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A natural complex product Yaocha reduces uric acid level in a live zebrafish model

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ABSTRACT

Introduction: This study was aimed to assess uric acid (UA)-lowering effect and its possible mechanisms of a natural complex product Yaocha in a live zebrafish model. **Methods:** The zebrafish high UA model was established by feeding 5 dpf zebrafish with both an uricase inhibitor potassium oxonate at 10 mM and an UA synthesis precursor xanthine sodium at 0.5 mM for 24 h. Yaocha was administered to the high UA zebrafish through soaking at 3 various concentrations, with allopurinol as a positive control. UA level, xanthine oxidase (XOD) activity, and mRNA expression of hypoxanthine guanine-phosphoribosyltransferases transferase (HPRT1) and organic anion transporter 1 (OAT1) were measured. **Results:** Yaocha effectively reduced UA level and inhibited xanthine oxidase (XO) activity in the high UA zebrafish. Yaocha could be a potential therapeutics for hyperuricemia through up-regulating HPRT1 and OAT1 gene expression and suppressing XO activity. **Discussion:** These results suggested that Yaocha hold a potential for high UA prevention and therapy, possibly through inhibiting UA production and promoting urate secretion and purine conversion.

1. Introduction

Hyperuricemia (HUA) is a common metabolic disease derived from the elevated uric acid (UA) production and/or the decreased renal excretion of UA (Liu, Zhang, Wang, & Liu, 2014). Approximately 5% ~ 12% of hyperuricemia will eventually develop into gout, a form of inflammatory arthritis characterized by recurrent attacks of a red, tender, hot, and swollen joint (Rott & Agudelo, 2003). HUA has also been linked to hyperlipidemia, obesity, insulin resistance diabetes, renal dysfunction, hypertension and metabolic syndrome (Akkasilpa, Avihingsanon, Hanvivadhanakul, & Wonchinsri, 2004; Choi, Atkinson, Karlson, & Curhan, 2005; Johnson et al., 2003; Peng et al., 2015; Soltani, Rasheed, Kapusta, & Reisin, 2013). Xanthine oxidase (XO) has been recognized as one of the promising targets for the treatment of hyperuricemia, inhibition of XO activity can effectively reduce UA biosynthesis (Gliozzi et al., 2016). Hypoxanthine guanine-phosphoribosyltransferases transferase (HPRT) is a crucial enzyme in the pathway of remedy synthetic purine metabolism. HPRT gene defect inhibits the purine conversion, which could increase UA production (Mak, Chi, Tsai, Lee, & Lin, 2000; Zuo et al., 2000). Organic anion transporter 1 (OAT1) is a member of the SLC22A family of transporters and is mainly expressed in the renal proximal basement membrane. The role of OAT1 in the process of urate handling is to mediate the secretion of urate into the urine (Zhang et al., 2018).

The current marketed drugs used to treat hyperuricemia are mainly to inhibit UA production or promote UA excretion, such as allopurinol, a XO inhibitors, and benzbromarone, which targets on suppressing renal urate reabsorption. However, these drugs have some undesirable and even serious side effects, such as stevens–Johnson syndrome, hypersensitivity syndrome, gastrointestinal reactions, mitochondrial dysfunction, liver and kidney function damage and other adverse reactions (Fritsch & Sidoroff, 2000; Hammer, Link, Wagner, & Bohm, 2001; Pacher, Nivorozhkin, & Szabo, 2006; Schlesinger, 2004), limiting their long-term administration. Clinically, it is an urgent need to develop more safe and effective drugs for treating HUA and gout. More and more natural products and extracts have been proven effective in

Abbreviations: dpf, days post fertilization; h, hour; DMSO, dimethyl sulfoxide; XO, xanthine oxidase; UA, uric acid; UOX, urate oxidase; HPRT1, hypoxanthine guanine-phosphoribosyltransferases transferase; OAT1, organic anion transporter 1.

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hyperuricemic control and gout (Ferrari et al., 2016; Hou et al., 2015; Xu et al., 2013; Zhao, Chen, & Wu, 2017). Natural products show significant advantages in the treatment of HUA because of its efficacy in reducing serum UA level and its minimal adverse effects.

Yaocha is a mixed natural product consisting of bonito peptides powder, tea flower powder and rooibos powder (BTR). Bonito peptides powder are refined and processed from the muscles of deep-sea skipjack tuna, which contains abundant anserine and carnosine. These dipeptides were shown to has hypouricemic activity with no undesirable side effects (Kubomura et al., 2016). Tea flower is a plant under a theaceae family that has been widely cultivated in the Asian region. Rooibos, the scientific name is *Aspalathus linearis*, a plant endemic to the Western Cape province of South Africa has gained international recognition as an herbal tea. Bonito peptides are oligopeptides powder, research shows that oligopeptides powder of marine fish can promot skin wound healing and eliminate inflammatory response and has better vasorelaxation, cholesterol reducing activities, and antioxidative effects (Liu et al., 2013; Pan et al., 2012; Xiong et al., 2018). Tea flower has better an antioxidant, anti-proliferative, antiallergic and antiobesity activities (Wang et al., 2017; Yang, Xu, Jie, He, & Tu, 2007; Yoshikawa et al., 2008). Rooibos possesses relatively potent antioxidant, antimutagenic, immune-modulating and chemopreventive actions (McKay and Blumberg, 2007), but for its role in anti-hyperuricemic is not clear.

