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Antihyperglycemic Activity of Aqueous Root Extract of *Rubia cordifolia* in Streptozotocin-Induced Diabetic Rats

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Abstract

The aim of the current study was to examine the antidiabetic action of *Rubia cordifolia* Linn (Rubiaceae) aqueous root extract (RCAREt) in streptozotocin (STZ)-induced diabetic rat model. STZ-induced diabetic rats were treated with RCAREt for 8 weeks (1 g kg^{-1} b.w., orally per day). Serum glucose, total cholesterol and triglycerides, hematological parameters, and liver and kidney transaminases in normal, STZ diabetic, and RCAREt-treated diabetic rats were measured. The observed hyperglycemia, hypertriglyceridemia, enhanced transaminases of liver and kidney, hypochromic microcytic anemia, and loss of body weight in STZ diabetic rats were normalized by RCAREt treatment, whereas the hypercholesterolemia was not rectified. The beneficial effect of RCAREt treatment might be due to different types of active principles, each with a single or a diverse range of biological activities.

Keywords: Antidiabetic, diabetes, *Rubia cordifolia*, transaminases.

Introduction

Diabetes mellitus is a heterogeneous metabolic disorder. In modern medicine, satisfactory effective therapy is not yet available to cure diabetes mellitus. Unfortunately, neither insulin injections nor oral antidiabetic drugs (sulfonlyureas, metformin, and acarbose) reinstate a normal pattern of glycemic control, whether used alone, in combination, or administered as a standard or intensive regimen (UKPDS Group, 1995).

Oral antihyperglycemic drugs play an important role in the treatment of non-insulin dependent diabetes mellitus (NIDDM) and have a characteristic profile of side effects (Rang & Dale, 1991). The yawning gap for additional agents to combat hyperglycemia and its accompanying complications presents an opening to revisit traditional antidiabetic plants (Gray & Flatt, 1997; Ladeji et al., 2003; Maiti et al., 2004).

Rubia cordifolia Linn (Rubiaceae) is a climbing plant that grows in the northwestern Himalayas and other hilly districts of India. All parts of the plant are used as medicines with the roots predominating. Advankar and Chitnis (1982) reported the anticancer activity of the methanol extract of *R. cordifolia*. Dried roots are used as astringent and diuretic. Traditionally, they are used in folklore medicine for treatment of dropsy, paralysis, jaundice, amenorrhea, and visceral obstructions (Nadkarni, 1982).

The alcohol extract of *R. cordifolia* is reported to possess anti-PAF activity (Tripathi et al., 1993) and potato-lipoxygenases inhibitory activity (Tripathi et al., 1995). Clinical trials by Ojha and Dwivedi (1996) revealed the beneficial action of *R. cordifolia* roots against non-healing diabetic foot ulcer by oral administration, and dipping the foot in *R. cordifolia* decoction healed 90% of the patients within 4–5 weeks of treatment.

The ethanol extract of aerial parts of the plant has hypoglycemic activity in albino rats (Anonymous, 1999). Ethno-medico-botanical studies of Bhakshu (2002) reported that ground root infusions of *R. cordifolia* given orally once a day for 40 days were used as a folk remedy for diabetes. Literature surveys have yielded

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scanty information on the pharmacological properties of *R. cordifolia* in diabetes. Thus, the current study was undertaken to examine the possible antidiabetic activity of aqueous root extract of *R. cordifolia* (RCAREt) in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Chemicals

The chemicals used in the current study were procured from Sigma Chemical Co. (St. Louis, MO, USA), Koch-Light Laboratories (Huntingdon, Cambridgeshire, England), and SISCO Research Laboratories (Maharashtra, India). An aqueous *R. cordifolia* root extract (brown, dry powder) was received as a gift from Chemiloids (manufacturers and exporters of herbal extracts; Vijayawada, Andhra Pradesh, India). Herb-to-product ratio is 10:1. The extract was suspended in distilled water prior to use.

Induction of diabetes

Two- to 3-month-old male albino Wistar rats of body weight 130–150 g were procured from Sri Venkateswara Enterprises (Bangalore, India), acclimatized for 7 days to our animal house, and maintained at standard conditions of temperature, relative humidity, light and dark cycles. The current work was carried out with a prior permission from our institutional animal ethical committee. Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared STZ (55 mg kg^{-1} body weight, in ice-cold 0.1 M citrate buffer, pH 4.5, in a volume of 0.1 ml per rat). Seventy-two hours after STZ administration, the serum glucose level of each rat was determined for confirmation of diabetes. A window period of 10 days was allowed before commencement of treatment. Rats with fasting blood glucose greater than 300 mg/100 ml were considered diabetic and included in the current study.

