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RhusCoriaria Effect on Serum Uric Acid Level and in Vivo Xanthine Oxidase Activity in Oxonate-Induced Hyperuricemic Mice

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ABSTRACT

Background: Genetic defects in purine metabolism pathway cause hyperuricemia and goutleading to renal dysfunction. The aim of this study was to determine the in vivo hypouricemic effect and mechanism of action of Rhuscoriaria (sumac-sumak) extract on oxonate-induced hyperuricemic mice and to compare it with hypouricemic effect of Allopurinol.

Materials and Methods*:* Forty Mice were divided into 6 groups and all, except the control group (group1) received an intraperitoneal potassium oxonate injection. One hour later, sumac extract with 250, 500 or 1000 mg/kg concentrations were given to mice in groups 2, 3 and 4, respectively, while group 5 received 10 mg/kg Allopurinol via gavage. Three hours after gavage, mice were anesthetized with ether and decapitated. Serum samples were collected and kept in -80 ^{0}C until laboratory measurements. Pieces of liver were removed and washed with phosphate buffer and homogenized with 100 mMTris-Hcl buffer, pH 7.5 to obtain supernatant. Xanthine oxidase activity was investigated in the supernatant.

Results: The results showed that sumac extract decreased uric acid level in a dose-dependent manner. In all doses, hypouricemic effect of sumac extract was less effective than Allopourinol. Regarding the average xanthine oxidase activity in liver tissue homogenate, no significant enzyme activity reduction was seen in oxonate-induced hyperuricemic mice except in Allopourinol group.

Conclusion: Hypouricemic action of Rhuscoriaria may be responsible for some of the beneficial effects of sumac extract on hyperuricemia and gout. This effect was dose-dependent and in all doses was less effective than that of Allopourinol.

KEYWORDS: Rhuscoriaria, Sumac, Uric acid, Xanthine oxidase, Mice
Runnning title: Effect of Rhuscoriaria on hyperuricemia.

INTRODUCTION

Hyperuricemia and gout are caused by genetic defects in purine metabolism pathway and also could be resulted from reduction in body fluids volume. Serum/plasma uric acid level above 7-8 mg/dl is defined as hyperuricemia. Primary hyperuricemia is hereditary or arises due to the severe prolonged high consumption of oysters and red meat diet. Secondary hyperuricemia arises from increased cellular metabolism such as leukemia and some anemias, cell degradation and cytolysis [1]. In leukemia and advanced lymphoma, hyperuricemia could happen alone or in combination with other electrolytes disturbances [2].

Xanthine oxidoreductase (XOR), a prototype of human molybdenum hydroxylase, is known as the responsible enzyme for hydroxylation of xanthine to uric acid as well as a drug target for hyperuricemia and gout [3]. In mammals two forms of enzymes are found. The form in normal physiological condition is xanthine dehydrogenase (XDH) and xanthine oxidase (XO) [4, 5].

The drug treatment of hyperuricemia and gout is based on using allopurinol (a xanthine oxidase inhibitor which decreases uric acid production) and Rasburicase [6-8]. Ethanol extract of *Biota orientalis* species also showed in vitro inhibitory effects against XO activity in mouse liver. This is an important indication that it might reduce serum uric acid levels by acting as the enzyme inhibitor [9, 10].

Many scientific research centers around the world are exploring medicinal plants due to global belief of their efficacy in treatment. Spices of the family Anacardiaceae [11] have long reputation in folk medicine for their nutritional value of edible fruits and seeds, and for variable ailments such as treatment of bowel complain, chronic wounds, pimples, boils, jaundice, hepatitis, and inflammatory conditions[12].

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Sumac is the common name for a genus (Rhus) that contains over 250 individual species of flowering plants in the family Anacardiaceae[13, 14]. Rhuscoriaria, mountain shrub with fruit bitter/sour flavor, mostly grows in the Mediterranean, South- East Asia, Italy and is famously used in Mediterranean and Middle East region as a spice, sauce, drink, etc [15].The sumac name is derived from ‘‘sumaga’’, meaning red in Syrian. In Persian folk medicine, Sumac (Rhuscoriaria) is believed to have atheroprotective effects and is consumed in some Persian dishes..

