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Lipid-lowering effect of *Rhus coriaria* L. (sumac) fruit extract in hypercholesterolemic rats

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Hyperlipidemia is a major risk factor for development of atherosclerosis. In the present study, the hypolipidemic effects of sumac (*Rhus coriaria* L.) fruits in high cholesterold diet (HCD)-fed rats was investigated. There was a significant ($p < 0.001$) increase in the levels of total cholesterol (TC) and triglycerides (TG) along with augmented activities of serum aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase. Treatment with aqueous methanol extract of sumac fruits reduced the above alterations observed in hypercholesterolemic rats. Sumac extract also reversed the hypertrophic cardiac histology. Furthermore, *in vivo* toxicological studies showed no evidence of acute toxicity of the extract in male Wistar rats. In conclusion, sumac fruit extract intervention minimized the lipid abnormalities and abnormal biochemical changes induced in HCD fed rats. This shows that sumac fruit extract possesses cardioprotective and hepatoprotective activities which will be beneficial in hypercholesterolemic condition.

1. Introduction

Atherosclerosis is the major cause of ischemic heart disease, stroke, and death (Lovegrove and Jackson 2000). It is well established that elevated serum lipid levels constitute a primary risk factor for atherosclerosis (Castelli et al. 1986). It has also been observed that hypercholesterolemia (HC) leads to intracellular lipid accumulation in cardiomyocytes and some alterations in the structure and properties of the myocardium in experimental rats (Hexeberg et al. 1993). Dietary plants with cholesterol lowering activity, in particular, are considered useful in preventing cardiovascular disorders such as atherosclerosis.

Sumac (*Rhus coriaria* L.; family Anacardiaceae) is commonly used in the Mediterranean region and Middle East as a spice and also as a medicinal herb (Sezik et al. 1991; Brunke et al. 1993). The spice is produced by grinding dried fruits. Sumac fruits contain flavonol, phenolic acids, hydrolysable tannins and anthocyanins (Guvenc and Koyuncu 1994; Mavlyanov et al. 1997). It has also been reported that the fruits of sumac contain organic acids, eg, malic, citric and tartaric acids (Brunke et al. 1993; Kossah et al. 2009). Fatty acids, vitamins and minerals exist in sumac fruits as well (Kossah et al. 2009). The fruits have been demonstrated to possess antimicrobial (Nassar-Abbas and Halkman 2004; Fazeli et al. 2007; Nimri et al. 1999); antioxidant (Ozcan 2003; Candan 2003; Candan and Sökmen 2004); hypoglycemic (Giancarlo et al. 2006) and hypouricemic (Candan 2003) properties. There are no studies providing evidence for its hypolipidemic and organ protective effects. The primary risk organs of hypercholesterolemia are the liver and heart (Suanarunsawat et al. 2010). Therefore, the present study was conducted to investigate the effects of *Rhus coriaria* fruit extract on serum lipids status, serum marker enzymes, and microscopic analysis of cardiac tissue in hypercholesterolemic condition in experimental rat model. Furthermore, there are few reports on the toxicological studies of *Rhus coriaria* L. fruits (Sökmen 2001), so possible acute *in vivo* toxicity of the extract was assessed in Wistar rats. A toxicological study was performed to identify any potential hazards that might arise from the plant's use.

Table 1: Effects of *Rhus coriaria* L. (sumac) fruits extract on serum lipids in rats fed on a high cholesterol diet (HCD). Values are expressed as mean ± S.E.M for six animals.

Groups	Dose (mg/kg/day)	TC (mg/dl)	TG (mg/dl)
Normal control	–	59.03 ± 4.95	80.25 ± 6.76
HCD control	–	$154.00 \pm 1.73^{\#}$	$140.67 \pm 7.04^{\#}$
Sumac extract	100	$102.07 \pm 6.57^{¥}$*	75.37 ± 5.82**
Sumac extract	200	$101.38 \pm 8.95^{¥}$**	62.03 ± 7.06**
Atorvastatin	50	$105.76 \pm 4.89^{\#}$*	72.04 ± 7.28**

TC = total cholesterol; TG = triglyceride; $^{¥}p < 0.01$, $^{\#}p < 0.001$ *vs.* normal control value, $^{*}p < 0.01$, $^{**}p < 0.001$ *vs.* HCD control value.

