Acta Poloniae Pharmaceutica – Drug Research, Vol. 70 No. 5 pp. 861–867, 2013 ISSN 0001-6837
Polish Pharmaceutical Society

RHUS CORIARIA AMELIORATES INSULIN RESISTANCE IN NON-INSULIN-DEPENDENT DIABETES MELLITUS (NIDDM) RATS

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Abstract: We have investigated the effect of methanolic extract of *Rhus coriaria* (RC) on hyperinsulinemia, glucose intolerance and insulin sensitivity in non-insulin-dependent diabetes mellitus (NIDDM) rats. NIDDM was induced by single intraperitoneal injection of streptozotocin (STZ, 100 mg/kg) to 2 days old rat pups. RC (200 mg/kg and 400 mg/kg) was administered orally once a day for 5 weeks after the animals were confirmed diabetic (i.e, 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. There was a significant increase in blood glucose, glycosylated hemoglobin (HbA_1c) and serum insulin levels were observed in NIDDM control rats. Treatment with RC reduced the elevated levels of blood glucose, HbA_1c and insulin in the NIDDM rats. An oral glucose tolerance test (OGTT) was also performed in the same groups, in which we found a significant improvement in glucose tolerance in the rats treated with RC. The insulin sensitivity was assessed for both peripheral insulin resistance and hepatic insulin resistance. RC treatment significantly improved insulin sensitivity index (K_{ITT}) which was significantly decreased in NIDDM control rats. There was significant rise in homeostasis model assessment of insulin resistance (HOMA-R) in NIDDM control rats whereas RC treatment significantly prevented the rise in HOMA-R in NIDDM treated rats. Our data suggest that methanolic extract of RC significantly delayed the onset of hyperinsulinemia and glucose intolerance and improved insulin sensitivity in NIDDM rats.

Keywords: *Rhus coriaria*, streptozotocin, hyperglycemia, hyperinsulinemia, insulin sensitivity

Type 2 diabetes mellitus (DM) is possibly the world's fastest growing metabolic disorder which results from a combination of genetic and acquired factors impairing β-cell functions (1) on one side, and insulin sensitivity on the other (2). The progression of type 2 DM begins with an impairment of glucose tolerance (3) and is often associated with a state of insulin resistance, which means insulin that is secreted by the β-cells and bound to liver, muscle and fat cells, is subnormally efficacious in carrying out its metabolic actions (4). In recent years, plant derived medicines have received great deal of attention compared to synthetic ones for the cure and prophylaxis of various diseases. Management of type 2 DM without any side effect is still a challenge to the medical system. The conventional pharmacological treatments for type 2 DM have a number of limitations, such as adverse effects and high rates of secondary failure (5). Medicinal plants with antidiabetic activities were used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have many side effects (6). It is assumed that herbal medicine can only be effective as an alternative to oral hypoglycemic agents in the treatment of type 2 DM, where pancreatic islets are not totally destroyed.

Rhus coriaria (RC) L. (Family: Anacardiaceae), commonly known as sumac (also spelled as sumach) is a well-known spice in the Middle-East and grown in the central region of Turkey (7). Previous report showed that sumac contains flavonols, phenolic acids, hydrolyzable tannins, anthocyans, and organic acids (8). Phytochemicals in RC are being used as antibacterial, antidiarrhoeal, antispasmodic, antiviral, astringent, candidicide, hepatoprotective, antigastric, anti-inflammatory,

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antioxidant, antiulcer, fungicide, cyclooxygenase-inhibitor and lipoxygenase inhibitor due to their contents of ellagic acid, gallic acid, quercetin, isoquercitrin, myricetin and tannic acid (9-11). Hypoglycemic efficacy of sumac (*Rhus coriaria* L.) has been investigated through inhibition of a glycoside hydrolase: α-amylase in the treatment and prevention of diabetes (12). Only a little is known about the antioxidative activity of methanolic extract of sumach fruit on oil stabilizing property (13). Methanolic extract (water-soluble part) of RC was found to be an uncompetitive inhibitor of xanthine oxidase and scavenger of superoxide radical (14). Pioglitazione is a member of the thiazolidinedione group, and because of its significantly positive effect on glycemic control, it is especially preferred in type 2 diabetic patients with a high cardiovascular disease risk. In the present study, pioglitazone has been used as a standard drug for comparing the data as its insulin sensitizing property is well known, its pharmacokinetic is well understood and its effect is predictable (15, 16). Recently we have reported antihyperglycemic, antidyslipidemic and antioxidant activity of methanolic extract of *Rhus coriaria* in STZ-induced type 2 diabetic rats (17).

