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Effects of Onion on Serum Uric Acid Levels and Hepatic Xanthine Dehydrogenase/Xanthine Oxidase Activities in Hyperuricemic Rats

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Abstract: The aim of this study was to investigate the effects of onion on serum uric acid levels and hepatic Xanthine Dehydrogenase/Xanthine Oxidase activities in normal and hyperuricemic rats. Hyperuricemia was induced by intraperitoneal injection of 250 mg kg⁻¹ potassium oxonate in rats. Oral administration of onion at 3.5 and 7.0 mg kg⁻¹ day⁻¹ for 7 days was able to reduce serum uric acid levels in hyperuricemic rats with no significant effects on the level of this compound in the normal animals. In addition, onion when tested *in vivo* on rat liver homogeneities elicited significant inhibitory actions on the Xanthine Dehydrogenase (XDH) and Xanthine Oxidase (XO) activities. This effect resulted less potent than that of allopurinol. However, the hypouricemic effect observed in the experimental animal did not seem to parallel the change in XDH and XO activities, implying that the onion might be acting via other mechanisms apart from simple inhibition of enzyme activities. Such hypouricemic action and enzyme inhibitory activity of onion makes it a possible alternative for allopurinol, or at least in combination therapy to minimize the side-effects of allopurinol, in particular in long-term application.

Key words: Onion, hyperuricemia, uric acid, xanthine dehydrogenase, xanthine oxidase

INTRODUCTION

Gout is a common metabolic disorder with a worldwide distribution. It is estimated that the overall incidence of this disease is about 1.4/1000 per annum (Nuki, 2006). The disease is characterized by abnormal high levels of uric acid in the bodies resulting from the deposition of urate crystals in the joint and kidney (Terkeltaub, 2006). That is way the control of uric acid production has been widely considered as a key factor in the prevention and treatment of these diseases (Liote, 2003). Uric acid is the end product of purine metabolism in humans, and its overproduction and/or underexcretion can lead to the incidence of hyperuricemia and as gout (Lee and Terkeltaub, 1996).

Plasma uric acid is derived from dietary purines, the catabolism of cellular nucleoproteins and purine nucleotides synthesized *de novo* (Nuki, 2006). The treatment and management of gout can not be effectively achieved through only diet therapy and omission of purine-rich foods (Franzese, 2004). Accordingly, the

inhibition of uric acid biosynthesis and overproduction will be one of the therapeutic approaches to treat gout.

Xanthine oxidase (XO) is a molybdenum-containing enzyme that catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid in the purine catabolic pathway. Therefore, the inhibition of XO activity will decrease the uric acid levels and results in an anti-hyperuricemic effect. Allopurinol is the sole XO inhibitor under the clinical application and has served as a dominant uric acid-lowering agent in the past three decades (Okamoto, 2008; Fels and Sundry, 2008). However, some severe adverse effects such as hepatitis, nephropathy, allergic reactions and 6-mercaptopurine toxicity limit the clinical use of allopurinol (Strazzullo and Puig, 2007) and it would be highly desired to search for new XO inhibitors, in particular from natural sources, as alternatives for allopurinol (Nguyen *et al.*, 2004; Wang *et al.*, 2004).

Flavonoids are a group of polyphenolic compounds that are ubiquitously distributed in various foods and beverages of plant origin (Lin *et al.*, 2002; Kinoshita *et al.*,

2006). There are many reports indicating the beneficial effects of these compounds. The most of the therapeutic properties of flavonoids have been ascribed to their antioxidant and enzyme inhibitory activities (Murota and Terao, 2003; Potapovich and Kostyuk, 2003). XO is one of the most important enzymes that are inhibited by some flavonoids (Van Hoorn *et al.*, 2002).

The average human intake of flavonoids has been estimated to be $\sim 25 \text{ mg day}^{-1}$ (Hertog *et al.*, 1993). Onion is a flavonoid-rich staple food and it has been shown that it ranked highest in quercetin content among 28 vegetables and 9 fruits assayed by Finnegan *et al.* (1992). Its major flavonoids have been identified as quercetin, quercetin-4'-glucoside and quercetin-3, 4'-diglucoside (Slimestad *et al.*, 2007; Caridi *et al.*, 2007). It has been shown that the bioavailability of flavonoids in onion is higher than that of other tropical vegetables and that onion intake leads to an increase in plasma quercetin levels (Miean and Mohamed, 2001).

