
**Regular Article**

Inhibition of 3,5,2',4'-tetrahydroxychalcone on production of uric acid in hypoxanthine-induced hyperuricemic mice

Yanfen Niu\textsuperscript{a,h,d,f,1}, Yuanfang Zhou\textsuperscript{b,c,1}, Hua Lin\textsuperscript{b}, Li-Hui Gao\textsuperscript{b}, Wenyong Xiong\textsuperscript{d}, Huajie Zhu\textsuperscript{e}, Cheng-Gang Zou\textsuperscript{a*}, Ling Li\textsuperscript{b*}

\textsuperscript{a}State Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming, 650091, PR China.

\textsuperscript{b}Biomedical Engineering Research Center, Kunming Medical University, Kunming, 650500, PR China.

\textsuperscript{c}The Second Affiliated Hospital of Kunming Medical University, Kunming 650101, PR China.

\textsuperscript{d}State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P.R.China

\textsuperscript{e}College of Pharmacy, HeiBei University, BaoDing, 071002, PR China.

\textsuperscript{f}Graduate University of Chinese Academy of Sciences, Beijing 100049, P.R. China.

\textsuperscript{1} These authors contributed equally to this work.
*Corresponding authors:

Prof. Ling Li, Ph.D.

Biomedical Engineering Research Center,
Kunming Medical University,
1168 Western Chunrong Road, Yuhua Street, Chenggong New City,
Kunming 650500, P.R. China

E-mail address: kmli62@163.com (L. Li)

Prof. Cheng-Gang Zou, Ph.D.

State Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming, Yunnan 650091, China.

E-mail: chgzou@ynu.edu.cn (C.G. Zou)
Abstract

The mechanism of 3,5,2',4'-tetrahydroxychalcone on lowering urate level is still unknown. Here we investigated the effects of 3,5,2',4'-tetrahydroxychalcone on urate levels, xanthine oxidase/xanthine dehydrogenase (XOD/XDH) activities in hypoxanthine-induced hyperuricemic mice, as well as the effects of 3,5,2',4'-tetrahydroxychalcone on the mRNA expression levels and content of phosphoribosyl pyrophosphate synthetase (PRPS), phosphoribosyl pyrophosphate amidotransferase (PRPPAT) and hypoxanthine-guanine phosphoribosyl transferase (HGPRT). Our results demonstrated that 3,5,2',4'-tetrahydroxychalcone (1.0, 2.0, and 4.0 mg/kg) reduced the uric acid levels in serum of the hyperuricemic mice in dose- and time-dependent manners. The activities of XOD/XDH in serum and liver were also significantly inhibited by 3,5,2',4'-tetrahydroxychalcone; In addition, 3,5,2',4'-tetrahydroxychalcone decreased the mRNA expression of HGPRT in brain and content of PRPS and PRPPAT in liver. These findings demonstrated that 3,5,2',4'-tetrahydroxychalcone suppresses uric acid production by affecting the critical enzymes, XOD/XDH, PRPS, PRPPAT and HGPRT in purine nucleotide metabolism.

Keywords: 3,5,2',4'-Tetrahydroxychalcone; xanthine oxidase/xanthine dehydrogenase (XOD/XDH); hypoxanthine-guanine phosphoribosyl transferase (HGPRT); phosphoribosyl pyrophosphate synthetase (PRPS); phosphoribosyl pyrophosphate amino-transferase (PRPPAT)
Gout and hyperuricemia are metabolic disorders associated with abnormal amounts of uric acid in the body. The overproduction of uric acid is one of the major factors in most patients with gout. Maintaining uric acid level < 6 mg/dl is important for prevention of gout. Many enzymes participated in purine metabolic pathway, such as xanthine oxidase/xanthine dehydrogenase (XOD/XDH), hypoxanthine-guanine phosphoribosyl transferase (HGPRT), phosphoribosyl pyrophosphate synthetase (PRPS), and phosphoribosyl pyrophosphate amino-transferase (PRPPAT).\textsuperscript{1} The dysfunction of these enzymes increases the production of uric acid, resulting in hyperuricemia.\textsuperscript{2} Particularly, the XOD has been recognized as one of the promising targets for the treatment of hyperuricemia. Allopurinol, a potent XOD inhibitor with a purine backbone, has been used clinically for more than 50 years.\textsuperscript{3,4} However, allopurinol induces serious side effects such as renal failure, impaired hepatic function and allergic reactions,\textsuperscript{5} limiting its clinical application. Recently, a new non-purine XOD inhibitor, Febuxostat, has been approved for management of gout in the European Union and USA.\textsuperscript{6} But a few side effects of febuxostat have been reported,\textsuperscript{7} such as liver function abnormalities, nausea, arthralgias, and rash. Therefore, novel alternatives to allopurinol with potent XOD inhibitory activity and less side effects are in great demand.

