH amide (1.7 µm, 2.1 mm × 50 mm); wavelength, 285 nm; mobile phase, 0.1% acetic acid water solution/acetonitrile = 10/90, *v/v*; flow rate, 0.3 mL/min; the column was maintained at 30°C.

Histopathology of renal tissues

The collected mice kidneys were fixed in 4% paraformaldehyde, cut, and embedded. The tissues were placed in xylene until transparent, immersed in wax, and cut into 5-µm slices. After treatment, the sections were sealed and the pathological morphology of the kidney tissue was observed using an Axio Imager D2 (Zeiss, Oberkochen, Germany).

Western blot analysis

An amount of 30 mg of tissue was placed in a 2.0-mL Eppendorf tube and 300 µL of radioimmunoprecipitation assay (RIPA) lysate (with 1 mM phenylmethylsulfonyl fluoride and phosphatase inhibitor) was added. The tissue was thoroughly broken on ice using a homogenizer, left on ice for 30 min, centrifuged at 12,000×*g* at 4°C for 10 minutes. BCA protein quantification working solution was configured and total protein was quantified to 50 µg.

For protein quantification, an appropriate amount of protein loading buffer was added to the EP tube, mixed, heated at 100°C in a water bath for 10 min, and stored at –80°C. After protein separation using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), the protein bands were transferred onto a PVDF membrane using electroporation. The target bands were cut and incubated at 4°C overnight with primary antibody. The following day, the bands were washed four times with PBST (8 min each) and incubated for 1 hour at room temperature with the secondary antibody. The bands were washed four times with PBST for 8 min each. Next, a chemiluminescent solution was prepared, and the bands were immersed in the developing solution and left for 1 min. The protein bands were imaged using the ChemiDoc MP Imaging System, and the gray level of the bands was quantified using ImageJ software.

Statistical analysis

The data are expressed as mean ± standard error of mean (SEM). The statistical software, Graph Pad Prism Version (8.1.1), was used to evaluate the significant difference between the means using one-way analysis of variance and *t* tests. $P < 0.05$ was considered statistically significant.

Results

HPLC analysis of the PCP

PCP has gathered attention because of its medicinal value. Gallic acid was selected as the standardized reference index for PCP because of its relatively stable content, excellent chemical stability, and biological activity[21]. The chromatogram indicated the gallic acid content of the PCP extract samples within 60 min (Figure 1). Quantification was performed using an external comparison method, and the gallic acid content of the sample was found to be 1.808%.

Effect of PCP on lowering UA and improving renal function in HUA mice

An investigation of the potential anti-hyperuricemic effects of PCP in a population of mice undergoing treatment for HUA was conducted. The body weight of the mice in the treatment group declined rapidly compared to that of the mice in the normal control group. Furthermore, careful examination of the kidney and spleen indices indicated marked variations between the control and treatment groups. These data suggest the potential for renal and splenic involvement in the treatment process. The serum levels of UA, creatinine, and BUN were substantially higher in HUA mice. These data indicate the successful establishment of HUA and potential kidney injury. However, the elevation in BUN, creatinine, and UA