Taking into account the high content of flavonoids in onion and the high daily consumption of this staple food, it is possible that onion acts as an inhibitor of XO activity and reduces uric acid levels. In the present *in vivo* study, therefore, the effects of onion intake on serum uric acid levels and hepatic XDH/XO activity in hyperuricemic rats have been investigated.

MATERIALS AND METHODS

Reagents: Xanthine, nicotinamide adenine dinucleotide (NAD⁺), uric acid and allopurinol were purchased from Sigma (St. Louis, Mo, USA). Potassium oxonate was purchased from Aldrich Inc. All other chemicals were purchased from Merck (Darmstadt, Germany). The reagents used were from of analytical grades.

Animals: Male Sprague-Dawley rats (6-8 weeks old, 180-200 g, n = 42) were obtained from the animal house of Tabriz University of Medical Sciences, Iran. They were fed with a standard laboratory diet and allowed food and water ad libitum for an acclimatization period of 7 days prior to the experiment. The temperature and humidity were kept at $18 \pm 1^\circ \text{C}$ and 50%, respectively and the lighting cycle was 07.00-19.00 h light and 19.00-07.00 h dark. Animals were handled with humane care in accordance with the National Institutes of Health guidelines.

Animal model of hyperuricemia in rats: An *in vivo* potassium oxonate-induced hyperuricemic animal model was used to study anti-hyperuricemic effects of drugs (Stavric *et al.*, 1975; Hall *et al.*, 1990). Briefly, 250

potassium oxonate dissolved in 0.9% saline solution was administrated intraperitoneally to each animal 1 h before oral administration of test compounds on the first, thirds and seventh days of the experiment. Whole blood sample was taken from each rat by cutting the tail tip 2 h after the test drug administration. Serum was obtained by centrifuging blood sample at 3000 rpm for 10 min and then was ultrafiltered using Ultrafree-MC centrifugal filter units (Millipore Corp., Bedford, MA, USA) at 7500 rpm for 1 h to remove proteins before analysis. The sera were stored at -20°C until use.

Study design and treatment of animals: Forty two rats were divided into seven equal groups. In group 1, the normal control group, each animal received only water as vehicle. In groups 2 and 3, the normal animals received 5.0 mg kg^{-1} allopurinol and 7.0 g kg^{-1} onion juice, respectively, without injection of potassium oxonate. In group 4, uricase inhibitor potassium oxonate (250 mg kg^{-1}) was administrated intraperitoneally 1 h before oral administration of the test compounds. In groups 5, 6 and 7, each animal received the same dose of potassium oxonate together with 5.0 mg kg^{-1} allopurinol, 3.5 g kg^{-1} onion juice or 7.0 g kg^{-1} onion juice, respectively. The administration of allopurinol and fresh onion juice (by oral gavage) was carried out daily for seven days, while potassium oxonate was given only on the first, third and seventh days of the experiment.

Preparation of the onion juice: The outer dry skins and any inedible outer portions of onion were removed and the remaining edible portion was weighted and completely blended in distilled water (1:1 w/v). The freshly prepared juicy sample was administrated to each animal by oral gavage administration.

Uric acid assay: The serum uric acid levels were determined by the phosphotungstic acid method as described elsewhere (Carroll *et al.*, 1971).

Assay of XO and XDH activities: The animals were killed between 09.00 and 10.00 am by cervical dislocation and their livers were immediately excised and placed in ice-cold isotonic potassium chloride solution (1.15% KCl w/v) containing 0.1 mM EDTA. The livers were then chopped into 4-5 volumes of 50 mM phosphate buffer (pH 7.4) and homogenized by a homogenizer fitted with a Teflon pestle. The homogenate was then centrifuged at 3000 g for 10 min, the lipid layer was carefully removed and the resulting supernatant fraction was further centrifuged at 10,000 g for 60 min at 4°C . The supernatant was used for enzyme assays.

The XO and XDH activities were assayed spectrophotometrically by monitoring the production of uric acid from xanthine. In the case of XDH, the assay mixture consisted of 50 μM xanthine, 50 μM phosphate buffer (pH 7.4), 200 μL NAD^+ and 100 μL of the enzyme solution. After preincubation at 37°C for 15 min, the reaction was initiated by the addition of the substrate solution. After 30 min, the reaction was terminated by adding 0.5 mL HCl (0.6 M) and the absorbance was measured at 290 nm using a Shimadzu 2550 UV/VIS spectrophotometer which was controlled by the Shimadzu UV Probe personal software package including kinetics software. The instrument was connected to a Shimadzu cell temperature control unit. XO activity was measured using a similar method described for XDH with the difference being that molecular oxygen was used in place of NAD^+ as electron acceptor.