3,5,2',4'-Tetrahydroxychalcone is one of chalcones (Fig 1). It has been reported to possess various pharmacological activities, such as antitumor,\textsuperscript{8} free radical scavenging\textsuperscript{9}, antibiosis\textsuperscript{10}, antiulcer\textsuperscript{11} and spasmolysis\textsuperscript{12}. Currently, there are few reports about their inhibitory effects against the XOD activity. In a previous study, we reported that 3,5,2',4'-tetrahydroxychalcone had an inhibitory effect on XOD with an
IC$_{50}$ value of 22.5 μM in vitro. Moreover, intragastric administration of 3,5,2’,4’-tetrahydroxylchalcone (2.0 mg/kg) was able to significantly reduce serum uric acid levels and inhibit hepatic XOD activities in potassium oxonate-induced hyperuricemic mice. The results indicate that 3,5,2’,4’-tetrahydroxylchalcone reduces the uric acid production by inhibiting the activity of XOD. However, effects of 3,5,2’,4’-tetrahydroxylchalcone on other key enzymes involved in the generation of uric acid, such as HGPRT, PRPS and PRPPAT, have not been completely clarified. In this study, we focused on effects of 3,5,2’,4’-tetrahydroxylchalcone on activities of XOD/XDH, as well as mRNA expression levels and content of HGPRT, PRPS and PRPPAT in hyperuricemic mice.

**MATERIALS AND METHODS**

**Reagents** 3,5,2’,4’-Tetrahydroxylchalcone was prepared by Prof. Huajie Zhu. Allopurinol was purchased from Guangdong P.D. Pharmaceutical Co., Ltd. Hypoxanthine was a product of Shanghai Crystal Pure Reagent Co., Ltd. The kits for measurement of XOD activity and uric acid levels were obtained from Nanjing Jiancheng Bioengineering Institute. The kits for measurement of HGPRT, PRPPAT and PRPS were bought from Shanghai Enzyme-linked Biotechnology Co., Ltd.

**Animals** Adult male KM mice (18–22 g) were purchased from the Chengdu Biological Technology Co., Ltd. (Certificate No. SCXK 2013-24). The animals were housed on a constant 12-h light/12-dark cycle in temperatures controlled central animal facility and allowed free access to solid food and tap water. They were allowed one week to adapt to the laboratory environment before used for experiment. All procedures were carried out in
accordance with the Institute Ethical Committee for Experimental Use of Animals.

**Effects of 3,5,2',4'-tetrahydroxychalcone on the uric acid levels in normal mice** Male mice were divided into six groups of ten mice. The mice were treated with 1.0% polyethyleneglycol 400 (PEG400), 3,5,2',4'-tetrahydroxychalcone (2.0, 10.0, 50.0 and 250.0 mg/kg) or allopurinol (1.0 mg/kg) dissolved in 1.0% PEG400, respectively. All drugs were given intragastrically twice daily for five doses (2.5 days). Whole blood samples were collected by orbit vein bleeding in mice after exposed to ether. Serum was separated by centrifuged (3000 rpm, 15 min). Mouse liver was excised and homogenized in 10 volumes of normal saline (NS) at 4℃. The homogenate was then centrifuged at 3600 rpm/min for 10 min, the supernatant was carefully removed for assays of uric acid. Uric acid levels in serum and supernatant were determined using kits of the phosphotungstic acid method.  