Previous studies have suggested that methanolic extracts of Rhuscoriaria L. fruits may be a source of natural antioxidants[17, 18].

The spice is used as a condiment and sprinkled over fish, chicken, grilled meat and salad, giving these dishes a lemony taste [19].

Properties and effects which are claimed for Rhuscoriaria include anti-microbial [20], anti-oxidant [21, 22], anti-diabetic [23], appetite stimulator, astringent [24] and stomach cleanser, diuretic, fever reducing, rheumatic complications treatment, urea excretion, digestion strengther and stomach bleeding stopple, menorrhagia reliever, diarrhea and decreasing sweating [25].The aqueous extract of Iranian R. coriaria L. fruits showed hepatoprotective activity against cytotoxicity of oxidative stress [26] and antibacterial activity against Salmonella typhimurium[27.

Studies on this plant have shown that its fruits [28] and leaves 29] have in vitro antioxidant properties and also it has already been reported in vitro hypoglycemic activity of the methanolic extract of fruits of Rhuscoriaria due to inhibition of a-amylase [9].

Phenolic acids, such as gallic acid, methyl gallate, or protocatechuic acid in R. coriaria support the folkloric use of this plant as spice, food preserving as well as wound cleaning[31] and justifies the previously reported pharmacological results [32].

To our knowledge, however, no other similar study has been done concerning the possible effect of Rhuscoriaria on serum uric acid level and xanthine oxidase activity.

MATERIALS AND METHODS

Animals

Male healthy mice (25-30 g)were purchased from the Laboratory Animal Center (Mashhad university of medical sciences), fed with standard chow diet and water. Theywere housed in iron cages and were allowed one week to adapt to their environment before experiments. All animals were kept on a 12-h light/12-h dark cycle, at a constant temperature of 25 °C. Animals received human care according to the criteria outlined in the ‘‘Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH).

Materials

Hydroalcoholic Rhuscoriaria extract was prepared by distillation soxhlet method, with 35.6% efficiency (26.7 g pure extract of 75 grams sumac). Seventy five grams of Rhuscoriaria fruit were twice refluxed with 70% ethanol for 1 hour. The ethanol extract was filtered and concentrated to remove ethanol at 50 °C under vacuum. The ethanol extract was partitioned with petroleum to delete any lipid-soluble substances. To obtain sumac extract at 250, 500 and 1000 mg/kg concentrations, 0.075, 0.15 and 0.3 mg of extract were dissolved in 0.3 ml distilled water, respectively. For 10 mg/kg concentration of Allopurinol, 0.01 g of tablets was resolved in 3 ml distilled water. To prepare oxonic acid in 280 mg/kg concentration, 0.1 g of potassium oxonate was resolved in 3 ml of sodium bicarbonate.

Study design

Forty adult male mice with an average weight of 30 g were divided into 6 groups. All mice except the control group (group 1) received 0.3 ml oxonic acid 0.03% via peritoneal injection to increase the serum urate level as described before [33, 34].

Rhuscoriaria extract at various concentrations and Allopurinol dissolved in distilled water were given orally to study groups. The volume of the suspension administered was based on body weight measured immediately prior to each dose.

Animals in group I (control group) were orally administered with 0.3 ml of 0.9% saline solution. The groups 2 to 4 were orally received Rhuscoriaria extract 250, 500 or 1000 mg/kg, respectively. For groups 5 and 6, 0.3 ml of Allopurinol at 10 mg/kg and 0.3 ml sodium bicarbonate 5% were administered, respectively.

Three hours after gavage, mice were anesthetized with ether and decapitated. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 3500 x g for 5 min (3-30k SIGMA German company) to obtain serum. The serum was stored at -80 °C for future laboratory measurements.

The effects of ethanolic extract of Rhuscoriaria fruits were measured on blood uric acid level and on liver homogenate xanthine oxidase activity by commercial kits.

Serum uric acid was determined by uricase-peroxidase method. This assay principally is based on the production of hydrogen peroxide from uric acid using uricase. Hydrogen peroxide then causes oxidative coupling of 3,5-dichloro-2-hydroxybenzene sulfonate (DHBS) and 4-aminoantipyrine, in the presence of peroxidase, forming a red colored quinoneimine dye. The intensity of the color produced is directly proportional to the concentration of uric acid in the sample, with maximum absorbance at 520 nm. Serum uric acid levels were determined with Biosystem kit (LOT Number .171 AA and BT 3000 Autoanalyser) [35].

