International Journal of Medical and Health Sciences

Journal Home Page: http://www.ijmhs.net ISSN:2277-4505

Original article

Hypouricemic Property of Crude Ethanolic Extract of Ginger Rhizome (*Curcuma longa*) on Potassium Oxonate-induced Hyperuricemic Mice

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ABSTRACT

Introduction: Several herbal medicines have hypouricemic effect due to their antioxidant property and inhibition of xanthine oxidase. *Curcuma longa*, known as turmeric, has several medicinal properties. Studies on its hypouricemic property are limited. This study aimed to determine the hypouricemic property of crude ethanolic extract of *C. longa* rhizome on potassium oxonate-induced hyperuricemic mice. **Materials and Methods:** Twenty male BALB/c mice were randomly assigned into four groups (n=5). At hour 0, the Vehicle Group (VG) received 0.5 ml of phosphate buffered solution (PBS) intraperitoneally (ip); Negative Group (NG) received 0.25 ml of PBS and 0.25 ml of potassium oxonate (200 mg/ml) ip; Treatment Group (TG) received similar dose of potassium oxonate and 0.25 ml of the crude ethanolic extract of *C. longa* rhizome (300 mg/ml) ip; and the Positive Group (PG) received similar dose of potassium oxonate and 0.25 ml of allopurinol (300 mg/ml) ip. Four hours later, blood collection was done and uric acid levels were determined. Statistical analysis done with *P-value* set at <0.05. **Results:** Major findings showed that the mean uric acid level of TG group was significantly lower than the NG group (477.6 ± 80.2 vs. 1,875.5 ± 429.5 µmol/l; *P<0.05*). The mean uric acid level of TG group was not significantly different when compared with VG (390.5 ± 22.7 µmol/l) and PG (325.6 ± 44.0 µmol/l; *P>0.05*). **Conclusion:** These findings showed that the crude ethanolic extract of *C. longa* rhizome has hypouricemic potential for pharmacologic application.

KEYWORDS: *Curcuma longa*, hyperuricemia, hypouricemic property, mice model

INTRODUCTION

Uric acid is the product of purine metabolism in the liver of humans. It is water-soluble and is excreted mainly in the urine through the kidneys and, to some extent, in the intestines. It circulates in the plasma in its water-soluble form as urate due to its high dissociation constant [1]. Hyperuricemia is a condition in which blood uric acid level is elevated. For males, hyperuricemia is present when serum uric acid level is ≥ 7.0 mg/dL. For females, the cut-off value is ≥ 6.0 mg/dL. Hyperuricemia is due to several genetic and dietary factors. This condition leads to different diseases such as gout and tophaceous gouty arthritis. These clinical conditions have high prevalence and remain to be the most common inflammatory arthritic diseases especially among men in the geriatric age group and in post-menopausal women [2].

In general, hyperuricemia results from an increase in the activity of xanthine oxidase, which leads to increase catabolism of purine from exogenous and endogenous sources and dysfunctional excretion of uric acid in the

kidneys and intestines [3]. Secondarily, hyperuricemia can also be due to disease conditions where renal excretion of uric acid is impaired like nephropathy, chronic renal failure, cardiac failure and other metabolic diseases. Four important transport proteins are involved in the secretion and re-absorption of urate in the proximal tubules of the kidneys: 1) urate transporter 1 (URAT1), 2) glucose transporter 9 (SLC2A9), 3) ATP-binding cassette, subfamily G2 (ABCG2), and 4) organic anion transporter 1 and 3 (OAT1 and 3). Mutation in these genes can cause impairment of renal excretion of uric acid leading to elevation of uric acid in the blood [4-7].

Hyperuricemia and its associated diseases continue to challenge the medical community with its high health care cost and its limited therapeutic interventions. The treatment of mild form of hyperuricemia is only through dietary restriction of purine-rich foods, which are degraded in the human body by xanthine oxidase to uric acid. While moderate to severe forms of hyperuricemia with clinical sequelae are treated with pharmacologic agent like allopurinol, which inhibits xanthine oxidase and lowers blood uric acid level. Being the main pharmacologic treatment of clinical conditions associated with hyperuricemia, allopurinol is widely used to lower blood uric acid level. However, its use is not without any complications. It is associated with mild adverse reaction such as mild hypersensitivity reaction to severe form of hypersensitivity reaction like Stevens-Johnson syndrome which could be fatal. Hence, the need for less toxic alternatives is imperative [2].