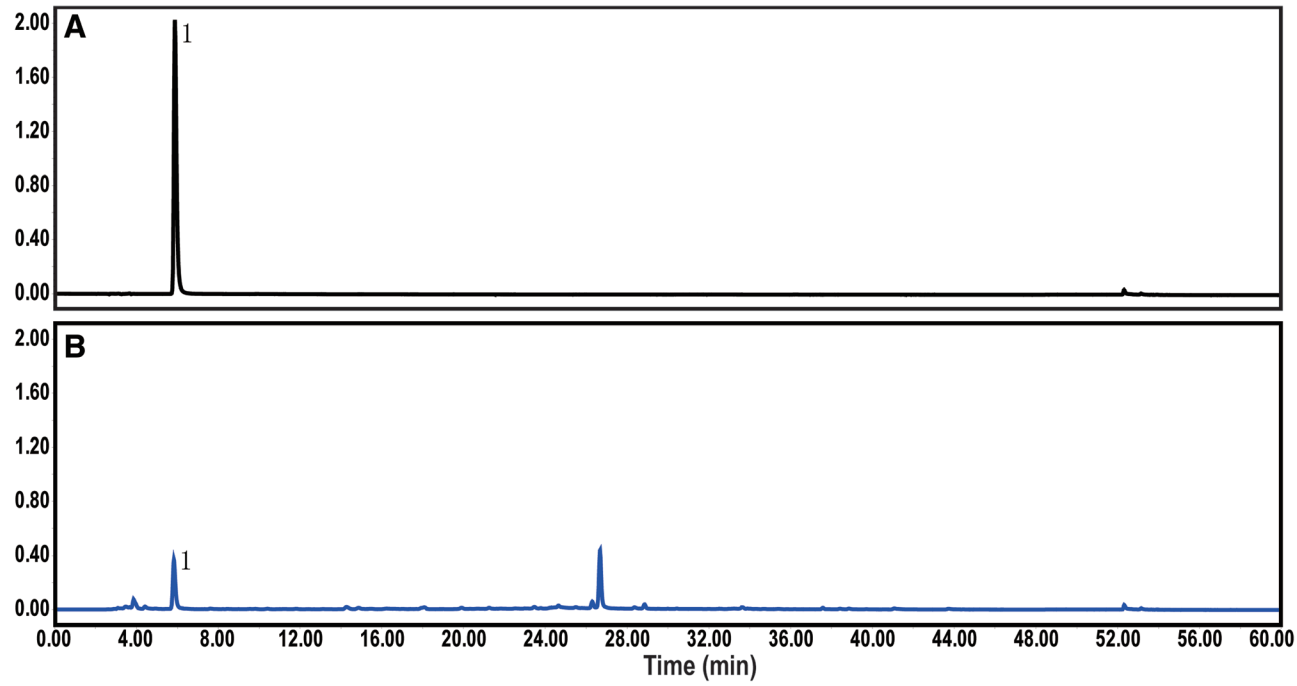

Figure 1. High performance liquid chromatography analysis of PCP. Quantifications were performed using external comparison method; gallic acid is 1.808% in the sample. (A) Reference, (B) Extracts of *Penthorum chinense* Pursh. sample (1. gallic acid). PCP: *Penthorum chinense* Pursh.

content, as well as the renal histological changes, were effectively reversed by PCP administration at doses of 300 and 600 mg/kg. Renal histological changes in HUA mice were marked (Figure 2). In the control group, the kidneys exhibited proximal tubule necrosis, inflammatory cell infiltration, inconspicuous boundaries, cytoplasmic vacuolation, and dilatation. However, comparative analysis revealed that PCP treatment, particularly at doses of 300 and 600 mg/kg, substantially improved glomerular atrophy and sclerosis, proximal tubule swelling, disordered arrangement, and infiltration of inflammatory cells. These histological changes indicate that PCP treatment effectively attenuated the pathological changes in the kidneys of HUA mice. These findings provide compelling evidence for the potential use of PCP in the treatment of HUA and emphasize the importance of understanding the underlying mechanisms of action in future research.

Effects of PCP on kidney UA excretion in HUA mice

In the response of the renal system to HUA, alterations in kidney transporters during episodes of renal tubular injury, a condition closely linked to this metabolic abnormality, were observed. Three key transporters, URAT1 and GLUT9, which are integral to UA reabsorption; and ABCG2, which plays a fundamental role in facilitating UA excretion, were studied. Analyses of renal tissues from these mice highlighted substantial differences in the expression and function of these transporters. The protein contents of URAT1 and GLUT9 were substantially increased in the control group. Conversely, the protein content of ABCG2, which is central to UA excretion, was markedly decreased. PCP effectively regulated the protein contents of URAT1, GLUT9, and ABCG2 in the kidneys of HUA mice (Figure 3). The administration of PCP at 600 mg/kg led to the downregulation of URAT1 and GLUT9 at the protein level. This resulted in the upregulation of ABCG2 and another important transporter, OAT1, at the protein level. These groundbreaking findings not only emphasize the intricate interplay between UA transporters and renal function but also offer compelling evidence for the potential therapeutic benefits of PCP in managing HUA. By modulating the protein content of URAT1, GLUT9, and ABCG2, PCP exerts a profound influence on the capacity of the kidneys to excrete UA, thereby alleviating the detrimental effects of HUA.

Effects of PCP on the gut microbiota diversity

Chao1 and Shannon indices, two widely used measures in diversity analysis, effectively reflected the α diversity within each sample. Compared to the normal group, the control group had a substantially lower Chao1 index and the Shannon index tended to decrease. These observations indicated a decrease in the α-diversity, which in turn indirectly reflects the decrease in microbial species in HUA mice (Figure 4A, B).

