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Inhibitory Effects of Tart Cherry (*Prunus cerasus*) Juice on Xanthine Oxidoreductase Activity and its Hypouricemic and Antioxidant Effects on Rats

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ABSTRACT

The aim of this study was to investigate the effect of tart cherry juice on serum uric acid levels, hepatic xanthine oxidoreductase activity and two non-invasive biomarkers of oxidative stress (total antioxidant capacity and malondialdehyde concentration), in normal and hyperuricemic rats. Tart cherry juice (5 ml/kg) was given by oral gavage to rats for 2 weeks. Allopurinol (5 mg/kg) was used as a positive control and was also given by oral gavage. Data showed that tart cherry juice treatment did not cause any significant reduction in the serum uric acid levels in normal rats, but significantly reduced ($P \leq 0.05$) the serum uric acid levels of hyperuricemic rats in a time-dependent manner. Tart cherry juice treatment also inhibited hepatic xanthine oxidase/dehydrogenase activity. Moreover, a significant increase ($P \leq 0.05$) in serum total antioxidant capacity was observed in tart cherry juice treated-rats in both normal and hyperuricemic groups. The oral administration of tart cherry juice also led to a significant reduction ($P \leq 0.05$) in MDA concentration in the hyperuricemic rats. Although the hypouricemic effect of allopurinol, as a putative inhibitor of xanthine oxidoreductase, was much higher than that of tart cherry, it could not significantly change anti-oxidative parameters. These features of tart cherry make it an attractive candidate for the prophylactic treatment of hyperuricaemia, particularly if it is to be taken on a long-term basis. Further investigations to define its clinical efficacy would be highly desirable.

INTRODUCTION

Tart cherry (*Prunus cerasus*) contains sensible amounts of anthocyanins in addition to other bioflavonoids such as chlorogenic acid, gallic acid, *p*-coumaric acid and quercetin (Seeram, Bourquin & Nair, 2001; Seymour *et al.*, 2007). Tart cherry anthocyanins possess a wide range of biochemical and pharmacological effects and have been recommended as chemopreventive agents or nutritional supplements (Kanga *et al.*, 2003; Kinoshita *et al.*, 2006). The predominant mechanism of their biological actions is thought to result from anti-oxidant activity, enzyme inhibition, and the capacity to scavenge free radicals (Lin *et al.*, 2002; Rackova *et al.*, 2007).

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Xanthine oxidoreductase (XOR) is one of the most important enzymes that are inhibited by some flavonoids (Potapovich & Kostyuk, 2003). XOR, a molybdenum-containing enzyme, is the key enzyme in the catabolism of purines that catalyses the oxidative hydroxylation of hypoxanthine to xanthine and xanthine to the terminal catabolite uric acid (Nguyen *et al.*, 2004). Overproduction of uric acid, characterised by hyperuricemia, is a common metabolic disorder and has been considered as an important risk factor for gout and may be associated with oxidative stress conditions (Strazzullo & Puig, 2007). It is worth noting that XOR occurs in two different forms. Xanthine dehydrogenase (XDH) is the prevalent operative form under physiological conditions. Under pathological conditions, however, in parallel to the degradation of ATP into adenine and xanthine, an extensive conversion of XDH to xanthine oxidase (XO) takes place. The latter uses molecular oxygen as an electron acceptor and leads to the formation of superoxide anion and hydrogen peroxide in parallel with uric acid production. Therefore, XOR can act as a source of reactive oxygen species (ROS) (Rackova *et al.*, 2007). ROS may be involved in the pathogenesis of various degenerative diseases because they induce damage to biological macromolecules such as proteins, fats and DNA (Choi, 2008). That is why the inhibition of XOR activity may decrease the level of uric acid and ROS production and result in anti-hyperuricemic and anti-oxidative effects.

Allopurinol is the sole XOR inhibitor under clinical application and has served as a dominant uric acid-lowering agent in the past four decades (Fels & Sundy, 2008). However, some severe adverse effects such as hepatitis, nephropathy and allergic reactions limit the clinical use of allopurinol (Nguyen *et al.*, 2004; Strazzullo & Puig, 2007). Therefore, a promising approach for hyperuricemia and its complications might be a combination therapy utilising dietary phytochemicals and hypouricemic pharmaceuticals at a suboptimal dosage to minimise any potential adverse side effects.