2. Investigations and results

2.1. Serum lipid profile in HCD-fed rats

The serum lipid profiles of the rats fed the high cholesterol diet (HCD) and treated with oral doses of an aqueous methanol extract of sumac fruits (100 and 200 mg/kg/day) for 15 days are summarized in Table 1. Serum total cholesterol (TC) and triglyceride (TG) levels in the HCD-fed control rats markedly increased, whereas oral treatment of both doses of the extract led to significant decrease in lipid levels. The lower dose of

Table 2: Effect of *Rhus coriaria* L. (sumac) fruits extract on serum level of high cholesterol diet (HCD)-induced injury marker enzymes. Values are expressed as mean ± S.E.M for six animals.

Groups	Dose (mg/kg/day)	ALT U/L	AST U/L	LDH U/L
Normal control	–	65.77 ± 6.50	141.78 ± 7.63	913.72 ± 26.72
HCD control	–	94.00 ± 2.45#	186.25 ± 2.17#	1336.39 ± 54.13#
Sumac extract	100	66.22 ± 3.87*	150.75 ± 12.27	1213.55 ± 83.80
Sumac extract	200	63.04 ± 2.51*	134.25 ± 8.94*	1096.67 ± 73.79
Atorvastatin	50	101.99 ± 8.04	157.84 ± 7.17	1145.67 ± 131.28

ALT = alanine transaminase; AST = aspartate transaminase; #$p < 0.05$ *vs.* normal control value, *$p < 0.01$ *vs.* HCD control value.

the extract (100 mg/kg/day) reduced serum TC and TG levels by 33.72% and 46.42%, respectively, while the higher dose (200 mg/kg/day) caused a non-significant greater reduction, i.e., 34.17% in TC and 55.92% in TG serum levels. The reductions in TC and TG levels were comparable to those achieved with atorvastatin.

2.2. Serum lipid profile in rats fed on normal diet

The effects of sumac extract on serum lipid profile in rats fed on normal diet were also studied. Supplementation of the extract caused a non-significant decrease in both TC and TG serum levels (data not shown). Atorvastatin also non-significantly lowered serum TC and TG concentrations in normal diet-fed rats.