However, the reports on the effect of RC on hyperinsulinemia, glucose intolerance and insulin sensitivity is still lacking in the literature. Therefore, the present study was designed to investigate the effect of RC on hyperinsulinemia, glucose intolerance and insulin sensitivity in NIDDM model of rats.

MATERIALS AND METHODS

Experimental 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

Rhus 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 RC seed was extracted with methanol at room temperature three times with 5 volumes of methanol (w/v) (9). The solvent was evaporated at 35-40°C under reduced pressure to give methanolic extract, yielding approximately 10% (w/w). 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. Pioglitazone was purchased from Sun Pharmaceuticals Ltd. The enzyme-linked immunosorbent assay (ELISA) kit for insulin assay was purchased from Mercodia (Uppsala, Sweden). All the other biochemicals and chemicals used for the experiment were of analytical grade.

Induction of diabetes

To induce NIDDM, STZ (100 mg/kg) in citrate buffer (pH-4.5) was administered intraperitoneally (*i.p.*) to 2 days old rat pups (18). Another group of pups received only citrate buffer on the 2nd day of their birth. Ninety 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 six groups comprising of six animals in each group as follows:

- Group I: Citrate control rats, received citrate buffer (0.1 mL/10 g, *i.p.*)
- Group II: NIDDM control rats, received streptozotocin in a single dose (100 mg/kg, *i.p.*)
- Group III: NIDDM-treated rats, received RC (200 mg/kg, *p.o.*)
- Group IV: NIDDM-treated rats, received RC (400 mg/kg, *p.o.*)
- Group V: only RC-treated rats, received RC (400 mg/kg, *p.o.*)
- Group VI: NIDDM treated rats, received pioglitazone (25 mg/kg, *p.o.*)

RC (200 mg/kg 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. Pioglitazone was suspended in 1% carboxymethyl cellulose (CMC) and given until the end of the study (5 weeks) to group VI. On the last day of the experiment, blood samples were collected by nicking the tip of tail for biochemical estimations.

Determination of blood glucose

Blood glucose level was estimated by glucose oxidase method (19) using a commercial diagnostic kit from Span diagnostic Ltd., Surat, India.

Determination of glycosylated hemoglobin (HbA_1c) level

Glycosylated hemoglobin level was estimated by Bannon method (20) using a commercial diagnostic kit from Monozyme India Ltd., Secunderabad, India.

Determination of insulin level

Plasma insulin level was estimated quantitatively by ELISA method of Morgan and Lazarow (21). For this purpose Insulin ELISA kit was used.

Determination of OGTT

OGTT was measured according to the method of Pari and Saravanan (22). Glucose solution (2 g/kg) was given to overnight fasted rats. Blood samples were taken at 0, 15, 30, 60 and 120 min after glucose administration. All the blood samples were collected for glucose estimation.

Determination of insulin sensitivity

Insulin tolerance test (ITT) is used to assess peripheral insulin resistance (23). This test measures insulin sensitivity using K_{ITT} as an index of insulin mediated glucose metabolism. Rats were fasted overnight before giving insulin challenge. Insulin (0.2 U/100 g body weight human regular insulin; Eli Lilly, Indianapolis, IN) was administered by slow *i.v.* injection through tail vein. Blood samples were collected at 0 min and then at 15, 30, 60 and 120 min after administration of insulin injection. Glucose was estimated by glucose oxidase-peroxidase method. K_{ITT} was determined from the slope of a linear portion of the regression line of natural logarithm of glucose *versus* time using the formula:

$$K_{ITT} = \frac{0.693}{t_{1/2}} \times 100$$

where $t_{1/2}$ represents the half-life of plasma glucose decay. The half-life of plasma glucose was obtained by plotting plasma glucose concentrations *versus* time on semilogarithmic graph paper.

HOMA-R was calculated using fasting blood glucose (FBG) and fasting insulin (FI) level and was used for the determination of hepatic insulin resistance (24). The insulin sensitivity level was calculated using the following formula:

HOMA-R = FI (mU/mL) × FBG (mg/dL)/405.