**Effects of 3,5,2',4'-tetrahydroxychalcone on the uric acid levels in hyperuricemia mice** Hyperuricemic mice were induced by a single intraperitoneal injection with hypoxanthine. Male mice were divided into normal group, the untreated hyperuricemic group and treated hyperuricemic groups. The normal and hyperuricemic control mice were treated with 1.0% PEG400, hyperuricemic mice were treated with 3,5,2',4'-tetrahydroxychalcone and allopurinol dissolved in 1.0% PEG400, respectively. For dose-effect study, 3,5,2',4'-tetrahydroxychalcone (1.0, 2.0, 4.0 mg/kg) or allopurinol (1.0 mg/kg) were administered intragastrically to mice twice daily for five doses (2.5 days). The last dose was given at 0.5 h before intraperitoneal injection with hypoxanthine (100 mg/kg) to increase serum uric acid levels. One hour after administration of the last dose, blood samples were collected to determine the level of serum urate. Liver and brain were taken and stored at -80℃ for RT-PCR and elisa examination. For time-course study, the hyperuricemic mice
received 3,5,2',4'-tetrahydroxychalcone at a single dose of 2.0 mg/kg by intragastrical administration. Blood samples were collected at 1, 2, 4 and 6 h after administration. Serum uric acid levels were determined by the phosphotungstic acid method. Food was withdrawn from the animals 2 h prior to blood sample collection. Mouse liver was taken and homogenized as described above. The XOD/XDH activities in serum and liver were determined.

**Assays of XOD / XDH activities** The activity of XOD was determined using the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer’s instruction. The XDH activity was assayed by monitoring uric acid formation using a spectrophotometric method as described elsewhere.3,16)

**Assays of mRNA expression levels and content of HGPRT, PRPPAT and PRPS**
Total RNA was extracted from excised brain or liver tissue using TRIzol reagent, according to other study.17) The cDNA synthesized was used directly for amplification by RT-PCR. The PCR amplifications were performed in 20 μL reaction mixture by cycler (Bio-Rad). The PCR specific procedure was: denaturing at 94°C for 1 min, annealing for 1 min at 60°C, and extension for 1 min at 72°C (HGPRT), denaturing at 94°C for 30 s, annealing for 30 s at 53°C, and extension for 5 min at 72°C (PRPPAT), denaturing at 94°C for 30 s, annealing for 30 s at 52°C, and extension for 5 min at 72°C (PRPS), a total of 35 PCR cycles were performed. The PCR primer sequences were designed by Primer Premier 5.0. The production sizes and the primer sequences used in the experiments are shown in Table 1. The PCR products were electrophoresed in 1.5% agarose gel with nucleic acid dyestuff and gels were photographed with Gel Doc (BIO-RAD Gel Doc XR). The intensities of HGPRT, PRPPAT and PRPS were expressed by their ratios to the intensities of mGAPDH. The contents of HGPRT and PRPPAT in liver as well as PRPS in brain were evaluated by
using commercially available Elisa kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.).

**Statistical analysis**  All data were expressed as mean ± S.E.M and statistical analysis was performed with an one-way analysis of variance followed by Dunnett’s multiple comparison tests using GraphPad Prism version 5.1. Statistical significance was accepted at the level of \( P< 0.05 \).

**RESULTS**

**Effects of the 3,5,2',4'-tetrahydroxychalcone on urate levels in normal and hyperuricemic mice** We, firstly, assessed the effects of 3,5,2',4'-tetrahydroxychalcone on serum and hepatic uric acid levels in normal mice. The 3,5,2',4'-tetrahydroxychalcone at the doses of 2.0, 10.0, 50.0 or 250.0 mg/kg did not influence the levels of uric acid in these mice (Fig.2).

After intraperitoneal injection of hypoxanthine (100 mg/kg), the serum uric acid of mice in model group were increased (from 135.0 ± 25.0 to 224.5 ± 48.1), indicating that the hyperuricemic model is successfully established. These mice were then orally administrated of 3,5,2',4'-tetrahydroxychalcone at a dosage of 1.0, 2.0 and 4.0 mg/kg. We observed a significant reduction of the serum uric acid levels, but not the hepatic uric acid levels in hyperuricemic mice (Fig.3 A-B).

We next evaluated its time-dependent effect. The results showed that 3,5,2',4'-tetrahydroxychalcone of 2.0 mg/kg markedly decreased the serum uric acid levels in a time-dependent manner. The onset of lowering serum and hepatic uric acid was 1 h after administration with 3,5,2',4'-tetrahydroxychalcone and the hypouricemic action could
be maintained for more than 2 h and 6 h, respectively, as shown in Table 2.

