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Antidiabetic activity of 50% ethanolic extract of *Ricinus communis* and its purified fractions

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ABSTRACT

We investigated the antidiabetic activity of 50% ethanolic extract of roots of *Ricinus communis* (RCRE) along with its bioassay-guided purification. Five-hundred milligram per kilogram body weight appeared to be the effective dose as it caused the maximum lowering of the fasting blood glucose, both in normal as well as type 1 diabetic animals. The maximum hypoglycemic effect was always observed at the 8th h up to which the study has been conducted.

Administration of the effective dose of RCRE to the diabetic rats for 20 days showed favorable effects not only on fasting blood glucose, but also on total lipid profile and liver and kidney functions on 10th and 20th day. RCRE was purified using silica gel column chromatography. Out of several different fractions tested, only one fraction (R-18) showed significant antihyperglycemic activity. RCRE seemed to have a high margin of safety as no mortality and no statistically significant difference in alkaline phosphatase, serum bilirubin, creatinine, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and total protein was observed even after the administration of the extract at a dose of 10 g/kg b.wt. Thus *R. communis* seems to have a promising value for the development of a potent phytomedicine for diabetes.

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1. Introduction

Insulin-dependent diabetes mellitus or type 1 diabetes is an autoimmune disorder caused by destruction of insulin producing β-cells when auto aggressive T-lymphocytes infiltrate the pancreas. This leads to hypoinsulinaemia and thus hyperglycemia (Bach, 1995). Hyperglycemic condition causes increased glycosylation leading to biochemical and morphological abnormalities due to altered protein structure which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiomyopathy (Arky, 1982).

Traditional medicines derived mainly from plants play major role in the management of diabetes mellitus (Ahmed et al., 2004; Karunanayake and Tennekoon, 1993; Patel and Srinivasan, 1997). World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for oral therapy (Day, 1998). Recently, Mankil et al. (2006) have reviewed many medicinal plants possessing experimental and clinical antidiabetic activity that have been used in traditional systems of medicine. The present work was undertaken to explore the antidiabetic potential of a plant, *Ricinus communis* in type 1 diabetic animals.

R. communis L. is called as Erandah in Sanskrit, Amudam in Telugu and Erandi or Arandi in Hindi and is also known as castor oil bean, castor oil plant and wonder tree. It is a member of family Euphorbiaceae, cultivated all over India for its seed oil. Castor beans have been used in classical Egyptian and Greek medicine and their use has been described in the Susruta and Ayurveda as early as sixth century B.C. (Olsnes et al., 1976). The use of different parts of this plant for the treatment of various diseases in traditional or folk remedies throughout the world has been reviewed (Scarpa and Guerci, 1982). The root of this plant is also useful as an ingredient of various prescriptions for nervous diseases and rheumatic affections such as lumbago, pleurodynia and sciatica (Nandkarni, 1954). In the Indian system of medicine, the leaf, root and seed oil of this plant have been used for the treatment of inflammation and liver disorders (Kirtikar and Basu, 1991) as they have been found to be hepatoprotective (Yanfg et al., 1987; Visen et al., 1992), laxative (Capasso et al., 1994) and diuretic (Abraham et al., 1986). The antifertility activity of 50% ethanolic extract of *R. communis* has also been reported (Sandhyakumary et al., 2003). Roots and aerial parts are useful in the treatment of diabetes (Pullaiah and Naidu, 2003). Fifty percent of ethanolic extract of the root, stem and leaves of this plant showed hypoglycemic

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activity (Dhar et al., 1968). Except the initial report of Dhar et al. (1968), there is no report of systematic investigation of antidiabetic activity of *R. communis*. Fifty percent of ethanolic extract of roots of *R. communis* (RCRE) had shown significant hypoglycemic activity in normal animals and antihyperglycemic activity in diabetic animals in our initial screening studies. Thus, the aim of the present study was to perform detailed studies on the antidiabetic activity of RCRE and further to purify it.