Protein determination: Protein concentration was determined spectrophotometrically by bicinchoninic acid kit using bovine serum albumin as the standard (Smith *et al.*, 1985).

RESULTS

The initial mean serum uric acid levels remained almost unchanged at $\approx 1.6 \text{ mg dL}^{-1}$ throughout the experiment in the control normal animals. The administration of either onion juice ($7.0 \text{ g kg}^{-1} \text{ day}^{-1}$) or allopurinol ($5.0 \text{ mg kg}^{-1} \text{ day}^{-1}$) did not cause any significant change in the serum uric acid levels in the normal rats (Table 1).

However, treatment with uricase inhibitor potassium oxonate (po) resulted in a significant elevation of serum uric acid levels reaching to 3.27 mg dL^{-1} on the 7th day of the intervention (Table 1). Following treatment of the hyperuricemic animals with onion juice at the doses of 3.5 and $7.0 \text{ g kg}^{-1} \text{ day}^{-1}$, the uric acid levels reduced compared to hyperuricemic control rats. This reduction was not statistically significant on the first day, but

when the onion was administered for 3 days, a significant reduction was observed in the uric acid levels. This became more obvious when onion juice at $7.0 \text{ g kg}^{-1} \text{ day}^{-1}$ was given for 7 days, so that, the uric acid levels of hyperuricemic rats reached to a level similar to that of the normal control animals. Oral administration of $5.0 \text{ mg kg}^{-1} \text{ day}^{-1}$ allopurinol also caused a significant reduction in the serum uric acid levels in the hyperuricemic rats compared to hyperuricemic control group. Unlike onion juice, the hypouricemic effect of allopurinol was observed even after 1 day of the drug administration and remained almost unchanged throughout the experiment period indicating the quicker onset of allopurinol action compared to that of onion juice. However, there were no significant differences between the inhibitory effects of allopurinol and onion juice on the 7th day of the treatment. It appears that onion juice exerts its effect in a dose- and time-dependent manner (Fig. 1).

In Table 2 the activity of XDH and XO in normal and oxonate-induced hyperuricemia rats together with the effects of oral administration of onion juice and allopurinol on hepatic enzyme activities after 7 days of intervention have been shown. In normal rats, the administration of onion at a daily dose of 7.0 g kg^{-1} caused only 11 and 6% inhibition on hepatic XDH and XO activities, respectively. The corresponding values following daily treatment with 5.0 mg kg^{-1} allopurinol were 12 and 10%, respectively.

It seems that oxonate administration can lead to an increase in both XDH and XO activities (Table 2). Unlike normal groups, in oxonate-pretreated rats both onion juice and allopurinol were able to produce significant decrease in hepatic XDH and XO activities. The reductions in liver XDH and XO activities in these hyperuricemic animals receiving $3.5 \text{ g kg}^{-1} \text{ day}^{-1}$ onion were 31 and 25%, respectively. A two fold increase in the dose of the onion juice resulted in only 5 and 8% increase in the inhibitory activities in rat livers, respectively. Treatment of the

Table 1: Hypouricemic effects of fresh onion juice and allopurinol on serum uric acid levels in normal and hyperuricemic rats

Treatment	Serum uric acid levels (mg dL^{-1})			
	Starting (0) day	1st day	3rd day	7th day
Normal animal				
Vehicle	1.60±0.08	1.59±0.08	1.59±0.08	1.55±0.10
Allopurinol (5 mg kg^{-1})	1.67±0.09	1.64±0.07	1.55±0.09	1.41±0.08
Onion (7 g kg^{-1})	1.66±0.08	1.61±0.08	1.58 ± 0.06	1.49±0.06
Hyperuricemic animal				
Po + vehicle	1.65±0.08	2.96±0.18 ^{###}	3.13±0.23 ^{###}	3.27±0.21 ^{###}
Po + allopurinol (5 mg kg^{-1})	1.59±0.10	1.62±0.08 ^{**}	1.56±0.09 ^{***}	1.48±0.06 ^{***}
Po + onion (3.5 g kg^{-1})	1.54±0.10	2.53±0.11 ^{###}	2.10±0.12 ^{##}	1.83±0.09 ^{###}
Po + onion (7 g kg^{-1})	1.61±0.10	2.48±0.15 ^{###}	1.88±0.10 ^{**}	1.71±0.09 ^{**}