There is significant interest in the direct antioxidant activities of anthocyanins, due to associations between consumption of anthocyanins-rich fruits such as tart and sweet cherry and decreased incidence of oxidative stress related diseases (Blando, Gerardi & Nicoletti, 2004; Kim *et al.*, 2005; Halvorsen *et al.*, 2006). However, indirect antioxidant action, such as the inhibition of ROS-producing enzymes, may be equally relevant to health benefits through a general reduction in oxidative stress *in vivo*. The objectives of this research were to determine the potential of tart cherry to inhibit hepatic XO and XDH activity in normal and hyperuricemic rats. The possible role of tart cherry in reducing serum uric acid levels and its effect on biomarkers of oxidative stress was also investigated.

MATERIALS AND METHODS

Chemicals

Potassium oxonate, xanthine, nicotinamide adenine dinucleotide (NAD^+), uric acid, allopurinol, tetraethoxypropane (TEP), trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), bicinchoninic acid kit and 6-mercaptopurine were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). All other reagents used were of analytical grades. Tart cherry (*Prunus cerasus*) was purchased from a wholesale market.

Test compound preparation

Tart cherry is commonly squeezed for its juice, following removal of its seeds. Allopurinol used as a positive control, was prepared in 0.9 % saline.

Animals

A total of 36 male Wistar rats (body weight: 180-200 g) were obtained from the animal house of Tabriz University of Medical

Sciences, Iran. They were fed with a commercial laboratory diet and allowed food and water *ad libitum* for an acclimatisation period of 1 week prior to the experiment. All animals were maintained on a 12 hour / 12 hour light / dark cycle and the temperature and humidity were kept at 18 ± 1°C and 50%, respectively. They were handled according to the recommendations of the Tehran University of Medical Science Ethics Committee.

Animal model of hyperuricemia in rats

Experimentally-induced hyperuricemia in rats (due to inhibition of uricase with potassium oxonate) was used to study anti-hyperuricemic and antioxidant effects of tart cherry (Hall *et al.*, 1990). Briefly, 250 mg / kg potassium oxonate (PO) dissolved in 0.9% saline solution was administrated intra-peritoneally to each animal 1 hour before oral administration of test compounds.

Experimental design

Rats were randomly divided into six equal groups (6 rats per group). In group 1, the normal group, each animal received only water as vehicle. Groups 2 and 3 received 5 ml / kg tart cherry juice and 5 mg / kg allopurinol respectively (without injection of PO). In group 4, the hyperuricemic control group, PO (250 mg / kg) was administrated intraperitoneally. In groups 5 and 6, each animal was first injected intraperitonially the same dose of PO 1 hour before oral administration of test compounds and then received 5 ml / kg tart cherry juice and 5 mg / kg allopurinol respectively. The freshly prepared juice samples were administered to the corresponding groups by oral gavage once a day for 2 weeks.

Sample preparation

Blood sample was taken from each rat by cutting the tail tip, 1 hour after administration of the test compound, at the pre-intervention, 1st, 7th and 14th days of the study. Serum was obtained by centrifuging blood sample at 6000 rpm for 10 minutes. For HPLC analysis, the serum was filtered using SPARTAN 13 / 0.45 RC, Watman. The sera were stored at -20 °C until used. At the end of the experiment, rats were anesthetised between 09.00 and 10.00 am. Their livers were removed, weighed and then rapidly washed in cold saline (0.9%) 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 homogenised by a homogeniser fitted with a Teflon pestle. The homogenate was then centrifuged at 3000 g for 10 minutes, the lipid layer was carefully removed, and the resulting supernatant fraction was further centrifuged at 15,000 g for 60 minutes at 4°C. The supernatant was stored at -80 °C until used.