2. Materials and methods

2.1. Reagents

Chemicals and reagents used in the present studies were of analytical grade, purchased from Sigma Chemical Co., USA, E Merck, Germany, Fine Chemicals, Fluka Chemicals, Lancaster, Spectrochem and SD Fine Chemicals, India. Organic solvents were freshly distilled prior to use. Thin layer chromatography (TLC) was carried out on commercially available flexible TLC silica gel (silica gel 60, F254) plates and compounds were visualized using short wave ultraviolet light. Alloxan monohydrate was purchased from Sigma–Aldrich Co., USA. One touch-glucometer (Accu-check sensor) of Roche Diagnostics, Germany and Uristix purchased from Bayer Diagnostics India Ltd. was used. Insulin from Abbot India was used. Insulin level of animals was measured using Rat Insulin Enzyme Linked Immunosorbent Assay (ELISA) kit form DRG Diagnostics, GmbH Germany. Total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglyceride (TG), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALKP), serum bilirubin (BIL), blood urea nitrogen (BUN) and creatinine (CRTN) was assayed using kits from Bayer Diagnostics-India Ltd. Total protein (TPR) was estimated using the Bradford kit purchased from Bangalore Genei. Roots of *R. communis* were collected from places nearby the campus of University of Delhi, Delhi.

2.2. Animals

Six to eight week old male Wistar rats (weighing 180 ± 20 g) obtained from Animal Research Facility of Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi, India were used. The animals were kept at 25–30° C and 45–55% relative humidity, acclimatized with free access to food (Golden feed, Delhi, India) and water ad libitum for 1 week under 12 h light, 12 h dark cycle. All animals were carefully monitored and all the experimental work with the animals was carried out after obtaining approval from the Institutional Animal Ethical Committee. For experimental purpose, animals were kept fasting overnight but had free access to water.

2.3. Preparation of 50% ethanolic extract

Roots of *R. communis* (2.5 kg) were washed with distilled water and shade dried. They were then cut into small pieces and ground in a grinder to be obtained in a powder form. The powder was extracted with 50% ethanol using a mechanical stirrer till exhaustion. The extract was filtered with Whatman filter paper (type 4) and the filtrate was concentrated under reduced pressure on rotavapor (BÜCHI, R-3000, Switzerland) at 40 °C temperature. The concentrated extract was further lyophilized. The lyophilized extract (300 g) was then used for the experiments.

2.4. Induction of experimental diabetes

Type 1 diabetes was induced by using alloxan monohydrate following the method of Sochor et al. (1985). Rats were starved for 24 h and diabetes was induced by a single subcutaneous injection of 150 mg/kg b.wt. alloxan monohydrate dissolved in freshly prepared 0.154 M sodium acetate buffer (pH 4.5). From next day onwards, the alloxan-induced rats were injected intraperitoneally with two units of insulin for the next seven days to reduce the mortality rate and stabilize the diabetic animals. The severity of diabetes was checked in alloxan diabetic rats by using urine glucose detection strips. Insulin was then withdrawn and the diabetic rats were randomly divided into different groups.

2.5. Determination of the effective dose

2.5.1. Normal animals

To find out the effective dose, six groups comprising of five animals each were used. Various doses (125, 250, 500, 750, 1000 and 2000 mg/kg b.wt.) of the root extract were administered orally to animals of groups I–VI, after drawing the initial blood sample. Results were compared with tolbutamide, which was administered orally to animals of group VII at a dose of 200 mg/kg b.wt. Further samples of blood were collected at 1, 2, 4, 6 and 8 h after the extract or drug administration (0 h). Animals of the control group were treated similarly but with distilled water instead of the extract.

2.5.2. Diabetic animals

Diabetic animals were similarly divided into eight groups of five animals each. Increasing doses of the root extract were administered to animals of group I–VI and tolbutamide was administered to group VII at a dose of 200 mg/kg b.wt. Additionally 500 mg/kg b.wt. tolbutamide was given to group VIII to observe the effect of increased dose of tolbutamide on FBG of diabetic animals. Animals of the control group were treated similarly but with distilled water instead of the extract/drugs.

2.6. Treatment of diabetic animals (for 20 days)

Three groups of six rats each were used in this experiment. Group I consisted of normal animals and served as normal healthy control group (NC). Groups II and III comprised of alloxan diabetic rats. Group II consisted of the untreated diabetic control (DC) animals, which were orally given distilled water daily for 20 days. Group III consisted of diabetic treated (DT) animals, which were treated daily with a single dose of 500 mg/kg b.wt. (effective dose) of the root extract for 20 days. FBG was determined on 0, 2nd, 5th, 7th, 10th, 15th and 20th day of the experiment. Also the effect of this dose on lipid profile was studied by collection of blood from tail on 0, 10th and 20th day of the experiment. TC was estimated by enzymatic method (Lopes-Virella et al., 1977), HDL–cholesterol by phosphotungstate method (Lopes-Virella et al., 1977; Allain et al., 1974; Richmond, 1973; Miller, 1977) and TG by enzymatic method (Trinder, 1969; Werner et al., 1981). VLDL (very low density lipoprotein) and LDL (low density lipoprotein) cholesterol were calculated by Friedewalds formula (Friedewald et al., 1972) as described below.