Mice liver were removed and washed with phosphate-buffered saline (PBS). Liver tissues were homogenized with 8 ml of 100mM Tris-Hcl buffer, pH 7.5 with Homogenizer Silent crusher (Heidolph German company), and centrifuged at 10000 x g, at 4 °C for 15 minutes. Supernatants were stored in -80 °C for xanthine oxidase activity.

The assay is based on a multi step enzymatic reaction in which xanthine oxidase first produces H_2O_2 during oxidation of hypoxanthine[36, 37].In the presence of horseradish peroxidase, the H_2O_2 reacts with ADHP(10-acetyl-3-7 diydroxyphenoxazine) in 1:1 stoichiometry to produce the highly fluorescent compound resorufin. Resorufin fluorescence was analysed with an excitation wavelength of 520-550 nm and an emission wavelength of 585-595 nm. Liver xanthine oxidase activity was measured by xanthine oxidase kit (Cayman Chemical Company, Item no.10010895) and UV Spectrophotometer (CF-9500, CECIL). Each assay was performed in duplicate.

Statistical analysis

All statistical analyses were carried out using SPSS 11.5 for Windows statistical software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of $p < 0.05$. Results were shown represent the mean ± standard error of the mean (S.E.M.). The statistical evaluation of the results was carried out utilizing two-tailed, paired Student's t-tests. Statistical significance was set at $p < 0.05$.

RESULTS

The in vivo hypouricemic effect of Rhuscoriaria extract (sumac-sumak) on oxonate-induced hyperuricemic mice was examined in this study. Varaince analysis showed significant differences in mean of serum uric acid between groups ($p < 0.05$) (Figure1).

Allopourinol decreased serum uric acid levels and showed significant difference with all other groups ($p < 0.05$).

In group 2, 250 mg/kg of sumac extract caused hyperuricemic effect and showed significant difference with Allopourinolhypouricemic effect ($p < 0.05$).

In Groups 3, 500 mg/kg of sumac extract showed significant difference with Allopourinolhypouricemic action ($p < 0.05$).

In group 4, 1000 mg/kg of sumac extract caused hupouricemic effect and showed no significant difference with Allopourinol group in reduction serum uric acid level (Figure1).

In group 6, increased serum uric acid level in induced hyperuricemic mice showed significant difference with Allopourinol group ($p < 0.05$).

Serum uric acid levels in each group were homogenous (Figure 2).

Normality of liver xanthine oxidase activity was examined by Shapiro-wilk test in the studied groups, showed all groups were normal other than groups 2 and 6. Non-parametric kruskal-wallis test indicated significant differences between groups in xanthine oxidase activity ($p < 0.05$).

Man-Whitney test showed significant differences in enzyme activity reduction between Allopourinol and other groups ($p < 0.05$) (Figure 3).

Because of limited number of groups and mice, with Bonferroni test significance level adjusted in 0.01, thus only differences between groups 5 and 6 were statistically significant ($p < 0.01$).

Between all studied groups, only Allopourinol group (group 5) showed significant reduction in liver enzyme activity ($p < 0.01$).

Enzyme activity in groups 2, 3 and 6 showed heterogeneity but homogeneity in the other groups was observed (Figure 4).