Statistical analysis

Data were expressed as the mean ± standard error (S.E.) of the mean. 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 RC on hyperglycemia in NIDDM rats

Table 1 shows the effect of RC on the blood glucose levels. Significant ($p < 0.001$) increase in blood glucose levels was observed in NIDDM control rats when compared with citrate control rats. Oral administration of RC at two doses (200 mg/kg

Table 1. Effect of *Rhus coriaria* on blood glucose and glycosylated hemoglobin levels in NIDDM rats.

Groups	Treatment	Blood glucose (mg/dL)	Glycosylated hemoglobin (%)
I	Citrate control	97.18 ± 3.02	5.70 ± 0.265
II	Streptozotocin (STZ 100 mg/kg, *i.p.*)	324.66 ± 10.87[x]	12.18 ± 0.322[x]
III	NIDDM + RC (200 mg/kg, *p.o.*)	139.65 ± 1.89[y]	8.97 ± 0.109[y]
IV	NIDDM + RC (400 mg/kg, *p.o.*)	117.08 ± 1.87[y]	6.83 ± 0.129[y]
V	Only RC (400 mg/kg, *p.o.*)	96.66 ± 1.89	5.90 ± 0.134
VI	NIDDM+ pioglitazone (25 mg/kg, *p.o.*)	104.47 ± 1.88[y]	6.27 ± 0.337[y]

The data are expressed as the mean ± S.E.; n = 6 in each group. [x]$p < 0.001$ compared with the corresponding value for citrate control animals (group I). [y]$p < 0.001$ compared with the corresponding value for NIDDM control animals (group II).

and 400 mg/kg) reduced the blood glucose levels significantly ($p < 0.001$) in a dose-dependent manner. On the other hand, pioglitazone treatment also significantly ($p < 0.001$) reduced blood glucose levels when compared with NIDDM control rats. Only RC treatment did not produce any significant change in the blood glucose levels when compared with citrate control rats.

Effect of RC on HbA_1c in NIDDM rats

Table 1 shows the effect of RC on glycosylated hemoglobin levels. Significant ($p < 0.001$) increase in HbA_1c levels were observed in NIDDM control rats when compared with citrate control rats. Oral administration of RC at two doses (200 mg/kg and 400 mg/kg) decreased the HbA_1c levels significantly ($p < 0.001$) in a dose-dependent manner. Treatment with pioglitazone significantly ($p < 0.01$) restored the increased levels of HbA_{1c} when compared with NIDDM control rats. There was no significant change in HbA_1c levels of only RC treated rats when compared with citrate control rats.

Effect of RC on insulin levels in NIDDM rats

Table 3 shows the effect of RC on insulin levels. Hyperinsulinemia was observed in NIDDM control rats when compared with citrate control rats. RC treatment significantly ($p < 0.001$) reduced the elevated levels of insulin when compared with NIDDM control rats. Pioglitazone treatment also significantly ($p < 0.001$) reduced the elevated levels of insulin when compared with NIDDM control rats. Only RC treatment did not induce any significant change in the levels of insulin when compared with citrate control rats.

Effect of RC on OGTT in NIDDM rats

Table 2 shows the blood glucose levels of citrate control, NIDDM control and NIDDM treated rats after oral administration of glucose (2 g/kg). In NIDDM control rats the peak increase in blood glucose levels were observed after 1 h. The blood glucose levels remained high over next one hour. RC and pioglitazone treated rats showed significant ($p < 0.001$) decrease in blood glucose levels at 1 and 2 h when compared with NIDDM control rats. Only RC treatment did not produce any significant change in the blood glucose levels at 1 and 2 h during OGTT when compared with citrate control rats.

Effect of RC on insulin sensitivity in NIDDM rats

Table 3 shows the levels of K_{ITT}, an index of peripheral insulin resistance and the levels of HOMA-R, an index of hepatic insulin resistance. NIDDM control rats showed significant decrease in K_{ITT} with significant increase in HOMA-R levels when compared with citrate control rats. Treatment with RC and pioglitazone significantly ($p < 0.001$) increased the levels of K_{ITT} and prevented an increase in HOMA-R levels in NIDDM rats when compared with NIDDM control rats. There was no significant change in the levels of K_{ITT} and HOMA-R in only RC treated rats and citrate control rats.

Table 2. Effect of *Rhus coriaria* on oral glucose tolerance test (OGTT) in NIDDM rats.