Zebrafish (*Danio rerio*) is emerging as a predictive vertebrate animal model for *in vivo* assessment of drug efficacy, toxicity and safety (He, Gao, Huang, & Li, 2014; Zhou, Guo, Zhang, & Li, 2014; Zhou, Xu, Guo, & Li, 2015; Zhu et al., 2014). An important advantage of the zebrafish model is that the morphological and molecular basis of tissues and organs is either identical or similar to mammals, including humans (Granato and Nusslein-Volhard, 1996). The sequence and presumed function of many genes that are important for vertebrates are conserved in the zebrafish (Howe et al., 2013). Zebrafish have xanthine dehydrogenase, uric acid enzyme, allantoase and allantoic acid enzyme which related to purine metabolism (Zhang et al., 2009). In the previous studies, zebrafish were used to study the urate oxidase (Andersen et al., 2006; Cao & Rong, 2010; Kuang, Rong, & Zhu, 2014) and zebrafish UA concentrations were determined by high performance liquid chromatography (HPLC) with electrochemical detection (Kirkwood et al., 2012). Compared with mammals, the major advantages of the zebrafish high uric acid model include lower labor intensity, less time and cost, and much less compound per test, and tiny body that could be handled in 96-well microplate, suitable for *in vivo* high throughput screening.

In this study, we assessed effects of a natural complex health product Yaocha reduces UA level in a live zebrafish model induced by potassium oxonate combined with xanthine sodium salt, we found that Yaocha could be a potential therapeutics for hyperuricemia through upregulating expression of HPRT1 and OAT1 genes and suppressing XO activity.

2. Materials and methods

2.1. Yaocha analyses

Yaocha is a mixed natural product consisting of Bonito peptides powder, tea flower powder and rooibos powder (BTR) at ratios of 2.5: 1: 1 and was provided by Yabao Pharmaceutical Group Co., Ltd. Flavonoids of rooibos powder were quantified using a Shimadzu UV-2550 ultraviolet spectrophotometer at an absorbance wavelength 360 nm. Saponin of tea flower powder separation was quantified using a Agilent HPLC with a C18 column (250 × 4.6 mm, 5 μm) and the column temperatures were kept at 40 °C. The mobile phase consisting of 65% (*v*/v) methanol and 35% phosphate buffer (0.01 mol/L KH_2PO_4, Adjust pH to 2.3), flow rate of elution was 1 mL/min and the detection wavelength was 230 nm (Kirkwood et al., 2012). The formula of Yaocha and the preparation methods of tea flower powder and rooibos powder have been applied for Chinese patent (China patent number: 201610951314.9).

2.2. Zebrafish care and maintenance

Adult AB strain zebrafish were housed in a light- and temperature-controlled aquaculture facility with a standard light: dark cycle of 14:10 h/day and fed with live artemia larvae twice daily and dry flake as supplement once a day. Zebrafish embryos were generated by natural pair-wise mating, on average, 200–300 embryos were generated. Both adults and embryos were maintained at 28 °C in fish water (0.2% Instant Ocean Salt in deionized water, pH 6.9–7.2, conductivity 480–510 μS/cm and hardness 53.7–71.6 mg/L $CaCO_3$). For experiments, the embryos were collected, washed and staged at 6 and 24 hpf (hours post fertilization) (Kimmel et al., 1995). All the experiments were carried out in accordance with international ethical guidelines for animal experiments, and the zebrafish facility at Hunter Biotechnology, Inc. is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.3. Chemicals and reagents

Potassium oxonate (lot #: K1516110) was purchased from Aladdin (Shanghai, China) and xanthine sodium salt (lot #: SLBL2532V) from Sigma-Aldrich (St. Louis, USA). Bonito peptides powder was bought from the ShangHai Lytone Biochemicals, Ltd. (Shanghai, China), Rooibos powder from the huangshan hualuyuan Biotechnology Co., Ltd. (Anhui, China), and tea flower powder from the ShangHai Royoung Biotech Co., Ltd. (Shanghai, China). BTR was a miture of bonito peptides powder, tea flower powder and rooibos powder (BTR) at ratios of 2.5: 1: 1. Amplex™ Red Uric Acid/Uricase Assay Kit and Amplex™ Red Xanthine/Xanthine Oxidase Assay Kit was ordered from Thermo Fisher Scientific (USA), and enhanced bicinchoninic acid (BCA) protein assay kit from TIANGEN Biotech (Beijin, China).

2.4. Determination of Yaocha testing concentrations

To determine test concentrations of a Yaocha, 5 dpf zebrafish were treated with Yaocha for 24 h, zebrafish high UA model was induced by the uricase inhibitor potassium oxonate combined with the UA synthesis precursor xanthine sodium salt. Thirty zebrafish were distributed into 6-well plates (Nest Biotech., Shanghai, China) in 3 mL fresh fish water. Mortality and toxicity was record at the end of treatment. In the initial tests, five concentrations (125, 250, 500, 1000 and 2000 mg/L) were used. Dead zebrafish was defined as the absence of heartbeat under a dissecting stereomicroscope. The test concentration induced neither death nor any visually observable adverse effect on zebrafish. Allopurinol were selected for the validation of the zebrafish high UA model, five concentrations at 13.6, 34, 65, 102 and 136 mg/L were tested.

2.5. Assessment of Yaocha effect on the high UA zebrafish

The effect of Yaocha on hyperuricemia was assessed in the live high UA zebrafish developed above. Thirty 5 dpf AB strain zebrafish were distributed into 6-well plates in 3 mL fresh fish water. Zebrafish were first treated with Yaocha at 3 concentrations (222, 667, 2000 mg/L), and then co-treated with 10 mM potassium oxonate (the optimum potassium oxonate concentration) in combination with 0.5 mM xanthine sodium salt (the optimum xanthine concentration) for 24 h. Zebrafish only treated with potassium oxonate and xanthine sodium salt served as the high UA model. The high UA zebrafish treated with 136 mg/L allopurinol (the optimum allopurinol concentration) were used as a positive control. After treatment, zebrafish UA was measured.

2.6. UA measurement

After treatment, zebrafish were randomly selected and placed in a 96-well plate, 3 zebrafish per well in 50 μL uric acid detection kit solution. UA was measured using AmplexTM Red Uric Acid/Uricase Assay Kit according to the manufacturer's instructions. The fluorescence intensity (S) was quantified in a multifunction microplate reader (MikroWin 2000, Berthold, Germany) using excitation in the range of 530–560 nm and emission detection at ~ 590 nm. In each assay, at least six wells per sample were measured for each dose and the results were averaged. The data were expressed as mean ± SEM and the effect of a test drug on UA was calculated based on the formula below: Efficacy (%) = [S(drug) - S(model)]/[S(model)] × 100%.