Uric acid determination

The serum uric acid levels were analysed by high performance liquid chromatography (HPLC) method using a system supplied by Waters Associates, Northwich, Cheshire which consisted of a Waters 515 pump, Waters 717 plus Autosampler, Waters 2487, Dual λ Absorbance Detector. The mobile phase was a mixture of 100 mM KH_2PO_4 (pH 3.5): Methanol (97:3, v / v). Separations were performed on a C-18 column (Perfectsil Target ODS-3 (5 μM), 250x4.6 mm) with a C-18 guard column (Perfectsil Target ODS-3 (5 μM), 10x4 mm). The effluent was monitored by UV detection at 290 nm at a flow rate of 1.0 ml / minutes. Standard solutions of uric acid in the range of 10 to 1000 μmol / L were prepared in mobile phase. Serum uric acid concentrations were expressed as μmol / L. 6-Mercaptopurine (1 mM) was used as the internal standard.

XO and XDH activities determination

The XO and XDH activities were measured spectro-photometrically by monitoring the

production of uric acid from xanthine according to Prajda and Weber's method (Prajda & Weber, 1975). In the case of XDH, the assay mixture consisted of 50 μM xanthine, 50 μM phosphate buffer (pH 7.4), 200 μM NAD^+, and 100 μl of the enzyme solution. After pre-incubation at 37°C for 15 minutes, the reaction was initiated by the addition of the substrate solution. After 30 minutes, 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. One unit (U) of activity was defined as 1 nmol of uric acid formed per minute at 37°C, pH 7.4.

Protein determination

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

Total antioxidant capacity (TAC) assay

Total serum antioxidant capacity assay was performed using TPTZ (tripyridyltriazine) reagent. This method measures the ability of the antioxidants contained in the sample to reduce ferrictripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) which absorbs light at 593 nm. TAC of serum was calculated by plotting a standard curve of absorbance against μmol/L concentration of Fe^{2+} standard solution (Benzie & Strain, 1996).

Lipid peroxide determination

Lipid peroxide in the serum was measured by the following procedure according to Yoshioka *et al.* (1979) method. Briefly, 0.5 ml serum was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube. 1ml of 0.67 % TBA was added to the mixture, shaken, and warmed for 60 minutes in a boiling water bath followed by rapid cooling. Then it was shaken into a 4 ml of n-butanol layer in a separation tube and MDA content in the serum was determined at 532 nm by spectrophotometer against n-butanol. The standards of 0.1 to 20 μmol/L tetraethoxypropane (TEP) were used. The results were expressed as μmol/L serum.

Statistical analysis

All the samples and standards were run in duplicate and the results are expressed as means ± standard deviation (SD). The statistical comparison between the experimental groups was performed by independent-sample *t*-test using SPSS 13.0 computer software. The probabilities of 5% or less ($P \leq 0.05$) were considered significant.

RESULTS

Effect of tart cherry juice and allopurinol on serum uric acid

The effects of the orally administered tart cherry juice and allopurinol on serum uric acid levels in normal and hyperuricemic rats are shown in Table 1. Tart cherry juice treatments did not cause any significant reduction in the serum uric acid levels in normal rats after 14 days, but allopurinol, as a putative inhibitor of XO, significantly reduced ($P \leq 0.01$) the levels of serum uric acid in the normal rats after 14 days. As also shown in Table 1, intraperitoneal injection of potassium oxonate (250 mg/kg) to control group markedly increased the serum uric acid levels, and reached to 214.36 ± 26.42 (μmol/L) at the end of the experiment. The uric acid level in normal rats was only 99.87 ± 17.44 (μmol/L). Following treatment of the hyperuricemic rats with tart cherry juice

Table 1. Effect of orally administered tart cherry juice and allopurinol on serum uric acid levels (a time-dependent study)

Treatment	*Uric acid (μmol/L)*			
	Pre-intervention day	*1st day*	*7th day*	*14th day*
Normal animal				
Vehicle	98.69 ± 31.45	98.09 ± 15.80$^{\#\#\#}$	96.31 ± 14.01$^{\#\#\#}$	99.87 ± 17.44$^{\#\#\#}$
Tart cherry juice(5ml/kg)	91.10 ± 24.35	90.57 ± 12.84$^{\#\#\#}$	91.96 ± 15.07$^{\#\#\#}$	95.22 ± 17.90$^{\#\#\#}$
Allopurinol(5mg/kg)	98.54 ± 27.43	90.29 ± 20.42$^{\#\#\#}$	81.28 ± 10.86$^{\#\#\#}$	53.03 ± 24.41$^{**,\#\#\#}$
Hyperuricemic animal				
Vehicle	87.65 ± 32.05	207.14 ± 33.41***	210.47 ± 34.63***	214.36 ± 26.42***
Tart cherry juice(5ml/kg)	90.87 ± 25.30	205.28 ± 42.18***	184.74 ± 31.22***	171.95 ± 25.78$^{***,\#}$
Allopurinol(5mg/kg)	90.85 ± 29.65	148.05 ± 13.61$^{***,\#\#}$	114.16 ± 25.62$^{\#\#\#}$	72.38 ± 19.24$^{*,\#\#\#}$

