Antidiabetic properties of the ethanolic extract of Rhus coriaria fruits in rats

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ABSTRACT

Background and the purpose of the study: Fruits of Rhus coriaria L. (Anacardiaceae) are traditionally used as a table spice in Iran and are highly recommended for diabetic patients. The purpose of this study was to determine the antidiabetic properties of the ethanolic extract of Rhus coriaria fruits and also its mechanisms of action.

Methods: The effects of ethanolic extract of Rhus coriaria fruits were measured on blood glucose, lipids and antioxidant enzymes by commercial kits. mRNA levels of insulin (INS) and glucose transporter type-4 (GLUT-4) genes were investigated by RT-PCR (Reverse transcription-polymerase chain reaction) technique. Moreover, its effects on intestinal α-glucosidases was measured using an in vitro method.

Results and Conclusion: Following a single dose administration of the extract it was found that extract could significantly reduce postprandial blood glucose by 24% (at 5 hrs). In the long term experiment, on the day of 21, postprandial blood glucose (PBG) was found to be significantly lower (by 26%) compared to diabetic control group. The plant extract raised markedly serum high-density lipoprotein (HDL) by 34% and also reduced low-density lipoprotein (HDL) by 32%. Also it had noticeable antioxidant effects by elevating superoxide dismutase (SOD) and catalase (CAT) activities by 46% and 77%, respectively. However it did not show a strong effect on glutathione peroxidase (GPX) activity. The extract inhibited maltase and sucrase activities by 44% and 27%, respectively. However it made no changes in the transcript levels of INS and GLUT-4 genes.

It can be concluded that constituents of Rhus coriaria fruits have effective components which can be utilized as useful herb for alleviation of diabetes complications.

Keywords: Antioxidant, Blood Glucose, α-Glucosidase, Lipids.

INTRODUCTION

Type 2 diabetes is a major public health concern, causing tens of millions of chronic illnesses and a significant number of deaths worldwide each year (1). It is characterized by hyperglycemia and profound alterations in the plasma lipids and lipoproteins profiles (2). Also antioxidant defense system which normally modulates the level of oxidative stress (3) is altered in diabetes (4) and it involves in the pathogenesis of diabetes and its complications (5). Therefore glycemic, lipids profiles and oxidative stress control is fundamental for the management of diabetes (6).

In Iran, Rhus coriaria is traditionally used as a table spice especially along with rich dishes and is highly recommended for adjustment of the blood lipids in diabetic patients.

Previous studies on this plant have shown that its fruits (7) and leaves (8) have in vitro antioxidant properties. Also it has already been reported that in vitro hypoglycemic activity of the methanolic extract of fruits of Rhus coriaria is due to inhibition of α-amylase (87% inhibition at 50 μg/ml) (9).

In this study the antihyperglycemic, hypolipidemic and anioxidant activities of the ethanolic extract of Rhus coriaria fruits on diabetic male Wistar rats was investigated in order to verify the use of this herbal medicine in treatment of type 2 diabetes.

MATERIAL AND METHODS

Chemicals and reagents
DEPC Water, Taq polymerase and RNA extraction kit were purchased from Cinnagen (Tehran, Iran). DNase I, RNase free kit was from Fermentas (Ontario, Canada), RT kit and Primers were from Bioneer (Daejon, Korea), dNTPs were from BioFlux (Tokyo, Japan). The kits for determination of glucose, triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) were from Chem Enzyme.

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Antioxidant, Blood Glucose, α-Glucosidase, Lipids.

In this study the antihyperglycemic, hypolipidemic and antioxidant properties of the ethanolic extract of \textit{Rhus coriaria} fruits were measured on blood samples of diabetic rats. The extract was found to significantly reduce blood glucose, lipids and antioxidant enzymes by commercial kits. mRNA levels of insulin (INS) and glucose transporter-4 (GLUT-4) were measured using an in vitro method.