The effects of 3,5,2',4'-tetrahydroxchalcone on XOD/XDH activity in hypoxanthine-induced hyperuricemic mice The blood and the liver collected from the 3,5,2',4'-tetrahydroxchalcone-treated mice, and then were used for determination of the activity of XOD and XDH. Interestingly, 3,5,2',4'-tetrahydroxchalcone significantly inhibited the activity of XOD and XDH in serum and liver of hyperuricemic mice. Whereas allopurinol inhibited the activity of XOD both in serum and liver, as well as the activity of XDH only in liver, not in serum of hyperuricemic mice, as reported in Fig.3 C-F. Moreover, the inhibition of 3,5,2',4'-tetrahydroxchalcone on the activity of XOD and XDH in serum could be maintained for 4 h and 6 h, respectively. However, there was no inhibition of 3,5,2',4'-tetrahydroxchalcone on the activity of XOD and XDH in liver after a single dose administration, as shown in Table 3-4. The action was weaker than equimolar allopurinol (positive control). Allopurinol inhibited the activity of XOD in serum and liver more than 6 h, and inhibited the activity of XDH in serum and liver for 2 h and 1 h, respectively.

Effect of 3,5,2',4'-tetrahydroxchalcone on the mRNA expressions and content of HGPRT, PRPPAT and PRPS in hyperuricemic mice As shown in Fig 4, treatment with 3,5,2',4'-tetrahydroxchalcone at the dose of 1.0, 2.0 and 4.0 mg/kg (twice daily for 2.5 days) decreased the mRNA levels of PRPPAT and PRPS in liver, but had no significant effect on the mRNA level of HGPRT in brain, which is similar to allopurinol.

Compared with the model group, 3,5,2',4'-tetrahydroxchalcone could decrease the content of hepatic PRPS at doses of 1.0, 2.0 and 4.0 mg/kg, and reduced the content of
hepatic PRPPAT/cerebral HGPRT at dose of 1.0 and 4.0 mg/kg, as shown in Fig 5.

DISCUSSION

Hyperuricemia in humans is classified into primary and secondary hyperuricemia. Primary hyperuricemia is caused by a high intake of purine-rich food, environmental factors and genetic effect. Secondary hyperuricemia refers to an acquired rise in the serum urates that is directly related to another disorder. Hypoxanthine, as a former of uric acid, is catalyzed by the xanthine dehydrogenase (XOR) to form uric acid. An injection of a large amount of hypoxanthine markedly increased the basic material in the synthesis of uric acid, and ultimately caused overproduction of uric acid and induced hyperuricemic state. Therefore, hypoxanthine-induced hyperuricemic mice might be close to the primary hyperuricimia in humans caused by a high intake of purine-rich food. In the present study, a model of hyperuricemic mice was successfully induced by an intraperitoneal injection with hypoxanthine of 100 mg/kg. And 3,5,2',4'-tetrahydroxychalcone was able to significantly reduce the uric acid levels in the hyperuricemic mice, further demonstrating that 3,5,2',4'-tetrahydroxychalcone inhibited the generation of uric acid.

It was worthy of note that 3,5,2',4'-tetrahydroxychalcone only lowered the serum urate levels in the hyperuricemic mice, but not reduced the serum and hepatic uric acid levels in normal mice after oral administration of 3,5,2',4'-tetrahydroxychalcone, even the dose up to 250 mg/kg. This characteristic of 3,5,2',4'-tetrahydroxychalcone could also be considered as an advantage. Indeed, elevated levels of uric acid in the circulation could give rise to gout and possibly other pathological conditions. But on the other hand, uric acid is a natural
antioxidant \(^3\) and plays an important role in the oxidative reaction, particularly its ability to inhibit DNA damage.\(^{22-23}\) Thus, a low level of uric acid in the circulation does not only reflect an inadvertent consequence of steady-state purine metabolism, but might actually be serving some important biological purposes. Thus, excessive lowering the uric acid level in the circulation beyond that of the normal range might even be counterproductive. These findings suggest that 3,5,2',4'-tetrahydroxychalcone has no effect on purine metabolism under physiological conditions and 3,5,2',4'-tetrahydroxychalcone maybe has less side effect. This feature of 3,5,2',4'-tetrahydroxychalcone makes it an attractive candidate for the prophylactic treatment of gout particularly if the compound is to be taken on a long-term basis. It has been reported that allopurinol at the dose of 10 mg/kg exerts its hypouricemic action in both normal and hypericemic animals.\(^{24}\) In the present study, allopurinol did not decrease the uric acid level in normal mice, which might be attributed to the used dose low to 1.0 mg/kg.