Natural products like *Curcuma longa*, also known as turmeric or "luyang dilaw", can be one of the possible candidates as hypouricemic agent. It is a perennial herb belonging to the family Zingeberaceae which is widely distributed in the tropical and subtropical regions of Asia. It has been reported to have several medicinal properties like antioxidant, anti-inflammatory, anti-protozoal, tumoricidal and antimicrobial, purportedly due to its curcurmin content [8-10]. However, studies regarding its hypouricemic property are limited in the literature.

Aside from the limited studies in the literature, animal models for gout are very difficult to replicate. Only hyperuricemic murine models are commonly used using potassium oxonate. Unlike humans, rodents can futher catabolize uric acid to allantoin because they produce uricase. Selective competitive inhibition of uricase in rodents by potassium oxonate induces hyperuricemia but not the more severe clinical sequelae of hyperuricemia such gout and gouty arthritis where urate crystal deposits become tophi over a longer period of time. However, potassium oxonate-induced hyperuricemia in rodents remains to be the most commonly used pre-clinical model in drug discovery from both natural and synthetic products as an alternative to allopurinol which competitively inhibits xanthine oxidase [11].

However, this model is not effective in simulating the chronic nature of the clinical diseases associated with hyperuricemia. The most appropriate use of this model is only in acute setting of inducing hyperuricemia, which can be done in several hours or up to seven days of observation. Even with this limitation, this model is still relevant especially in determining the probable mechanism of action of both natural and synthetic products being tested for their hypouricemic property when compared with the xanthine oxidase inhibitor, allopurinol. The mechanism of action of allopurinol as competitive inhibitor of xanthine oxidase is already well established. It is considered to be the most effective and commonly used drug to lower blood uric acid levels in patients with hyperuricemia who are not responsive in dietary treatment of hyperuricemia and its associated diseases [12].

Other drugs used in lowering blood levels of uric acids in hyperuricemic patients do not belong in the group of xanthine oxidase inhibitors like allopurinol and are not used commonly due to their renal and gastrointestinal toxicities. They are called uricosuric drugs because they increase renal and gastrointestinal excretion of uric acid. These drugs include probenecid and benzbromorone [13, 14]. Colchicine, a nonsteroidal anti-inflammatory drugs, and corticosteroids are concommitantly used in hyperuricemic patients with inflammatory conditions to control the symptoms of the disease and are not responsible for lowering blood uric acid level [15] .

The medical community is only equipped with limited pharmacological drugs with considerable safety to lower blood uric acid level in patients with hyperuricemia. Hence, this study aimed to determine the hypouricemic property of crude ethanolic extract of *Curcuma longa* rhizome on potassium oxonate-induced hyperuricemic mice.

MATERIALS AND METHODS

This study was approved by the Pamantasan ng Lungsod Maynila (PLM)-University Research Center and the PLM-College of Medicine Research Committee after technical and ethical review for the use of animals in this study. It was registered with the Research Implementation and Development Office (RIDO) of the College of Medicine, University of the Philippines-Manila. The guidelines for animal care and use for this study followed the Philippines Association of Laboratory Animal Science. This study used a post-test only experimental design for animal ethical consideration.

Plant Material and Extraction

The plant was procured from the Bureau of Plant Industry (Manila, Philippines). It was sent for taxonomic authentication at De La Salle University (Manila, Philippines). The rhizome part of the plant was used for this study. The rhizome was washed with tap water and dried with clean cloth. Thin 1-cm slices were prepared and dried under shade for 7 days. Five hundred grams of dried rhizome were pulverized with mortar and pestle and soaked in 95% ethyl alcohol for 48 hours at 4^0 C. The mixture was filtered with Whatman™ filter paper no. 5. The filtrate containing the organic solvent was concentrated using rotary evaporator (IKA® RV10 Digital, Selangor, Malaysia) under reduced pressure. The semi-solid residue was placed in glass container to make a final concentration of 300 mg/ml using distilled water. The extract was kept at 4^0 C until further use.