**Measurement of RE effects on oral glucose tolerance**

NC and DC Groups received distilled water orally. Animals of REa+D and REb+D groups received orally RE, 200 and 400 mg/kg BW respectively. To group Ac+D was given the reference drug, acarbose, (20 mg/kg BW). Thirty min later, each rat received orally a carbohydrate solution (equal proportion of maltose and sucrose) (2 g/kg BW). PBG of each animal was determined at 0 min, just before carbohydrate solution loading, and at 30, 60 and 120 min after that, using a glucometer (On Call Now, San Diego, USA).

**Gene expression analysis**

Pancreas and heart left ventricle tissues of DC, NC and REb+D groups were dissected from rats immediately after sacrificing, placed in liquid nitrogen, and then stored at -70°C until used. Total RNA of tissues were extracted by RNX-Plus kit according to its manual. The DNA free RNA was prepared prior to RT-PCR using DNase I, RNase-free kit. For reverse transcription to obtain cDNA, AccuPower RT PreMix kit, 50 ng/μl template RNA and 25 ng/μl oligo dT18 were used. The primers were as follows: INS F, 5′-TTCTTCTACACACCCAG-3′; INS R, 5′-GCAGTAGTTCTCCAGTTG-3′ (155-bp) GLUT-4 F, 5′-AGGCAACCGTCCTCTTT-3′; GLUT-4 R 5′-GACAGAAAGGGCAACAGAAGC-3′ (318-bp); and B-act F, 5′-AGCCATGTACGTAGCCA-3′; B-act R, 5′-TCTCAGCTGTTGTTGGAAC-3′ (248-bp). For PCR reaction, 500 ng of the cDNA was added to a PCR reaction mixture consisting of 10X PCR buffer (2.5 μl), 50 MmMgCl₂ (0.75 μl), 10 mM dNTPs (0.5 μl), 10 pM of paired primers (0.5 μl of each), 0.25 units of Taq polymerase and distilled water in a total volume of 25 μl. The reaction mixture was overlaid in a PCR thermal cycler for 35 cyclic reactions. PCR products were run on 1.5% agarose gel.
agarose gels, stained with ethidium bromide and photographed. Images of radiographs were analyzed with TotalLab v1.10 using 1D analysis.

In vitro inhibition assay for rat intestinal sucrase and maltase activities

The inhibitory effects of RE on rat intestinal sucrase and maltase activities were determined using a literature method (14).

Statistical analyses

All data are presented as means±S.D. for six rats in each group. Comparison between groups and between time points was made by one-way analysis of variance (ANOVA) followed by Duncan’s test using SPSS (SPSS Inc, Chicago, USA).

RESULTS AND DISCUSSION

Short-term effects of RE on PBG levels

Administration of the extract at the dose of 400 mg/kg reduced the glucose level at 5 hrs time point compared to both initial level and DC group (Table 1).

Effects of RE on oral glucose tolerance

As shown in table 2, diabetic rats treated with 200 and 400 mg/kg RE showed remarkable decrease in blood glucose at the first hour after carbohydrate solution loading compared to DC group; however they were not as effective as acarbose.

Long-term effects of RE on PBG

The RE at the dose of 400 mg/kg induced significant reductions in PBG levels in diabetic rats, after 7, 14 and 21 days of treatment (Table 3), compared to initial time point. The data of REb+D and Met+D groups were significantly different from those of DC group (p<0.0001).

Effects of RE on rat intestinal sucrase and maltase activities

The crude ethanolic extract of Rhus coriaria fruits showed strong inhibitory effects for both sucrase and maltase activities, 25.38%±2.05 and 44.33%±1.14 respectively.

Effects of RE on INS and GLUT-4 genes expression

Densitometric scanning revealed no increase in INS (p<0.156) and GLUT-4 (p>0.564) transcripts by RE, as compared to DC group.

