Original article

Effect of TongFengNing Decoction on Uric Acid Levels and Xanthine Oxidase Activity in Hyperuricemia Rats

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Highlights

The effect of TongFengNing Decoction (TD) on xanthine oxidase (XOD) activity and mRNA expression in the liver and small intestine of a hyperuricemia (HUA) rat model was explored as the possible mechanism of action for reducing serum uric acid (SUA). The experimental results show that TD reduces SUA levels in HUA model rats by promoting uric acid excretion and inhibiting XOD activity and mRNA expression. These results demonstrate the probable mechanism by which TD lowers uric acid, highlight its clinical application, and reflect the therapeutic advantage of using traditional Chinese medicine.

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Abstract

Objective: To observe the effect of TongFengNing Decoction (TD) on uric acid levels, xanthine oxidase (XOD) activity, and XOD mRNA expression of hyperuricemia (HUA) model rats. **Methods**: 90 rats were randomly divided into 6 groups (n=15), and the HUA model in all groups except the blank group was established by administering hypoxanthine (HX) by gavage and injecting potassium oxonate (OAPS) intraperitoneally. Rats in all TD groups and allopurinol group were administered multiple doses of TD and a single dose of allopurinol by gavage twice daily for 21 days, while the blank group and the model group were administered normal saline. On the 7th, 14th, and 21st days of drug intervention, serum uric acid (SUA), urine uric acid (UUA), intestinal uric acid (IUA), as well as XOD activity and mRNA expression in the liver and small intestine were measured in randomly selected 5 rats of each group. **Results**: On the 14th and 21st days of intervention, all TD dose groups and the allopurinol group showed decreased SUA and IUA levels, increased UUA levels, as well as decreased XOD activity and mRNA expression in the liver and small intestine, compared with the model group ($P < 0.05$). The low- and high-dose TD group and the allopurinol group showed increased SUA and IUA levels, as well as XOD activity and mRNA expression in the liver and small intestine, and decreased UUA levels, compared with the moderate-dose TD group ($P<0.05$). Upon extending the drug intervention time of each TD dose group, SUA and IUA levels, XOD activity, and XOD mRNA expression in the liver and small intestine decreased and UUA levels increased ($P < 0.05$). **Conclusion**: TD reduces SUA levels in HUA model rats, which promotes uric acid excretion and inhibits XOD activity and XOD mRNA expression to reduce uric acid production. The reduction in uric acid level by the intermediate dose of TD was better than that by allopurinol and the low and high doses of TD.

Keywords: TongFengNing, Hyperuricemia, Xanthine oxidase, Uric acid, Traditional Chinese medicine

摘要

目的：观察痛风宁对 HUA 大鼠尿酸、黄嘌呤氧化酶（xanthine oxidase，XOD）活性及 XOD mRNA 的影响。

方法：90 只 SD 大鼠随机分为 6 组（每组 15 只）。除空白组外，各组大鼠均以次黄嘌呤（HX）灌胃和氧嗪酸钾（OAPS）腹腔注射建立 HUA 大鼠模型。低、中、高剂量痛风宁组、别嘌醇组大鼠分别用不同剂量的相应药液灌胃，空白组与模型组用等量生理盐水灌胃，2 次/d，共 21d。在干预后的第 7 、14 、21 d 每组随机取 5 只大鼠，分别测定血尿酸（serum uric acid，SUA）、尿尿酸（urine uric acid，UUA）、肠道尿酸（Intestines uric acid，IUA）以及肝脏、小肠组织 XOD 活性及其 mRNA 表达情况。

结果：干预第 14 天和第 21 天，各剂量痛风宁组和别嘌呤醇组与模型组相比均显示 SUA、IUA 水平降低，UUA 水平升高，肝脏和小肠组织内 XOD 活性和 XOD mRNA 表达降低（$P < 0.05$）。低、高剂量痛风宁组和别嘌呤醇组与中剂量痛风宁组相比，SUA、IUA 水平升高，肝脏和小肠组织内 XOD 活性和 XOD mRNA 表达增加，UUA 水平降低（$P < 0.05$）。随着各剂量痛风宁组药物干预时间延长，SUA、IUA 水平、肝脏和小肠组织内 XOD 活性、XOD mRNA 表达持续降低，UUA 水平升高（$P < 0.05$）。