Animal Care and Experimentation

Twenty male BALB/c mice were procured from the Animal Laboratory of the National Institutes of Health at the

University of the Philippines-Manila and acclimatized for two weeks. Standard mice pellets and water were given ad libitum. The animal care followed the guidelines of the Philippine Association of Laboratory Animal Science. On the day of experiment, these mice were randomly assigned into four groups. At hour 0, Vehicle Group (VG; n=5) received 0.5 ml of PBS ip; Negative Group (NG; n=5) received 0.25 ml PBS and 0.25 ml of potassium oxonate (Sigma® Laboratories, Mumbai, India) with a concentration of 200 mg/ml ip; Treatment Group (TG; n=5) received similar dose of potassium oxonate and 0.25 ml of the crude aqueous ethanolic extract of *C. longa* rhizome (300 mg/ml) ip; and the Positive Group (PG; n=5) received similar dose of potassium oxonate and 0.25 ml of allopurinol (Prinol®, Hizon Laboratory, Antipolo, Philippines) (300 mg/ml) ip.

After four hours, blood collection was done through cardiac puncture under anesthesia using Ketamine HCl (Etamine®, International Apex Pharmaceutical, Inc., Pasig City, Philippines) in combination with Xylazine (Sedazine®, MO, USA) at 10/0.5 mg/Kg BW. After blood collection, all mice were sacrificed and properly disposed. Serum was separated from whole blood by centrifugation at 10,000 rpm. The serum uric acid levels were determined using the uricase method (Cypress® Diagnostics, Langdorp, Belgium) and run using a spectrophotometer (StatFax® Semi-automated machine, CA, USA). The results were presented in mean ± SD and subjected to statistical analysis using SPSS® software. Statistically significance was set at *P-value* of < 0.05.

RESULTS

This study showed that potassium oxonate was able to induce hyperuricemia in mice as shown by the mean serum uric acid level of 1,875.5 ± 429.5 µmol/l in the Negative Group. The serum uric acid level of the Treatment Group was significantly lower than the Negative Group (477.6 ± 80.2 vs. 1,875.5 ± 429.5 µmol/l; $P < 0.05$) as shown in Table 1. The mean serum uric acid level of the TG group was not significantly different when compared with the Vehicle Group (390.5 ± 22.7 µmol/l) and the Positive Group (325.6 ± 44.0 µmol/l; $P > 0.05$).

Table 1:Comparative results of mean serum uric acid levels among the different groups

Groups	Mean Serum Uric Acid (µmol/l)	*P-value*
Vehicle Group (VG)	390.5 ± 22.7	> 0.05*
Negative Group (NG)	1,875.5 ± 429.5	< 0.05
Treatment Group (TG)	477.6 ± 80.2	< 0.05†
Positive Group (PG)	325.6 ± 44.0	> 0.05‡

*VG compared with TG and PG; †TG vs. NG; ‡PG vs. TG and VG

DISCUSSION

The abnormal level of uric acid in the blood, which leads to hyperuricemia, contributes to the development of many inflammatory diseases in humans. It causes the deposition of urate crystals in the development of tophaceous gouty arthritis. It can cause nephropathy leading to chronic renal failure. It can also affect the cardiovascular system and develops into cardiovascular diseases, which may lead to cardiac failure. It is also associated with various metabolic diseases. Pharmaceutical drugs are available to decrease the blood uric acid level with various mechanisms.

These drugs are categorized into: 1) xanthine oxidase inhibitors like allopurinol, oxypurinol, tisopurine, febuxostat, and topinostat and 2) uricusoric agents like benzimidazole, benzbromorone, and probenecid. The former competitively inhibits xanthine oxidase that decreases the level of uric acid in the blood. The latter increases the excretion of uric acid in the kidneys and gastrointestinal tract by up regulation of the expression of uric acid-associated transport proteins. However, due to safety issues and adverse reactions of these drugs, the pharmacologic treatment option for hypeuricemia is limited [16].