All values are expressed as mean ± SD (n=6). Independent-sample *t*-test was used for statistical significance assessment. *indicates $P\leq0.05$, **indicates $P\leq0.01$ and ***indicates $P\leq0.001$ vs normal control group, $^{\#}$ indicates $P\leq0.05$, $^{\#\#}$ indicates $P\leq0.01$ and $^{\#\#\#}$ indicates $P\leq0.001$ vs hyperuricemic control group.

Table 2. Effect of orally administered tart cherry juice and allopurinol on hepatic XO and XDH activity after 14 days

Treatment	*Activity (U/mg protein)*		*Inhibition %*	
	XO	*XDH*	*XO*	*XDH*
Normal animal				
Vehicle	2.50 ± 0.63	3.80 ± 0.38	-	-
Tart cherry juice(5ml/kg)	2.17 ± 0.68	2.95 ± 0.49$^{**,\#}$	13.20	22.36
Allopurinol(5 mg/kg)	1.17 ± 0.28$^{**,\#\#\#}$	1.47 ± 0.40$^{***,\#\#\#}$	53.20	61.31
Hyperuricemic animal				
Vehicle	2.49 ± 0.52	3.82 ± 0.62	-	-
Tart cherry juice(5ml/kg)	1.99 ± 0.15$^{\#}$	2.69 ± 0.32$^{***,\#\#}$	20.08	29.58
Allopurinol(5 mg/kg)	1.05 ± 0.18$^{***,\#\#\#}$	1.27 ± 0.33$^{***,\#\#\#}$	57.83	66.75

All values are expressed as mean ± SD (n=6). Independent-sample *t*-test was used for statistical significance assessment. **indicates *P*d"0.01 and ***indicates $P\leq0.001$ vs normal control group, $^{\#}$ indicates $P\leq0.05$, $^{\#\#}$ indicates $P\leq0.01$ and $^{\#\#\#}$ indicates $P\leq0.001$ vs hyperuricemic control group.

and allopurinol for 14 days, the uric acid levels were significantly reduced compared to hyperuricemic control rats ($P\leq0.05$ and $P\leq0.001$, respectively).

The results also indicate that tart cherry juice and allopurinol exert their hypo-uricemic effects in a time-dependent manner.

Effect of tart cherry juice and allopurinol on hepatic XO and XDH activity

The effects of the orally administered tart cherry juice and allopurinol on hepatic XO and XDH activity after 14 days are shown in Table 2. The oral administration of tart

cherry juice to normal rats caused 13.20% and 22.36% inhibition on hepatic XO and XDH activity respectively. Allopurinol significantly inhibited the mean activity of XO and XDH by 53.20% ($P \leq 0.01$) and 61.31% ($P \leq 0.001$) in the normal rats after 14 days.

In hyperuricemic rats, tart cherry juice treatment caused 20.08% ($P \leq 0.05$) and 29.58% ($P \leq 0.01$) inhibition on hepatic XO and XDH activity compared to hyperuricemic control rats. Treatment of the hyperuricemic animals with allopurinol significantly inhibited ($P \leq 0.001$) both XO and XDH activity (57.83% and 66.75% respectively).

Effect of tart cherry juice and allopurinol on biomarkers of oxidative stress

The present investigation also tested the efficacy of orally administered tart cherry and allopurinol on two serum non-invasive biomarkers of oxidative stress (total antioxidant capacity and malondialdehyde concentration). As shown in Figure 1, a significant increase ($P \leq 0.05$) in serum total antioxidant capacity (FRAP value) was observed in tart cherry juice treated normal rats after 14 days compared to normal control group (340 ± 37.41 *vs* 275 ± 43.24 μmol / L). A similar effect was obtained in hyperuricemic animals following tart cherry juice administration compared to hyperuricemic control group (Figure 1).