结论：痛风宁能降低 HUA 模型大鼠 SUA，这可能与其促进尿酸排泄，同时可抑制 XOD 活性并干预 XOD mRNA 的表达以减少尿酸生成有关。其中痛风宁中剂量降尿酸效果优于低、高剂量及别嘌醇。

关键词：痛风宁；高尿酸血症；黄嘌呤氧化酶；尿酸；中医药

Abbreviation: TD: Tongfengning Decoction, HUA: hyperuricemia, XOD: xanthine oxidase, HX: hypoxanthine, OAPS: potassium oxonate, SUA: serum uric acid, UUA: urine uric acid, IUA: intestinal uric acid.

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Background

Hyperuricemia (HUA) is a purine metabolic disease caused by overproduction, inadequate excretion of serum uric acid (SUA), or both, which is the principal biochemical basis for the onset of gout and is closely related to chronic nephrosis and heart cerebrovascular disease [1]. Western medicine currently uses drugs such as allopurinol, febuxostat, benzbromarone, and probenecid to treat HUA and gout [2]. However, these drugs have a high price and several adverse reactions; long-term use can damage the stomach, liver, and kidneys. As summarized by our research team, TongFengNing Decoction (TD) is a recipe shown to be effective in treating gout following years of clinical practice under the guidance of the concept of "Bi syndrome caused by internal dampness" [3]. Previous clinical studies have confirmed that TD can reduce the level of SUA in patients with gout [4,5]. Animal studies have shown that TD can reduce SUA levels in rabbits, chickens, and HUA model mice with acute gouty arthritis [6-8], but the specific mechanism by which uric acid is lowered is still unclear. Modern medical research has confirmed that xanthine oxidase (XOD) is a key enzyme in the production of uric acid. Numerous drugs reduce uric acid mainly by interfering with the XOD expression pathway, by competitive inhibition of XOD activity, or both [9]. In this study, we assessed the effect of TD on the levels of SUA, urine uric acid (UUA), and intestinal uric acid (IUA), as well as on XOD activity and mRNA expression in the liver and small intestine of HUA rats. By studying the possible mechanism of TD-mediated reduction of uric acid, we hope to lay a foundation that will popularize the clinical application of this decoction.

Materials

Animals

Healthy male SD rats (90 total, weighing 200 ± 20 g, SPF grade, and 3 months old) were provided by Shanghai Slack Laboratory Animals Co., Ltd. (Shanghai, China, license No: SCXK (Shanghai) 2012-0002). These rats were purchased and raised at the Experimental Animal Center of Fujian University of Traditional Chinese Medicine, (Fuzhou, China, Certificate No: SYXK (Min) 2014-0001). These rats were maintained in a room under the following conditions: A temperature of 22 ± 2 °C, humidity of 60 ± 10%, and a 12-h light/dark cycle.

Drugs

Test drug: PThe TD test drug consisted of Tufuling (*Smilacis glabrae rhizoma*), Bixie (*Dioscoreae spongiosae rhizome*), Zexie *(Alismatis rhizome*), Cangzhu (*Atractylodis rhizoma*), Jinqiancao (*Lysimachiae herba*), Danshen (*Salviae miltiorrhizae radix etrhizoma*), Chuanniuxi (*Cyathulae radix*), Qinjiao (*Gentianae macrophyllae radix*), Zhongjiefeng (*Sarcandrae herba*), Huangbo (*Phellodendri chinensis cortex*). They were purchased from the National Medical Hall of Fujian University of Traditional Chinese Medicine, concentrated to a drug solution containing 2 g/mL of crude drug according to the established process.

Positive drug: Allopurinol (100 mg/tablet) was purchased from Chongqing Qingyang Pharmaceutical Co., Ltd. (batch number: 141208).