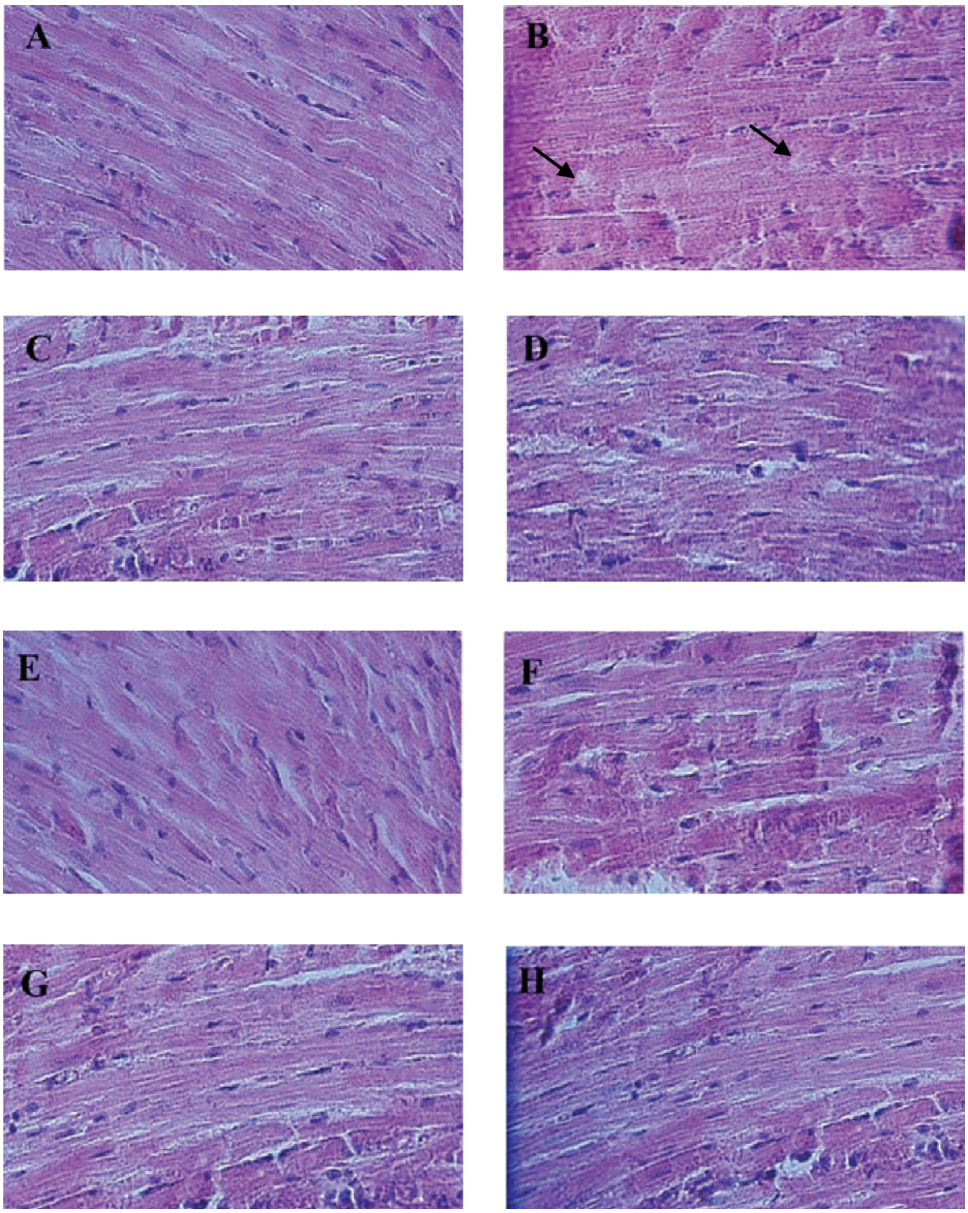

Fig.: Histopathological observation of heart tissue: (A) Group I: normal control showing normal myocytes; (B) Group IV: high cholesterol diet (HCD) control showing marked hypertrophy with cellular and nuclear changes and lipid droplets (arrow); (C and D) Group II and III: sumac extract (100 and 200 mg/kg/day) supplemented groups showing mild cellular atrophy; (E) Group V: sumac extract (100 mg/kg/day) treated HCD-fed rats showing a mild reversal of hypertrophic changes with fewer lipid droplets; (F) Group VI: sumac extract (200 mg/kg/day) treated HCD-fed rats showing a moderate reversal of hypertrophic changes with very few lipid droplets; (G) Group VII: atorvastatin (50 mg/kg/day) treated HCD-fed rats showing moderate reversal of hypertrophic changes with few lipid droplets and (H) Group VIII: atorvastatin treated normal fed rats showing near normal myocyte with mild cellular atrophy (H & E staining, 400 Magnification)

Table 3: Histopathological evaluation of left ventricular myocytes obtained from control and experimental groups on score base.