The xanthine dehydrogenase (XOR) is a key enzyme playing an important role in purine metabolic pathway. XOR exists in two alternative forms of the same gene product, XOD and XDH.\(^{25}\) Both XOD and XDH can catalyze the terminal two steps of the uric acid generation (hypoxanthine \(\rightarrow\) xanthine \(\rightarrow\) uric acid) in the human body. In a previous study, we found that 3,5,2',4'-tetrahydroxychalcone could reduce the uric acid levels in hyperuricemic mice induced by potassium oxonate, implicating that the hypouricemic mechanism is probably related to the inhibition of XOD activity.\(^{13}\) In the dose-effect study, oral administration of 3,5,2',4'-tetrahydroxychalcone (1.0, 2.0, 4.0 mg/kg) also significantly inhibited the activity of XOD and XDH in serum or liver of hypoxanthine-induced hyperuricemic mice in a dose-dependent manner. In the time-effect study, after a single 2.0
mg/kg dose of the compound (1, 2, 4, 6 h), the activities of XOD and XDH attenuated in serum, which sustained over 4 h for XOD and 6 h for XDH. These findings suggested that the compound attenuated the overproduction of uric acid induced by substantial hypoxanthine via blocking the activities of XOD/XDH, suppressing the increase of serum uric acid. These further demonstrated in vivo that 3,5,2',4'-tetrahydroxychalcone was an XOR inhibitor. However, the enzyme inhibition was not observed in liver when a single dose given, which was probably associated with the lower accumulated concentration of the compound in the liver after a single dose. The deduction needs to be further identified.

PRPS, PRPPAT and HGPRT are very important enzymes involved in the synthetic metabolism of purine. Defects of these enzymes may cause hyperuricemia. PRPS controls the production of PRPP, an important regulatory substrate in the synthesis of purine, pyrimidine, and pyridine nucleotides. Overactivity of PRPS leads to generating a great of PRPP and subsequently excessive producing uric acid, in association with hyperuricemia and gout. PRPPAT is the rate-limiting enzyme in the biosynthesis of uric acid. The elevated activity and/or concentration of PRPPAT and the increase in the affinity of PRPPAT binding to PRPP reduce the sensitive of feedback inhibition on purine nucleoside, resulting in overproduction of uric acid. HGPRT is a transferase enzyme that catalyzes conversion of hypoxanthine into inosine monophosphate (IMP); and guanine to guanosine monophosphate (GMP). It is a pivotal enzyme in the salvage synthesis of nucleotides, which provides cells with alternatives to the energy-expensive de novo synthesis of nucleotides, and plays a critical role in the maintenance of intracellular purine nucleotide pools. Partial HGPRT deficiency causes purine overproduction, which results in increased uric acid biosynthesis. In
the present study, hypoxanthine induced an augment of PRPS and HGPRT contents and an increase in tendency of PRPPAT, but no changes in the mRNA expression of all three enzymes. After administration of 3,5,2',4'-tetrahydroxchalcone, the contents of PRPS, PRPPAT and HGPRT were significantly attenuated to nearly normal levels, suggesting that 3,5,2',4'-tetrahydroxchalcone suppresses these changes induced by hypoxanthine. Moreover, 3,5,2',4'-tetrahydroxchalcone markedly decreased the mRNA levels of PRPS and PRPPAT, by a maximum of 28.3% and 34.9%, respectively, at the dose of 2.0 mg/kg. These findings indicate that 3,5,2',4'-tetrahydroxchalcone has a wide influence on enzymes participated in purine metabolism, inhibiting the overproduction of uric acid. However, it is unclear whether the activities of PRPS, PRPPAT and HGPRT are affected by 3,5,2',4'-tetrahydroxchalcone. More evidence needs to be done.

As uric acid is oxidized by uricase into allantoin, the uricase activity influences serum uric acid levels. Therefore, it is necessary to study the effect of 3,5,2',4'-tetrahydroxchalcone on the activity of uricase. In our study, we found that 3,5,2',4'-tetrahydroxchalcone had an inhibitory rate of -5.0 % against uricase at the concentration of 120 mg/L (the data are not listed) \textit{in vitro}. Whereas, potassium oxonate, an uricase inhibitor, markedly suppressed the activity of uricase at the same concentration, with an inhibitory rate of 99.0 %. These indicated that the observed hypouricemic action might not be related to increasing the uricase activity.