Reagents

Reagents utilized in this study include: Potassium oxonate (OAPS), hypoxanthine (HX), sodium carboxymethylcellulose (CMC-Na) (Aladdin Industrial Corporation); uric acid assay kit, XOD kit (Nanjing Institute of Bioengineering); RNA Isolater, HiScript® II RT SuperMix, PCR Mix (Vazyme); analytical grade of isopropanol, analytical grade of chloroform (National Pharmaceutical Group Chemical Reagent Co., Ltd.); agarose (OXOID), DNA marker, and nucleic acid dye solution (Vazyme).

Preparation of experimental reagents

The experimental reagents utilized in these experiments were prepared as follows:

(1) 0.5% CMC-Na solution: CMC-Na powder (5 g) was added to 995 mL physiological saline and stirred to dissolve completely to prepare a 0.5 % CMC-Na solution.

(2) OAPS solution: OAPS powder was added to 0.5% CMC-Na solution to prepare the OAPS solution with a concentration of 50 g/L.

(3) HX solution: CMC-Na solution was added (0.5%) to HX powder to prepare a solution with a concentration of 25 g/L HX.

(4) Allopurinol suspension: An allopurinol pill was ground into a powder and dissolved in 0.5% CMC-Na solution to prepare a 2 g/L allopurinol suspension.

Instrument

The instruments utilized in this study included a low-temperature high-speed centrifuge (Germany Eppendorf 5417R), a spectrophotometer (US Thermo NanoDrop 2000), a PCR instrument (US Applied Biosystems 9700 PCR System), a gel imager (US Biorad GelDoc XR), a horizontal electrophoresis system (US BIO, USA-RAD), and a multi-function microplate reader (Austria TECAN Infinite M200).

Methods

Animal grouping

Three-month-old SPF healthy male SD rats (90 total) were randomly divided into 6 groups with 15 rats in each group, namely: the Blank group, the model group, the low-, moderate-, and high-dose TD groups, and the allopurinol group.

Animal model establishment and identification

Rats in all groups except the blank group were administered 500 mg/kg HX (1 mL/kg) once a day by gavage and intraperitoneally injected with 100 mg/kg OAPS (0.5 mL/kg) to establish HUA rat model. SUA

levels were measured in blood from the canthus in rat eyes on the 21st day for model identification.

Drug intervention

The 22nd day of modeling was the 1st day of drug intervention by gavage with doses according to the equivalent dose conversion of human and animal body surface area [10]. After the model was successfully identified, rats in the low-, moderate-, and high-dose TD groups were administered 7.65 g/kg/d, 15.3 g/kg/d, and 30.6 g/kg/d of TD, respectively, by gavage twice daily for 21 days. Rats in the allopurinol group were administered 20.8 mg/kg/d of allopurinol suspension, while those in the blank group and the model group were administered the same amount of normal saline. In order to maintain a stable high level of SUA status, rats in all groups except the blank group were administered 500 mg/kg HX by gavage every morning and intraperitoneally injected with 100 mg/kg OAPS.

Specimen Collection

On the 7th, 14th, and 21st days after drug intervention, 24-h urine samples were collected from 5 rats, which were randomly selected from each group by the metabolic cage. Rats were anesthetized with 3 mL/kg 10% chloral hydrate intraperitoneally, blood samples were collected from the abdominal aorta and centrifuged (3,000 rpm, 20 min), then the serum was packed separately and stored at -20°C. Intestinal cannula irrigation with saline, beginning at the duodenum, was collected as the rinse solution at the end of the colon [10], centrifuged (3,000 rpm, 5 min), and the supernatant collected as the extract, The liver tissue was placed on ice, rinsed with normal saline, frozen with liquid nitrogen, and transferred to the -80° C freezer.

Index detection

Detection of SUA, UUA, and IUA SUA, UUA, and IUA levels were detected at different time points by enzyme colorimetry according to the instructions of the uric acid assay kit.

Detection of XOD activity XOD activity in the liver and small intestine was measured according to the instructions in the kit. OD levels were measured by spectrophotometer and used to calculate XOD activity levels.

