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Original article

Saponins extracted from *Dioscorea collettii* rhizomes regulate the expression of urate transporters in chronic hyperuricemia rats

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ABSTRACT

Objective: The current study aimed to investigate whether the saponins, bioactive component of effects of *D. collettii*, could reduce the serum uric acid level in a hyperuricemic mouse via regulation of urate transporters.
Methods: Chronic hyperuricemia model was established by combine administration of adenine (100 mg/kg) and ethambutol (250 mg/kg). In the model group, the serum uric acid (SUA), urine uric acid (UUA) volume, and 24-h UUA values increased significantly, while the uric acid clearance rate (CUr) and creatinine clearance rate (CCr) values decreased. Further, the model groups showed significantly lower expression of organic anion transporter 1 (OAT1) and organic anion transporter 3 (OAT3) and significantly higher expression of renal tubular urate transporter 1 (URAT1), glucose transporter 9 (GLUT9) and URAT1 mRNA than the normal control group.
Results: Saponins administration was found to have a dose-dependent effect, as evidenced by the increase in the 24-h UUA, CUr and CCr values; the decrease in SUA; the decrease in the renal expression of URAT1 mRNA and URAT1 and GLUT9 proteins; and the increase in the renal expression of the OAT1 and OAT3 proteins.
Conclusion: The saponins extracted from *D. collettii* rhizomes had an obvious anti-hyperuricemic effect through downregulation of the URAT1 mRNA and the URAT1 and GLUT9 proteins and upregulation of the OAT1 and OAT3 proteins.

1. Introduction

Hyperuricemia is an emerging risk factor characterized by increase urate and a decrease glomerular filtration rate and renal tube excretion of urate. The transport of uric acid is regulated by several genes and proteins. Urate transporter 1 (URAT1, encoded by *SLC22A12*) and glucose transporter 9 (GLUT9, encoded by *SLC2A9*) mainly mediate the re-absorption of uric acid while the organic anion transporter (OAT) 1 and 3 are encoded by *SLC22A6* and *SLC22A8*, respectively, and these transporters are involved in the regulation of uric acid excretion [1,2]. Dysregulation of these proteins could affect urine excretion and lead to increase level of uric acid in the serum. Therefore, urate transport proteins in the kidney have become the major target for the treatment of urinary diseases [3].

Renal factors contributing to hyperuricemia include single or combined effects of decreased glomerular filtration rate, drug-induced increases in renal urate reabsorption, and altered expression and/or function of renal urate transporters [4]. For many years, the accepted human model of renal urate handling consisted of four components: glomerular filtration, reabsorption from the glomerular ultrafiltrate, subsequent secretion, and then postsecretory reabsorption [5]. The central role of *SLC22A12* and *SLC2A9* in proximal tubular reabsorption of urate in humans has been underlined by the linkage of marked functional deficiencies of these transporters to hypouricemia of renal etiology [6,7]. GWAS identification indicated that renal urate transporters contribute to the regulation of SUA levels [8,9], and genetic loss of function studies demonstrated that GLUT9 (SLC2A9) and URAT1 (SLC22A12) play significant roles in renal urate reabsorption, as hypouricemia and have high fractional excretion of uric acid (FE_{UA}) were appeared in patients with disruptive mutations of these

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transporters [7,10–13]. Organic anion transporters (OAT) are another urate transporters that genetically linked to hyperuricemia and gout [9], and it also associated with the increased risk of hyperuricemia and goat that induced by diuretics treatment [14,15]. Therefore, it has been acknowledged the importance that identification and development of effective agent for urate-lowering therapy to improve risk stratification for patients with hyperuricemia.

Rhizomes from *Dioscorea collettii* contain dioscin, protodioscin, gracillin and protogracill, and they are commonly used for the treatment of gout and hyperuricemia in Traditional Chinese Medicine [16–19]. A literature reported that *D. collettii* and its components have anti-inflammatory, anti-tumor, anti-hyperuricemia, immunomodulatory, and lipid-regulating effects [20]. In addition, our previous studies have demonstrated that the saponins present in *D. collettii* rhizomes could decrease the serum uric acid level in hyperuricemic rats. The main underlying mechanism that is most likely to be involved in this therapeutic effect is the increased excretion of uric acid [21,22]. The previous findings have indicated that the saponins extracted from *D.* collettii may promote the re-absorption of uric acid and reduce the secretion of serum uric acid by regulating the expression of the proteins involved in urate transport. The aim of the current study was to investigate whether the therapeutic effect of *D. collettii* saponins on hyperuricemia is mediated by regulation of renal urate transporters in hyperuricemic rat model.

