Antihyperglycemic, antidyslipidemic and antioxidant activity of *Rhus coriaria* in STZ-induced type 2 diabetic rats

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The potential role of methanolic extract of *Rhus coriaria* L. on hyperglycemia, dyslipidemia and lipid peroxidation (LPO) was studied in type 2 diabetic rats. Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 100 mg/kg) to 2 days old rat pups. *R. coriaria* (200 and 400 mg/kg) was administered orally once a day for 5 weeks after the animals were confirmed diabetic (that is, 90 days after STZ injection). A group of citrate control rats were also maintained which has received citrate buffer on the 2nd day of their birth. Administration of *R. coriaria* extract showed a significant *(P < 0.001)* decrease in blood glucose and tissue MDA levels as compared to type 2 diabetic control rats. The levels of total cholesterol (TCh), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and very low density lipoproteins cholesterol (VLDL-C) were also significantly *(P < 0.001)* decreased by *R. coriaria* treatment when compared with type 2 diabetic control rats. In contrast the high density lipoprotein cholesterol (HDL-C) levels were significantly *(P < 0.001)* increased after treatment with *R. coriaria* extract when compared with type 2 diabetic control rats. These results revealed that *R. coriaria* possesses significant antihyperglycemic and antidyslipidemic activity along with potent antioxidant potential in type 2 diabetic mellitus.

Key words: Antidyslipidemic, antihyperglycemic, diabetes mellitus, *Rhus coriaria*, streptozotocin.

INTRODUCTION

Type 2 diabetes mellitus (DM) is one of the leading problems for the primary health-care system, within which most patients with this disease are treated. Epidemiologic data indicates that an increased level of glucose and lipids is an important predictor of future diabetic complications (O’Keefe and Bell, 2007). Prevention of the development of type 2 DM and its complications is the only way of preventing the problem from becoming too great for society to bear. Dyslipidemia is very early feature of metabolic syndrome and is the most important risk factor for cardiovascular disease (CVD) in diabetes (Niemeijer-Kanters et al., 2001). The key feature of diabetic dyslipidemia is the elevation of serum total cholesterol (TCh), triglycerides (TG) and low-density lipoprotein (LDL) levels associated with a reduced high-density lipoprotein (HDL) cholesterol. However pharmacological interventions are needed in more severe cases of dyslipidemia and hyperglycemia (Brunzell and Ayyobi, 2002).

In recent years, there has been a noteworthy increase in the use of indigenous drugs for the cure and prophylaxis of various diseases. The conventional pharmacological treatments for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure (Kim et al., 2006). Recently, attention to natural products has increased once again, but there is a need for thoroughly controlled studies on the effectiveness and potential risks of treatment with such products. Medicinal plants with antidiabetic activities are being used for many centuries and sometimes as
regular constituents of the diet, it is assumed that they do not have many side effects (Halim, 2002). Therefore, investigation on such agents from traditional medicinal plants has become more important.

*R. coriaria* L. (Anacardiaceae), commonly known as sumac (also spelled as sumach) is a well-known spice in the Middle-East and grown in the central and northern regions of Turkey (Çinbilgel and Gökçeoğlu, 2010; Ocak and Tokur, 2000). In Turkey, a garnish of *R. coriaria* is used to give a bitter taste in salads and dishes (Çakılcıoğlu and Türkoglu, 2010). In folk medicine, it is used for the treatment of indigestion, anorexia, diarrhoea, haemorrhage, hyperglycemia, antipyretic, diarrhoea, digestive, disinfect wounds, gingival, hemorrhoids, throat inflammations, rheumatism, weeping skin lesions and used as an antiseptic or to increase saliva (Fakir et al., 2009; Wetherilt and Pala, 1994). *R. coriaria* extracts were reported to have strong antimicrobial effects (Dıgrak et al., 2001). Previous studies showed that *R. coriaria* results in moderate nitrate values (Çakılcıoğlu, 2011). Recently, the hypoglycaemic efficacy of sumac (*R. coriaria* L.) has been investigated through inhibition of a glycoside hydrolase: alpha-amylase in the digestive, disinfect wounds, gingival, hemorrhoids, throat inflammations, rheumatism, weeping skin lesions and used as an antiseptic or to increase saliva (Çakılcıoğlu and Khatun, 2011). Sumac extract seemed to be promising source of natural antioxidants (Ozcan, 2003). However, no scientific studies of *R. coriaria* on hyperglycemia, dyslipidemia and lipid peroxidation in type 2 diabetic rats have been carried out until now. Since hyperglycemia, dyslipidemia and lipid peroxidation are implicated in type 2 diabetes, therefore the present study was designed to investigate the effects of *R. coriaria* on blood glucose, serum lipid profile and tissue lipid peroxidation (LPO) in type 2 diabetic rats.