2.7. XO activity assay

To investigate the possible biochemical mechanism by which Yaocha treatment resulted in a reduced UA in the high UA zebrafish, the model zebrafish were treated with BTR mixture at 3 concentrations (33, 100, 300 mg/L). After treatment, zebrafish were homogenized in 10 volumes of 0.9% ice-cold sodium chloride. The homogenate was centrifuged at 12,000 ×*g* at 4 °C for 10 min. The supernatant fraction was centrifuged at 12,000 ×*g* at 4 °C for 10 min once again and the resting supernatants were used to detect XO activity by a fluorometric assay with an Amplex™ Red Xanthine/Xanthine Oxidase Assay Kit as recommended by the manufacturer. XO activity was corrected by protein content. The protein content was determined by the BCA protein assay and bovine serum albumin was used as the standard.

2.8. Quantitative PCR analysis

After treatment, total RNA was extracted from thirty homogenized zebrafish per group using Trizol reagent (Invitrogen Life Technologies). The quality of RNA samples was evaluated using the methods from NanoDrop 2000 instruction (Thermo Scientific). Total RNA concentration was determined by absorbance at 260 nm and RNA purity was confirmed by A260 nm/A280 nm ratio. About 2 μg total RNA of each sample was used for cDNA synthesis using FastQuant RT Kit (With gDNase) (Tiangen). The primers used in this study were as follows, β-actin: 5'-TCG AGC AGG AGA TGG GAA CC-3′ and 5′- CTC GTG GAT ACC GCA AGA TTC-3′; OAT1: 5′-GGA CGA TAT CCT GCC AGC TC-3' and 5′- CGT CCT GTA AGG CCA GAT CC-3′; HPRT: 5′-TTG CAG TAG CTT GTC GGT GT-3′ and 5'-CAG ACG TTC AGT TCG GTC CA-3'; GLUT9: 5′- TGG ACA AAG CAG GCA GGA AA-3′ and 5′- TCA GGC CGT CTG TTA GGG TA-3′. Q-PCR amplifications were carried out with a CFX Connect detection system (Biorad) using the iTaq Universal SYBR Green Supermix (Biorad) in which there was 3 technical or biological replicates. The PCR protocol used was: 2 min at 95 °C, 40 cycles of 5 s 95 °C, and 30 s at 60 °C. Melting curve analysis was performed to check the specificity of the primers. Expression data was normalized against the expression of β-actin and the relative quantification of each gene mRNA level among groups was calculated using the 2-△△Ct method (Sharif et al., 2015).

2.9. Statistical analysis

All experiments results were repeated at least three times and all data were expressed as mean ± SEM. One-way ANOVA followed by the Dunnett's test was used to compare differences among groups. All statistical analyses were performed using the SPSS 16.0 software (SPSS, Chicago, IL, USA), and $p < 0.05$ was considered statistically significant. Figures were generated with GraphPad Prism 5 Software (GraphPad, Inc., San Diego, CA, USA).

3. Results

3.1. UV and HPLC analyses of Yaocha

According to ultraviolet spectrophotometer analyses, flavonoids were showed in rooibos powder with the ratio of 1.34%. According to HPLC analysis, total saponin were showed in tea flower powder with the ratio of 1.0% ~ 1.5%, and tea flower saponin with the ratio of 0.4% ~ 0.8% (Fig. 1).

3.2. Effects of Yaocha on the high UA zebrafish

The mortality of Yaocha-treated zebrafish were shown in Tables 1, three testing concentrations of Yaocha at 222, 667, and 2000 mg/L were selected and assessed. As demonstrated in Fig. 2A, allopurinol markedly reduced UA level in the model zebrafish. As compared with

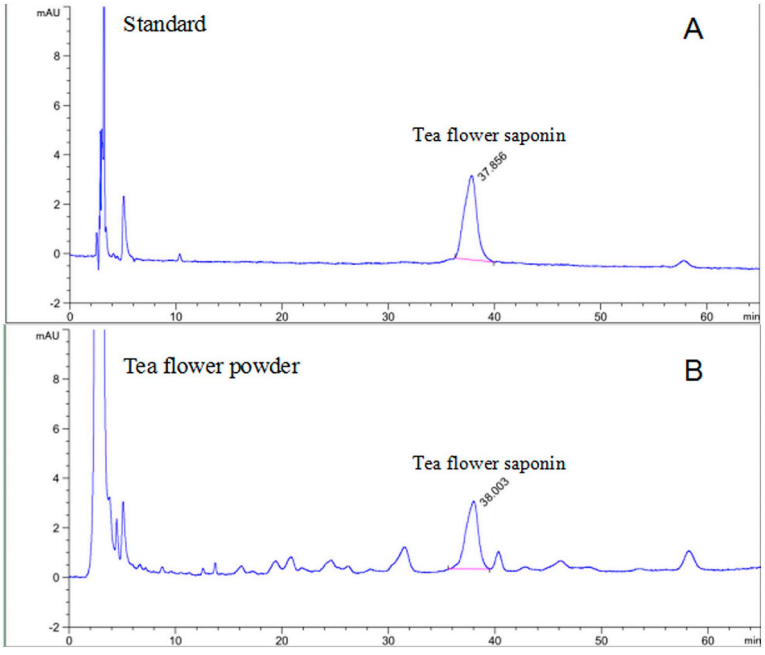

Fig. 1. HPLC chromatograms of standard Tea flower saponin (A) and Tea flower powder (B).

Table 1
Mortality in zebrafish treated with Yaocha and BTR mixture ($n = 30$).