Effects of RE on blood lipids

Treatment with RE (400 mg/kg) strongly increased the level of HDL, in a way that it was comparable to NC rats (p>0.988). Also it reduced the

Table 1. Acute effect of the ethanolic extract of Rhus coriaria fruits on postprandial blood glucose levels in Alloxan-diabetics rats.

| Groups   | Dose (mg/kg BW) | Blood Glucose (mg/dl) |
|----------|-----------------|------------------------|
|          |                 | 0  | 30  | 60  | 120 |
| NC       | -               | 72±2.8  | 133.8±31.1 | 126.2±28.5 | 90.6±6.1 |
| DC       | -               | 157.6±10 | 347.2±19.9 | 308.6±12.1 | 247±9.3  |
| REa+D    | 200             | 155.4±8.4 | 318.4±18.3 | 257.6±14.7 | 236±12.8  |
| REb+D    | 400             | 175±9.7  | 328.8±13.3 | 232.3±10.8 | 232±16.0  |
| Ac+D     | 20              | 154.4±6.8 | 234.4±10.5 | 172±6.2 | 162.8±5.6  |

NC indicates Normal Control; DC, Diabetic Control; RE+D, Diabetic rats treated with ethanolic extract of Rhus coriaria; Ac+D, Diabetic rats treated with Acarbose. *p<0.0001, *p>0.106 and *p>0.065 vs. DC.
Table 3. Glycemic control by ethanolic extract of Rhus coriaria fruits in Alloxan-diabetic rats after 3 weeks treatment.

| Groups     | Dose (mg/kg BW) | Postprandial blood glucose (mg/dl) Days after single dose treatment daily |
|------------|-----------------|----------------------------------------------------------------------------|
|            |                 | 0   | 7   | 14   | 21   |
| NC         | -               | 76.8±2.8<sup>a</sup> | 82±5<sup>c</sup>d | 77±6<sup>c</sup>d | 83.5±5<sup>c</sup>d |
| DC         | -               | 324.8±7.2<sup>a</sup> | 347±10.3<sup>b</sup> | 364±8.1<sup>a</sup> | 353.3±11.1<sup>a</sup> |
| RE<sub>a</sub>+D | 200            | 337.4±15.4<sup>a</sup> | 319.2±11.9<sup>c</sup>d | 311.6±19.4<sup>c</sup>d | 314.7±22.7<sup>c</sup>d |
| RE<sub>b</sub>+D | 400            | 331.4±19.6<sup>a</sup> | 282.2±17.7<sup>c</sup>d | 269.8±16.3<sup>c</sup>d | 261.6±22.4<sup>c</sup>d |
| Met+D      | 100             | 318.2±10.2<sup>a</sup> | 251.3±6.7<sup>c</sup>d | 213.1±5.9<sup>c</sup>d | 190.2±8.2<sup>c</sup>d |

NC indicates Normal Control; DC, Diabetic Control; RE+D, Diabetic rats treated with ethanolic extract of Rhus coriaria fruits; Met+D, Diabetic rats treated with Metformin. *p<0.0001, †p<0.004 and ‡p<0.141 vs. Time 0; §p<0.0001, ¶p<0.001 and ‖p<0.065 vs. DC.

Table 4. Effects of the ethanolic extract of Rhus coriaria fruit on plasma lipids of Alloxan-diabetic rats, after 21 days of treatment.

| Groups     | Dose (mg/kg BW) | Serum lipids (mg/dl) |
|------------|-----------------|----------------------|
|            |                 | TG | TC | HDL | LDL | VLDL |
| NC         | -               | 82.2±5.6<sup>a</sup> | 81.4±2.8<sup>a</sup> | 45.8±3.2<sup>a</sup> | 19.2±4<sup>a</sup> | 16.4±1.1<sup>a</sup> |
| DC         | -               | 144.2±5.3<sup>a</sup> | 112.6±9<sup>a</sup> | 35.5±5.6<sup>a</sup> | 48.8±13.8<sup>a</sup> | 28.8±1<sup>a</sup> |
| RE<sub>a</sub>+D | 200            | 141.6±7.5<sup>a</sup> | 111.7±4.8<sup>a</sup> | 40.8±8.3<sup>a</sup> | 42.6±9.5<sup>a</sup> | 28.3±1.5<sup>a</sup> |
| RE<sub>b</sub>+D | 400            | 137.4±6.1<sup>a</sup> | 107.8±6.4<sup>a</sup> | 47.2±7<sup>a</sup> | 33.2±6.5<sup>a</sup> | 27.4±1.2<sup>a</sup> |
| Met+D      | 100             | 131.4±3.3<sup>a</sup> | 102.1±6.7<sup>a</sup> | 37.1±3.7<sup>a</sup> | 38.3±9.8<sup>a</sup> | 26.3±6<sup>a</sup> |

NC indicates Normal Control; DC, Diabetic Control; RE+D, Diabetic rats treated with ethanolic extract of Rhus coriaria fruits; Met+D, Diabetic rats treated with Metformin. *p<0.0001, †p<0.001, ‡p<0.031 and †p<0.086 vs. DC; ¶p<0.988 vs. NC.