Allopurinol is the main choice among the different xanthine oxidase inhibitors. Due to its severe adverse drug reactions, research on natural products with hypouricemic property is being advocated [17].

This study used the model of inducing hyperuricemia through intraperitoneal administration of potassium oxonate in mice. The various treatments in the research experiment were likewise through the intraperitoneal route. Although, oral administration would be the best route to simulate the oral administration of these drugs like allopurinol and plant extracts in humans, we used the intraperitoneal route for easy and standard method of inducing hyperuricemia using potassium oxonate in an acute setting using mice model. The bioavailability may be different when given orally. Intraperitoneal injection remains an easy and reliable method for inducing hyperuricemia in murine models. It can also be used for screening of various products for hypouricemic property exploratory experimental studies.

Flavonoids from *C. longa* and most plants are one of the groups of phytochemicals that provide antioxidant properties of most plant-based sources. In a study by Sarawek et al., flavonoids show significant inhibition of xanthine oxidase in an *in vitro* experiment. However, in an *in vivo* study, oral administration of various phytochemicals in semi-pure form like flavonoids among others shows no

hypouricemic property using a murine model of hyperuricemia [18]. Flavonoids and other antioxidant phytochemicals undergo extensive metabolism in the intestine and liver. This results in the inability of these phytochemicals to lower serum uric acid level [19]. The use of intraperitoneal route avoids the possible decrease in bioavailability of phytochemical in crude extract form in studies involving hypouricemic property using potassium oxonate-induced hyperuricemia in murine models.

In this study, the crude aqueous ethanolic extract of *Curcuma longa* rhizome was shown to have a hypouricemic property comparable with allopurinol, which could be used as a potential alternative in the treatment of hyperuricemia. Although the mechanism of action of allopurinol is well understood, the mechanism by which the crude aqueous ethanolic extract of *Curcuma longa* rhizome cannot be ascertained to the inhibition of xanthine oxidase as being the enzyme target of allopurinol. Numerous studies on various natural products coming from plants and plant-derived products have hypouricemic property.

Highly purified bioactive compounds from these plants show competitive inhibition of xanthine oxidase enzyme in both and *in vitro* and *in vivo* studies. However, these natural products are not being used in clinical practice except for some compounds, which are incorporated or are used as single formulation in supplements. These products claim to lower uric acid level in hyperuricemic patients but they are categorized as adjuncts or food supplements with no approved therapeutic claims. Most of these natural products report that the uric acid lowering property is secondary to the antioxidant property of these products which are mostly plant-based or derived from various plants [16].

Phytochemical screening of various extracts of *Curcuma longa* rhizome shows the following groups of compounds: 1) alkaloids, 2) tannins and phenols, 3) proteins, 4) flavonoids, 5) steroids and triterpenoids, 6) glycosides, and 7) carbohydrates. Several studies also identified the primary bioactive compounds present in *Curcuma longa* rhizome. These include: 1) α-phelandrene, 2) sabinene, 3) cineol, 4) borneol, 5) zingiberene, 6) sequiterpenes, and 7) curcumins. The most active group of primary bioactive compounds and the most extensively studied are the curcumins. Several curcumins are reported in the literature.

These include: 1) curcumin, 2) demethoxycurcumin, 3) bisdemethoxycurcumin, and 4) bisabocurcumin.

These studies show that the antioxidants present in *Curcuma longa*, especially its curcumin content, are reported to be the most important bioactive compounds. They are responsible for the purported medical properties of the plant. Studies using plant-based products tested for hypouricemic property show that the uric acid lowering effect is due to its antioxidant property [11, 20-23]. This could be the reason for the observed blood uric acid lowering property of the crude ethanolic extract of *Curcuma longa* used in this study, which contains significant amount of flavonoids and curcumins.