Further comparison of the control and PCP groups indicated that the Chao1 and Shannon indices were increased in the PCP-treated group. Diversity and gut microbiota in HUA mice were therefore effectively restored using PCP administration. A more detailed examination of the genus level indicated great variation between the control and normal groups (Figure 4C–F). *Alistipes* and *Akkermansia* were markedly lower in the control group than in the normal group (Figure 4G–I). The distribution of *Alistipes* in the gut microbiota of mice was positively correlated with serum BUN. Distribution of the intestinal bacterium, *Akkermansia,* was negatively correlated with serum creatinine levels. To provide a comprehensive visual representation of the changes in the gut microbiota composition, we created a heat map of the top 14 genera (Figure 4D), which indicates that the gut microbiota composition in the control group underwent

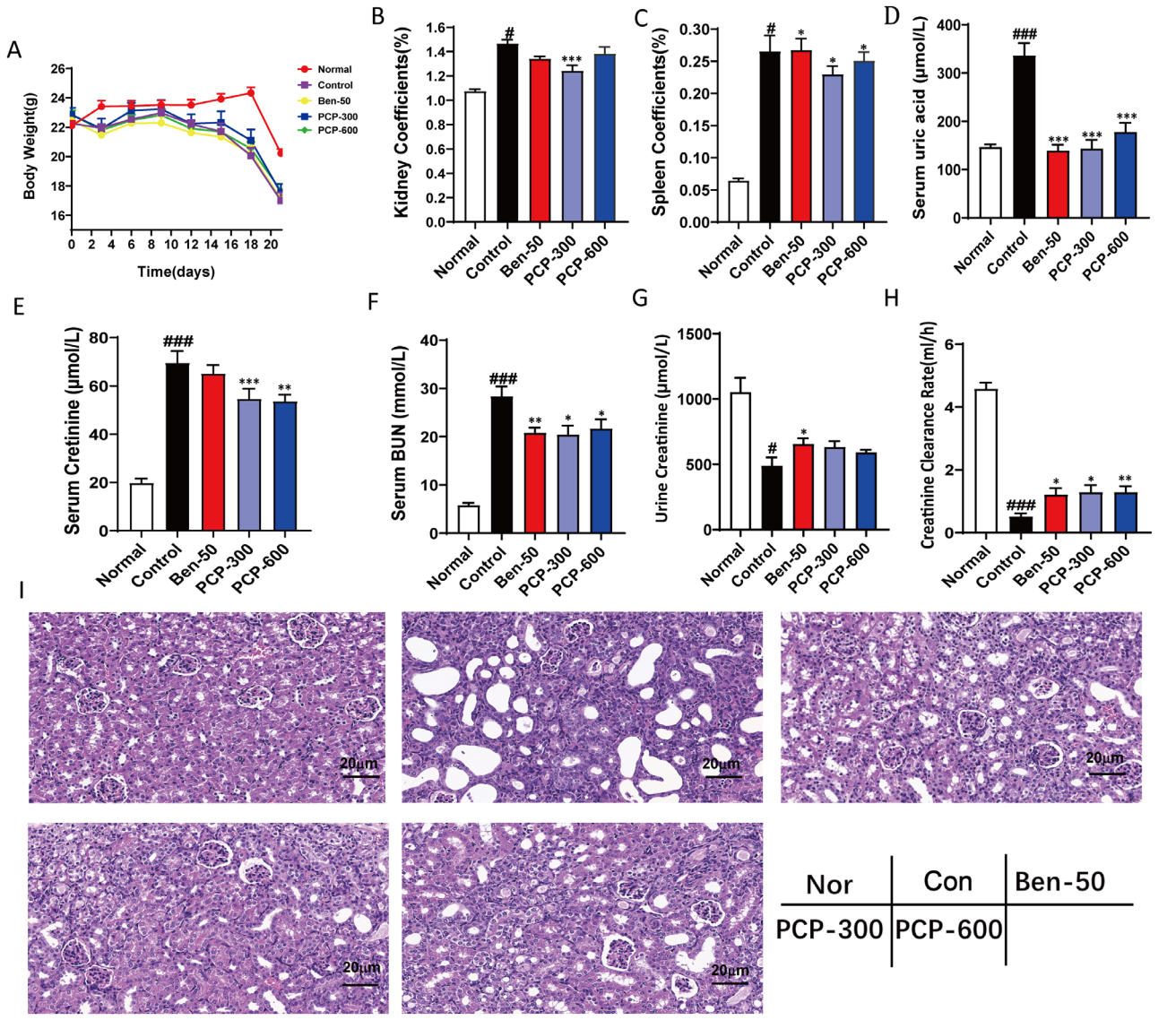

Figure 2. Effect of PCP on lowering uric acid and improving renal function in HUA mice. (A) Body weight, (B) kidney coefficient, (C) spleen coefficient, (D) serum uric acid, (E) serum creatinine, (F) serum blood urea nitrogen (BUN), (G) urine creatinine, (H) creatinine clearance, and (I) representative HE photomicrographs. Nor: blank control group; Mod: control group; Ben-50: benzbromarone 50 mg/kg; PCP-300: extracts of *Penthorum chinense Pursh.*; low-dose group 300 mg/kg; PCP-600: extracts of *Penthorum chinense Pursh.*, high-dose group 600 mg/kg. Data are expressed as mean ± SEM ($n = 6$). Compared with normal group, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$; compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. HE: Hematoxylin and eosin; HUA: Hyperuricemia; PCP: *Penthorum chinense* Pursh.; SEM: Standard error of mean.