**MATERIALS AND METHODS**

**Animals**

Healthy albino Wistar rats were kept for breeding. The animals were maintained under controlled condition of illumination (12 h light/12 h darkness) and temperature 20-25°C. They were housed under ideal laboratory conditions, maintained on standard pellet diet (Lipton rat feed, Ltd; Pune) and water *ad libitum* throughout the experimental period. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi, India.

**Preparation of plant extract**

*R. coriaria* L. seeds were collected freshly from the local market (Khari Bawli, Old Delhi) and identified by Dr. M. P. Sharma in the department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India. Dried *R. coriaria* seed was extracted with methanol at room temperature three times with 5 volumes of methanol (w/v). The solvent was evaporated under reduced pressure below 50°C to give a methanolic extract. A dark semi-solid (greenish black) material was obtained. It was stored at 4°C until used. When needed the residual extract was suspended in distilled water and used in the study.

**Drugs and chemicals**

Streptozotocin was procured from Sigma Chemicals, USA. Glucose, cholesterol and triglycerides kits were purchased from the Span Diagnostics Ltd. (India). All the other biochemicals and chemicals used for the experiment were of analytical grade.

**Induction of type 2 diabetes mellitus**

Type 2 diabetes mellitus was induced by single intraperitoneal injection of STZ (100 mg/kg, dissolved in citrate buffer, pH-4.5) to 2 days old rat pups (Murali et al., 2003). Another group of pups received only citrate buffer on the 2nd day of their birth. 90 days after STZ treatment, development of diabetes was confirmed by measuring blood glucose level. Rats with fasting blood glucose levels of 200 mg/dl or higher were considered to be diabetic.

**Experimental design**

The rats were divided into five groups comprising of six animals in each group as follows:

- **Group I:** Citrate control rats, received citrate buffer (0.1 ml/10g, i.p)
- **Group II:** Type 2 diabetic control rats, received STZ in a single dose (100 mg/kg, i.p)
- **Group III:** Only *R. coriaria* treated rats, received *R. coriaria* (400 mg/kg, p.o)
- **Group IV:** Type 2 diabetic treated rats, received *R. coriaria* (200 mg/kg, p.o)
- **Group V:** Type 2 diabetic treated rats, received *R. coriaria* (400 mg/kg, p.o)

*R. coriaria* (200 and 400 mg/kg) was dissolved in water and given until the end of the study (5 weeks) to group III, IV and V animals. On the last day of experiment, blood samples were collected by nicking the tip of tail for biochemical estimations. Later the animals were sacrificed and pancreas was removed, cleaned and washed in ice-cold normal saline for biochemical study.

**Determination of blood glucose**

Blood glucose level was estimated by glucose oxidase method (Braham and Trinder, 1971) using a commercial diagnostic kit from Span diagnostic Limited, Surat, India.

**Determination of lipid profile**

Lipid profile such as serum cholesterol (TCh and HDL) (Wybenga et al., 1970; Warnick et al., 1985), serum triglycerides (TG) (Bucolo and David, 1973) were estimated using diagnostic kits Span diagnostic Limited, Surat, India. Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated using Friedewald et al. (1972) equation.