Experimental design

In the current study, a total of 24 rats (8 normal and 16 STZ diabetic surviving rats) were used. The rats were divided into three groups of eight each, normal untreated; STZ diabetic untreated; STZ diabetic treated with RCAREt. The dose of the RCAREt in the current study is based on the earlier reports (Anonymous, 1999) of the ethanol extract of aerial parts of the plant. RCAREt was administered orally at a dosage of 1 g kg^{-1} body weight per day for 8 weeks. Body weight was monitored at biweekly intervals.

Sample collection

Blood was collected into Eppendorf tubes from 12-h fasted rats with capillary tube through retino-orbital plexus. Blood collected in Eppendorf tubes containing EDTA (10 mg/ml) was used for hematological analysis. The blood collected without anticoagulant was used for estimation fasting glucose and analysis of lipid profile.

Hematological and lipid profile measurement

Serum glucose level was estimated by glucose oxidase (GOD)-perioxidase (POD) enzymatic method using the Span Diagnostic kit (Span Diagnostic Ltd, Udhna, India) (Trinder, 1969). Red blood cells were counted by the method of Davidsohn and Peter (1963); Hemoglobin (Hb) and packed cell volume (PCV) was estimated by following Henry (1984). Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), and Mean Cell Hemoglobin Concentration (MCHC) are calculated from RBC count, Hb, and PCV (Chessbrough & Arthur, 1976; Henry, 1984). Accurex enzymatic diagnostic kit was used for estimating cholesterol (Allain et al., 1974) and triglycerides (Foosati & Prencipe, 1982).

Animal sacrifice

Animals from each experimental group were starved for 16 h and sacrificed by cervical dislocation. Liver and kidneys were removed, washed thoroughly with ice-cold saline, suspended in 0.15 M potassium chloride, and frozen at $-80^{\circ}C$ until assays were carried out.

Liver and kidney transaminases

The frozen tissues (liver and kidney) were thawed, and 10% tissue homogenate was prepared in ice-cold 0.1 M Tris HCl buffer, pH 7.4, and centrifuged at 12,000 rpm for 45 min in a Sigma laboratory centrifuge (model 3K18, rotor no. 12150). Alanine aminotransferase (ALT) and aspartate aminotransferase (APT) in the clear supernatant of the cytosolic fraction were assayed by following the method of Reitman and Frankel (1990).

Statistical analysis

All results are expressed as means $\pm$ SE. Statistically elevation of the data was performed by Student's *t*-test; p values were calculated to assess the significance of the changes observed.

Results

Clinical symptoms such as loss of body weight, weakness, polyphagia, polydepsia, and polyuria observed in the diabetic untreated group were rectified in the diabetic treated group. A significant reduction in body weight ($p < 0.05$) was observed in the diabetic untreated group,

Table 1. Effect of *R. cordifolia* aqueous root extract (RCAREt) treatment on fasting serum glucose in streptozotocin-induced diabetic rats.

Groups (n = 8)	Fasting serum glucose (mmol l^{-1})				
	Initial (10 days after confirmation of diabetes)	Second week	Fourth week	Sixth week	Eighth week
Normal control	3.36 ± 0.01	3.38 ± 0.01	3.43 ± 0.01	3.55 ± 0.01	3.64 ± 0.01
Diabetic untreated	16.54 ± 0.25	$20.02 \pm 0.26^*$	$24.86 \pm 0.28^*$	$28.18 \pm 0.31^*$	$28.68 \pm 0.29^*$
Diabetic treated	17.21 ± 0.21	$15.29 \pm 0.25^*$	$12.52 \pm 0.26^*$	$10.53 \pm 0.28^*$	$5.03 \pm 0.33^*$

Values are given as mean ± SE, groups of eight animals each. Diabetic untreated group was compared with corresponding normal control groups. Diabetic treated groups were compared with corresponding diabetic untreated groups.
$^*p < 0.001$.

whereas diabetic rats treated with RCAREt showed improvement in body weight similar to normal untreated group during the experimental period of 8 weeks.

Serum glucose level

Table 1 shows the effect of RCAREt treatment on serum glucose level. Normal control rats remained persistently euglycemic throughout the course of the study. In the diabetic untreated group, the serum glucose level gradually increased during the experimental period from 16.54 to 28.68 mmol l^{-1}, whereas in the RCAREt treated diabetic group a significant antihyperglycemic effect was evident from the second week onwards and reached near normal values by 8 weeks of treatment.

Hematological parameters

The significant decrease in Hb (16.7%), PCV (25.1%), MCV (26.0%), and MCH (22.5%) observed in diabetic untreated animals compared with normal controls was found to be rectified and reached near normal values in the diabetic treated group, whereas no significant alterations were observed in RBC count and MCHC in both the diabetic untreated and treated groups compared with normal control group (Table 2).