Concentrations (mg/L)	Mortality (%)	
	Yaocha	BTR mixture
250	0	0
300	0	0
500	0	100
1000	0	100
1500	0	100
2000	0	100

Table 2
XO activity in zebrafish treated with BTR mixture.

Group	Concentration(mg/L)	Xanthine oxidase activity (U/g protein , mean ± SE)
Control	–	8.84 ± 0.13**
High UA Model	–	10.35 ± 0.06
Allopurinol	136	9.30 ± 0.20*
BTR mixture	33.3	8.29 ± 0.04***
	100	9.31 ± 0.10***
	300	10.71 ± 0.13

Compared with Model: *p < 0.05, ***p < 0.001.

the model group, UA level in the model zebrafish were reduced by 17% ($p < 0.01$), 33% ($p < 0.001$), 41% (p < 0.001), 42% (p < 0.001) and 46% (p < 0.001) when treated with allopurinol at concentrations of 13.6 mg/L, 34 mg/L, 68 mg/L, 102 mg/L and 136 mg/L, respectively.

As expected, after a 24 h co-treatment, UA level in the model zebrafish were significantly higher than in the normal zebrafish ($p < 0.001$) (Fig. 2B), suggesting the model was successfully established. As showed in Fig. 2C, Yaocha treatment at concentrations of 222, 667, and 2000 mg/L led to UA reduction by 17% ~ 39% in the high UA zebrafish ($p < 0.01$ - $p < 0.001$), suggesting that Yaocha had a significant anti-hyperuricemic activity, although not as strong as allopurinol.

3.3. Effects of Yaocha on xanthine oxidase

The levels of XO in our experimental animals were measured to explore the potential mechanism responsible for the lower UA level in Yaocha-treated high UA zebrafish. The maximum test concentration was 300 mg/L for BTR mixture (Table 1). Compared with normal group, XO activity (p < 0.001) of high UA model zebrafish were significantly elevated (Table 2). The positive control drug allopurinol had a marked inhibition on XO activity in the high UA zebrafish ($p < 0.05$). Significantly lower XO levels were also observed in the high UA zebrafish treated with BTR mixture at concentrations of 33.3 and 100 mg/L (p < 0.01), but not at the concentration of 300 mg/L.

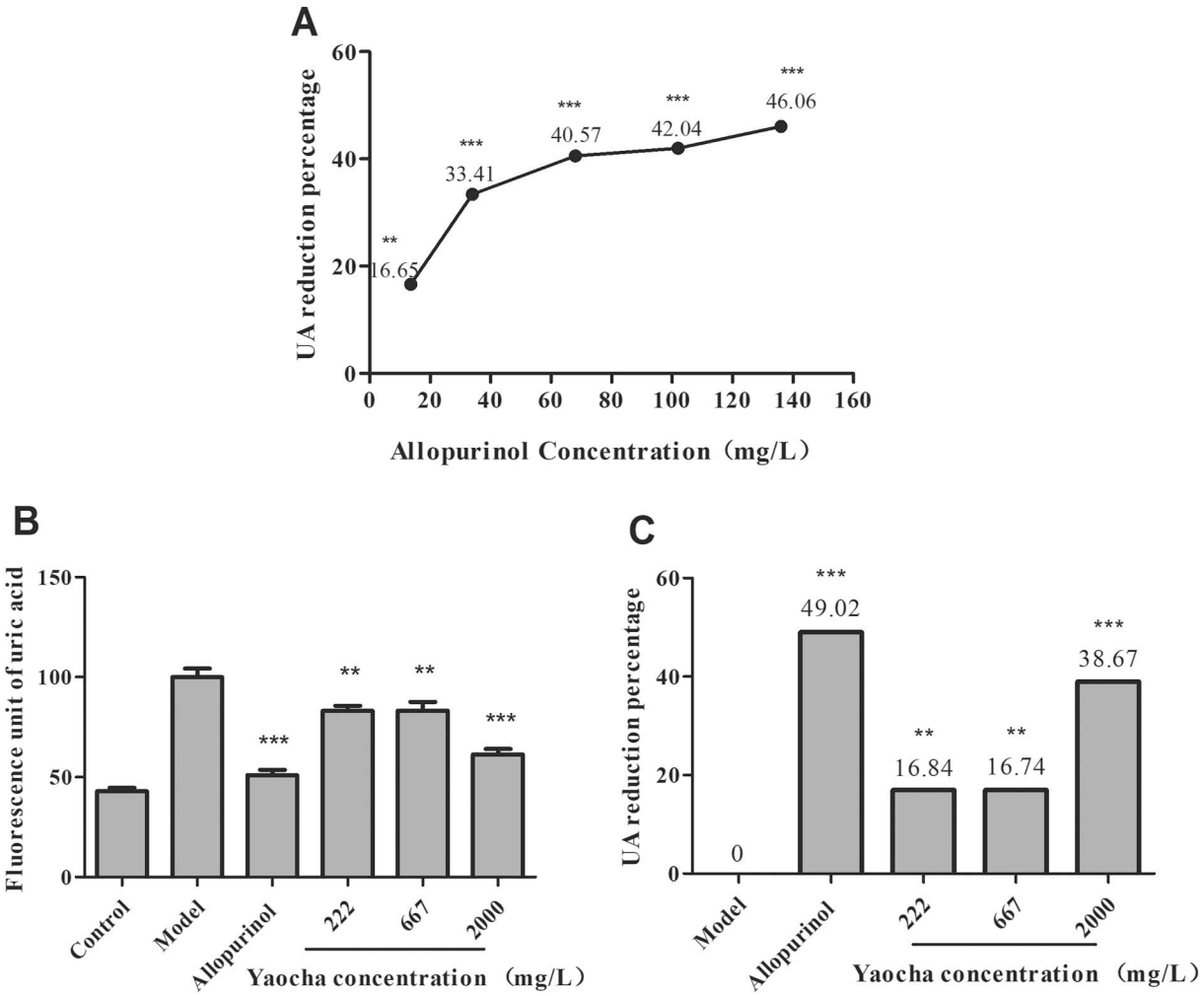

Fig. 2. Allopurinol (A) and Yaocha (B and C) significantly reduced levels of UA in the high UA zebrafish. Compared with the high UA zebrafish model: **$p < 0.01$, ***$p < 0.001$.