Groups	Treatment	Blood glucose (mg/dL)				
		0 min	15 min	30 min	60 min	120 min
I	Citrate control	82.82 ± 1.67	112.20 ± 2.05	149.49 ± 2.92	124.14 ± 1.69	98.43 ± 1.28
II	Streptozotocin (STZ 100 mg/kg, *i.p.*)	256.56 ± 5.84^x	301.51 ± 7.72^x	328.78 ± 5.69^x	342.92 ± 9.78^x	318.18 ± 6.83^x
III	NIDDM + RC (200 mg/kg, *p.o.*)	137.32 ± 1.92^y	164.07 ± 1.81^y	184.30 ± 1.51^y	165.36 ± 1.52^y	148.90 ± 2.69^y
IV	NIDDM + RC (400 mg/kg, *p.o.*)	114.71 ± 1.83^y	130.31 ± 2.23^y	155.45 ± 2.41^y	137.79 ± 1.90^y	120.02 ± 1.61^y
V	Only RC (400 mg/kg, *p.o.*)	83.14 ± 1.73	115.32 ± 2.91	157.69 ± 2.63	140.73 ± 2.92	96.08 ± 2.26
VI	NIDDM + pioglitazone 25 mg/kg, *p.o.*)	104.02 ± 1.37^y	128.75 ± 2.57^y	128.75 ± 2.57^y	138.85 ± 2.92^y	113.35 ± 2.82^y

The data are expressed as the mean ± S.E.; n = 6 in each group. $^x p < 0.001$ compared with the corresponding value for citrate control animals (group I). $^y p < 0.001$ compared with the corresponding value for NIDDM control animals (group II).

Table 3. Effect of *Rhus coriaria* on insulin levels, K_{ITT} and HOMA-R in NIDDM rats.

Groups	Treatment	Insulin level (mU/L)	K_{ITT}	HOMA-R
I	Citrate control	13.13 ± 0.245	10.15 ± 0.162	3.14 ± 0.037
II	Streptozotocin (STZ 100 mg/kg, *i.p.*)	24.09 ± 0.329[x]	4.61 ± 0.162[x]	19.28 ± 0.541[x]
III	NIDDM + RC (200 mg/kg, *p.o.*)	19.09 ± 0.222[y]	6.66 ± 0.115[y]	6.57 ± 0.060[y]
IV	NIDDM + RC (400 mg/kg, *p.o.*)	15.89 ± 0.200[y]	8.72 ± 0.135[y]	4.59 ± 0.056[y]
V	Only RC (400 mg/kg, *p.o.*)	12.88 ± 0.235	10.19 ± 0.197	3.088 ± 0.068
VI	NIDDM + pioglitazone (25 mg/kg, *p.o.*)	14.53 ± 0.123[y]	9.18 ± 0.198[y]	3.74 ± 0.065[y]

The data are expressed as the mean ± S.E.; n = 6 in each group. [x]$p < 0.001$ compared with the corresponding value for citrate control rats (group I). [y]$p < 0.001$ compared with the corresponding value for NIDDM control rats (group II).

DISCUSSION

Diabetes mellitus, the most common endocrine disease, is not a single disease but a group of disorders of varying etiology and pathogenesis. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (25). Diabetes mellitus is the most common serious metabolic disorder and it is considered to be one of the five leading causes of death in the world (26). STZ is frequently used to induce DM in experimental animals (27, 28). 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 (29, 30). Several lines of evidences indicate that the free radicals may play an essential role in the mechanism of β-cell damage and diabetogenic effect of STZ (31).

The method of NIDDM induction was first described by Portha et al. (32). At 8–10 weeks of age and thereafter, neonatal rats treated with STZ manifest hyperglycemia, an impaired response to the glucose tolerance test (33) and loss of β-cell sensitivity to glucose (34). People who develop diabetes usually pass through the phases of excessive adipogenesis, nuclear peroxisome proliferator-activated receptor (PPAR) modulation, insulin resistance, hyperinsulinemia, pancreatic β-cell stress and impaired glucose postprandial and fasting levels (35, 36). Hyperinsulinemia has generally been considered a marker of insulin resistance, i.e., a decrease in the effect of insulin to stimulate glucose uptake at a given serum insulin concentration (37). Those considered as at risk for developing type 2 DM tend to exhibit a constellation of risk factors i.e., abdominal obesity, hypertension, dyslipidemia, and insulin resistance (38-40). Hence, in addition to glycemic control, management of hyperinsulinemia is also essential for controlling insulin resistance and limiting the complications of NIDDM.