Liver and kidney transaminases

STZ-induced diabetes resulted in a significant rise in the activities of Alanine aminotransferase (ALT) and Aspartate aminotransferase AST both in the liver (823.3 ± 27.8 and 723.1 ± 48.5 pkat/mg protein, $p < 0.001$) and kidney [149.0 ± 31.2 ($p < 0.01$) and 567.2 ± 15.1 ($p < 0.001$) pkat/mg protein] compared with normal control group of liver (493.1 ± 0.3 and 432.0 ± 4.7) and kidney (57.2 ± 7.7 and 343.3 ± 19.5 pkat/mg protein). Diabetic rats treated with RCAREt showed significant decline in the activities of ALT and AST in the liver [416.1 ± 13.2 and 271.2 ± 7.7 ($p < 0.001$) pkat/mg protein] and AST of kidney [450.5 ± 8.9 ($p < 0.001$) pkat/mg protein] compared with the diabetic untreated group.

Serum total cholesterol and triglycerides

In diabetic untreated rats, serum total cholesterol and triglycerides were found to be significantly increased (4.38 ± 0.08 and 2.12 ± 0.01 mmol l^{-1} $p < 0.001$) when compared with normal untreated rats (3.68 ± 0.1 and 1.28 ± 0.02 mmol l^{-1}). RCAREt-treated diabetic rats (4.52 ± 0.15 and 1.27 ± 0.0 mmol l^{-1}) showed no significant alteration in serum cholesterol level, whereas there was a significant decrease in triglycerides compared with the diabetic untreated group.

Table 2. Effect of *R. cordifolia* aqueous root extract (RCAREt) treatment on hematological parameters.

Parameters	Normal control (n = 8)	Diabetic untreated (n = 8)	Diabetic treated (n = 8)
Hemoglobin (mmol l^{-1})	2.18 ± 0.04	$1.73 \pm 0.07^*$	$2.17 \pm 0.04^*$
Red blood cells (millions/mm^3)	4.89 ± 0.11	4.96 ± 0.15	4.98 ± 0.02
Packed cell volume (%)	46.75 ± 2.13	$35.00 \pm 0.94^*$	$45.75 \pm 1.57^*$
Mean cell volume (μm^3)	96.24 ± 5.33	$71.27 \pm 3.34^\dagger$	$93.35 \pm 5.29^\dagger$
MCH (pg)	2.89 ± 0.08	$2.24 \pm 0.04^*$	$2.85 \pm 0.11^*$
MCHC (g%)	30.60 ± 1.4	32.10 ± 2.25	30.92 ± 1.13

Values are mean ± eight individual animals.
$^*p < 0.001$; $^\dagger p < 0.01$.

Discussion

RCAREt exhibited significant antihyperglycemic activities in STZ-induced hyperglycemic rats. Even though the antihyperglycemic effect was evident by 2 weeks of treatment, the euglycemic state was achieved by 8 weeks of treatment. To the best of our knowledge, this is the first report on antihyperglycemic effect of roots of *R. cordifolia.*

Muscle wasting, negative nitrogen balance, and enhanced gluconeogenesis are characteristic features of uncontrolled diabetes (Buse et al., 1972). The observed elevation in transaminase activities (AST and ALT) in liver and kidney in diabetic untreated rats of the current study are inconsistent with earlier reports (Ghosh & Suryawanshi, 2001), which is an indication of increased protein degradation leading to enhanced gluconeogenesis and ketogenesis (Maiti et al., 2004). The restoration of activities of AST and ALT of liver and AST of kidney to their respective normal levels after supplementation with RCAREt further strengthens the antidiabetogenic effect of this extract.

Clinical symptoms of diabetes (such as loss of body weight, weakness, polyphagia, polydepsia, and polyuria) were significantly rectified in the diabetic treated group. Overcoming the weight loss of diabetic rats by RCAREt treatment may be due to antihyperglycemic effect, thus, enhancing glucose utilization by controlling the protein degradation.

The hematological alterations observed in the current study revealed that STZ-induced diabetes resulted in hypochromic microcytic anemia reflected by decreased Hb, MCH, PCV, and MCV without change in RBC count when compared with that of the normals. The hematological alterations are rectified in the RCAREt-treated group.

The roots of *R. cordifolia* are valued in Ayurveda in the maintenance of general health and normal blood circulation. Studies by Nadkarni (1982) revealed that the biologically active constituents of *R. cordifolia* facilitate the free circulation of blood by removing congestion and improving the quality of blood.

The marked increase in serum total cholesterol and triglycerides in the diabetic untreated rats are in agreement with earlier findings (Burcelin et al., 1995). Abnormalities of plasma lipid and lipoprotein metabolism are very common in diabetes (Alan, 1996) and have long been thought to play a role in atherogenesis in diabetes. RCAREt treatment showed a lipid-lowering activity reflected by significant decrease in serum triglycerides, with no significant alteration in total cholesterol compared with the diabetic untreated group. The decrease of serum Triglycerides (TG) level is an important finding of this experiment.

In conclusion, the antihyperglycemic, hypotriglyceridemic, and other beneficial effects of RCAREt treatment might be due to different types of active principles acting individually or synergistically each with a single or a diverse range of biological activities.

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