Tropical Journal of Pharmaceutical Research September 2016; 15 (9): 1883-1887
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v15i9.10

Original Research Article

Effect of Dioscorea tokoro Makino extract on hyperuricemia in mice

Yang Fei[1], Dan Ye[2], XiaoFen Fan[3] and FengQin Dong[2]*

[1]Department of Metabolism and Endocrinology, People's Hospital of Fuyang City, Zhejiang Province, Fuyang, [2]Department of Endocrinology and Metabolism, Hangzhou, China; [3]Department of Traditional Chinese Medicine, The First Affiliated Hospital of Medical School of Zhejiang University, Zhejiang Province, Hangzhou, China

For correspondence: **Email: yangfei133494@sina.com; **Tel:** +86 0571 63101305*

Received: 24 March 2016 *Revised accepted: 11 August 2016*

Abstract

Purpose: *To investigate the anti-hyperuricemic effect of Dioscorea tokoro Makino extract (DTME) in potassium oxonate-induced hyperuricemic mice.*
Method: *The effect of DTME was investigated in the hyperuricemic mice induced by potassium oxonate. DTME. The extract was administered to the mice daily at doses of 220, 440 and 880 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. Simultaneously, protein levels of urate transporter 1 (URAT1) and organic anion transporter 1 (OAT1) in the rat kidney were analyzed by Western blotting.*
Results: *Compared with control, a high dose of DTME inhibited xanthine oxidase (XOD) activity in both serum (18.12 ± 1.33 U/L) and in liver (70.15 ± 5.20 U/g protein) ($p < 0.05$); decreased levels of serum uric acid (2.04 ± 0.64 mg/L) ($p < 0.05$), serum creatinine (0.35 ± 0.18 µmol/L) and blood urea nitrogen (BUN) (8.83 ± 0.71 mmol/L) ($p < 0.05$). Furthermore, the extract increased levels of urine uric acid (38.34 ± 8.23 mg/L), urine creatinine (34.38 ± 1.98 mmol/L), down regulated of URAT1 and up regulated of OAT1 protein expressions ($p < 0.05$) in the renal tissue of hyperuricemic mice.*
Conclusion: *DTME improves renal dysfunction in rats by regulating renal urate transporters in hyperuricemic rats. This may find therapeutic application in antihypertensive therapy.*

Keywords: *Dioscorea tokoro Makino, Hyperuricemic, Renal urate transporters, Uric acid, Creatinine*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Hyperuricemia is one of the most common and extensive metabolic diseases in populations, characterized by high uric acid level in the blood, causing deposition of urate crystals in the joints and kidneys, and is well known as important risk factor for gouty arthritis, uric acid nephrolithiasis, cardiovascular and renal disease, especially hypertension [1,2]. Despite advances in the use of anti-hyperuricemic agents for the treatment of hyperuricaemia and gout, allopurinol as a frequently used xanthine oxidase (XOD) inhibitor could induce severe hypersensitivity (such as a mild exanthema) and agranulocytosis, and aggravate renal toxicity by impairing pyrimidine metabolism [3,4]. Therefore, it is necessary to study available anti-hyperuricemic agents, especially herbal medicine [5].

Dioscorea tokoro Makino is widely used as a traditional Chinese herb for its efficiencyin treating hyperuricemia, gout and inflammatory arthritis in China [6], but its actual mechanisms in the hypouricemic process remains unclear. This study is to investigate therapeutic effects of

DTME on XOD activity and urate excretion in experimental hyperuricemia mice.

EXPERIMENTAL

Plant material and extraction

Samples of *Dioscorea tokoro* Makino were collected from Bozhou City, Anhui Province in China in May 2015. Taxonomic identification of the plant was performed by Professor HeHuang of Zhejiang University in China. A voucher specimen (no. DTME 20150517) was deposited in the Herbarium of College of Pharmacy, Zhejiang University, China for future reference.

A whole plant of *Dioscorea tokoro* Makino was dried in an oven at 100 ^{o}C for 12 h. The aqueous extract of DTM was obtained by steeping the dried *Dioscorea tokoro* Makino in water at 60 ^{o}C three times, each for 1 h before first drying in an oven and then freeze-drying the last extract thus obtained. One gram powder was obtained from about 1.5 g dried sample, a yield of 66.7 %.

Animals and experimental procedures

Male ICR mice, weighing 18 - 22 g, were purchased from Animal Experimental Center, Zhejiang University, China. The rat experiment was approved by the Animal Care and Use Committee of Zhejiang University (approval ref no. 20121012) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [7].