Detection of the expression of XOD mRNA in the liver and small intestine 1. Extraction of RNA: 1) 100 mg of liver and small intestine tissues were fully ground in liquid nitrogen and put into 2 EP tubes. RNA isolater (1 mL) was added to both tubes and left at room temperature for 15 min after mixing. 2) Each tube was treated with 1 mL Trizol and 200 μL of chloroform, subjected to 15 s of sharp whirlpool oscillates, placed at room temperature for 10 min, and centrifuged (12,000 rpm, 15 min). 3) The supernatants (500 μL) were transferred into 2 new EP tubes and an equal volume of isopropyl alcohol was added. The tubes were placed at -20°C for 30 min, then centrifuged (13,000 rpm, 10 min). 4) The supernatant was discarded and the sediment was retained. Pre-cooled 75% ethanol (1 mL) was added to each tube to oscillate and wash the RNA precipitation, then centrifuged (7,500 rpm, 5 min). 5) The supernatant was discarded and the RNA pellet dissolved in solution. After the UV spectrophotometer was adjusted to zero, the RNA sample (1 μL) was added to the test bench, the OD 260 nm and OD 280 nm values were recorded, and the RNA concentrations were calculated. 6) The RNA solution was centrifuged (7,500 rpm, 1 min), the residual liquid was absorbed, and the RNA pellet was let stand at room temperature for 3 min to dry. The RNA samples were then stored at - 80°C until use.

2. Reverse transcription: HiScript® II RT SuperMix was used to carry out reverse transcription in preparation for PCR as follows: 1) The DNA was prepared by placing the removal solution on ice, adding 4×gDNA wiper Mix (6 μL), total RNA (according to the sample concentration) and ddH_2O (total volume to 24 μL). The mixture was shaken to disperse the constituents evenly, centrifuged for several seconds, put into the PCR instrument, and set at 42℃ for 2 min to remove the DNA reaction. 2) The RT reaction liquid was prepared on ice, 5×RT SuperMix II (6 μL) and added to generate the first reaction liquid (26 μL), mixed by shaking, then centrifuged for several seconds, placed in the instrument, reverse transcribed at 50°C for 15 min, then inactivated at 85°C for 2 min; 3) The cDNA was stored at -20°C or used immediately to perform a PCR reaction.

3. Design and synthesis of primers: Primer design and synthesis were commissioned by Kingsry Biotechnology Co.; LTD. GAPDH was used as an internal reference (Table 1).

4. Polymerase chain reaction (PCR): 1) The reactants were added into the PCR reaction system as follows: 2×rTaq PCR Mix (10 μL), 10 μmol/L upstream primer (0.4 μL), 10 μmol/L downstream primers (0.4 μL), cDNA template (4 μL) and 20 μL ddH_2O. 2) PCR amplification was performed as follows: Pre-degeneration: 94°C for 5 min, Degeneration: 94°C for 30 s, Annealing: 58°C for 30 s, Extension: 72°C for 20 s, 2→4 (30 cycles), Extension: 72°C for 7 min. PCR amplification products (4 μL) were obtained after the reaction was complete and a 10% agarose gel was prepared for electrophoresis and product detection.

5. Agarose gel electrophoresis identification: 1) TAE (50×, 20 mL) was diluted to 1000 mL and 0.8 g agarose was dissolved in 80 mL of this solution. Gelred nucleic acid dye (10 μL) was added after boiling, and 750 mL of TAE was poured into the electrophoresis chamber. 2) When the agarose solution was cooled, it was poured into the assembled electrophoresis plate. After the gel solidified, the comb was pulled out and the TAE electrophoresis solution was added. 3) DNA marker (4 μL) and PCR sample (4 μL) were added in sequence. 4) Electrophoresis was performed at 120 V for 30 min and the gel strips were imaged using a Gel imager.

Statistical analysis

This experiment used SPSS 20.0 software for statistical analysis. All measurement data are expressed as mean± SD. Group statistical comparisons were assessed by

one-way analysis of variance (ANOVA). The LSD method was used when the variance is homogeneous. Otherwise the Tamhane's T2 (M) method was applied. Differences were considered significant when the *P* value was less than 0.05.