**Determination of LPO**

LPO was estimated by thiobarbituric acid (TBA) reaction with...
Table 1. Effect of *Rhus coriaria* on blood glucose and MDA levels in type 2 diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose (mg/dl)</th>
<th>MDA (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Citrate control</td>
<td>84.99±3.97</td>
<td>0.802±0.05</td>
</tr>
<tr>
<td>II</td>
<td>STZ-diabetic (100 mg/kg, i.p)</td>
<td>275.93±8.09**</td>
<td>1.32±0.055**</td>
</tr>
<tr>
<td>III</td>
<td><em>R. coriaria</em> (400 mg/kg, p.o)-treated controls</td>
<td>87.83±3.12</td>
<td>0.771±0.044</td>
</tr>
<tr>
<td>IV</td>
<td>STZ-diabetic + <em>R. coriaria</em> (200 mg/kg, p.o)</td>
<td>136.82±2.20 x</td>
<td>1.12±0.056 x</td>
</tr>
<tr>
<td>V</td>
<td>STZ-diabetic + <em>R. coriaria</em> (400 mg/kg, p.o)</td>
<td>114.18±2.04 x</td>
<td>1.08±0.029 x</td>
</tr>
</tbody>
</table>

The data are expressed in mean ± SEM; n=6 in each group. **P < 0.001 compared with the corresponding value for citrate control rats (group I). *P < 0.001 compared with the corresponding value for STZ-diabetic rats (group II).

Table 2. Effects of *Rhus coriaria* on total cholesterol (TCh), HDL-cholesterol (HDL-C) and triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) levels in type 2 diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>TCh (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C</th>
<th>VLDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Citrate control</td>
<td>76.85 ± 1.13</td>
<td>36.08 ± 1.39</td>
<td>87.57 ± 2.55</td>
<td>51.65 ± 1.21</td>
<td>17.51 ± 0.51</td>
</tr>
<tr>
<td>II</td>
<td>STZ-diabetic (100 mg/kg, i.p)</td>
<td>181.45 ± 1.54**</td>
<td>20.79 ± 0.73**</td>
<td>169.56 ± 3.24**</td>
<td>143.95 ± 3.76**</td>
<td>33.13 ± 0.45**</td>
</tr>
<tr>
<td>III</td>
<td><em>R. coriaria</em> (400 mg/kg, p.o)-treated controls</td>
<td>83.23 ± 1.35</td>
<td>35.26 ± 0.88</td>
<td>80.62 ± 1.86</td>
<td>60.64 ± 1.17</td>
<td>16.12 ± 1.37</td>
</tr>
<tr>
<td>IV</td>
<td>STZ-diabetic + <em>R. coriaria</em> (200 mg/kg, p.o)</td>
<td>118.43 ± 1.62 x</td>
<td>27.04 ± 1.25 x</td>
<td>119.83 ± 1.95 x</td>
<td>89.06 ± 1.33 x</td>
<td>23.96 ± 0.59 x</td>
</tr>
<tr>
<td>V</td>
<td>STZ-diabetic + <em>R. coriaria</em> (400 mg/kg, p.o)</td>
<td>111.75 ± 2.69 x</td>
<td>31.76 ± 1.34 x</td>
<td>102.09 ± 1.34 x</td>
<td>82.59 ± 1.42 x</td>
<td>20.42 ± 0.67 x</td>
</tr>
</tbody>
</table>

The data are expressed in mean ± S.E.; n=6 in each group. **P < 0.001 compared with the corresponding value for citrate control rats (Group I). *P < 0.001 compared with the corresponding value for STZ-diabetic rats (Group II).

Statistical analysis

Data were expressed as the mean ± standard error (S.E) of the means. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The Tukey-Karmer post hoc test was applied to identify significance among groups. P < 0.05 was considered to be statistically significant.