The uricase inhibitor potassium oxonate was used to induce hyperuricemia in mice according to previous reports [8]. Sixty mice were divided into six groups: normal group, model group, model plus allopurinol group, model plus high dose, plus middle dose and plus low dose of DTME groups. Each group had 10 mice.

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 DTME group rats were treated with 880, 440 and 220 mg/kg DTME, respectively. Normal and control group rats were treated with water (10 ml/kg).

Except the normal mice, other rats were all orally administered 250 mg/kg oxonate once daily for 7 consecutive days to induce hyperuricemia. The drugs (allopurinol and DTME) were dispersed in water and were orally administered once daily from day 1 to day 10, while the normal mice was treated with a similar vehicle.

Biochemical analysis

After 10 days of treatment, diets were removed from the cages 12 h before the animals were sacrificed by cervical vertebrae. Blood samples were collected by eyeball removal 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 method using commercially available kits (Shenzhen XinBoSheng Biological Technology Co. Ltd, China) according to the manufacturers' instructions.

Western blotting in kidney tissues

Kidney samples were homogenized and lysed in SDS-PAGE sample buffer, centrifuged and the supernatant recovered. Samples were run on 10 % SDS polyacrylamide gels, electroblotted onto nitrocellulose membranes. Immunoblotting was assayed using anti-URAT1, anti-OAT1 antibodies (Santa Cruz Biotech, USA). Detection was done using an enhanced chemiluminescence detection kit (Wuhan Boster Biological technology Ltd., Hunan, China). The band density were quantified using Lab works (GelPro 4.0, Media Cybernetics, LP) via calculating the average optical density in each field.

Data analysis

All data were analyzed using SPSS 16.0 (SPSS Inc, Illinois, Chicago, USA) and are expressed as mean ± standard error of mean (SEM). The analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of DTME on serum and urinary levels of uric acid and creatinine, and blood urea nitrogen (BUN)

As shown in the Table 1, after orally administration with potassium oxonate, the level of serum uric acid (S_{UA}) in model group rats were significantly higher than those in normal group ($p < 0.05$), which indicated that the model was successful for inducing hyperuricemia in rats. Over a period of 10 days of treatment, compared with model group, the levels of S_{UA} were suppressed significantly ($p < 0.05$) by DTME treatment at the dose of 220 - 880 mg/kg, while the levels of urinary uric acid (U_{UA}) were increased significantly ($p < 0.05$). High dose of

Table 1: Effect of DTME on serum and urinary levels of UA and Cr, and BUN in hyperuricemic mice (n = 10)

Group	Dose (mg/kg)	S_{UA} (mg/L)	U_{UA} (mg/L)	S_{Cr} (μmol/L)	U_{Cr} (mmol/L)	BUN (mmol/L)
Normal	-	1.42±0.18*	35.25±5.73*	0.29±0.11*	41.56±3.42*	7.58±0.85*
Model	-	6.13±0.76	12.68±4.79	0.95±0.38	17.33±4.68	16.43±1.13
Allopurinol	5	1.85±0.37*	19.26±5.28	0.34±0.22*	24.26±3.32*	9.37±1.14*
DTME-L	220	3.73±1.14*	19.79±6.21	0.56±0.21*	18.13±2.79	13.38±0.68*
DTME-M	440	2.85±0.91*	28.65±7.55*	0.45±0.21*	26.72±3.02*	11.37±0.74*
DTME-H	880	2.04±0.64*	38.34±8.23*	0.35±0.18*	34.38±1.98*	8.83±0.71*

Data were expressed as mean ± SEM; $p < 0.05$ compared with model group. DTME-L: low dose of DTME, DTME-M: middle dose of DTME, DTME-H: high dose of DTME

Table 2: Effect of DTME on XOD activities in the serum and liver of hyperuricemic mice (n = 10)

Group	Dose (mg/kg)	Serum XOD (U/L)	Liver XOD (U/g protein)
Normal	-	16.27±1.15*	65.34±3.68*
Model	-	26.58±1.46	84.53±4.56
Allopurinol	5	18.43±1.24*	37.38±4.59*
DTME-L	880	23.83±1.36	79.63±5.27
DTME-M	440	22.65±1.42	76.14±5.34
DTME-H	220	18.12±1.33*	70.15±5.20*

Data are expressed as mean ± SEM; $p < 0.05$ compared with model group. DTME-L: low dose of DTME, DTME-M: middle dose of DTME, DTME-H: high dose of DTME

DTME also had significant effects on serum and hepatic XOD activities in hyperuricemic mice as showed in Table 2. Allopurinol at dose of 5 mg/kg significantly suppressed hepatic XOD activity of hyperuricemic mice ($p < 0.01$). Compared with the normal group, the levels of BUN and serum creatinine (S_{Cr}) were suppressed significantly (both $p < 0.05$) by DTME treatments at a dose of 220 – 880 mg/kg, and conversely, the degree of promotion of urinary creatinine (U_{Cr}) levels induced by DTME 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 DTME-treated mice were higher than those in allopurinol-treated mice, DTME dose-dependently enhances the UA excretion.