Table 1. Primer sequences

Name	Primer sequences	Amplification products
XOD	F: 5TCTTCCTGGCTGCTTCTATCTT3	284 bp
	R: 5TGTTCTGTGGTATGTTCCTCCT3	
GAPDH	F: 5ACGGCAAGTTCAACGGCACAG3	124 bp
	R: 5GAAGACGCCAGTAGACTCCACGAC3	

Results

Effects of TD on the levels of SUA, UUA, and IUA in each group of rats over time

As shown in Table 2, Table 3, and Table 4, the SUA and IUA of the model groups were increased significantly on the 7th, 14th and 21st days of the intervention ($P < 0.05$), but there was no significant difference in UUA ($P > 0.05$) compared to the blank group. Compared with the model group, SUA and IUA decreased and UUA increased in each dose of the TD group on the 14th and 21st days of the intervention ($P < 0.05$), while SUA and IUA in the allopurinol group decreased at all time points ($P < 0.05$) and there was no significant difference in UUA ($P > 0.05$). Compared with the moderate-dose TD group, SUA of the allopurinol group decreased on the 7th day of the intervention ($P < 0.05$), then the SUA and IUA were increased and UUA was decreased on the 14th and 21st days of the low- and high-dose TD groups and the allopurinol group ($P < 0.05$). Over time, SUA and IUA continued to decrease in TD groups at each dose ($P < 0.05$). The UUA on the 14th day of intervention was higher than that on the 7th day of intervention ($P < 0.05$) and there was no significant difference between the 21st day and the 14th day of intervention ($P > 0.05$).

Effects of TD on XOD activity in the liver and small intestine of each group of rats over time

As shown in Table 5, XOD activity in the liver and small intestine increased in the model group on the 14th and 21st days of intervention ($P < 0.05$). Compared with the model group, XOD activity in the liver and small intestine decreased at each dose within the TD group and the allopurinol group on the 14th and 21st days of intervention ($P < 0.05$). Compared with the moderate-dose TD group, XOD activity in the liver and small intestine increased in the low- and high-dose TD group and the allopurinol group ($P < 0.05$). Over time, the activity of XOD in the liver and small intestine at each dose within the TD group and allopurinol group decreased ($P < 0.05$).

Effects of TD on XOD mRNA in the liver and small intestine of each group rats over time

As shown in Table 6, the expression of XOD mRNA in the liver and small intestine increased in the model group on the 14th and 21st days of intervention ($P < 0.05$). Compared with the model group, the expression of XOD mRNA in the liver and small intestine decreased at each dose within the TD groups and allopurinol group at each time point ($P < 0.05$). Compared with the moderate-dose TD group, the expression of XOD mRNA in the liver and small intestine increased in the low- and high-dose TD groups and the allopurinol group at different time points ($P < 0.05$). Over time, the expression of XOD mRNA in the liver and small intestine at each dose within the TD groups and the allopurinol group decreased ($P < 0.05$).

Table 2. Comparison of SUA in each group rats over time ($\bar{x}\pm s$, n=5, μmol/L)

Groups	7 d	14 d	21 d
1	115.12±19.30	115.00±21.53	114.20±17.56
2	409.63±17.98▲	474.30±20.01▲	547.16±20.66▲
3	397.14±11.97	330.75±10.79★▼■	260.19±10.88★▼■
4	395.69±13.88	250.90±3.75★■	150.58±11.02★■
5	366.78±20.17	304.38±9.01★▼■	185.23±11.15★▼■
6	275.94±11.98★▼	271.81±4.33★▼	250.06±9.11★▼

Note: ▲Compared with the blank group ($P < 0.05$); ★compared with the model group ($P < 0.05$); ▼compared with the moderate-dose TD group ($P < 0.05$); ■compared with each time point ($P < 0.05$). 1 Blank group; 2 Model group; 3 Low dose TD group; 4 moderate dose TD group; 5 High dose TD group; 6 Allopurinol group.