The oral administration of tart cherry juice for 14 days also led to a significant reduction ($P \leq 0.05$) in MDA concentration in the hyperuricemic rats (Figure 2). Allopurinol was not able to reduce significantly the elevated level of MDA in these animals. However, neither of these treatments could reduce the MDA level in the normal groups compared to the normal control value.

DISCUSSION

Hyperuricemia is a metabolic disorder which may play an important role in the development of gout and several oxidative stress diseases such as cancer and cardiovascular diseases (Nuki, 2006; Strazzullo & Puig, 2007). At the present, allopurinol, a purine analogue, which causes inhibition on XOR, is the only drug with clinical application known to lower uric acid production (Fels & Sundy, 2008). However, allopurinol has also been associated with some serious side effects such as hepatitis, nephropathy and allergic reactions (Nguyen *et al.*, 2004; Strazzullo & Puig, 2007). To be acceptable to the public as a treatment and especially as a preventative, any approach needs to involve minimal risks. Most dietary phytochemicals, including anthocyanins, have in comparison to pharmaceuticals a greatly reduced risk profile even when consumed at higher dosages (Hou, 2003; Bobe *et al.*, 2006).

Tart cherry (*Prunus cerasus*) is one of the major sources of anthocyanins and contains between 100 and 400 mg of anthocyanins / kg of fresh weight (Seeram *et al.*, 2001). Presently, *in vivo* investigation has assessed the effects of tart cherry juice in comparison to allopurinol on biomarkers of oxidative stress, serum uric acid levels and hepatic XO and XDH activity in normal and hyperuricemic rats.

We found that the orally administered tart cherry juice could not cause any significant reduction in the serum uric acid levels in normal rats after 14 days, but allopurinol, as a putative inhibitor of XO, significantly reduced ($P \leq 0.01$) the levels of serum uric acid in the normal rats after 14 days. In hyperuricemic rats, tart cherry juice and allopurinol significantly decreased the uric acid levels compared to hyperuricemic control rats. However, the effect of allopurinol, as a reference drug on reducing uric acid level was more potent and reduced serum uric acid levels to a lower level than that of normal levels. Kong *et al.*(2004) have also shown that the water extract of Ermiao wan (a Chinese herbal medicine used in the

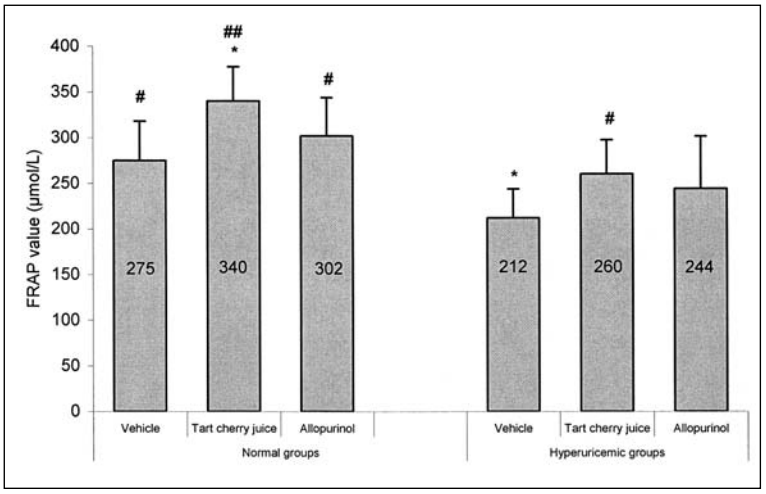

Figure 1. Effect of orally administered tart cherry juice and allopurinol on serum total antioxidant capacity (FRAP value) after 14 days (mean±SD, n=6)

Note: * indicates $P \leq 0.05$ vs normal control group, # indicates $P \leq 0.05$, ## indicates $P \leq 0.01$ and ### indicates $P \leq 0.001$ vs hyperuricemic control group.