RESULTS

Effect of *R. coriaria* on hyperglycemia

Table 1 shows the effect of *R. coriaria* on blood glucose levels. Significant (P < 0.001) increase in blood glucose levels were observed in type 2 diabetic control rats when compared with citrate control rats. Oral administration of *R. coriaria* at two doses (200 and 400 mg/kg) reduced blood glucose levels significantly (P < 0.001) in a dose-dependent manner. Higher dose of *R. coriaria* (400 mg/kg) was the most effective dose in decreasing blood glucose levels. Only *R. coriaria* (400 mg/kg) treatment did not produce any significant change in the levels of blood glucose as compared to citrate control rats.

Effect of *R. coriaria* on lipid profile

Table 2 shows the effect of *R. coriaria* on serum malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids (Ohkawa et al., 1979). Tissue was homogenized in chilled 0.1 M potassium chloride (KCl) solution. Aliquot of 1 ml of the suspension medium was taken from the supernatant obtained after the centrifugation of 10% w/w tissue homogenate at 10,000 rpm. 0.5 ml of 30% TCA followed by 0.5 ml of 0.8% TBA was then added to it. The tubes were kept in shaking water bath for 30 min at 80°C. After 30 min of incubation tubes were taken out and kept in ice cold water for 10 min. These were then centrifuged at 3000 rpm for 15 min. The absorbance of supernatant was read at 540 nm at room temperature against appropriate blank. The concentration of MDA was measured from the standard calibration curve prepared by using tetra-ethoxy propane. Protein was estimated by the method of Lowry et al. (1951). Lipid peroxidation (LPO) was expressed as nmols of MDA per milligram of protein.
lipid profile. A significant increase \((P < 0.001)\) in TCh, TG, LDL-C and VLDL-C levels were observed in type 2 diabetic control rats when compared with citrate control rats. Administration of \(R.\ coriaria\) at the doses of 200 and 400 mg/kg significantly \((P < 0.001)\) reduced the elevated levels of these cholesterols and triglycerides when compared with type 2 diabetic control rats. However, the HDL-cholesterol levels were significantly \((P < 0.001)\) decreased in type 2 diabetic control rats when compared with citrate control rats. Treatment with \(R.\ coriaria\) significantly \((P < 0.001)\) increased the HDL-cholesterol levels \((P < 0.001)\). Higher dose treatment of \(R.\ coriaria\) \((400 \text{ mg/kg})\) was most effective in improving lipid profile. There was no significant change in the levels of cholesterol and triglyceride in only \(R.\ coriaria\) \((400 \text{ mg/kg})\) treated rats and citrate control rats.

**Effect of \(R.\ coriaria\) on LPO levels**

Table 1 shows the levels of malondialdehyde (MDA), a secondary product of LPO in pancreatic tissue homogenate. STZ treatment resulted in a significant \((P < 0.001)\) increase in MDA levels in type 2 diabetic control rats when compared with citrate control rats. Treatment with \(R.\ coriaria\) \((200 \text{ and } 400 \text{ mg/kg})\) showed significant \((< 0.001)\) decrease in the levels of MDA when compared with type 2 diabetic control rats. The higher dose of \(R.\ coriaria\) \((400 \text{ mg/kg})\) treatment was found to be more effective in decreasing MDA levels. Only \(R.\ coriaria\) \((400 \text{ mg/kg})\) treatment did not produce any significant change in the levels of MDA as compared to citrate control rats.

**DISCUSSION**

Type 2 DM is a chronic metabolic disorder characterized by hyperglycemia and dyslipidemia due to decreased secretion of insulin, insulin insensitivity or both (George and Ludvik, 2000; Nyholm et al., 2000). Either of the factors causes disturbances in carbohydrate, lipid and protein metabolism. The management of dyslipidemia, a well recognized and modifiable risk factor among patients with type 2 DM, is an important element in the multifactorial approach to prevent CVD (Schwartz, 2006). Diabetic dyslipidemia is characterized by hypertriglyceridaemia, low levels of HDL-C and the presence of small, dense LDL-C particles. Increased secretion of VLDL-C from the liver is a central feature of dyslipidemia and is linked significantly to the low HDL-C and abnormal LDL-C (Chahil and Ginsberg, 2006).