Urate transporters, including urate-anion transporter 1 (URAT1), glucose transporter 9 (GLUT9), organic aniontransporter 3 (OAT3), and so on, play an important role in the excretion of uric acid in kidney. Recently, we demonstrated that
3,5,2',4'-tetrahydroxychalcone possessed potent uricosuric effects associated with inhibiting urate reabsorption by down-regulating the protein expression of GLUT9 in kidney,\textsuperscript{33} other transporters need to be done for more evidence. This finding suggested that the hypouricemic action of 3,5,2',4'-tetrahydroxychalcone might be related to the enhancement of urate excretion.

Taken together, hypouricemic action of 3,5,2',4'-tetrahydroxychalcone might be associated with inhibiting the generation of uric acid via targeting the key enzymes in the purine metabolism and enhancing the excretion of uric acid by inhibiting the protein expression of GLUT9 in kidney. 3,5,2',4'-tetrahydroxychalcone, as a potential agent with dual mechanisms of hypouricemic action, is worthy of further development for hyperuricemia and gout.

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\textbf{Conflict of interest} The Authors declare no conflict of interest.
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22) Burkhardt S, Reiter RJ, Tan DX, Hardeland R, Cabrera J, Karbownik M. DNA oxidatively damaged by chromium(III) and H(2)O(2) is protected by the antioxidants
melatonin, N(1)-acetyl-N(2)-formyl-5-methoxykynuramine, resveratrol and uric acid.


Figure Legends

Fig.1. The Chemical structure of 3,5,2',4'-Tetrahydroxychalcone

Fig.2. Effects of 3,5,2',4'-tetrahydroxychalcone on uric acid levels in the normal mice.
CON – normal control, Data are expressed as mean ± SEM for 10 mice in each group. $P > 0.05$ vs. control group.

Fig.3. The dose-dependent hypouricemic effects of 3,5,2',4'-tetrahydroxychalcone on serum uric acid (A), hepatic uric acid (B), serum XOD activities (C), hepatic XOD activities (D), serum XDH activities (E), hepatic XDH activities (F) in hyperuricemic mice induced by hypoxanthine. CON – normal control, M – hyperuricemic control. Data are expressed as mean ± SEM for 10 mice in each group. **$P<0.01$ vs. control group; $^*P<0.05$, $^{**}P<0.01$ vs. hyperuricemic group; $^*P<0.05$ vs. allopurinol group.

Fig.4. Effects of 3,5,2',4'-tetrahydroxychalcone on brain mRNA levels of HGPRT(A), hepatic mRNA levels of PRPS(B) and PRPPAT (C) in hyperuricemic mice induced by hypoxanthine. CON – normal control, M – hyperuricemic control. Data are expressed as mean ± SEM for 10 mice in each group. $P>0.05$ vs. control group; $^*P<0.05$ vs. hyperuricemic group; $P>0.05$ vs. allopurinol group.
Fig. 5. Effects of 3,5,2',4'-tetrahydroxychalcone on brain HGPRT(A), hepatic PRPS(B) and PRPPAT (C) content in hyperuricemic mice induced by hypoxanthine. CON – normal control, M – hyperuricemic control. Data are expressed as mean ± SEM for 10 mice in each group. $P>0.05$ vs. control group; $^*P<0.05$ vs. hyperuricemic group; $P>0.05$ vs. allopurinol group.
Figures

Fig 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Table 1
Sequences of primers used for RT-PCR gene expression studies.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence of primers (5’-3’)</th>
<th>Product size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mHGPRT</td>
<td>R: GTTCTTTGCTGACCTGCTG</td>
<td>308</td>
</tr>
<tr>
<td></td>
<td>F: GCCTGTATCCAACACTTCG</td>
<td></td>
</tr>
<tr>
<td>mPRPS</td>
<td>R: AAGTTTAGCAACCAGGAGACC</td>
<td>370</td>
</tr>
<tr>
<td></td>
<td>F: CCCGAATCCACTGAAGAA</td>
<td></td>
</tr>
<tr>
<td>mPRPPAT</td>
<td>R: CGAGGAATGTGGGTGTTT</td>
<td>301</td>
</tr>
<tr>
<td></td>
<td>F: TACGAATGGCTGGCAGTT</td>
<td></td>
</tr>
<tr>
<td>mGAPDH</td>
<td>R: TGAGGCCGCTGCTGATATGT</td>
<td>496</td>
</tr>
<tr>
<td></td>
<td>F: CAGTCTTCTGGGTCAGTGAT</td>
<td></td>
</tr>
</tbody>
</table>
**Table 2**