Data represent mean±SD (n = 6). For statistical significance, Mann-Whitney U test was used. *p<0.05 (compared to hyperuricemic control group). **p<0.01 (compared to hyperuricemic control group). ***p<0.001 (compared to hyperuricemic control group). #p<0.05 (compared to normal control group). ##p<0.01 (compared to normal control group). ###p<0.001 (compared to normal control group)

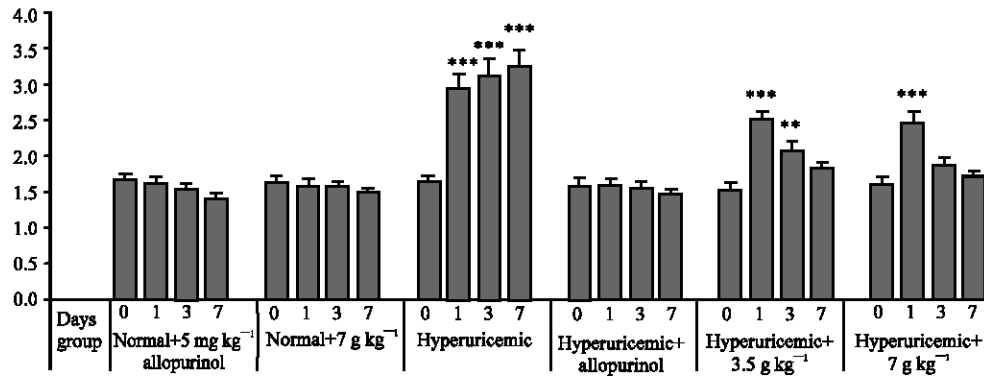


Fig. 1: Time-dependent effects of fresh onion juice and allopurinol on serum uric acid levels. Data represent mean±SD (n = 6). For statistical significance, Mann-Whitney U-test was used. *p<0.05, **p<0.01, ***p<0.001 when the values obtained from the treated animals were compared to pre-intervention values in each group

Table 2: Effects of fresh onion juice and allopurinol on hepatic XDH and XO activities in normal and hyperuricemic rats

Treatments	Enzyme activity (nmol uric acid min ⁻¹ mg protein ⁻¹)		Inhibition (%)	
	XDH	XO	XDH	XO
Normal animal				
Vehicle	2.75±0.34	2.18±0.38	-	-
Allopurinol (5 mg kg ⁻¹)	2.40±0.36	1.96±0.31	12	10
Onion (7 g kg ⁻¹)	2.44±0.29	2.04±0.54	11	6
Hyperuricemic animal				
Po + vehicle	3.15±0.31	2.49±0.30	-	-
Po + allopurinol (5 mg kg ⁻¹)	1.84±0.32***,####	1.44±0.30***,####	42	41
Po + onion (3.5 g kg ⁻¹)	2.18±0.34**,#	1.88±0.25*,#	31	25
Po + onion (7 g kg ⁻¹)	2.0±0.29**,#	1.67±0.47*,,##	36	33

Data represent mean±SD (n = 6). For statistical significance, Mann-Whitney U-test was used. *p<0.05 (compared to hyperuricemic control group). **p<0.01 (compared to hyperuricemic control group). ***p<0.001 (compared to hyperuricemic control group). #p<0.05 (compared to normal control group). ##p<0.01 (compared to normal control group). ###p<0.001 (compared to normal control group)

hyperuricemic animals with 5.0 mg kg⁻¹ day⁻¹ allopurinol also reduced XDH and XO activities. The corresponding values were found to be 42 and 41% reduction in XDH and XO activities, respectively. It appears that the inhibitory effect of allopurinol on XDH and XO activities is higher than that of onion even at the higher dosage of onion used.