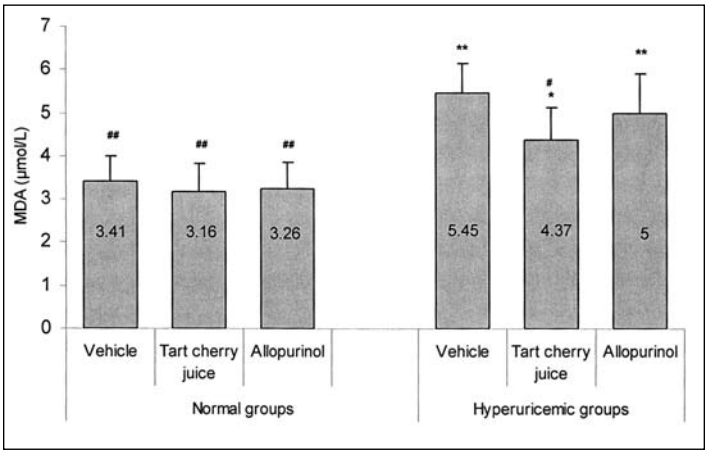

Figure 2. Effect of orally administered tart cherry juice and allopurinol on MDA concentration after 14 days (mean±SD, n=6)

Note: * indicates $P \leq 0.05$ and ** indicates $P \leq 0.01$ vs normal control group, # indicates $P \leq 0.05$ and ## indicates $P \leq 0.01$ vs hyperuricemic control group.

treatment of acute gout) and allopurinol have less inhibitory effects on serum uric acid levels in normal mice compared with those animals pre-treated with potassium oxonate (2004). In fact, this property of tart cherry juice could be considered its advantage. Although elevated levels of uric acid in the blood circulation could give rise to gout and possibly other pathological conditions (Nuki, 2006), the antioxidant action of uric acid, particularly its ability to inhibit DNA damage, is also well documented (Stinefelt *et al.*, 2005). Thus, excessive lowering of the uric acid level in blood circulation beyond

that of the normal range might even be counter productive (Wang *et al.*, 2004). These data also indicate that tart cherry juice might bring fewer side effects when compared to allopurinol in the treatment of hyperuricemia and oxidative stress related diseases. Jacob *et al.*(2003) found that healthy women (aged 20 to 40 years) who consumed two servings or 280 grams of cherries after an overnight fast showed a 15 % reduction in uric acid levels. Other researchers reported that daily cherry consumption helped to relieve gout attacks and the pain associated with arthritis. The researchers concluded that compounds in cherries may inhibit inflammatory pathways associated with gout (Blau, 1950; Jacob *et al.*, 2003).

We also demonstrated that tart cherry juice exerts its hypouricemic effect in a time-dependent manner. As it is obvious in Table 1, the reduction of serum uric acid levels in tart cherry juice treated-hyperuricemic rats was not statistically significant on the 1st and 7th day, but after 14 days of intervention, a significant ($P \leq 0.05$) reduction was observed in the uric acid levels; however, the serum uric acid levels did not yet reach the normal level in these animals. Unlike tart cherry juice, the hypouricemic effect of allopurinol was statistically significant ($P \leq 0.01$) even after 1 day of the drug administration, indicating the quicker onset of allopurinol action compared to that of tart cherry juice.

The hypouricemic property of tart cherry juice observed in this study can be explained at least in part by its inhibitory effect on hepatic XO and XDH activity. Numerous studies have showed that phenolic compounds found in plants, such as anthocyanins and quercetin, which are structurally related to xanthine, inhibit XO and XDH activity, thus reducing hyperuricemia (Lin *et al.*, 2002; Nguyen *et al.*, 2004; Wang *et al.*, 2004; Zhao *et al.*, 2006; Mo *et al.*, 2007). However, there is no apparently parallel relationship between the extent of the hypouricemic action and the reduction in the enzyme activity. Moreover, the extent of reduction in XO and XDH activity elicited by allopurinol was much higher than that observed with the tart cherry administration in both normal and hyperuricemic groups. Therefore, the observed hypouricemic effect can not be entirely attributed to this mechanism. 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 metabolising 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 XO and XDH inhibitory activity (Wang *et al.*, 2004; Zhao *et al.*, 2006).