In a published US patent involving the rhizome of *Curcuma longa*, crude ethanolic extract using 40% ethanol shows the highest inhibition of xanthine oxidase as compared with 60% and 100% ethanol using an *in vitro* assay. It also shows significant lowering of blood uric acid level accompanied by decrease in blood levels of superoxide radical and hydrogen peroxide [22]. These findings suggest that antioxidant may play a role in inhibiting the action of xanthine oxidase by hypouricemic agents. Although the mechanistic pathway on how the antioxidant property of *Curcuma longa* inhibits xanthine oxidase is unclear, it is possible that the antioxidant property affects the enzymatic activity of xanthine oxidase. Uric acid is not just an excretory product of purine metabolism. It is also considered to be both antioxidant and pro-oxidant in the balance of oxidative environment in the body. At lower or normal levels, uric acid is considered antioxidant. While at high or abnormal level, it is pro-oxidant promoting oxidative stress, which underlies the development of various cardiovascular diseases secondary to hyperuricemia [23].

In mammals, xanthine oxidase, also known as xanthine oxidoreductase, catalyzes the last two steps in the catabolism of purine bases to uric acid. The xanthine dehydrogenase form of xanthine oxidoreductase enzyme especially in the liver acts upon this reaction. This also can be easily converted to xanthine oxigenase by the oxidation of the sulfhydryl residues and/or proteolysis in the non-hepatic tissues and in the blood, which catalyzes the same reaction. However, the action of xanthine oxidoreductase in these sites produces reactive oxygen species (ROS) like superoxide dismutase and hydrogen peroxide. The production of these ROS is mostly due to the activity of xanthine oxidase form of the xanthine oxidoreductase enzyme. Superoxide dismutase and peroxidase enzymes in normal physiologic condition can neutralize these ROS.

This allows the balance between the activity of xanthine oxidase form in non-hepatic tissues and blood and the activity of xanthine dehydrogenase form in hepatic tissue. In hyperuricemia, a vicious cycle is created by a high level of uric acid. It increases the activity of xanthine oxidase form of the enzyme, which keeps the level of uric acid at an abnormal value. In the presence of antioxidant compounds like flavonoids and curcumin in the *Curcuma longa*, the xanthine dehydrogenase form of the enzyme in hepatic tissue and the xanthine oxigenase form in non-hepatic tissues and in the blood become less active. Thus, the formation of uric acid or hyperuricemia is prevented [24].

In conclusion, this study showed that the crude ethanolic extract of *Curcuma longa* rhizome has hypouricemic property in potassium oxonate-induced hyperuricemic mice similar to allopurinol, a known xanthine oxidase inhibitor, used to treat hyperuricemia and its associated clinical diseases. This plan-based product can be used in the research and development of hypouricemic drugs for natural products, which are inexpensive, and with high safety profile.

REFERENCES

1.Singh JA, Reddy SG, Kundukulam J. Risk factors for gout and prevention: a systematic review of the literature. Curr Rheumatol 2011; 32: 192-202.

2.Roddy E, Choi H. Epidemiology of gout. Rheuma Dis Clin North Am 2014; 40: 155-175.

3.Hu OH, Zhu JX, Ji J, et al. *Fructus gardenia* extract ameliorates oxonate-induced hyperuricemia with renal

dysfunction in mice by regulating organic ion transporters and mOIT3. Molecule 2013; 18: 8976-8993.

4.Li JM, Zhang X, Wang X, Xie XW, Kong LD. Protective effects of cortex fraxini coumarines against oxonate-induced hyperuricemia and renal dysfunction in mice. Euro J Pharmacol 2011; 666:196-124.

5.Anzai N, Ichida, K, Jutabha P, Kimura, T, Babu E, Jin, CJ, Srivastava S, Kitamura K, Hisatome I, Endou H, Sakurai H. Plasma urate level is directly regulated by a voltage-driven urate efflux transporter URATv1 (SLC2A9) in humans. J Biol Chem 2008; 283: 26834–26838.

6.Enomoto A, Endou H. Roles of organic anion transporters (OATs) and a urate transporter (URAT1) in the pathophysiology of human disease. Clin Exp Nephrol 2005; 9: 195–205.