Table 3. Comparison of UUA in each group rats over time
($\bar{x}$±s, n=5, μmol/L)

Groups	7 d	14 d	21 d
1	1326.40±53.68	1371.59±32.57	1320.55±29.46
2	1324.10±38.86	1333.18±38.86	1229.2±44.46
3	1345.38±86.52	1629.85±76.26★▼▪	1703.72±88.19★▼
4	1355.67±89.25	1922.37±79.09★▪	1992.20±70.58★
5	1341.50±62.45	1792.20±52.86★▼▪	1863.45±46.27★▼
6	1289.76±56.46	1359.98±59.28▼	1307.18±73.55▼

Note: ▲Compared with the blank group ($P < 0.05$); ★compared with the model group ($P < 0.05$); ▼compared with the moderate-dose TD group ($P < 0.05$); ▪compared with each time point ($P < 0.05$). 1 Blank group; 2 Model group; 3 Low dose TD group; 4 moderate dose TD group; 5 High dose TD group; 6 Allopurinol group.

Table 4. Comparison of IUA in each group rats over time
($\bar{x}$±s, n=5, μmol/L)

Groups	7 d	14 d	21 d
1	28.97±7.41	26.92±3.32	27.89±5.28
2	70.29±8.69▲	87.29±10.44▲	99.38±10.78▲
3	63.79±6.57	51.29±3.97★▼▪	43.18±2.71★▼▪
3	60.63±6.81	40.73±2.28★▪	28.35±1.90★▪
5	61.30±8.60	47.87±1.24★▼▪	41.41±1.88★▼▪
6	54.05±5.96★	53.01±6.41★▼	49.89±1.07★▼

Note: ▲Compared with the blank group ($P < 0.05$); ★compared with the model group ($P < 0.05$); ▼compared with the moderate-dose TD group ($P < 0.05$); ▪compared with each time point ($P < 0.05$). 1 Blank group; 2 Model group; 3 Low dose TD group; 4 moderate dose TD group; 5 High dose TD group; 6 Allopurinol group.

Table 5. Comparison of XOD activity in liver and small intestine of each group rats over time
($\bar{x}$±s, n=5, U/g protein)

Groups	Liver		Small Intestine	
	14 d	21 d	14 d	21 d
1	53.78±2.71	54.26±4.53	22.72±4.06	23.59±2.20
2	79.35±1.78▲	88.87±4.64▲	38.83±3.34▲	42.27±2.44▲
3	73.66±2.12★▼	65.02±1.60★▼▪	30.26±2.27★▼	28.65±1.11★▼▪
4	65.98±1.58★	56.04±1.01★	25.83±1.82★	24.58±1.22★
5	67.02±1.48★▼	60.36±1.11★▼▪	29.97±1.27★▼	27.41±1.01★▼▪
6	65.39±2.08★▼	61.83±1.17★▼▪	29.52±1.13★▼	27.52±1.23★▼▪

Note: ▲Compared with the blank group ($P < 0.05$); ★compared with the model group ($P < 0.05$); ▼compared with the moderate-dose TD group ($P < 0.05$); ▪compared with each time point ($P < 0.05$). 1 Blank group; 2 Model group; 3 Low dose TD group; 4 moderate dose TD group; 5 High dose TD group; 6 Allopurinol group.

Table 6. Comparison of relative gray value of XOD and GAPDH in liver and small intestine
($\bar{x}$±s, n=5)

Groups	Liver		Small Intestine	
	14 d	21 d	14 d	21 d
1	1.21±0.009	1.26±0.011	1.13±0.002	1.28±0.054
2	1.82±0.005▲	1.85±0.008▲	1.65±0.000▲	1.61±0.238▲
3	1.49±0.009★▼	1.23±0.011★▼▪	1.31±0.017★▼	1.15±0.027★▼▪
4	1.31±0.012★	0.95±0.013★▪	1.09±0.000★	0.90±0.000★▪
5	1.40±0.015★▼	1.13±0.006★▼▪	1.14±0.000★▼	0.98±0.074★▼▪
6	1.26±0.009★▼	1.03±0.015★▼▪	1.12±0.000★▼	0.93±0.000★▼▪

Note: ▲Compared with the blank group ($P<0.05$); ★compared with the model group ($P<0.05$); ▼compared with the intermediate-dose TD group ($P<0.05$); ▪compared with each time point ($P<0.05$). 1 Blank group; 2 Model group; 3 Low dose TD group; 4 moderate dose TD group; 5 High dose TD group; 6 Allopurinol group.