2. Material and methods

2.1. Animals

SPF male SD rats, (weighting 200 ± 20 g) provided by the Anhui Medical Experimental Animal Center (Hefei, China) (certificate no. SCXK [Anhui] 2011-002) were used. All the animals were housed under standard conditions (temperature, 22 ± 3 °C; relative humidity, 40%–60%) under a 12-h light-dark cycle, and were given free access to tap water and food. All the studies were carried out in accordance with the guidelines of the Institutional Animal Care Committee. The experimental protocols described in this study were approved by the Ethics Review Committee for Animal Experimentation of Anhui University of Chinese Medicine and were conducted in compliance with the guidelines for laboratory animal care of NIH (NIH publication no. 85–23, revised 1985).

2.2. Plant material and extraction

Rhizomes of *D. collettii* were purchased from the First Affiliated Hospital of Anhui University of Chinese Medicine. The plant species was authenticated by Prof. Jianli Zhou of the Department of Pharmacognosy, School of Pharmacy, Anhui University of Chinese Medicine, Hefei, China, and a voucher specimen was stored in our laboratory (Department of Pharmacology, Anhui University of Chinese Medicine, Hefei, China). The subjected component was extracted. Briefly, air-dried and sliced rhizomes of *D. collettii* were extracted twice for 1.5 h each time, using 70% ethanol (eight times the volume of the rhizomes) at 80 °C. The extracts were filtered, and the filtrate was evaporated till the alcohol could not be tested. Then, water was added to the filtrate and mixed. This solution was then extracted four times with water-saturated butanol, combined butanol filtrate, and vacuum dried till the butanol was eliminated. The precipitate obtained was recrystallized twice with 95% ethanol and washed with ether. The content of saponins in the extract was determined using UV–vis spectrophotometry with diosgenin as the standard reference. Main compounds of yam saponins were dioscin, protodioscin, gracillin, and protogracill (Fig. 1) [23].

The HPLC analyses were performed with HPLC instrument (Essentia CTO-16, Shimadzu, Japan) equipped with a quaternary solvent delivery system, an autosampler, a column oven and a diode array UV/Vis detector. The column (Sinochrom ODS-BP C_{18}, 4.6 mm × 4.6 mm, 5 μm, Dalian Elite analytical Instruments Co, Ltd.) was eluted isocratically with a binary mixture of acetonitrile and water at a total flow rate of 1.0 ml/min. Elution was monitored at 215 nm on the diode array detector, the injection volume was 20 μl, and separations were carried out isothermally at 30 °C in a heated chamber.

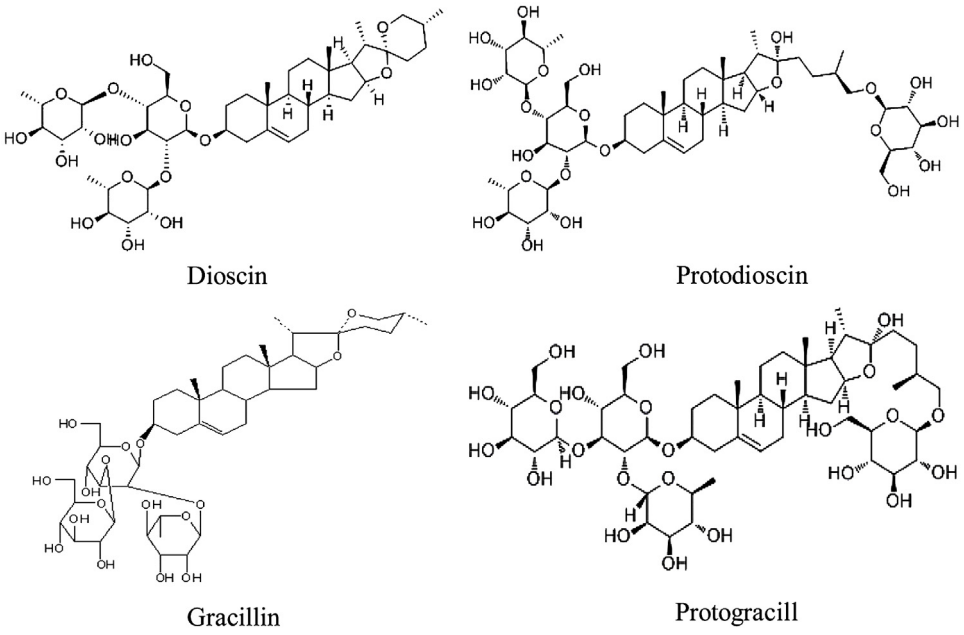

Fig. 1. Chemical structure of dioscin, protodioscin, gracillin and protogracill. The extracted saponins were separated and four main compounds were identified: dioscin, protodioscin, gracillin and protogracill.