As shown in Figures 1 and 2, intraperitoneal injection of potassium oxonate to rats caused a significant decrease in serum total antioxidant capacity and increase in serum MDA levels. Potassium oxonate, a selectively competitive uricase inhibitor, blocks the effect of hepatic uricase and produces hyperuricemia in rodents (Hall *et al.*, 1990). Though serum uric acid is considered to be an antioxidant within its normal physiological conditions, increased uric acid level in pathological states has been associated with increased production of oxygen free radicals, due to the conversion of XDH to XO that plays a pivotal role in progression of oxidative stress condition (Maia *et al.*, 2007). In the present study, uric acid was positively correlated with MDA (Figure 3). It is well known that XO is a source of ROS and this may explain the link between hyperuricemia and oxidative stress-induced diseases (Nguyen *et al.*, 2004; Maia *et al.*, 2007; Strazzullo & Puig, 2007). Moreover, serum uric acid elevation may promote oxygenation of LDL-C and facilitate lipid peroxidation (Johnson *et al.*, 2003).

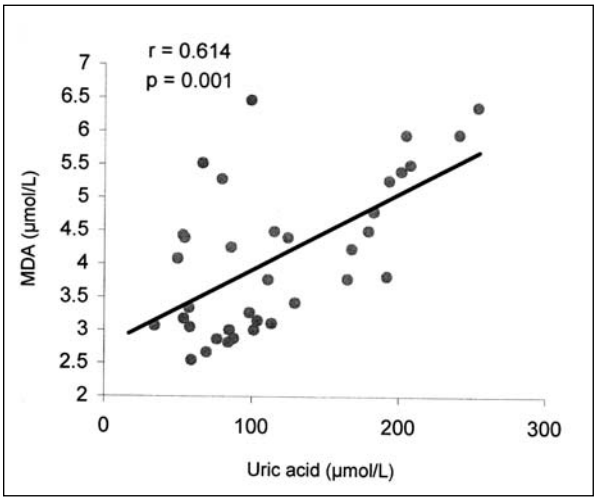

Figure 3. Linear regression between serum uric acid levels and MDA concentration

From the above discussion, we can assume that serum uric acid through lipid peroxidation, might be working towards the etiopathogenesis of oxidative stress diseases and its serum level may be a deciding factor for progression of the disease.

Following treatment of the normal and hyperuricemic rats with tart cherry juice, a significant increase ($P \leq 0.05$) in serum total antioxidant capacity was found. Tart cherry juice was also able to inhibit lipid peroxidation. It is worth noting that tart cherry juice exerts mostly its reducing effect on elevated MDA concentration in hyperuricemic rats rather than on the normal MDA concentration. Studies have identified the active antioxidants within tart cherries as eight polyphenolic compounds, including anthocyanins, chlorogenic acid, gallic acid, *p*-coumaric acid and quercetin (Chaovanalikit & Wrolstad, 2004). These phytochemicals suppress destructive oxygen free radicals. An over abundance of free radicals can damage all components of the cell, including proteins, fats and DNA, contributing to the development of several pathological conditions (Kinoshita *et al.*, 2006; Vijayalakshmi & Chandrasekhar, 2008). In this study, allopurinol could not significantly increase serum total anti-oxidant capacity and decrease serum MDA concentration in normal and hyperuricemic rats. However, the inhibition of XO by allopurinol was previously reported to decrease the level of ROS production and reduce the hepatic injury associated with liver transplantation (Lee & Lee, 2006).

In conclusion, tart cherry juice prevented oxidative stress by enhancing total antioxidant capacity and decreasing lipid peroxidation. Moreover, the hypouricemic and XOR inhibitory action of tart cherry juice has been confirmed in this study. Taking into account that tart cherry as a fruit can be used safely long term, this feature of tart cherry makes it a possible alternative for allopurinol, or at least in combination therapy to minimise the side-effects of allopurinol. Therefore, the use of suboptimal dosages of allopurinol in combination with dietary changes may provide a safer approach for prevention and treatment of hyperuricemia. Furthermore, the search for other safe anti-hyperuricemic and anti-oxidative foods and diets must continue.

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