marked changes compared to that in the normal group. PCP could therefore regulate the relative abundance of *Prevotellaceae UCG 001*, which is involved in UA metabolism, and *Lachnospiraceae_NK4A136_group*, which is associated with SCFA production. *Alistipes* and *Akkermansia* are both anti-inflammatory bacteria. PCP administration partially reversed the dysbiosis of these bacterial taxa. These findings collectively indicate that PCP can improve the composition of the gut microflora, thus offering a potential treatment option for HUA mice.

Effect of PCP on NLRP3/ASC/caspase-1 signaling pathways in the kidney

To elucidate whether PCP has an anti-inflammatory effect and to ascertain the possible mechanism through which it might achieve this, the NLRP3/ASC/Caspase-1 signaling pathway was investigated. The NLRP3 inflammasome, which is a multiprotein complex comprising NLRP3, ASC, and caspase-1, is instrumental in orchestrating the release of inflammatory factors, including IL-6, IL-1β, and IL-18. In the control group, there was a marked increase in the protein levels of NLRP3, ASC, caspase-1, and inflammatory factors, IL-6, IL-1β, and IL-18 (Figure 5). When the test samples were pretreated with 600 mg/kg of PCP, the contents of NLRP3, ASC, caspase-1, and IL-6, IL-1β, IL-18 were substantially reduced. PCP effectively mitigated renal inflammation and was associated with the suppression of NLPR3 inflammation, implying the mechanism behind the anti-inflammatory properties of PCP. This study provides valuable insights into inflammation and points to a potential new avenue for therapeutic intervention.

Discussion

In recent years, HUA has surged rapidly in China, a phenomenon primarily attributed to the dramatic changes

