

## Original Research Article

# Anti-hyperuricemic effect of *Plantago depressa* Willd extract in rats

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### Abstract

**Purpose:** To investigate the effects of *Plantago depressa* Willd. extract (PDWE) on hyperuricemia in rats.

**Methods:** The effect of PDWE was investigated in hyperuricemic rats induced by potassium oxonate. PDWE were fed to hyperuricemic rats daily at a dose of 160, 320 and 640 mg/kg for 10 days; allopurinol (5 mg/kg) was given as positive control. Serum and urine levels of uric acid and creatinine were determined by colorimetric method.

**Results:** PDWE inhibited xanthine oxidase (XOD) activity in serum ( $16.36 \pm 1.16$  U/L,  $p < 0.05$ ) and liver ( $72.15 \pm 5.26$  U/g protein,  $p < 0.05$ ), and also decreased levels of serum uric acid ( $2.43 \pm 0.59$  mg/L,  $p < 0.05$ ), serum creatinine ( $0.42 \pm 0.15$   $\mu$ mol/L) and blood urea nitrogen (BUN,  $9.58 \pm 0.72$  mmol/L,  $p < 0.05$ ), but increased levels of urine uric acid ( $39.23 \pm 8.22$  mg/L,  $p < 0.05$ ) and urine creatinine ( $32.24 \pm 1.69$  mmol/L,  $p < 0.05$ ) in the renal tissue of hyperuricemic rats.

**Conclusion:** PDWE exerts uricosuric action by regulating renal urate transporters to ameliorate renal dysfunction in hyperuricemic rats.

**Keywords:** *Plantago depressa* Willd., Hyperuricemic, Renal urate transporters, Renal dysfunction, Uricosuric action

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## INTRODUCTION

Hyperuricemia is characterized by abnormally high levels of uric acid in the blood. In general, the upper end of the normal range is 360  $\mu$ mol/L (6 mg/dL) for women and 416  $\mu$ mol/L (7.0 mg/dL) for men.<sup>1</sup> Hyperuricemia has also emerged as a metabolic disease which threatens human health, and is considered as an important risk factor for gout and may be associated with metabolic syndromes, stroke incidence, chronic kidney disease and cardiovascular disease [1,2]. Current anti-hyperuricemic agents in use include: xanthine oxidase (XOD) inhibitors, which inhibit

the activity of XOD, an enzyme involved in purine metabolism, of which allopurinol is the most often prescribed; uricosuric agents, which act on the proximal tubules in the kidneys to interfere with the absorption of uric acid from the urine back into the blood, and include probenecid, benzbromarone and sulfapyrazone; and the enzyme urate oxidase, including the recombinant form rasburicase, which catalyzes the oxidation of uric acid to the more soluble allantoin which is more readily excreted via the kidneys [3-5]. However, these existing anti-hyperuricemic agents possess undesirable effects. For example, allopurinol, which is the drug of choice,

has side effects including gastrointestinal irritation, bone marrow suppression, hypersensitivity syndromes, hepatitis and worsening renal function, which are unable to be tolerated by approximately 5 % of patients [6]. Uricosuric agents are used in patients with allopurinol-allergic syndromes as well as in under excretors with normal renal function and no history of urolithiasis. However, uricosuric agents, such as benzbromarone, also have issues. Though benzbromarone is still marketed in several countries by other drug companies, it was withdrawn by Sanofi-Synthelabo in 2003 after reports of serious hepatotoxicity. Similarly, the uricosuric agents probenecid and sulfinpyrazone have been reported to be nephrotoxic when used to treat hyperuricemia associated with moderate chronic renal insufficiency. Enzyme therapy using urate oxidase is only for treating severe hyperuricemia and is not widely used. Thus, anti-hyperuricemic agents have been limited in their clinical use due to their severe side effects. Therefore, it is important to search for alternative anti-hyperuricemic agents with more favorable toxicological profiles, and in particular from natural sources [7].

*Plantago depressa Willd.* is widely used as a traditional Chinese herb for its efficiency in treating gouty diseases such as hyperuricemia, gout and inflammatory arthritis in China [8-9], but its actual mechanisms in the hypouricemic process remains unclear.