Effect of DTME on organic anion transporter 1 (OAT1) and urate transporter 1 (URAT1)

As shown in Fig. 1, DTME dose-dependently decreased protein expressions of URAT1 and enhance OAT1 expressions in renal tissue of hyperuricemia mice. Compared with model group, protein expressions of URAT1 were significantly decreased in DTME-treated mice while the levels of OAT1 expression were significantly increased (both $p < 0.05$).

DISCUSSION

Hyperuricemia is a major risk factor for gout and chronic nephritis in clinical practice. Recently, the therapeutic agents for lowering serum uric acid are limited because of their undesirable adverse effects. Potassium oxonate is usually used to prepare a rodent model of hyperuricemia by inhibiting uricase [8].

Some studies also demonstrated that traditional Chinese medicine could down-regulate hepatic XOD and enhance renal urate excretion in hyperuricemic mice [9]. However, in the previous reports [10], inhibitory effect of Simiao pill on XOD was lower than that of allopurinol. Therefore, to reverse the complicated pathologic state of hyperuricemia in the early phase, DTME was employed to enhance renal urate excretion. Compared with model group, the levels of serum uric acid were suppressed significantly by DTME treatment at the dose of 220 - 880 mg/kg, while the levels of urinary uric acid were increased significantly ($p < 0.05$). High dose of DTME also had significant effects on serum and hepatic XOD activities in hyperuricemic mice.

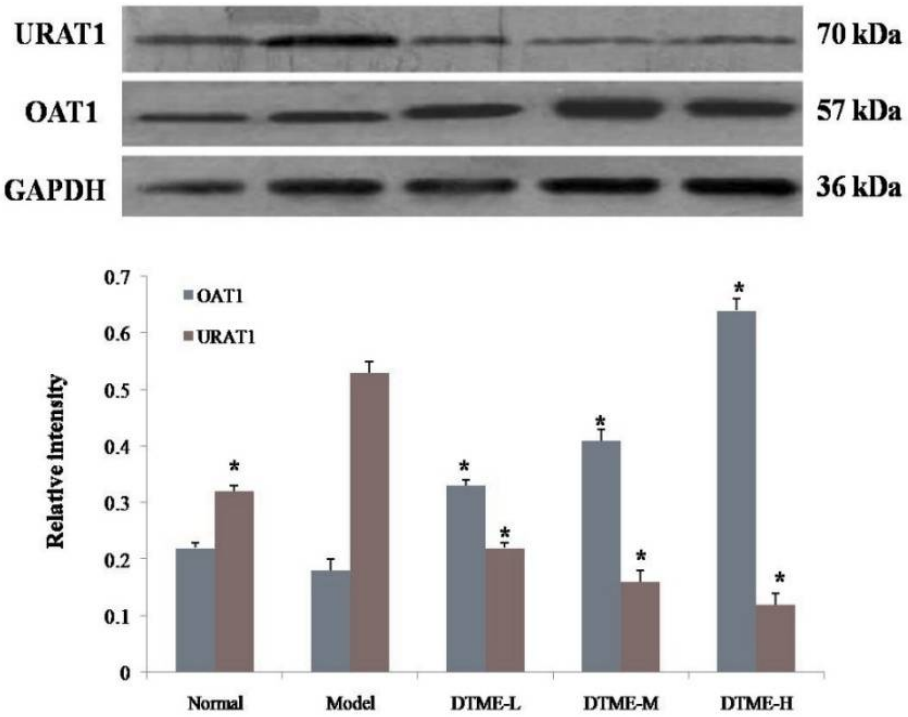

Figure 1: Protein expression of OAT1 and URAT1 in renal tissues by Western blot analysis. Representative immunoblot results. Densitometric analysis of blots for OAT1 and URAT1 protein. Values are mean ± SEM of 8 animals. Asterisks denote significance levels; $^{*}p < 0.05$, compared with model group

Increasing clinical reports have shown that hyperuricemia is associated with not only gout, but also chronic nephritis and renal dysfunction [11]. BUN and S_{Cr} levels are useful indicators of renal function.