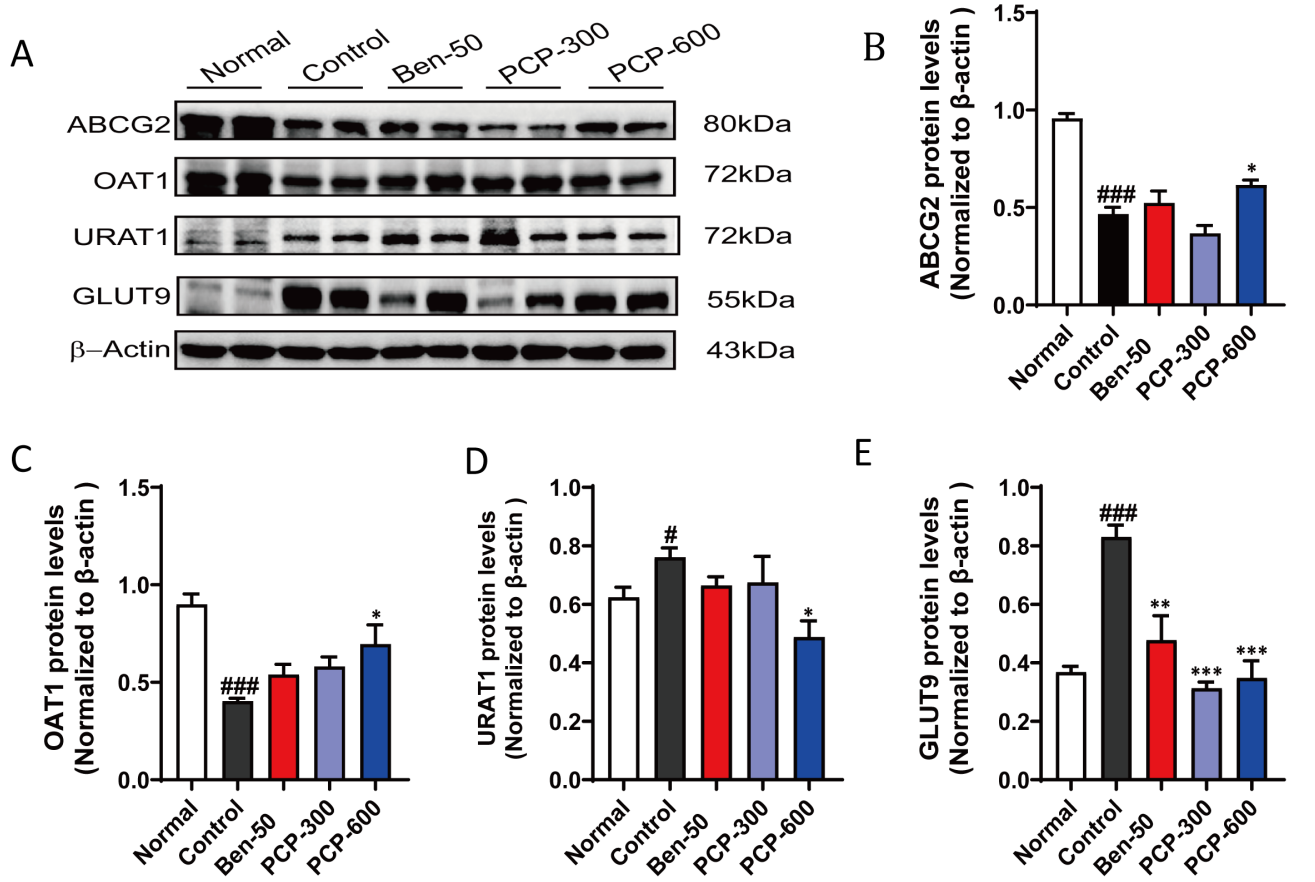

Figure 3. Effects of PCP on kidney uric acid excretion in HUA mice. (A) PCP downregulated URAT1 (600 mg/kg) and GLUT9 (300 and 600 mg/kg) protein levels in the kidneys of PO/AD-induced mice and upregulated ABCG2 and OAT1 protein levels (600 mg/kg). (B) ABCG2 protein levels compared to β-actin; (C) protein levels of OAT1 to β-actin; (D) URAT1 protein levels to β-actin; (E) protein levels of GLUT9 to β-actin. Nor: blank control group; Mod: control group; Ben-50: benzbromarone 50 mg/kg; PCP-300: extracts of *Penthorum chinense Pursh.*; low-dose group 300 mg/kg; PCP-600: extracts of *Penthorum chinense Pursh.*, high-dose group 600 mg/kg. Data are expressed as mean ± SEM ($n = 6$). Compared with normal group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$; compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. AD: Adenine; HUA: Hyperuricemia; PCP: *Penthorum chinense* Pursh.; PO: Potassium oxonate; SEM: Standard error of mean.

in dietary habits and lifestyles that have occurred in the country[22]. Given these circumstances, there is an urgent need to intervene using treatments that have fewer side effects and are more effective in curbing this disorder. One potential remedy is PO, a uricase inhibitor with a chemical structure that mirrors the purine ring of UA. This structural similarity allows PO to competitively bind to uricase, thereby inhibiting its function and increasing the blood UA levels[23]. Another intriguing compound that induces renal histopathological variations is AD[24]. PO and AD were found to elevate serum UA, creatinine, and BUN levels. The synergistic effect of PO and AD provides a firm basis for establishing a stable HUA model. The results indicate that PO and AD substantially increased serum UA levels compared to the control group, thus successfully establishing an HUA model.

PCP is renowned for its economic and industrial value[25] and has been found to exhibit potent antihyperuricemic effects and can ameliorate renal impairment. Administration of 300 and 600 mg/kg/day PCP water extract substantially reduced serum UA, BUN, and creatinine concentrations, indicating its efficacy in improving both HUA and kidney function.

Previous studies have indicated that HUA, a key factor in the progression of gout, is primarily attributed to the overproduction of UA and its insufficient excretion from the body. Renal UA transporters play a pivotal role in this process and have been implicated in the pathogenesis of HUA. Of these transporters, URAT1 is the primary UA reabsorption transporter in renal tubular epithelial cells and its function is primarily associated with the reabsorption of UA in the kidneys. GLUT9, another essential transporter, is predominantly expressed in the kidneys and is specifically responsible for the reabsorption of UA, and has emerged as a new target for the treatment of HUA[26]. These studies provide compelling evidence for the regulation of UA excretion and the development of HUA by URAT1 and GLUT9.

A high-dose PCP water extract substantially downregulated URAT1 protein content in the kidneys. Low and high doses of PCP aqueous extract inhibited renal GLUT9 expression. ABCG2, a high-volume UA efflux transporter, is expressed in proximal convoluted tubule membranes. Reduced ABCG2 expression results in UA excretion, which increases renal excretion and worsens renal injury.