This study is to investigate therapeutic effects of PDWE on XOD activity and urate excretion in experimental hyperuricemia mice.

## EXPERIMENTAL

### Plant material and extraction

Samples of *Plantago depressa Willd.* were collected from Guilin City, Guangxi Province in China in September 2015. Taxonomic authentication of the plant was performed by Professor Li He of Hubei University of Arts and Science in China. A voucher specimen (no. PDWE 20150922) was deposited in the Herbarium of College of Pharmacy, Hubei University of Arts and Science, China for future reference.

The whole plant of *Plantago depressa Willd.* was dried in a drying oven at 100 °C for 12 h. An aqueous extract of DTM was obtained by steeping the dried *Plantago depressa Willd.* in water at 60 °C three times, each for 1 h in an oven and then freeze-drying the last extract thus

obtained. One gram powder was obtained from about 2.0 g dried sample, i.e., a yield of 50.0 %.

### Animals and experimental procedures

Male SD rats, weighing 180 - 220 g, were purchased from Animal Experimental Center, Wuhan University, China. The rat experiment was approved by the Animal Care and Use Committee of Hubei University of Arts and Science (approval ref no. 20111016) and was carried out in compliance with Directive 2010/63/EU on the Handling of Animals used for Scientific Purposes [10].

Potassium oxonate was used to induce hyperuricemia in mice as previously reported [11,12]. Sixty rats were divided into six groups of ten rats each: normal group, model group, allopurinol group, high dose, middle dose and low dose of PDWE groups. Model rats were treated with 250 mg/kg oxonate. Allopurinol group rats were treated with allopurinol (5 mg/kg); high dose, middle dose and low dose of PDWE group rats were treated with 640 mg/kg, 320 mg/kg and 160 mg/kg PDWE respectively. Normal group rats and negative control group rats were administered water.

Except normal mice group, others were orally administered 250 mg/kg oxonate once daily for 7 consecutive days to induce hyperuricemia. The drugs (allopurinol and PDWE) were dispersed in water and were orally administered once daily from day 1 to day 10.

### Biochemical analysis

After 10 days of treatment, animals were sacrificed by cervical vertebra. Blood samples were collected and centrifuged at 3500 × g for 20 min to obtain serum. The levels of XOD activities in serum and liver, serum and urinary levels of uric acid (UA), creatinine (Cr) and blood urea nitrogen (BUN) were determined by colorimetric methods using commercially available kits (purchased from Nanjing Jiancheng Biological Technology Co., Ltd., China) according to the manufacturers' instructions.

### Data analysis

All statistics were analyzed using Statistical Package SPSS 18.0 (SPSS Inc, Illinois, Chicago, USA) and are expressed as mean ± standard error of mean (SEM), and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Effect of PDWE on serum and urinary levels of UA, Cr, and BUN

The  $S_{UA}$  level of model rats is significantly higher than those of normal rats after orally administered with potassium oxonate ( $p < 0.05$ ). Over a period of 10 days of treatment, the  $S_{UA}$  level was suppressed significantly ( $p < 0.05$ ) by PDWE treatment at the dose of 160 - 640 mg/kg, while the levels of  $U_{UA}$  were increased significantly ( $p < 0.05$ ) compared with model group (Table 1). High dose of PDWE also had significant effects on serum and hepatic XOD activities in hyperuricemic mice as showed in Table 2. Allopurinol at the dose of 5 mg/kg significantly suppressed hepatic XOD activity of hyperuricemic rats ( $p < 0.01$ ). Compared with the normal group, the levels of BUN and  $S_{Cr}$  were suppressed significantly (both  $p < 0.05$ ) by PDWE treatments at a dose of 160 - 640 mg/kg. Conversely,  $U_{Cr}$  levels at the treated doses was approximately 3 times more than that of allopurinol at a dose of 5 mg/kg and approximately 4 times more than that of the model group. Although both  $S_{UA}$  and  $U_{UA}$  levels in PDWE-treated rats were higher than those in allopurinol-treated mice, PDWE dose-dependently enhanced UA excretion.