Criteria	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Structural changes	0	1	1	3	2	1	1	2
Cellular atrophy	0	1	1	0	0	0	1	0
Cellular hypertrophy	0	0	0	3	2	1	0	1
Lipid droplet	0	0	0	3	2	1	0	1
Nuclear changes	0	0	0	3	2	1	0	2
Cytoplasmic changes	0	0	0	3	2	1	0	2

Scores for assessment of microscopic myocytes damage: none (0); mild (1); moderate (2); severe (3) ***Group I***: normal control ***Group II* and *III***: normal diet fed rats supplemented with sumac extract (100 and 200 mg/kg/day, respectively, PO) ***Group IV*:** high cholesterol diet (HCD) control ***Group V* and *VI*:** HCD fed group treated with sumac extract (100 and 200 mg/kg/day, respectively, PO) ***Group VII* and *VIII*:** normal diet and HCD fed groups, respectively, treated with atorvastatin (50 mg/kg/day, PO)

2.3. Evaluation of injury marker enzymes in HCD-fed rats

The levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as liver marker enzymes in control and experimental groups are presented in Table 2. Aminotransferases were increased ($p < 0.05$) in HCD-fed rats. On administration of both doses of sumac extract, the above changes were reverted to near normal. The serum levels of AST and ALT were higher in atorvastatin-treated HCD-fed rats compared to sumac extract-treated groups. Also, HCD significantly raised serum lactate dehydrogenase (LDH) as cardiac injury marker enzyme. Sumac extract treatment caused a non-significant reduction in the enzyme level.

2.4. Histopathological observation of heart tissue

Histopathological changes of the left ventricle of control and experimental group hearts are presented in the Fig. (Photomicrographs A to H) and Table 3. Normal control animals showed normal myocardium fibers (A) whereas a structural analysis of cardiac tissue revealed a varying degree of abnormal changes in HCD-fed rats. In this group, thickening of cardiac cells, cardiac muscle hypertrophy, lipid droplets, nuclear and cytoplasmic changes were seen (B). In rats fed on normal diet when supplemented with sumac extract 100 and 200 mg/kg/day (C and D, respectively), no obvious changes, except a mild cellular atrophy, was observed while sumac extract treatment in HCD-fed rats caused a mild (at dose 100 mg/kg/day) (E) or moderate (at dose 200 mg/kg/day) (F) reversal of hypertrophic changes. Also, treatment with the lower dose of the extract in HCD-fed rats showed fewer lipid droplets interspersed within myocardium fibers while treatment with the higher dose demonstrated very few lipid droplets. Administration of atorvastatin (50 mg/kg/day) to normal diet-fed rats showed near normal myocytes with a mild atrophy (H) while in HCD-fed rats, it caused a moderate reversal of hypertrophic changes and also a marked decrease in fat droplets (G).

2.5. Acute toxicity of the extract

Oral treatment of rats with ascending doses (0.5, 1, 3, 5 g/kg/day) of sumac fruit extract showed no lethality or adverse toxic signs during the experimental period so LD50 could not be determined. AST, ALT, and LDH serum levels were obtained in control and experimental groups (data not shown). No significant differences were observed among control and test groups.

3. Discussion

Hyperlipidemia is the most important risk factor for atherosclerosis (Wouters et al. 2005) and it has been shown that atherosclerosis could be suppressed by regulating the level of serum cholesterol. Recently various plants were shown to be helpful in lowering serum cholesterol levels (Choudhary et al. 2005; Bursill et al. 2007; Tan et al. 2011). In the present study, the effects of aqueous MeOH extract of *Rhus coriaria* L. (sumac) fruits on the serum lipid profile of hypercholesterolemic rats were investigated. Rats fed with a diet supplemented with 4% cholesterol and 1% cholic acid for 30 days served as the experimental animal model. Cholic acid improves cholesterol absorption by its emulsifying property and also has an inhibitory action on hepatic cholesterol 7-α hydroxylase activity (Beynen et al. 1986).