High-dose PCP water extract effectively upregulated renal ABCG2 protein content. OAT1, another critical transporter, plays an integral role in the excretion of UA in the basolateral side of proximal tubules and is closely

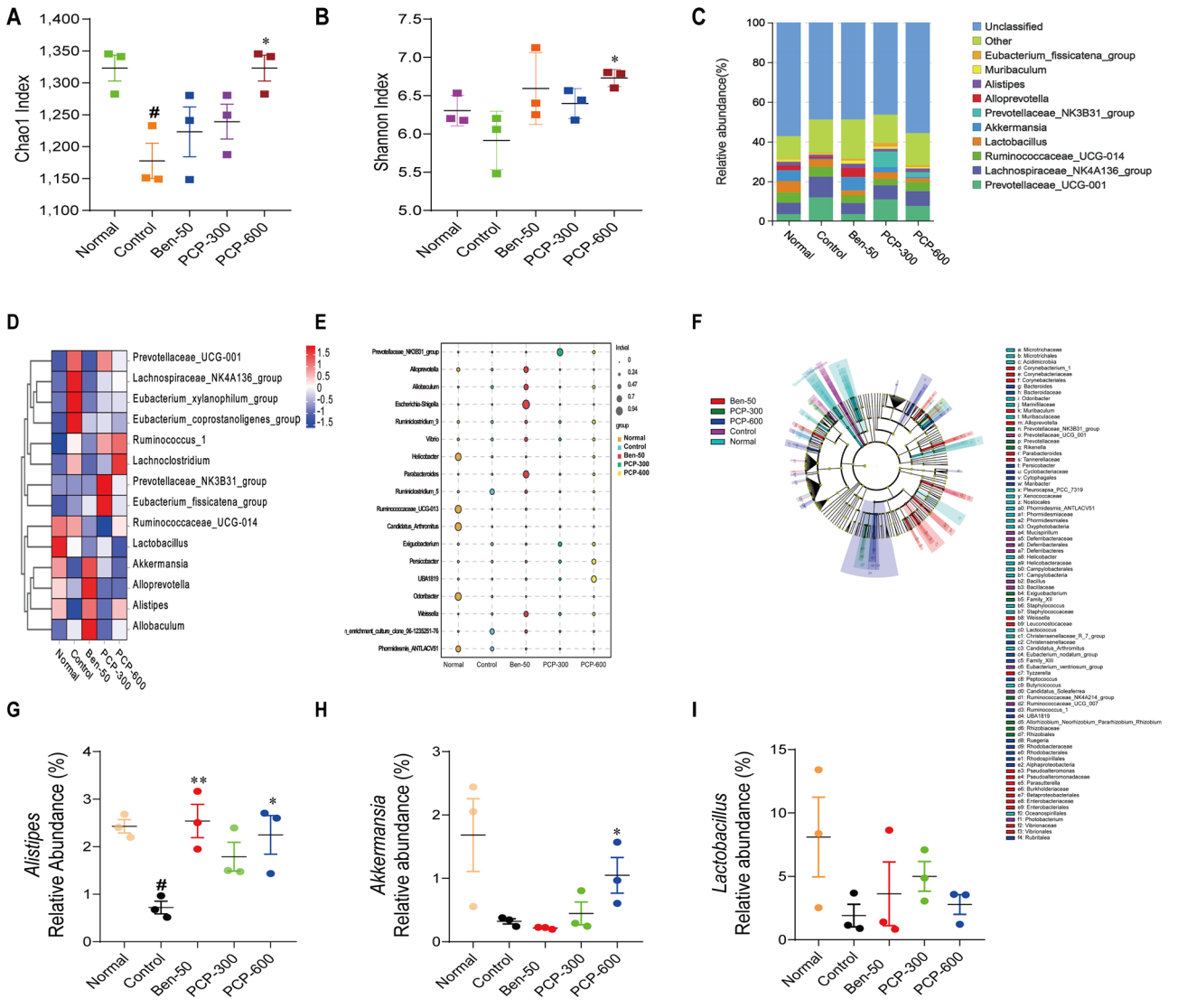

Figure 4. Effects of PCP on the gut microbiota diversity. (A) Chao1 index, (B) Shannon index, (C) structural changes in gut microbiota, (D) intestinal flora heat map, (E) indicator, (F) linear discriminant analysis effect size, (G) relative abundance of *Alistipes*, (H) relative abundance of *Akkermansia*, and (I) relative abundance of *Lactobacillus*. Nor: blank control group; Mod: control group; Ben-50: benzbromarone 50 mg/kg; PCP-300: extracts of *Penthorum chinense Pursh.*; low-dose group 300 mg/kg; PCP-600: extracts of *Penthorum chinense Pursh.*, high-dose group 600 mg/kg. Data are expressed as mean ± SEM ($n = 3$). Compared with normal group, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$; compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$. PCP: *Penthorum chinense* Pursh.; SEM: Standard error of mean.

related to the progression of CKD[27]. In the current study, the aqueous extract of PCP had no impact on the OAT1 transporter, which is therefore not a target of action for the PCP aqueous extract. The findings highlight the beneficial effects of high-dose PCP aqueous extract in reducing URAT1 and GLUT9 protein expression in the kidneys and augmenting ABCG2 protein levels, which in turn lessens UA reabsorption and increases its excretion. These changes could potentially attenuate the development of HUA and its associated health complications.