STZ is frequently used to induce diabetes in experimental animals through its toxic effects on pancreatic \(\beta\)-cells (Yamagishi et al., 2001; Szkudelski, 2003). Although, it is generally accepted that the cytotoxicity produced by STZ depends on DNA alkylation and subsequent activation of poly ADP-ribose synthetase that causes rapid and lethal depletion of NAD in pancreatic islets (Bolzan and Bianchi, 2002). Several lines of evidences indicate that the free radicals play an essential role in the mechanism of \(\beta\)-cell damage and diabetogenic effect of STZ (Okhuwa et al., 1995).

Effective control of hyperglycemia is a key step in preventing or reversing diabetic complications and improving the quality of life in both types 1 and 2 diabetic patients (Diabetes Control and Complications Trial Research Group, 1993; DeFronzo, 1999). Type 2 diabetic control rats showed significant hyperglycemia after 90 days of STZ treatment. Results of the present study showed that methanolic extract of \(R.\ coriaria\) treated rats showed significantly reduced elevated blood glucose levels. The mechanism(s) by which the extract induced decrease in blood glucose levels were not investigated in the present study, it could be due to increased insulin production and release or improvement in the insulin sensitivity. However, further studies are required to determine the exact mechanism(s) and site of action of \(R.\ coriaria\) extract.

Type 2 diabetes mellitus is one of the most common human metabolic disorders and is often associated with profound alterations in the plasma lipid and lipoprotein profile with an increased risk of coronary heart disease. Hypercholesterolemia and hypertriglyceridaemia have been reported to occur in STZ-induced diabetic rats (Ahmad et al., 2001). Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of CVD and related complications (Brown et al., 1993). In this experiment we have shown that administration of \(R.\ coriaria\) extract to type 2 diabetic rats reverses hyperglycemia. Evidence is presented to show that, in addition to the antihyperglycemic activity of \(R.\ coriaria\) extract, it also possess lipid lowering properties. In the present study when \(R.\ coriaria\) was given to type 2 diabetic rats, the serum TCh, TG, LDL-C and VLDL-C levels were significantly reduced. We observed significant increase in HDL-C levels in the same groups. This finding is in favour of \(R.\ coriaria\), since low HDL-C levels are considered as a risk for coronary heart disease. HDL-C is strongly protective against atherosclerosis. An important mechanism underlying this protective effect is the role of HDL-C in the removal of excess cholesterol from the peripheral tissues. But in addition, HDL-C also protects by inhibiting oxidation of lipoproteins such as LDL-C in the pathogenesis of atherosclerosis (Witzum and Steinberg, 1991).

There is evidence that hyperglycemia in diabetes mellitus results in oxidative stress through an increase in reactive oxygen species (ROS) production and a decrease in ROS scavenging capacity (Abdel-Wahab and Abd-Allah, 2000). In many tissues, antioxidant therapy has been shown to reduce or retard the damaging effect of ROS in diabetes and diabetes related complications (Yavuz et al., 2003; Bonnefont- Rousselot, 2004). The
elevated levels of MDA are attributed to the enhanced production of ROS (superoxide radicals, hydrogen-peroxide and hydroxyl radicals). Some recent studies have shown that *R. coriaria* has antioxidant properties and prevents lipid peroxidation (Candan and Sokmen, 2004). In the present study we observed a significant increase in the levels of MDA, a secondary product of LPO, in pancreas of type 2 diabetic rats. The *R. coriaria* treatment to such rats has shown significant decrease in the levels of MDA suggesting its possible role in scavenging hydroxyl and peroxyl radicals.

Further pharmacological evaluations are required to identify and isolate the active principles in the plant with antihyperglycemic, antidiyslipidemic and antioxidant activity as well as elucidating their mechanisms of action. If these results are extrapolated in humans then *R. coriaria* might prove useful in the treatment and/ or prevention of type 2 diabetes mellitus.

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**REFERENCES**