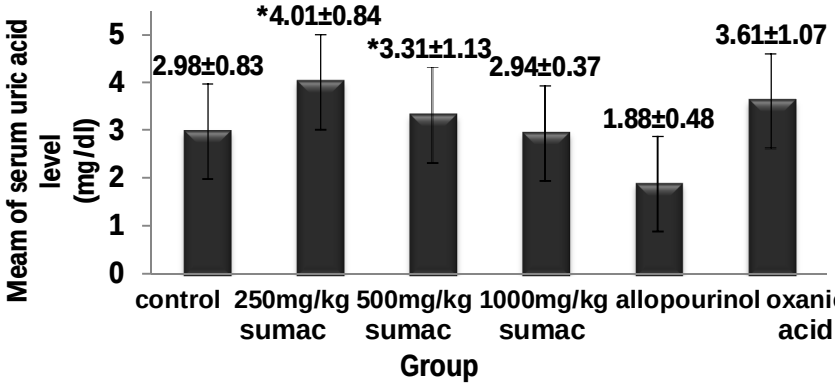

Figure 1. Effect of intraperitoneal administration of Rhus coriaria hydroalcoholic extract and Allopurinol on serum urate levels in hyperuricemic mice pretreated with the potassium oxonate. The hyperuricemic mice were produced by potassium oxonate pretreatment as described in Methods and Material. They were intraperitoneally administered with Rhus coriaria hydroalcoholic extract or Allopurinol at the different doses, respectively. Data represent mean values (±S.E.M.) of serum urate levels (mg/dl) in the groups of animals (n = 6).* $P < 0.05$ vs. Allopurinol group

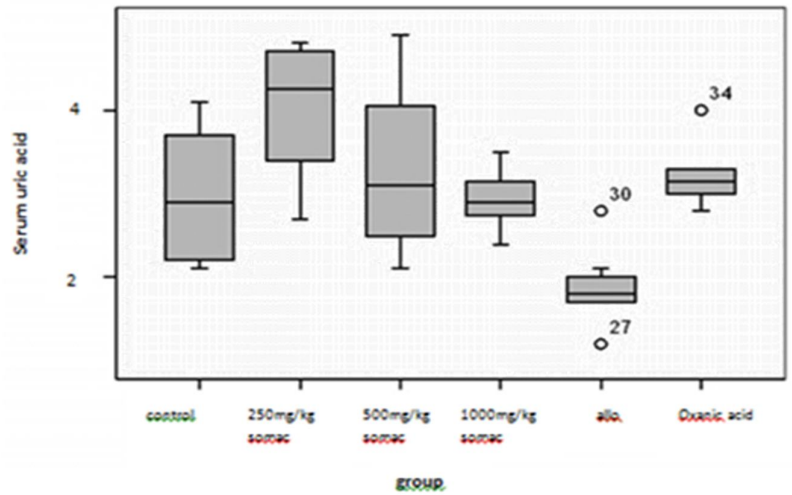

Figure 2. Box Plot chart for serum uric acid levels in each group shows homogeneity between groups.

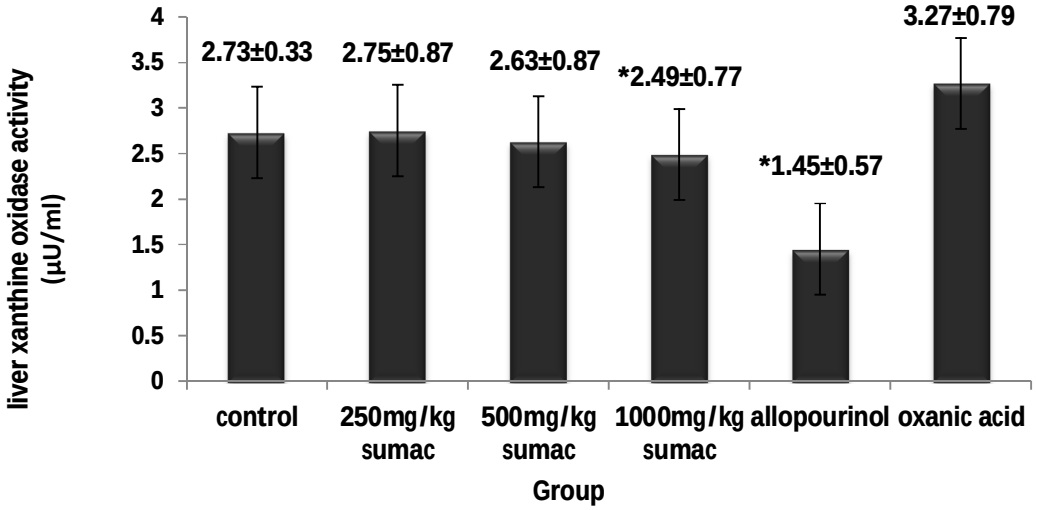

Figure 3. Effect of intraperitoneal administration of Rhus coriaria hydroalcoholic extract and Allopurinol on liver xanthine oxidase activity in hyperuricemic mice pretreated intraperitoneal with the potassium oxonate. The hyperuricemic mice were produced by potassium oxonate pretreatment as described in Methods and Material. They were orally administered with Rhus coriaria hydroalcoholic extract or Allopurinol at the different doses, respectively. Data represent mean values (±S.E.M.) of xanthine oxidase activity (µU/ml) in the groups of animals (n = 6).* $P < 0.05$ vs. hyperuricemic oxonate group