Inflammation is a critical feature of HUA-induced renal injury. High UA levels stimulate inflammatory cells and activate inflammatory reactions, resulting in further renal injury[28]. HUA induces inflammatory cytokine production. The concentrations of inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and IL-18, were substantially upregulated in HUA mice and downregulated by the treatment with PCP aqueous extract.

Excessive UA can activate the NLRP3 signaling pathway. The NLRP3 inflammasome is a nucleotide oligomerization domain (NOD)-like receptor protein, a family of multiprotein complexes that includes three proteins: NLRP3, ASC, and caspase-1[29]. The expressions of NLRP3, ASC, caspase-1, and IL-6, IL-1β, IL-18 were upregulated in HUA mice. However, the administration of the PCP aqueous extract effectively reversed these trends. PCP aqueous extract attenuated the inflammatory response by suppressing the NLRP3 pathway in HUA.

The NLRP3/ASC/Caspase-1 inflammasome is a critical intracellular platform that mediates the activation of pro-inflammatory cytokines, such as IL-1β, IL-6, and IL-18, and is implicated in various inflammatory diseases[30]. The relationship between this inflammasome and differentially expressed intestinal bacteria is complex and multifaceted. Intestinal bacteria influence the

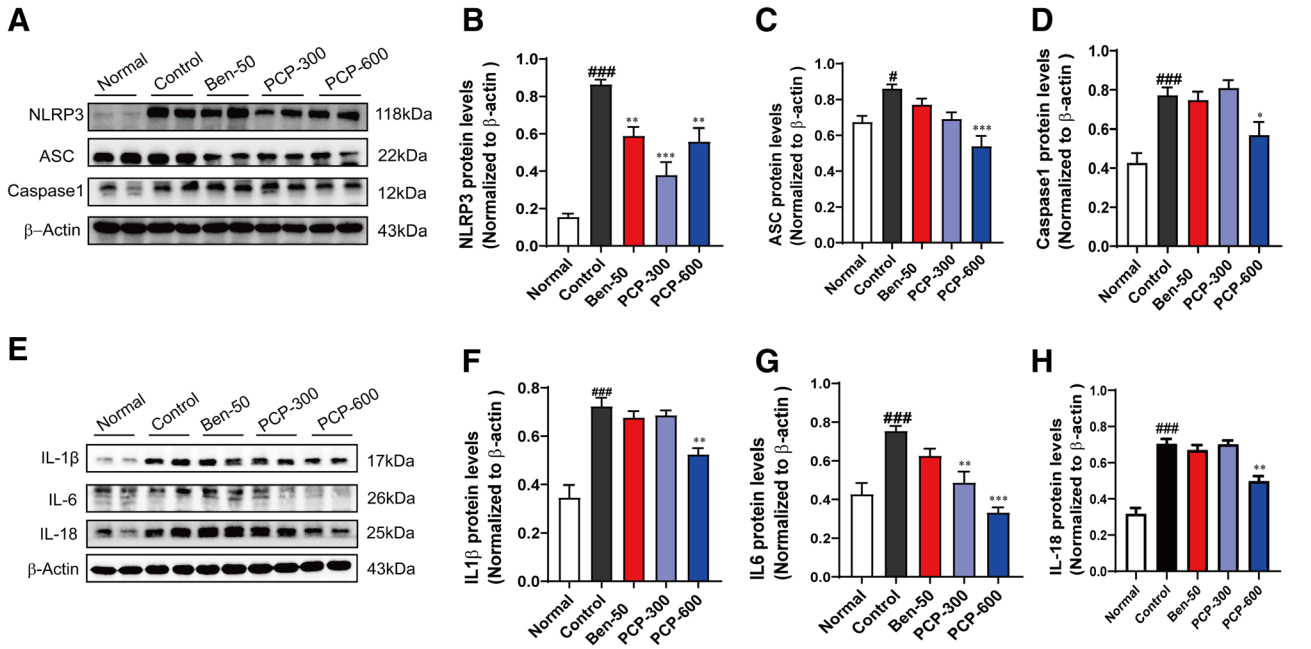

Figure 5. Effect of PCP on NOD-like receptor family, pyrin domain-containing protein 3 (NLRP3)/apoptosis-associated speck-like protein containing a CARD (ASC)/caspase-1 signaling pathways in kidney. (A) PCP (600 mg/kg) downregulated NLRP3/ASC/caspase-1 protein levels; (B) the protein levels of NLRP3 to β-actin; (C) ASC protein level compared with β-actin; (D) protein levels of caspase-1 to β-actin; (E) PCP (600 mg/kg) downregulated IL-1β and IL-6 protein levels; (F) IL-1β protein level to β-actin; (G) the protein level of IL-6 to β-actin; (H) IL-18 protein level to β-actin. Nor: blank control group; Mod: control group; Ben-50: benzbromarone 50 mg/kg; PCP-300: extracts of *Penthorum chinense Pursh.*, low-dose group 300 mg/kg; PCP-600: extracts of *Penthorum chinense Pursh.*, high-dose group 600 mg/kg. Data are expressed as mean ± SEM ($n = 6$). Compared with normal group, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$; compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. IL: Interleukin; PCP: *Penthorum chinense* Pursh.; SEM: Standard error of mean.