Discussion

Owing to changes in lifestyle and diet, the number of HUA patients in China has been gradually increasing [11] and the occurrence of gout associated with HUA is becoming ever more acute. Therefore, there is an urgent need to prevent and treat the disease. The pathogenesis of HUA based on principles of Traditional Chinese Medicine can be summarized as follows: 1) There is an abundance of phlegm and dampness in obese people, whose diets often include greasy and sweet food, and the long-term existence of this condition can damage the spleen; 2) The absorptive, metabolic, and digestive dysfunctions of the small intestine cause body fluid transportation disturbances; 3) The irregular flow of the liver qi leads to the dysfunction of the spleen in transportion and the Sanjiao in gasification; 4) The deficiency of kidney qi causes disorder of transpiration and vaporization and disturbance of ascending lucidity and descending turbidity of the kidney. All the above factors can cause "internal dampness" to breed and become pathogenic heat, which leads to stagnation of Qi and blood, pathogenic dampness, and heat obstruction of the veins and limbs, which eventually induces gout. This is precisely the same as the modern medicine pathogenesis of gout, so we believe that “Bi Syndrome Caused by Internal Dampness Accumulation”.

In recent years, clinical reports on the treatment of HUA and gout with traditional Chinese herbs have been published [12,13]; they have received increasing attention because of the advantages they offer through multi-target and multi-way regulation with minimal side effects. Years of clinical practice have demonstrated that TD is a clinically effective prescription for treating gout. Our previous animal studies and clinical research of gout have proven that it can significantly decrease the level of SUA, but the specific mechanism of TD on reducing uric acid is still unclear. Therefore, it is difficult to provide a reliable basis for its popularization and application.

There are two sources of uric acid in the human body: exogenous and endogenous. Exogenous mainly refers to food sources that undergo multiple rounds of hydrolysis to form nucleotides and other products, which are further decomposed into purines in intestinal mucosal cells. The main endogenous source is the de novo synthesis pathway. The liver is the main site of the de novo synthesis pathway, followed by the small intestinal mucosa and the thymus. XOD plays a vital role in uric acid production as a rate-limiting enzyme in the human uric acid production pathway [14]. In the metabolic pathway of human purine nucleosides, a non-purine precursor substance is continuously transformed into purine nucleotides in vivo, decomposing into xanthine and HX to form uric acid after continuous oxidation by XOD. Therefore, when the activity of XOD strengthens, uric acid production in the human body increases; when the activity of XOD is inhibited, uric acid production in vivo is greatly reduced. Therefore, inhibiting the activity of XOD is an effective way to reduce SUA and decrease uric acid production in vivo. Now that modern medicine has made it clear that XOD activity is related to the production of uric acid, the TD-mediated reductions in SUA levels through inhibition of XOD activity and down-regulation of XOD mRNA are significant.

These results indicate the SUA and IUA of the model group increased over time compared with the blank group, while there was no significant difference in UUA, indicating that the HUA model rat was successfully established and uric acid excretion was not impaired. In view of the significant decrease in SUA levels after 14 days the intervention, we chose the 14th and 21st days post-intervention to observe the XOD activity and mRNA expression. On these days, the SUA and IUA levels were decreased, the UUA level was increased, and the XOD activity and XOD mRNA expression were decreased in the liver and small intestine, suggesting this as the mechanism by which TD may decrease the level of SUA in HUA rats. TD also promotes uric acid excretion, to some extent, lending to the purpose of reducing uric acid. The effect of reducing uric acid after 21 days of intervention is better than that for 14 days, suggesting that it can be used for long-term control of uric acid levels. The inhibition of XOD activity and the down-regulation of XOD mRNA expression on 14th and 21st days after intervention were better in the moderate-dose TD group than in the low- and high-dose TD groups and the allopurinol group, indicating that after a certain period of intervention, the effect of reducing uric acid is better with TD than with allopurinol. This may be related to the multi-target intervention of the active ingredients in treating gout, it may also be related to the promotion of uric acid excretion and inhibition of uric acid synthesis. As for the dosage of TD, the moderate dose was better than the low or high doses. This may be because the low dose results in a lower drug concentration in the blood and a limited effect of the active ingredient of the drug, while the high dose leads to greater antagonism of the active ingredient of the drug. However, the specific mechanism and the optimal dose for reducing uric acid require further study.