The time-dependent hypouricemic effects of 3,5,2',4'-tetrahydroxychalcone on the serum/hepatic uric acid levels in the hyperuricemic mice induced by hypoxanthine. (\(\overline{\text{x}}\pm s\), \(n=10\))

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg</th>
<th>Dose μmol/kg</th>
<th>Serum (μmol/L)</th>
<th>Uric acid</th>
<th>Hepatic (μmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 h</td>
<td>2 h</td>
<td>4 h</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>—</td>
<td>—</td>
<td>130.0 ± 12.9</td>
<td>133.5 ± 10.0</td>
<td>131.8 ± 11.2</td>
</tr>
<tr>
<td><strong>Hyperuricemia</strong></td>
<td>—</td>
<td>—</td>
<td>221.6 ± 41.4#</td>
<td>223.1 ± 40.1#</td>
<td>220.2 ± 39.4#</td>
</tr>
<tr>
<td>3,5,2',4'-Tetrahydroxychalcone</td>
<td>2.0</td>
<td>7.3</td>
<td>174.9 ± 28.4*</td>
<td>175.5 ± 32.6^c</td>
<td>199.4 ± 25.8</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>1.0</td>
<td>7.3</td>
<td>149.9 ± 21.7**</td>
<td>156.9 ± 21.9**</td>
<td>193.0 ± 29.4</td>
</tr>
</tbody>
</table>

\# \(P<0.01\) vs. control group\[     \* \(P<0.05\) \]  \** \(P<0.01\) vs. hyperuricemic group\[ \Pp 0.01\) vs. allopurinol group\[ LSD test one-way analysis of variance. \]
Table 3 The time-dependent effects of 3,5,2',4'-Tetrahydroxychalcone on the serum/hepatic xanthine oxidase (XOD) in the hyperuricemic mice (n=10 ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg</th>
<th>Dose μmol/kg</th>
<th>Serum (U/L)</th>
<th>XOD Serum</th>
<th>XOD Hepatic [U/g prot]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>9.9 ± 0.8</td>
<td>9.8 ± 0.6</td>
<td>9.9 ± 0.6</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>—</td>
<td>—</td>
<td>11.0 ± 2.2</td>
<td>11.0 ± 2.1</td>
<td>10.7 ± 2.2</td>
</tr>
<tr>
<td>3,5,2',4'-Tetrahydroxychalcone</td>
<td>2.0</td>
<td>7.3</td>
<td>9.1 ± 0.9* *</td>
<td>9.4 ± 0.9* *</td>
<td>9.1 ± 1.0* *</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>1.0</td>
<td>7.3</td>
<td>7.6 ± 0.8**</td>
<td>7.7 ± 0.8**</td>
<td>7.6 ± 0.9**</td>
</tr>
</tbody>
</table>

*P<0.05 vs. control group **P<0.01 vs. hyperuricemic group ***P<0.01 vs. allopurinol group LSD test one-way analysis of variance.
Table 4  The time-dependent effects of 3,5,2',4'-Tetrahydroxychalcone on the serum/hepatic xanthine oxidase (XDH) in the hyperuricemic mice (n=10 ± SEM).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Serum (μ mol acid/min.L)</th>
<th>XDH</th>
<th>Hepatic (μ mol acid/min.g prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>6.3 ± 0.7</td>
<td>6.2 ± 0.5</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>—</td>
<td>7.3 ± 0.7**</td>
<td>7.1 ± 0.5##</td>
<td>7.1 ± 0.4##</td>
</tr>
<tr>
<td>3,5,2',4'-Tetrahydroxychalcone</td>
<td>2.0</td>
<td>6.2 ± 0.6**</td>
<td>6.7 ± 0.6**</td>
<td>6.3 ± 0.8*</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>1.0</td>
<td>6.2 ± 1.4*</td>
<td>5.7 ± 0.7**</td>
<td>6.0 ± 0.6**</td>
</tr>
</tbody>
</table>

## P<0.01 vs. control group; *P<0.05; **P<0.01 vs. hyperuricemic group; ***P<0.01 vs. allopurinol group. LSD test one-way analysis of variance.