NLRP3/ASC/caspase-1 inflammasome through several mechanisms. First, the composition of gut microbiota can affect the activation of NLRP3. This activation can be a host defense mechanism against pathogenic bacteria but may also contribute to inflammation in the context of dysbiosis, where the balance of the gut microbiota is disrupted. Second, the NLRP3/ASC/caspase-1 inflammasome responds to changes in the gut microbiota[31]. This protective effect may be due to the early activation of NLRP3 and ASC in intestinal epithelial cells, which helps limit bacterial replication and spread, thereby reducing the severity of intestinal inflammation. Third, the inflammasome can be activated by bacterial components, such as lipopolysaccharides (LPS) or flagellin, which are recognized as pathogen-associated molecular patterns (PAMPs). For example, polymorphisms in NLRP3 have been linked to susceptibility to HUA, which is associated with a dysregulated immune response in the gut microbiota. Furthermore, activation of the NLRP3 inflammasome can be influenced by the epigenetic control of ASC, which may have implications in the regulation of inflammation in the context of heart failure and other diseases[32]. The gut microbiota can influence inflammasome activation, and in turn, the inflammasome can shape the gut microbial environment. This interplay is essential for maintaining intestinal homeostasis and has implications in the pathogenesis of inflammatory diseases.

UA (70%) is excreted through the kidneys and 30% is excreted from the intestines. High UA levels aggravate the renal burden and result in functional impairment of the kidney. A large amount of evidence has shown that kidney injury leads to gut microbiota dysregulation[33]. Gut microbiota plays an important role in UA excretion and are a main component of the intestine. Gut microbiota maintains human health, most of which are anaerobic bacteria, such as *Bacteroidetes* and *Firmicutes*[34–35]. Recently, the gut microbiota has been identified as a potential target for HUA. Regarding the alteration of the gut microbiota, the PCP aqueous extract may be another key factor contributing to the alleviation of HUA and inflammation in the kidney. The abundance of *Alistipes* and *Bacteroides* was aqueous downregulated in HUA mice, and the abundance of *Lachnospiraceae_NK4A136_group, Akkermansia,* and *Prevotellaceae_UCG-001* was substantially downregulated. After PCP aqueous extract (600 mg/kg) administration, the abundance of the gut microbiota recovered to that of the normal group.

Lactobacillus is a probiotic found in the gut that has an anti-hyperuricemic effect and can degrade UA into urea. Administration of PCP aqueous extract upregulates the abundance of *Lactobacillus*. Treatment with PCP aqueous extract to relieve HUA is considered a potentially effective mechanism. However, whether the beneficial effect of the PCP aqueous extract is mediated by the gut microbiota should be further explored using fecal microbiota transplantation experiments.

Conclusions

In conclusion, PCP extract decreased UA production and increased UA excretion in HUA mice. Moreover, PCP decreases inflammation to alleviate renal damage, and its possible mechanisms include changes in UA transporters and suppression of the NLRP3 inflammasome. PCP altered

the gut microbiota structure and increased the abundance of beneficial bacteria, such as *Lactobacillus* and *Alistipes*. Whether the anti-hyperuricemic and anti-inflammatory effects of PCP are mediated by the gut microbiota remains to be elucidated. This study provides an effective and novel treatment for HUA and presents a new application of PCP.

Conflict of interest statement

Tao Wang is an editorial board member of this journal. The other authors declare no conflict of interest.

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Author contributions

Yang Wang, Dan Wang, Yi Zhang, and Tao Wang participated in research design. Jing Xie, Siya Yan, Meifang Cheng, Yan Jing, Ke Li, Fengyan Yang, Qian Chen, Jian Li, and Yuzheng Wu participated in the performance of the research and analyzed the data. Yang Wang and Dan Wang were responsible for writing the manuscript. Yi Zhang and Tao Wang checked the manuscript. Tao Wang and Dan Wang provided the funding.

Ethical approval of studies and informed consent

Animal experiments were approved by the Science and Technological Committee and the Animal Use and Care Committee of TJUTCM (TCM-LAEC2022060).

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Data availability

All data generated or analyzed during this study are included in this published article.

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