Table 5. Changes in the activities of superoxide dismutase, glutathione peroxidase and catalase of erythrocytes of Alloxan-diabetic rats made ethanolic extract of Rhus coriaria fruits.

| Groups     | Dose (mg/kg BW) | Blood antioxidant enzymes |
|------------|-----------------|--------------------------|
|            |                 | SOD (U/g Hb) | GPX (U/g Hb) | CAT (K/g Hb) |
| NC         | -               | 2978±95.2<sup>a</sup> | 55.6±7.2<sup>a</sup> | 1.441±0.09<sup>a</sup> |
| DC         | -               | 1916.8±22.1<sup>a</sup> | 31.91±5.54<sup>a</sup> | 0.508±0.031<sup>a</sup> |
| RE<sub>a</sub>+D | 200            | 2455.7±83.4<sup>a</sup> | 30.84±6.62<sup>a</sup> | 0.755±0.053<sup>a</sup> |
| RE<sub>b</sub>+D | 400            | 2793.8±90.2<sup>a</sup> | 35.45±1.7<sup>a</sup> | 0.901±0.069<sup>a</sup> |
| Met+D      | 100             | 2048.1±19.8<sup>a</sup> | 39.33±4.59<sup>a</sup> | 0.533±0.034<sup>a</sup> |

NC indicates Normal Control; DC, Diabetic Control; RE+D, Diabetic rats treated with ethanolic extract of Rhus coriaria fruits; Met+D, Diabetic rats treated with Metformin. *p<0.0001, †p<0.004 and ‡p<0.107 vs. DC.

Effects of RE on antioxidant enzymes activities
As presented in table 5, the RE-treated groups showed no remarkable change in GPX activity, however a strong increase was observed in SOD and CAT activities at the dose of 400 mg/kg compared to DC group.

**DISCUSSION**

Previous findings demonstrated that some genus of *Rhus* like *Rhus chirindensis* (15) and *Rhus chinensis* (16) have hypoglycemic properties. In the Alloxan-induced diabetic rats, administration of the extract of *Rhus coriaria* fruits produced a statistically significant acute and long-term decrease in postprandial blood glucose concentration, which were not comparable to the effects of the reference drugs. It has already been reported that *rhus coriaria* might have hypoglycemic activity by inhibition of alpha-amylase, a glycoside hydrolase (9).Results of this study showed no change in the INS and GLUT-4 genes expression, so antihyperglycemic effects of *Rhus coriaria* fruits may be related to modulation of insulin secretion or action (17). OGTT results and the ability of inhibiting the α-glucosidases activities by the extract indicate that control of postprandial glucose level might be mediated through the inhibition of carbohydrate digestion or absorption (16).

Oral administration of ethanolic extract of *Rhus*
coriaria fruits increased SOD and CAT levels of the red blood cells. This finding upholds the in vitro antioxidant activity of Rhus coriaria demonstrated previously (7). Antioxidant activity of Rhus coriaria could also be useful for prevention of diabetes complications due to hyperglycemia (11). This study aside from Rhus coriaria antihyperglycemic and antioxidant activities indicated that extract may also reverse dyslipidemia associated with diabetes by increasing the HDL level and decreasing the LDL level similar to some other herbs previously described (18).

In conclusion, the use of Rhus coriaria in Iranian folk medicine as a hypolipidemic agent has a significant correlation with the scientific data generated in this study. Also the present study explains two other properties of Rhus coriaria fruits on diabetic rats which can improve the life of type 2 diabetic patients: mild antihyperglycemic and potent antioxidant peroperties. Further studies are required to show the active constituent(s) of Rhus coriaria.

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