DISCUSSION

Hyperuricemia is a metabolic disorder which may play an important role in the development of gout, nephrolithiasis, hypertension and hyperlipidemia (Choi and Ford, 2007). As hyperuricemia is characterized by elevated production of uric acid, this disorder is most often controlled by reducing uric acid production. In man, uric acid is produced as the major end-product of purine metabolism. The key enzyme in this pathway is XO. This enzyme catalyzes the conversion of hypoxanthine to xanthine, which is then further oxidized to uric acid. Therefore, the inhibition of XO can be used as an effective therapeutic approach to treat hyperuricemia. At the present, allopurinol is the only XO inhibitor with

clinical application. Due to some serious side effects of this drug (Strazzullo and Puig, 2007) many attempts are made to find a safer alternative for allopurinol particularly from natural sources (Kong *et al.*, 2004; Zhu *et al.*, 2004; Yu *et al.*, 2007). In this study, it was shown that the oral administration of onion as a flavonoid-rich food can reduce the elevated uric acid levels in hyperuricemic rats in a dose- and time-dependent manner. This reductive effect of onion juice on the uric acid levels was found to be almost similar to that of allopurinol. Interestingly, although both onion juice and allopurinol had significant reductive effects on the serum uric acid levels in the hyperuricemic animals, the uric acid concentration was not significantly altered in the normal rats by either onion juice or allopurinol administration. Kong *et al.* (2004) have also shown that the water extract of Ermiao wan (a Chinese herbal medicine used in the treatment of acute gout) and allopurinol have less inhibitory effects on serum uric acid levels in normal mice compared with those animals pretreated with potassium oxonate. However, the inhibitory effects of both Ermiao wan extract and allopurinol on serum uric acid levels in normal rats reported by Kong *et al.* (2004) were statistically

significant, bearing in mind that they used allopurinol at 10 mg kg⁻¹ compared with the dose of 5 mg kg⁻¹ used in the present study. On the other hand, this property of onion juice could be considered as an advantage for this staple food. Although the elevated levels of uric acid in the circulation could give rise to gout and possibly other pathological conditions (Nuki, 2006; Terkeltaub, 2006) the antioxidant action of uric acid, particularly its ability to inhibit DNA damage, is also well documented (Muraoka and Miura, 2003; Stinefelt *et al.*, 2005). Thus, excessive lowering of the uric acid level in the circulation beyond that of the normal range might even be counter productive (Wang *et al.*, 2004). Taking into account that onion as a food can be used safely long-term; this feature of onion makes it an attractive candidate for the treatment of hyperuricemia and gout.

The hypouricemic property of onion juice observed in this study could be explained by the inhibitory effects of its flavonoids on XDH and XO activities; however, it can not be entirely attributed to this mechanism. Both onion juice and allopurinol administration showed almost similar hypouricemic action. In spite of this, the extent of reduction in XDH and XO activities elicited by allopurinol was much higher than that observed with the onion juice administration (Table 2). Therefore, there is not apparently a parallel relationship between the extent of the hypouricemic action and the reduction in the enzyme activity. Similar results have been reported by others (Kong *et al.*, 2004; Wang *et al.*, 2004; Zhu *et al.*, 2004). According to these studies, the involvement of other possible mechanisms such as enhanced uric acid clearance or actions on other purine metabolizing enzymes can not be ruled out (Kong *et al.*, 2004; Wang *et al.*, 2004). This could be further supported by the existence of some hypouricemic compounds including natural products that are devoid of XDH and XO inhibitory activities (Zhao *et al.*, 2006; Kong *et al.*, 2004; Wang *et al.*, 2004).

It seems that the inhibitory effects of both onion juice and allopurinol on XDH and XO activities in the potassium oxonate-induced hyperuricemia are more dominant than their effects on the normal activities of the either two forms of the enzyme (Table 2). It is well known that XO and XDH are inducible enzymes (Yoshisue *et al.*, 2000). The results obtained in the present study showed that Potassium oxonate caused an increase in XDH and XO activities. In fact, onion juice and allopurinol exert mostly their inhibitory actions on this induced activity rather than on the normal XDH and XO activities.

CONCLUSION

As a conclusion, onion is able to reduce serum uric acid levels in hyperuricemic rats with no significant

effects on the level of this compound in normal animals. This property can be considered as an advantage for the onion. As onion is a common component of the usual diets in almost throughout the world, this natural food could be served as a possible alternative for allopurinol, or at least in combination therapy to minimize the side-effects of allopurinol, in particular in long-term application. Although the mechanism of the hypouricemic action of onion is not completely understood, this effect could be attributed in part to its inhibitory effects on XDH and XO activity.

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