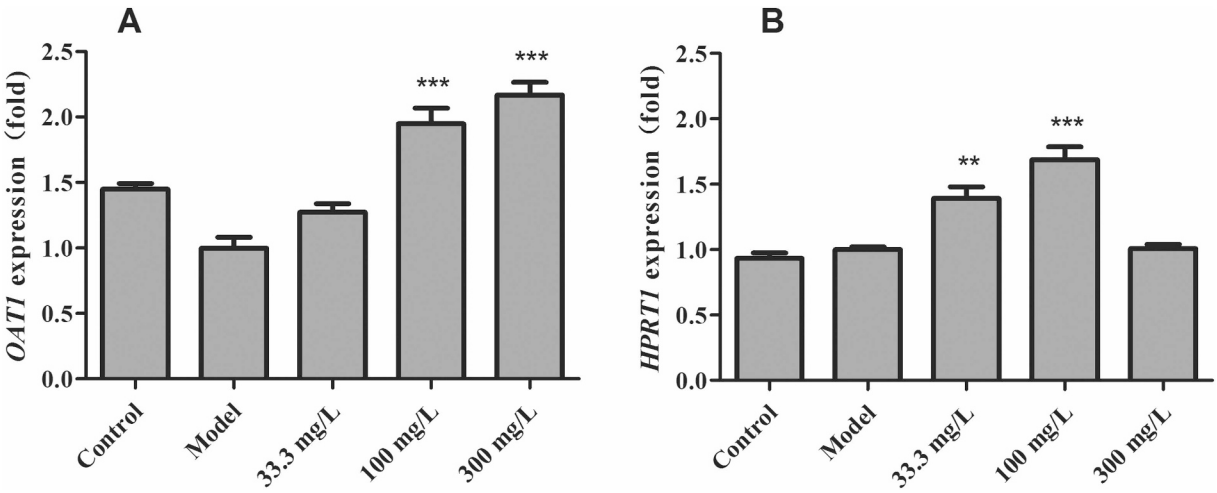

Fig. 3. Relative levels of OAT1 (A) and HPRT1 (B) gene expression in zebrafish treated with BTR mixture, measured by quantitative-PCR. β-actin gene was used as a house-keeping control gene for expressional level normalization. Compared with the high UA zebrafish model: **p < 0.01, ***p < 0.001.

3.4. Effects on the gene expression of zebrafish OAT1 and HPRT1

The effects of BTR mixture on the gene expression of OAT1 and HPRT1 in the high UA zebrafish were demonstrated in Fig. 3. Significantly reduced levels of OAT1 were observed in the high UA zebrafish ($p < 0.05$), as compared with normal control zebrafish. BTR mixture at 100 and 300 mg/L significantly up-regulated the expression of OAT1 of high UA zebrafish ($p < 0.001$). Moreover, BTR mixture at 33.3 and 100 mg/L effectively increased HPRT1 mRNA expression when compared with the high UA zebrafish model ($p < 0.01$, $p < 0.001$, respectively).

4. Discussion

Uric acid is the final product of purine nucleotide catabolism in human which can be modulated by different factors such as diet or drugs (Sinha et al., 2009). Hypoxanthine and xanthine are the intermediate products of this catabolism. Xanthine oxidase catalyzes the final two reactions in the biochemical chain that leads to UA formation: the conversion of hypoxanthine to xanthine and xanthine to UA (Bove et al., 2017). Increased UA production and/or reduced UA excretion could lead to hyperuricemia and gout.

UA is degraded to (*S*)-allantoin in most vertebrate species through a three-step enzymatic pathway. In the first step, UA (urate at neutral pH) is converted to 5-hydroxyisourate (HIU) in an oxygen-dependent reaction. In the second step, HIU is hydrolysed to 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU), which is decarboxylated to give CO_2 and (*S*)-allantoin in the third step (Cendron et al., 2007; Doniselli, Monzeglio, Palù, Alessandro, & A., & Percudani, R., 2015; Ramazzina, Folli, Secchi, Berni, & Percudani, 2006). The pathway was lost in hominoids (lesser and great apes, including humans), predisposing humans to hyperuricemia and gout (Marchetti et al., 2016). In previous studies, the HIU hydrolase (Urah) and OHCU decarboxylase (Urad) enzymes of the urate degradation pathway have been characterized in the vertebrate zebrafish (Doniselli et al., 2015). Potassium oxonate has been used to establish hyperuricemia model in rodents because it can efficiently and directly inhibit uricase, the enzyme converting UA into allantoin (Stavric, Clayman, Gadd, & Hébert, 1975; Zhao, Chen, & Wu, 2017). Xanthine sodium salt is a UA synthesis precursor, which is subsequently converted to UA by the action of the XO.

In this study, we observed that potassium oxonate at an optimum concentration of 10 mM in combination with a xanthine sodium salt at an optimum concentration of 0.5 mM for a 24-h exposure time induced significantly increased UA level in the larval zebrafish, with elevated XO activity and lower OAT1 mRNA expression, indicating that potassium oxonate and xanthine sodium salt co-treatment may produce more UA in zebrafish. The zebrafish high UA model was validated with allopurinol which is a clinically approved drug by FDA (Federal Drug Administration of the United States) for treating gout and remains a cornerstone in the therapy of primary and secondary hyperuricemia (Pacher et al., 2006). The UA level and XO activity were markedly reduced in the high UA zebrafish after treatment with allopurinol in a dose-dependent manner as expected.