## DISCUSSION

Uric acid is the final oxidation product of purine metabolism and is excreted in urine in humans,

who have lost hepatic uricase activity during evolution. As a consequence, humans have higher serum uric acid levels compared with most mammals. Several factors including high-protein diet, alcohol consumption, high cell turnover, and renal failure, can result in elevated uric acid levels. In recent decades, hyperuricemia has received increasing attention as a major public health problem because of its high prevalence and the associated increases in the risk of hypertension, cardiovascular disease, diabetes and chronic kidney disease [11,12].

Some studies also demonstrated that traditional Chinese medicine could down-regulate hepatic XOD and enhance renal urate excretion in hyperuricemic mice [13]. However, in our clinical practice and previous reports, inhibitory effect of traditional Chinese medicine simiao pill on XOD was lower than that of allopurinol. Therefore, to reverse the complicated pathologic state of hyperuricemia in the early phase, PDWE was used to enhance renal urate excretion. Compared with model group, the levels of  $S_{UA}$  were suppressed significantly by PDWE treatment at the dose of 160 - 640 mg/kg, while the levels of  $U_{UA}$  were increased significantly.

High dose of PDWE also had significant effects on serum and hepatic XOD activities in hyperuricemic mice. Increasing clinical reports have shown that hyperuricemia associated with an increasing risk of not only gout, but also chronic nephritis and

**Table 1:** Effect of PDWE on serum and urinary levels of UA, Cr and BUN (n = 10)

Group	Dose (mg/kg)	$S_{UA}$ (mg/L)	$U_{UA}$ (mg/L)	$S_{Cr}$ ( $\mu$ mol/L)	$U_{Cr}$ (mmol/L)	BUN (mmol/L)
Normal	-	1.26 $\pm$ 0.14	37.23 $\pm$ 5.59	0.23 $\pm$ 0.11	45.26 $\pm$ 3.27	8.59 $\pm$ 0.78
Negative control	-	6.34 $\pm$ 0.72	13.28 $\pm$ 4.36	0.84 $\pm$ 0.27	18.34 $\pm$ 4.38	17.44 $\pm$ 1.18
Allopurinol	5	1.94 $\pm$ 0.32	18.64 $\pm$ 5.13	0.35 $\pm$ 0.16	25.14 $\pm$ 3.25	9.84 $\pm$ 1.16
PDWE-L	160	3.75 $\pm$ 1.25	20.14 $\pm$ 6.15	0.61 $\pm$ 0.17	17.38 $\pm$ 2.27	14.25 $\pm$ 0.62
PDWE-M	320	2.64 $\pm$ 0.85	28.25 $\pm$ 7.13	0.54 $\pm$ 0.25	24.52 $\pm$ 2.87	12.34 $\pm$ 0.68
PDWE-H	640	2.43 $\pm$ 0.59	39.23 $\pm$ 8.22	0.42 $\pm$ 0.15	32.24 $\pm$ 1.69	9.58 $\pm$ 0.72

Data are expressed as mean  $\pm$  SEM;  $p < 0.05$  compared with model group. **Key:** PDWE-L = low dose of PDWE; PDWE-M = middle dose of PDWE; PDWE-H = high dose of PDWE

**Table 2:** Effect of PDWE on XOD activity in serum and liver of hyperuricemic mice (n = 10)

Group	Dose (mg/kg)	Serum XOD (U/L)	Liver XOD (U/g protein)
Normal	-	15.28 $\pm$ 1.25	59.46 $\pm$ 3.38
Model	-	29.26 $\pm$ 1.38	82.16 $\pm$ 4.26
Allopurinol	5	18.24 $\pm$ 1.23	35.33 $\pm$ 4.19
PDWE-L	160	25.21 $\pm$ 1.25	76.46 $\pm$ 5.25
PDWE-M	320	23.24 $\pm$ 1.17	74.18 $\pm$ 5.34
PDWE-H	640	16.36 $\pm$ 1.16	72.15 $\pm$ 5.26

Data is expressed as mean  $\pm$  SEM;  $p < 0.05$  compared with model group. **Key:** PDWE-L = low dose of PDWE; PDWE-M = middle dose of PDWE; PDWE-H = high dose of PDWE

renal dysfunction [14]. BUN and  $S_{Cr}$  levels are useful indicators of renal function. Renal damage could be accompanied by an increase in BUN and  $S_{Cr}$  indicating reduced urea and creatinine clearance [15]. Compared with the normal group, the levels of BUN and  $S_{Cr}$  were suppressed significantly by PDWE treatments at a dose of 160 - 640 mg/kg, and conversely, the degree of promotion of  $U_{Cr}$  levels induced by PDWE at the treated doses was approximately 3 times more than that of allopurinol.