In terms of composing prescriptions for TD, the whole prescription is comprised of the Chinese medicine herbs such as Tufuling, Bixie, Zexie, Jinqiancao and Cangzhu, etc. This prescription can remove dampness, promote diuresis, lower urinary turbidity, and swelling and pain relief. Several pharmacological studies have confirmed that total saponins from Bixie can reduce SUA levels, while increasing UUA concentrations and uric acid excretion in HUA model rats [15]. An aqueous solution of Tufuling can also reduce SUA levels and inhibit XOD activity in HUA model mice [16]. Ethanol extracts of Zexie can reduce the levels of SUA and serum creatinine in HUA model rats and inhibit the activity of XOD [17]. Ethanol extracts of Zhongjiefeng can significantly decrease model mouse SUA with significant anti-inflammatory activity [18]. (Figure 1, Figure 2, Figure 3, Figure 4)

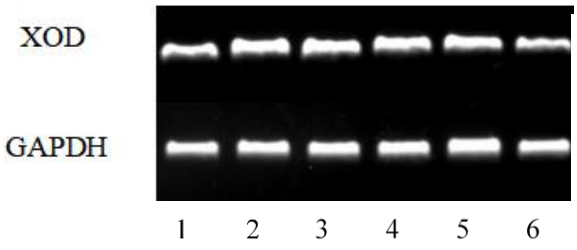

Figure 1. The expression of XOD mRNA in liver of each group on the 14th day after intervention of TD

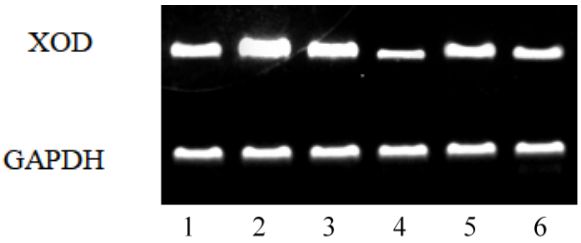

Figure 2. The expression of XOD mRNA in liver of each group on the 21st day after intervention of TD

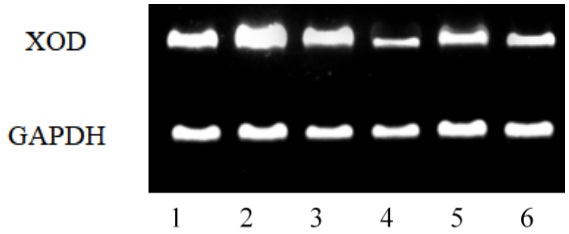

Figure 3. The expression of XOD mRNA in the small intestine of each group on the 14th day after intervention of TD

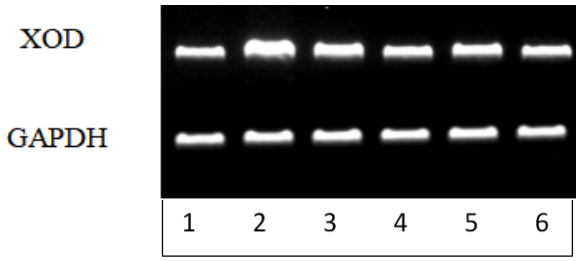

Figure 4. The expression of XOD mRNA in the small intestine of each group on the 21st day after intervention of TD

Note: 1 Blank group; 2 Model group; 3 Low dose TD group; 4 moderate dose TD group; 5 High dose TD group; 6 Allopurinol group

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