7.Woodward OM, Köttgen A, Coresh J, Boerwinkle E, Guggino WB, Köttgen M. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. Proc Natl Acad Sci USA 2009; 106: 10338–10342.

8.Dall'Acqua S, Stocchero M, Boschiero I, et al. New findings on the *in vivo* antioxidant activity of *Curcuma longa* extract by an integrated ^{1}H NMR and HPLC-MS metabolomic approach. Fitoterapia 2016; 109: 125-136.

9.Ramasewak RS, DeWitt DL, Nair MG. Cytotoxicity, antioxidant and anti-inflammatory activities of curcurmins I-III from *Curcuma longa*. Phytomedicine 2000; 7: 303-308.

10.Gupta A, Mahajan S, Sharma R. Evaluation of antimicrobial activity of *Curcuma longa* rhizome against *Staphylococcus aureus*. Biotechnology Report 2015; 6: 51-55.

11.Ma L, Zhang S, Yuan Y, Gao J. Hypouricemic actions of exopolysaccharide produced by *Cordyceps militaris* in potassium oxonate-induced hyperuricemic mice. Curr Microbiol 2014; 69: 852-857.

12.Stamp LS, Chapman PT, Palmer SC. Allopurinol and kidney function: an update. Joint Bone Spine. 2016; 83: 19-24.

13.Murugaiyah V, Chan K-L. Mechanisms of anti-hyperuricemic effect of *Phyllanthus niruri* and its lignin constituents. J Ethnopharmacol 2009; 124: 233 -239.

14.Yu Z, Fong WP, Cheng CHK. The dual actions of morin (3,5,7,2,4-pentahydroxyflavone) as a hypouricemic agent: uricosuric effect and xanthine oxidase inhibitory activity. J Pharmacol Exp Ther 2006; 316: 169-175.

15.Ferrari FC, Lima RDCL, Filha ZSF, et al. Effects of *Pimenta pseudocaryophyllis* on gout: anti-inflammatory and anti-hyperuricemic effects through xanthine oxidase and uricosuric action. J Ethnopharmacol 2016; 180:37-42.

16.Wang Y, Zhu JX, Kong LD, Yang C, Cheng CH, Zhang X. 2004. Administration of procyanidins from grape seeds reduces serum uric acid levels and decreases hepatic xanthine dehydrogenase/oxidase activities in oxonate-treated mice. Basic Clin Pharmacol Toxicol 2004; 94: 232–237.

17.Sarawek S, Feistel B, Pischel V. Flavanoids of *Cynara scolymus* possess potent xanthine oxidase inhibitory activity in vitro but are devoid of hypouricemic effects in rats after oral administration. Planta Med 2008; 74:221-227.

18.Kong AN, Owuor E, Yu R, et al. Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (APE/EpRE). Drug Metab Rev. 2001; 33:255-271.

19.Eigner D, Scholz D. *Ferula asa-toetida* and *Curcuma longa* in traditional medical treatment and diet in Nepal. J Ethnopharmacol 1999; 67: 1-6.

20.Balogun E, Hoque M, Gong P, et al. Curcumin activates the haem oxygenase I gene via regulation of Nrf2 and the antioxidant element. Biochem J. 2003; 371:887-895.

21.Xiao YC, Jing X, Min Y, et al. Bisabocurcumin, a new skeleton curcuminoid from the rhizome of *Curcuma longa* L. Chi Chem Lett 2011; 22: 1457-1460.

22.Agarwal KC. Development of biochemically standardized extracts from fresh rhizome of turmeric (*C. longa*) for the treatment of disease caused by hyperuricemia. US Patent No. 20100015260. Published January 21, 2010. [cited 2016 March]. Available from http://www.faqs.org/patents/app/20100015260.

23.Kostić DA, Dimitrijević DS, Stojanović GS. Xanthine oxidase: isolation, assays of activity and inhibition. J Chem 2015; 20: 1-8.

24.Enroth C, Eger BT, Okamoto K. Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion. Proc Nat Acad Sci 2000; 97: 10723-10728.

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