NIDDM control rats exhibited persistent hyperglycemia. RC treatment to NIDDM rats reduced the elevated blood glucose levels. In diabetes, there is an increased glycosylation of a number of proteins including hemoglobin and β-crystalline of lens (41). Measurement of glycosylated hemoglobin (HbA_1c) has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes (42). HbA_1c levels increased in NIDDM control rats when compared with citrate control rats. Agents with antioxidant or free radical scavenging property may inhibit oxidative reactions associated with protein glycation (43). Previous studies have shown that RC has antioxidant properties and prevents lipid peroxidation (14). Sumac (RC) extract seemed to be promising source of natural antioxidants (44). Administration of RC to NIDDM rats reduced the glycosylation of hemoglobin by virtue of its free radical scavenging property and thus decreased the levels of HbA_1c. A decrease in blood glucose levels might also contribute to decreased levels of glycated hemoglobin in RC treated NIDDM rats.

Hyperinsulinemia appears to be a compensatory mechanism that responds to increased levels of circulating glucose and is often associated with the progression to insulin resistance (45). The β-cells normally compensate insulin resistance by secreting higher amount of insulin to maintain glucose homeostasis. Bonora et al. (46) has reported that hyperinsulinemia is associated with decreased hepatic insulin clearance and hypersecretion of β-cells in mild glucose intolerance obese subjects. Results of the present study clearly showed that hyperinsulinemia (as evident by increased serum insulin levels) was seen in NIDDM control rats. Therefore, the hyperinsulinemia in NIDDM rats could be either due to decreased hepatic clearance of insulin or by down-regulation of insulin receptors and desensitizing postreceptor pathways (47), resulting in decreased insulin binding and degradation. Despite high insulin levels (hyperinsulinemia), the glucose levels were greater in NIDDM control rats than NIDDM treated rats. However, RC treatment was found to be effective in reducing insulin levels of NIDDM rats thereby preventing hyperinsulinemia. It seems that RC exerts antihyperglycemic effect by attenuating hyperinsulinemia.

An insulin-resistance state is a key phase of metabolic syndrome, constituting the major risk factor for the development of glucose intolerance and diabetes mellitus (48). Thus, interventions to decrease insulin resistance may postpone the development of diabetic complications. When animals were subjected to OGTT, increased blood glucose levels were found increased in time and were maintained until 2 h in NIDDM rats. In the present investigation, the rate of glucose disposal was found to be significantly decreased in NIDDM control rats. Treatment with RC significantly improved glucose tolerance, as indicated by reduction in peak blood glucose levels at 1 and 2 h in NIDDM treated rats during OGTT. RC might enhance glucose utilization by peripheral tissues and increasing the glycogen stores in the liver due to restoration of delayed insulin response because it significantly decreased the blood glucose levels in glucose loaded rats.

Our results showed that RC decreased blood glucose levels, prevented hyperinsulinemia and improved glucose tolerance in NIDDM rats. These results suggest that RC can improve insulin sensitivity. Thus K_{ITT} and HOMA-R levels were determined to check insulin sensitivity. K_{ITT} is used to assess peripheral insulin resistance (23) whereas HOMA-R is a useful clinical index of hepatic insulin resistance (49). The results obtained clearly showed that K_{ITT} was significantly improved by RC treatment to NIDDM rats. Additionally, RC treatment significantly prevented the rise in HOMA-R in NIDDM treated rats. These findings suggest that RC is pharmacologically effective in improving insulin sensitivity.

In the present study, the higher dose treatment of RC showed most significant results in decreasing the levels of blood glucose and glycosylated hemoglobin and preventing hyperinsulinemia as compared to low dose treatment. The higher dose of RC treatment showed most significant results in improving glucose tolerance and insulin sensitivity as compared to low dose treatment. Protective effect of RC was comparable to the standard drug (i.e., pioglitazone). If these results are extrapolated in humans, then RC might prove useful in the treatment and/or prevention of hyperinsulinemia, impaired glucose tolerance, insulin resistance and in addition being an effective means of controlling hyperglycemia.

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Received: 28. 02. 2013