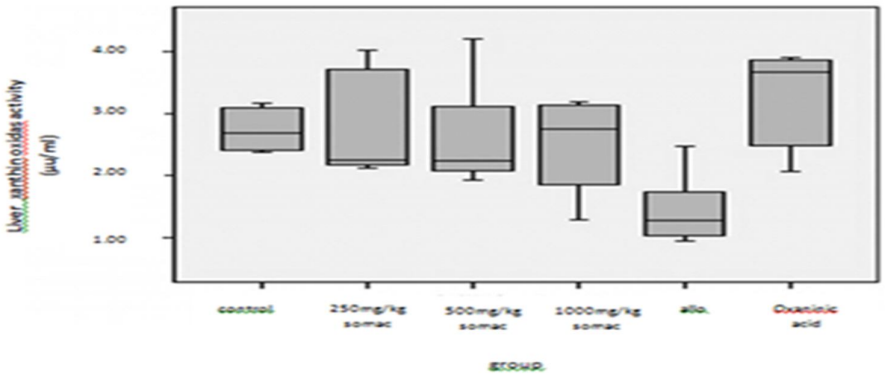

Figure 4. Box Plot chart for Liver xanthine oxidase activity showed heterogeneity between groups 2, 3 and 6 but homogeneity between other groups.

DISCUSSION

This research was done to find a plant compound to reduce serum uric acid levels. Uric acid-lowering effects of other herbs such as Biota Orientalis have been reported [33,37]. In a recent research, experimental animal model of hyperuricemia induced by potassium oxonate as an uricase inhibitor has been used to study drug action [38]. It was reported that intraperitoneal administration of some flavonoids such as rutin significantly reduced small and large intestinal transit in mice after one houre[39].

Xanthine oxidase/xanthine dehydrogenase is the key enzyme in purines' hepatic metabolic pathways. The reaction is the conversion of hypoxanthine to xanthine and uric acid [3]. Controlling uric acid levels is the most important factor in hyperuricemia prevention and treatment [40].Rasboricase and allopurinol are the xanthine oxidase enzyme inhibitors in clinical use. Allopurinol was the first uric acid lowering drug for the past 4 decades utilized in the treatment of hyperuricemia and gout [3]. Unfortunately allopurinol causes some deleterious side effects such as allergic reactions, gastrointestinal intolerance, and central nervous system dysfunction, and it possess some medical constraints [1, 41]. It is a hope to create a scientific source for persons studying in the fileld of ethnobotany, pharmacology and chemistry by comparing knowledge resulted from traditionally used herbs with previous laboratory studies.This research focused on finding an alternative or complementary plant origin drugs. Research on evaluation of this plant extractand it´s activities necessitates focusing on it´s crucial phytoconstituents, as well as, it´s possible toxicity. As we are aware, no similar study like this to investigate the effects of sumac extract on serum uric acid levels has been done, despite the fact that this herb has been traditionally used for several centuries in some parts of the word in combination with the diets that are meat-based. In book "zakhirekharazmshahi"[42], consumption of sumac plant with red meat has been emphesized, as the major risk factor for gout.

In our study, we observed a hypouricemic effect for sumac, even though it was not as strong as Allouporinol.But due to the observed relative increase in the hypouricamic effects of sumac extract by increased gavaged doses to rats, it could be expected to have a significant hypouricemic effect comparable to allopurinol by administrating higher concentrations of sumac extract. The other possibility is to conduct more investigations to extract the effective compound of sumac which can reduce serum uric acid with a hope to provide a safer drug for hyperuricemia as well as gout.

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