## CONCLUSION

The findings of this study reveal that PDWE successfully treated hyperuricemic in rats by regulating renal urate transporters. Thus, the plant has the potential to be developed for clinical application in future.

## DECLARATIONS

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### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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## REFERENCES

1. Bieber JD, Terkeltaub RA. Gout: on the brink of novel therapeutic options for an ancient disease. *Arthritis & Rheumatism*. 2004; 50: 2400-2414.

2. Choi HK, Atkinson K, Karlson EW, Willett W, Curhan G. Purine-rich foods. *N Engl J Med*. 2004; 350: 1093-1103.
3. Chen IH, Chang JM, Chen HC. Allopurinol-induced severe hypersensitivity with Kaohsiung J Med Sci. 2005; 21: 228 - 232.
4. Horiuchi H, Ota M, Nishimura S, Kaneko H, Komoriya K. Allopurinol induces renal toxicity by impairing pyrimidine metabolism in mice. *Life Sciences* 2000; 66: 2051-2070.
5. Mari E, Ricci F, Imberti D, Gallerani M. Agranulocytosis: an adverse effect of allopurinol treatment. *Italian J Med*. 2011; 5: 120-123
6. Sabina EP, Nagar S, Rasool M. A role of piperine on monosodium urate crystal-induced inflammation-an experimental model of gouty arthritis. *Inflammation*. 2011; 34: 184-192.
7. An J, Yang HJ, Park K, Lee J, Kim BW. Reparatory and preventive effects of oriental herb extract mixture (OHEM) on hyperuricemia and gout. *Food Sci Biotechnol* 2010; 19: 517-524.
8. Sweeney AP, Wyllie SG, Shalliker RA, Markham JL. Xanthine oxidase inhibitory activity of selected Australian native plants. *J Ethnopharmacol* 2001; 75: 273-277.
9. Liote F. Hyperuricemia and gout. *Curr Rheumatol Rep*. 2003; 5: 227-234.
10. European Commission [homepage on the internet]. Directive 2010/63/EU on the protection of animals used for scientific purposes [cited 2013 Jan 16]. Available from:[http://ec.europa.eu/environment/chemicals/lab\\_animals/legislation\\_en.htm](http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm).
11. Li JM, Zhang X, Wang X, Xie YC, Kong LD. Protective effects of cortex fraxini. *Eur J Pharmacol*. 2011; 666: 196-204.
12. Wang X, Wang CP, Hu QH, Lv YZ, Zhang X, Ouyang Z, Kong LD. The dual actions of Sanmiao wan as a hypouricemic agent: down-regulation of hepatic XOD and renal mURAT1 in hyperuricemic mice. *J Ethnopharmacol* 2010; 128: 107-115.
13. Hu QH, Jiao RQ, Wang X, Kong LD. Simiao pill ameliorates urate under-excretion and renal dysfunction in hyperuricemic mice. *J Ethnopharmacol* 2010; 128: 685-692.
14. Kong LD, Yang C, Ge F, Wang HD, Guo YS. A Chinese herbal medicine Ermiao wan reduces serum uric acid level and inhibits liver xanthine dehydrogenase and xanthine oxidase in mice. *J Ethnopharmacol* 2004; 93: 325-330.
15. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS. Uric acid and incident kidney disease in the community. *J Am Soc Nephrol*. 2008; 19: 1204-1211.
16. Anzai N, Endou H. Urate transporters: an evolving field. *Seminars in Nephrology* 2011; 31: 400-409.
17. Sato M, Wakayama T, Mamada H, Shirasaka Y, Tamai I. Identification and functional characterization of uric acid transporter Urat1 (Slc22a12) in rats. *Biochim Biophys Acta*. 2011; 1808: 1441-1447.