Natural products have been demonstrated as effective preventive and therapeutic agents for HUA (Lee et al., 2017; Wang et al., 2010; Zhang, Deng, Wu, Zheng, & Zhong, 2018). Here we showed that the natural complex health product Yaocha decreased UA level in the high UA zebrafish, implying that Yaocha could be a potential therapeutics for treating HUA disease. The major components of Yaocha are bonito peptides powder, tea flower powder and rooibos powder (BTR). The elevated XO activity was seen in the high UA zebrafish, and the XO activity was markedly diminished after BTR treatment, indicating that BTR / Yaocha reduced UA formation probably mainly through inhibiting XO activity. These results were supported by a few earlier investigations that flavonoids and saponins obtained from natural products inhibited XO activity *in vivo* (Lin, Qu, & Liang, 2011; Nagao, Seki, & Kobayashi, 1999). Others have also reported that rooibos contains abundant flavonoids (Kazuno et al., 2005) and tea flower contains abundant saponin (Yoshikawa et al., 2008).

HPRT is a central enzyme in purine salvage pathway. When this enzyme is lacking, purine is broken down but not recycled, producing abnormally high levels of UA. Complete or severe deficiency of HPRT activity causes Lesch Nyhan disease (LND), characterized by hyperuricemia, severe action dystonia, choreoathetosis, ballismus, cognitive and attention deficit and self-injurious behavior (Torres et al., 2017). Partial deficiency of HPRT enzyme activity is characterized by consequences of overproduction of UA and variable spectrum of neurological manifestations, without the manifestations of self-injurious behavior (Nguyen et al., 2017). OAT1 plays important roles in regulating urate excretion on the basolateral side (Kimiyoshi et al., 2003), Zebrafish, *hprt1* and *oatx* genes have been characterized and they show 91% and 46% identities with human HPRT1 and OAT1 sequences, respectively. In this study, potassium oxonate and xanthine sodium salt induced a significant down-regulation of OAT1 mRNA levels, while BTR mixture markedly up-regulated the mRNA expression, in agreement with other studies (Zhu et al., 2017). Though potassium oxonate and xanthine sodium salt had no effect on HPRT1 mRNA levels, BTR mixture treatment obviously up-regulated HPRT1 gene expression at low

and middle concentrations, whereas the high concentration almost had no effect. It seems that the concentration-response curve of BTR on HPRT1 is bell-shaped, which suggesting BTR might have a bidirectional regulating effect on HPRT1 gene expression probably due to its complex and diverse composition from the natural products. The bioactivity and mechanism of different components in BTR might be various or some interacting, so the dose-response curve of BTR might differ with pure compounds. Further study is in plan in our laboratory to learn more about the effects and mechanisms of Yaocha/BTR on HPRT1 and Lesch Nyhan disease.

5. Conclusions

The natural complex health product Yaocha/BTR lowered UA level in the high UA zebrafish model mainly through up-regulating HPRT1 and OAT1 gene expression and inhibiting XO activity and thus probably reducing UA production and promoting urate secretion and purine conversion. Yaocha could be a potential preventive and therapeutic natural product for hyperuricemia. The zebrafish high UA model developed in this report could speed up the screening and discovery process of hyperuricemia therapeutics.

Competing interests

The authors have no competing interests.

Acknowledgments

Yi Liu and Yong Zhang designed the research; Xiao-yun Xiong, Jun Liang, Sheng-Ya Guo, and Ming-Zhu Dai performed the research; Xiao-yun Xiong, Jun Liang and Jia-Li Zhou analyzed the data; Xiao-yun Xiong and Sheng-Ya Guo wrote the paper. This work was supported by National Key Research and Development Program of China (No. 2016YFD0400602) and National Science & Technology Major Projects of China (No. 2017ZX09301-059).

References

Akkasilpa, S., Avihingsanon, Y., Hanvivadhanakul, P., & Wonchinsri, J. (2004). Clinical manifestations of patients with hyperuricemia. *Journal of the Medical Association of Thailand, 87*(Suppl 2), S41–S44.

Andersen, O., Aas, T. S., Skugor, S., Takle, H., van Nes, S., Grisdale-Helland, B., ... Terjesen, B. F. (2006). Purine-induced expression of urate oxidase and enzyme activity in Atlantic salmon (Salmo salar). Cloning of urate oxidase liver cDNA from three teleost species and the African lungfish Protopterus annectens. *The FEBS Journal, 273*, 2839–2850.

Bove, M., Cicero, A. F., Veronesi, M., & Borghi, C. (2017). An evidence-based review on urate-lowering treatments: Implications for optimal treatment of chronic hyperuricemia. *Vascular Health and Risk Management, 13*, 23–28.

Cao, Z., & Rong, J. (2010). *The study on the properties of Uricase from zebrafish.* (Hubei Agricultural Sciences).

Cendron, L., Berni, R., Folli, C., Ramazzina, I., Percudani, R., & Zanotti, G. (2007). The structure of 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline decarboxylase provides insights into the mechanism of uric acid degradation. *Journal of Biological Chemistry, 282*(25), 18182–18189.

Choi, H. K., Atkinson, K., Karlson, E. W., & Curhan, G. (2005). Obesity, weight change, hypertension, diuretic use, and risk of gout in men: The health professionals follow-up study. *Archives of Internal Medicine, 165*, 742–748.

Doniselli, N., Monzeglio, E., Palù, D., Alessandro, M., & A., & Percudani, R. (2015). The identification of an integral membrane, cytochrome curate oxidase completes the catalytic repertoire of a therapeutic enzyme. *Scientific Reports, 5*(1), 13798.

Ferrari, F. C., Lemos Lima Rde, C., Schimith Ferraz Filha, Z., Barros, C. H., de Paula Michel Araujo, M. C., & Antunes Saude-Guimaraes, D. (2016). Effects of Pimenta pseudocaryophyllus extracts on gout: Anti-inflammatory activity and anti-hyperuricemic effect through xantine oxidase and uricosuric action. *Journal of Ethnopharmacology, 180*, 37–42.