In the present study, the levels of TC and TG were markedly increased in HCD-fed rats. The high concentrations of TC and TG were significantly reduced after oral administration of sumac fruit extract (33.72% and 46.42%, respectively, with the lower dose; and a greater reduction, i.e. 34.17% and 55.92%, respectively, with the higher dose). No significant differences in efficacy were noted between the two dose groups. Our findings indicated that the extract was more efficacious in reducing TG values than TC levels. The efficacy of sumac extract in lowering serum lipids was comparable to that of atorvastatin. Results obtained by doing experiments in rats fed on normal diet indicated that the extract could only produce a minor lipid lowering activity in normolipidemic rats.

It is widely known that both the liver and heart are at risk in patients with hypercholesterolemia (Suanarunsawat et al. 2010). Our results showed that HCD suppressed hepatic and cardiac functions as expressed by an augmentation of serum AST, ALT and LDH. Sumac extract treatment was able to normalize the high serum levels of AST and ALT whereas atorvastatin-treated HCD-fed rats showed higher enzyme levels as could be anticipated (Liu et al. 2010). Serum levels of LDH were also lowered by treatment with sumac extract in HCD-fed rats, however, the values did not return to normal levels.

Dietary cholesterol exerts a major influence on the physiology and pathology of the cardiovascular system (Simons 1986). The cardiac abnormalities induced by HCD are confirmed by histopathological findings. HCD-fed rat heart was found to exhibit marked hypertrophy with cellular and nuclear enlargement. Cardiac hypertrophic changes in hypercholesterolemic rats have been reported earlier (Deepa and Varalakshmi 2004; Sudhahar et al. 2007). Sumac extract treatment caused a mild (at lower dose) to moderate (at higher dose) reversal of the hypertrophic changes. In addition, the number of lipid droplets in HCD-fed rat heart increased. Hexeberg et al. (1993) reported that in high-cholesterol fed rats, lipids accumulated in the myocardial cells. Increase in the accumulation of lipid droplets may lead to swelling of mitochondria as well as some other pathological changes (Melax and Leeson 1975). Sumac extract treatment, by decreasing the plasma cholesterol levels, proved to be effective in alleviating the cardiac damage associated with hypercholesterolemia. A reduction in lipid droplets in cardiac tissue on

treatment with sumac extract was demonstrated. Antioxidant activity of sumac fruits MeOH extract against lipid peroxidation and free radicals has also been reported previously (Candan and Sökmen 2004; Pourahmad et al. 2010). Taken together, these findings indicate that sumac fruit extract may prevent the development of atherosclerosis. It also possesses cardioprotective and hepatoprotective activities which will be beneficial in hypercholesterolemic condition. The bioactive component(s) responsible for the lipid-lowering effect of sumac fruits is not currently identified, however, since the whole fruit powder is used as a spice and dietary supplement, its beneficial properties may be of clinical value.

To investigate any potential hazards that might arise from the plant's use, toxicological studies were also performed. No evidence of acute toxicity was found in male Wistar rats. Reports of sumac toxicity are very rare. In the only toxicological report found in the literature, *Rhus coriaria* L. fruit extract did not show toxicity in a brine shrimp bioassay (Sökmen 2001).

In summary, sumac (*Rhus coriaria* L.) fruit extract was able to decrease high serum lipid concentrations, alleviate the abnormally elevated cardiac lipid levels and modulate some enzymic indices and microscopic changes in the hypercholesterolemic conditions. The use of sumac for its beneficial properties in preventing atherosclerotic cardiovascular diseases seems to be devoid of toxicity.

4. Experimental

4.1. Plant materials

Dried sumac (*Rhus coriaria* L.) fruits were purchased from a local market in Tehran, Iran, and authenticated by Dr Gholamreza Amin at the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. A voucher specimen (PMP-615) was deposited at the Herbarium of the Department of Pharmacognosy.

4.2. Extract preparation

Sumac dried fruits were ground into powder using a household flour mill. The fruit powder (8 g) was extracted with 100 ml of 80% (v/v) aqueous MeOH (Merck, Germany) by cold maceration for 72 h. The extract was then filtered and concentrated to dryness *in vacuo* at 45 °C. After the extraction process, the percent yield of the dried crude extract was 40.8% of sumac dried fruits. The resulting extract was kept in the dark at 4 °C until tested. The extract was dissolved in distilled water for experimental use.