Renal damage are accompanied by an increase in BUN and S_{Cr} indicating reduced urea and creatinine clearance [12]. Compared with the normal group, the levels of BUN and S_{Cr} were suppressed significantly (both $p < 0.05$) by DTME treatments at dose of 220 – 880 mg/kg, and conversely, the U_{Cr} levels induced by DTME at the treated doses was approximately 3 times more than that of allopurinol.

The major regulator of SUA is renal excretion of uric acid. This renal exchange is mediated by specialized molecules expressed in renal proximal tubule cells, in which Urate transporter 1 (URAT1) and organic anion transporter 1 (OAT1) have been considered to play an important role in UA handling [13]. URAT1 is an important determinant of urate reabsorption. It is also a drug target which is inhibited by uricosuric drugs, such as benzbromarone, probenecid and losartan [14]. OAT1 mediate the active uptake of organic anions and controls the final exit into the urine via ATP-powered transporters and bi-directional exchangers [15]. To evaluate the mechanism of DTME on the increase of UA clearance, effects of DTME on the URAT1 and OAT1 activation were examined. DTME dose-dependently prevent protein expressions of URAT1 and enhance OAT1 expressions in renal tissue of hyperuricemia mice (both $p < 0.05$). These results are in accordance with the uricosuric effects of DTME on promotion of the UA excretion mentioned above and it also suggest that DTME inhibit oxonate-induced accumulation of S_{UA} and reduction of urine volume mediated by the URAT1 and OAT1 signaling targets.

CONCLUSION

DTME has a potent uricosuric effect by regulating renal urate transporters URAT1 and OAT1 in hyperuricemic mice. It should be developed into an agent for the treatment of hyperuricemia.

DECLARATIONS

Acknowledgement

This study was supported by Zhejiang Provincial Medical and Health Science and Technology (fund number 2013KYA062).

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.

REFERENCES

1. *Chen JH, Chuang SY, Chen HJ, Yeh WT, Pan WH. Serum uric acid level as an independent risk factor for all-cause, cardiovascular, and ischemic stroke mortality: a Chinese cohort study. Arthrit Care Res 2009; 61: 225-232.*
2. *Choi HK, Ford ES. Prevalence of the metabolic syndrome in individuals with hyperuricemia. Am J Med. 2007; 120: 442-447.*
3. *Chen IH, Kuo MC, Hwang SJ, Chang JM, Chen HC. Allopurinol-induced severe hypersensitivity with acute renal failure. Kaohsiung J Med Sci 2005; 21: 228-232.*
4. *Horiuchi H, Ota M, Nishimura S, Kaneko H, Kasahara Y, Ohta T, Komoriya K. Allopurinol induces renal toxicity by impairing pyrimidine metabolism in mice. Life Sciences 2000; 66: 2051-2070.*
5. *Ahmad NS, Farman M, Najmi MH, Mian KB, Hasan A. Pharmacological basis for use of Pistacia integerrima leaves in hyperuricemia and gout. J Ethnopharmacol. 2008; 117: 478-482.*
6. *Chen GL, Zhu LR, Na S, Li L. Effect of total saponins of Dioscorea tokoro Makino on hyperuricemia and renal tubular uric acid transporter1 expression. Chi. J Chi. Mate Medi. 2013; 38: 2348-2353.*
7. *European Commission [homepage on the internet]. Directive 2010/63/EU on the protection of animals used for scientific purposes [cited 2013 Jan 16]. Availablefrom:http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm.*
8. *Li JM, Zhang X, Wang X, Xie YC, Kong LD. Protective effects of cortex fraxini coumarines against oxonate-induced hyperuricemia and renal dysfunction in mice. Eur J Pharmacol. 2011; 666: 196-204.*
9. *Hu QH, Jiao RQ, Wang X, Kong LD. Simiao pill ameliorates urate under excretion and renal dysfunction in hyperuricemic mice. J Ethnopharmaco. 2010; 128: 685-692.*
10. *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 Ethnopharmaco. 2004; 93: 325-330.*
11. *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.*
12. *Hoffmann D, Fuchs TC, Henzler T, Matheis KA, Herget T, Dekant W, Hewitt P, Mally A. Evaluation of a urinary kidney biomarker panel in rat models of acute and subchronic nephrotoxicity. Toxicology 2010; 277: 49-58.*
13. *Anzai N, Endou H. Urate transporters: an evolving field. Seminars in Nephrology 2011; 31: 400-409.*
14. *Richette P, Bardin T. Gout. Lancet 2011; 375: 318-328.*
15. *I-Sheikh AAK, Masereeuw R, Russel FGM. Mechanisms of renal anionic drug transport. Eur J Pharmacol. 2008; 585: 245-255.*