Fritsch, P. O., & Sidoroff, A. (2000). Drug-induced Stevens-Johnson syndrome/toxic epidermal necrolysis. *American Journal of Clinical Dermatology, 1*, 349–360.

Gliozzi, M., Malara, N., Muscoli, S., & Mollace, V. (2016). The treatment of hyperuricemia. *International Journal of Cardiology, 213*, 23–27.

Granato, M., & Nusslein-Volhard, C. (1996). Fishing for genes controlling development. *Current Opinion in Genetics & Development, 6*, 461–468.

Hammer, B., Link, A., Wagner, A., & Bohm, M. (2001). Hypersensitivity syndrome during therapy with allopurinol in asymptomatic hyperuricemia with a fatal outcome. *Deutsche Medizinische Wochenschrift, 126*, 1331–1334.

He, J. H., Gao, J. M., Huang, C. J., & Li, C. Q. (2014). Zebrafish models for assessing developmental and reproductive toxicity. *Neurotoxicology and Teratology, 42*, 35–42.

Hou, P. Y., Mi, C., He, Y., Zhang, J., Wang, S. Q., Yu, F., ... Wu, X. H. (2015). Pallidifloside D from Smilax riparia enhanced allopurinol effects in hyperuricemia mice. *Fitoterapia, 105*, 43–48.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., & Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature, 496*, 498–503.

Johnson, R. J., Kang, D. H., Feig, D., Kivlighn, S., Kanellis, J., Watanabe, S., ... Mazzali, M. (2003). Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension, 41*, 1183–1190.

Kazuno, S., Yanagida, M., Shindo, N., & Murayama, K. (2005). Mass spectrometric identification and quantification of glycosyl flavonoids, including dihydrochalcones with neutral loss scan mode. *Analytical Biochemistry, 347*, 182–192.

Kimiyoshi, I., Makoto, H., Hiroaki, K., Michio, T., Yasunori, U., Tatsuo, H., & Hitoshi, E. (2003). Urate transport via human PAH transporter hOAT1 and its gene structure. *Kidney International, 63*, 143–155.

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics, 203*, 253–310.

Kirkwood, J. S., Lebold, K. M., Miranda, C. L., Wright, C. L., Miller, G. W., Tanguay, R. L., ... Stevens, J. F. (2012). Vitamin C deficiency activates the purine nucleotide cycle in zebrafish. *The Journal of Biological Chemistry, 287*, 3833–3841.

Kuang, H., Rong, J., & Zhu, Y. (2014). *Cloning and prokaryotic expression of urate acid oxidase gene of zebrafish.* (Sichuan Journal of Zoology).

Kubomura, D., Yamada, M., & Masui, A. (2016). Tuna extract reduces serum uric acid in gout-free subjects with insignificantly high serum uric acid: A randomized controlled trial. *Biomedical reports, 5*, 254–258.

Lee, Y. S., Son, E., Kim, S. H., Lee, Y. M., Kim, O. S., & Kim, D. S. (2017). Synergistic uric acid-lowering effects of the combination of Chrysanthemum indicum Linne flower and Cinnamomum cassia (L.) J. *Persl Bark Extracts., 2017*, 1–9.

Lin, L. P., Qu, W., & Liang, J. Y. (2011). Triterpene saponins with XOD inhibitory activity from the roots of Ilex pubescens. *Chinese Chemical Letters, 22*, 697–700.

Liu, W. Y., Lin, F., Rui-Zeng, G. U., Jun, L. U., Lin, D., & Cai, M. Y. (2013). *Vasorelaxation and cholesterol reducing activities of marine collagen oligopeptides.* (Food & Fermentation Industries).

Liu, H., Zhang, X. M., Wang, Y. L., & Liu, B. C. (2014). Prevalence of hyperuricemia among Chinese adults: A national cross-sectional survey using multistage, stratified sampling. *Journal of Nephrology, 27*, 653–658.

Mak, B. S., Chi, C. S., Tsai, C. R., Lee, W. J., & Lin, H. Y. (2000). New mutations of the HPRT gene in Lesch-Nyhan syndrome. *Pediatric Neurology, 23*, 332–335.

Marchetti, M., Liuzzi, A., Fermi, B., Corsini, R., Folli, C., Speranzini, V., et al. (2016). Catalysis and structure of zebrafish urate oxidase provide insights into the origin of hyperuricemia in hominoids. *Scientific Reports, 6*(1), 38302.

McKay, D. L., & Blumberg, J. B. (2007). A review of the bioactivity of south African herbal teas: Rooibos (Aspalathus linearis) and honeybush (Cyclopia intermedia). *Phytotherapy Research, 21*, 1–16.

Nagao, A., Seki, M., & Kobayashi, H. (1999). Inhibition of xanthine oxidase by flavonoids. *Bioscience, Biotechnology, and Biochemistry, 63*, 1787–1790.

Nguyen, K. V., Silva, S., Troncoso, M., Naviaux, R. K., & Nyhan, W. L. (2017). Lesch-nyhan disease in two families from chiloé island with mutations in the\\r, hprt1\\r, gene. *Nucleosides, Nucleotides & Nucleic Acids, 36*(7), 1–11.

Pacher, P., Nivorozhkin, A., & Szabo, C. (2006). Therapeutic effects of xanthine oxidase inhibitors: Renaissance half a century after the discovery of allopurinol. *Pharmacological Reviews, 58*, 87–114.

Pan, X. C., Wen-Jin, G. E., Liu, Y., Feng-Mei, Y. U., Rui-Zeng, G. U., Yong, M. A., & Cai, M. Y. (2012). *On anti-oxidative effects of Oral Administrationof marine collagen peptides.* (Journal of Preventive Medicine Information).

Peng, T. C., Wang, C. C., Kao, T. W., Chan, J. Y., Yang, Y. H., Chang, Y. W., & Chen, W. L. (2015). Relationship between hyperuricemia and lipid profiles in US adults. *BioMed Research International, 2015*, 127596.