4.3. Animal model

Male Wistar rats weighing 130–150 g were purchased from Iran Pasture Institute. The rats were cared for in accordance with guidelines of the Institutional Animal Ethics Committee. The rats were housed in a controlled environment with approximate room temperature at 25 ± 2 °C, with 12 h/12 h day-night cycle and had free access to water and commercially prepared pellet chow.

4.4. Experimental hyperlipidemic diet

The high-cholesterol diet was prepared by mixing the normal basal diet with 4 g% cholesterol powder and 1 g% cholic acid. It was then pelleted by using corn oil.

4.5. High-cholesterol diet-induced hyperlipidemic model

In order to induce hyperlipidemia, the animals were fed a high-cholesterol diet for 30 days. To identify the induction of hyperlipidemia, on day 15 blood was collected from the tail vein to analyze lipid profile. The rats were divided into eight groups of six animals each. ***Group I*****:** normal diet fed rats served as normal control; ***Group II*** and ***III***: normal diet fed rats received sumac extract (100 and 200 mg/kg/day, respectively, PO); ***Group IV***: HCD fed rats served as HCD control; ***Group V*** and ***VI***: HCD fed rats treated with sumac extract (100 and 200 mg/kg/day, respectively, PO); ***Group VII*** and ***VIII***: normal diet and HCD fed rats, respectively, treated with atorvastatin (50 mg/kg/day, PO). The treatments commenced 15 days after the start of experimental period.

4.6. Tissue sampling

At the end of experimental period, and after 12 h fasting, the rats were anesthetized with diethyl ether and blood was collected from the heart. All the animals were then sacrificed by cervical decapitation. Heart tissues were immediately excised and rinsed in ice cold physiological saline. The left ventricle of heart tissues were set aside for histopathological processing. The blood samples were centrifuged at 3000 g and 4 °C for 10 min. The serum was stored at −70 °C for later biochemical analysis.

4.7. Biochemical analysis

Serum levels of TC, TG, AST, ALT and LDH were determined by enzymatic colorimetric methods using commercial kits (Pars Azmoun Co., LTD, Iran). Serum AST and ALT were measured to evaluate liver function and serum LDH was also measured to assess cardiac function.

4.8. Histopathological studies

Portions of heart tissues were fixed in 10% formalin in phosphate buffered saline (PBS). The washed tissues were dehydrated in the ascending grades of ethanol and finally cleared in xylene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 μm thickness, stained with haematoxylin and eosin (H&E). The sections were then submitted for blinded histopathologic examination and grading. Ten random fields with objective of ×40 viewed under an Olympus photomicroscope (PROVIS AX70, Japan) equipped with a digital camera (DP11, Japan) for histopathological changes. Scores were calculated by averaging the mean score of each of the following pathological characteristics: no abnormality (0); mild (1); moderate (2); and severe (3) lesions.

4.9. Acute toxicity determination

This study aimed to evaluate the possible toxic effect of the extract following 14 days oral administration of different doses of the extract of *Rhus coriaria* L. fruits in rats. Male Wistar rats (100–110 g) were divided into five groups of three animals each. Control group was treated orally with distilled water (vehicle) while test groups were treated with 0.5, 1, 3 and 5 g/kg body weight of the extract. Toxicity of the extract was evaluated by side-cage observation, serum marker enzymes (AST, ALT, and LDH) measurement and incident of lethality.

4.10. Statistical analysis

The values are expressed as mean ± S.E.M. The data were analyzed by one-way ANOVA using SPSS® version 11.5, and the differences between the means were assessed using Tukey post hoc test. A non-parametric test was also used whenever needed. Statistical significance was considered at $p < 0.05$.

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