Ramazzina, I., Folli, C., Secchi, A., Berni, R., & Percudani, R. (2006). Completing the uric acid degradation pathway through phylogenetic comparison of whole genomes. *Nature Chemical Biology, 2*(3), 144–148.

Rott, K. T., & Agudelo, C. A. (2003). *Gout. Jama, 289*, 2857–2860.

Schlesinger, N. (2004). Management of acute and chronic gouty arthritis: Present state-of-the-art. *Drugs, 64*, 2399–2416.

Sharif, F., Steenbergen, P. J., Metz, J. R., & Champagne, D. L. (2015). Long-lasting effects of dexamethasone on immune cells and wound healing in the zebrafish. *Wound Repair and Regeneration, 23*, 855–865.

Sinha, S., Singh, S. N., & Ray, U. S. (2009). Total antioxidant status at high altitude in lowlanders and native highlanders: Role of uric acid. *High Altitude Medicine & Biology, 10*, 269–274.

Soltani, Z., Rasheed, K., Kapusta, D. R., & Reisin, E. (2013). Potential role of uric acid in metabolic syndrome, hypertension, kidney injury, and cardiovascular diseases: Is it time for reappraisal? *Current Hypertension Reports, 15*, 175–181.

Stavric, B., Clayman, S., Gadd, R. E. A., & Hébert, D. (1975). Some in vivo effects in the rat induced by chlorprothixene and potassium oxonate. *Pharmacological Research Communications, 7*(2), 117–124.

Torres, R. J., Puente, S., Menendez, A., & Fernandez-Garcia, N. (2017). Unapparent hypoxanthine-guanine phosphoribosyltransferase deficiency. *Clinica Chimica Acta, 472*, 136–138.

Wang, Y., Ren, N., Rankin, G. O., Li, B., Rojanasakul, Y., Tu, Y., & Chen, Y. C. (2017). Anti-proliferative effect and cell cycle arrest induced by saponins extracted from tea (Camellia sinensis) flower in human ovarian cancer cells. *Journal of Functional Foods, 37*, 310–321.

Wang, X., Wang, C. P., Hu, Q. H., Lv, Y. Z., Zhang, X., Ouyang, Z., & Kong, L. D. (2010). The dual actions of Sanmiao wan as a hypouricemic agent: Down-regulation of hepatic XOD and renal mURAT1 in hyperuricemic mice. *Journal of Ethnopharmacology, 128*, 107–115.

Xiong, X. Y., Liu, Y., Shan, L. T., Xu, Y. Q., Liang, J., Lai, Y. H., & Hsiao, C. D. (2018). Evaluation of collagen mixture on promoting skin wound healing in zebrafish caused by acetic acid administration. *Biochemical and Biophysical Research Communications, 505*, 516–522.

Xu, W. A., Yin, L., Pan, H. Y., Shi, L., Xu, L., Zhang, X., & Duan, J. A. (2013). Study on the correlation between constituents detected in serum from Rhizoma Smilacis Glabrae and the reduction of uric acid levels in hyperuricemia. *Journal of Ethnopharmacology, 150*, 747–754.

Yang, Z., Xu, Y., Jie, G., He, P., & Tu, Y. (2007). Study on the antioxidant activity of tea flowers (Camellia sinensis). *Asia Pacific Journal of Clinical Nutrition, 16*(Suppl 1), 148–152.

Yoshikawa, M., Wang, T., Sugimoto, S., Nakamura, S., Nagatomo, A., Matsuda, H., & Harima, S. (2008). Functional saponins in tea flower (flower buds of Camellia sinensis): Gastroprotective and hypoglycemic effects of floratheasaponins and qualitative and quantitative analysis using HPLC. *Yakugaku Zasshi, 128*, 141–151.

Zhang, Y., Cong, Y., & Zhang, S. C. (2009). The genes encoding purine metabolism enzymes in amphioxus:Structure and evolution. *Periodical of Ocean University of China, 39*, 96–104.

Zhang, Y., Deng, L., Wu, C., Zheng, L., & Zhong, G. (2018). Konjac glucomannan improves hyperuricemia through regulating xanthine oxidase, adenosine deaminase and urate transporters in rats. *Journal of Functional Foods, 48*, 566–575.

Zhao, R., Chen, D., & Wu, H. L. (2017). Pu-erh ripened tea resists to hyperuricemia through xanthine oxidase and renal urate transporters in hyperuricemic mice. *Journal of Functional Foods, 29*, 201–207.

Zhou, J., Guo, S. Y., Zhang, Y., & Li, C. Q. (2014). Human prokinetic drugs promote gastrointestinal motility in zebrafish. *Neurogastroenterology and Motility, 26*, 589–595.

Zhou, J., Xu, Y. Q., Guo, S. Y., & Li, C. Q. (2015). Rapid analysis of hypolipidemic drugs in a live zebrafish assay. *Journal of Pharmacological and Toxicological Methods, 72*, 47–52.

Zhu, L., Dong, Y., Na, S., Han, R., Wei, C., & Chen, G. (2017). Saponins extracted from Dioscorea collettii rhizomes regulate the expression of urate transporters in chronic hyperuricemia rats. *Biomedicine & Pharmacotherapy, 93*, 88–94.

Zhu, J. J., Xu, Y. Q., He, J. H., Yu, H. P., Huang, C. J., Gao, J. M., ... Li, C. Q. (2014). Human cardiotoxic drugs delivered by soaking and microinjection induce cardiovascular toxicity in zebrafish. *Journal of Applied Toxicology, 34*, 139–148.

Zuo, M., Nishio, H., Lee, M. J., Maejima, K., Mimura, S., & Sumino, K. (2000). The C677T mutation in the methylene tetrahydrofolate reductase gene increases serum uric acid in elderly men. *Journal of Human Genetics, 45*, 257–262.