- VLDL: TG/5.
- LDL: TC–(HDL+VLDL).

ALKP, BIL, BUN, CRTN, SGOT, SGPT, TPR were also estimated on 0, 10th and 20th day to study the effect of the extract on chronic administration. ALKP was estimated by PNPP method (Klin, 1972; Saligman et al., 1950), BUN by the UV method (Talke and schubert, 1965), BIL by Jendrassik and Grof method (Jendrassik and Grof, 1938; Gambino, 1965) and CRTN by picrate method (Henry et al., 1974). SGOT and SGPT were measured by UV kinetic (Expert panel of the IFCC on Enzymes, 1976) method while TPR was measured by Bradford Macro method (Bradford, 1976). Body weight and urine sugar were estimated on 0, 10th and 20th day. At the end of the experiment, the rats were sacrificed and their liver and kidneys were weighed. The weight of liver and of two-kidneys expressed as percentage body weight was also calculated.

2.7. Estimation of serum insulin levels

Three groups (1–3) of six rats each were used in this experiment. Group 1 served as normal healthy control (NC), groups 2 and 3 were served as untreated diabetic control (DC) and diabetic treated (DT) animals, respectively. Treated diabetic and untreated diabetic control animals were given RERC at a dose of 500 mg/kg b.wt. and distilled water, respectively for the period of 20 days. Blood was collected from the tail vein and the serum insulin levels were determined on 0, 7th, 15th and 20 day of the experiment using rat insulin ELISA kit.

2.8. Phytochemical investigation of 50% ethanolic extract

Phytochemical tests were carried out for various constituents of RCRE using the following chemicals and reagents. Dragendoff's reagents were used for alkaloids, frothing test for saponins, Liebermann–Burchard test for sterols, $FeCl_3$ for tannins, Molisch reagent for carbohydrates, ninhydrin for proteins and vanillin/sulfuric acid for terpenoids (Wagner et al., 1984; Evans, 2002; Cannell, 1998; Barbehenn, 1995).

2.9. Purification of RCRE

RCRE was purified (275 g) using silica gel (60–120 mesh size) column chromatography. The column was packed in petroleum ether (60–80 °C) and was eluted using solvents of increasing polarity, such as petroleum ether, ethyl acetate, methanol and water. Fractions of 500 ml were collected and similar fractions were combined on the basis of their TLC pattern.

2.10. Testing the activity of different fractions

For testing the antihyperglycemic activity of the fractions obtained from the 50% ethanolic extract, 21 groups of diabetic animals with five animals in each group were taken. Different fractions were administered orally at a single dose of 500 mg/kg b.wt. to the overnight fasted rats in group I–XX. Blood was collected at 0, 1, 2, 4, 6 and 8 h after the administration of each fraction. Animals in the control group were given distilled water only.

2.11. Acute toxicity

To study the acute toxicity of RCRE, five groups (four animals in each group) of animals were taken. Graded doses (2.5, 5, 7.5 and 10 g/kg b.wt.) of RCRE were orally administered to the overnight fasted rats of group I–IV, because these doses were 5, 10, 15 and 20 times of the effective dose of root extract. The control group was given only distilled water instead of RCRE. After administration of RCRE, the rats were observed for gross behavioral, neurological, autonomic and toxic effects continuously for 2 h and then at 6 h intervals up to three days, and daily once, up to a week. Blood was collected from tail just before the administration of RCRE and at 24 and 72 h after the administration of RCRE for the estimation of different biochemical parameters. ALKP, BIL, CRTN, SGOT, SGPT and TPR were measured by the same methods as described under the long-term treatment.

2.12. Statistical analysis

Results are presented as mean ± S.E.M. The statistical analysis involving two groups was evaluated by means of Student's *t*-test whereas analysis of variance (ANOVA) followed by Dunnet's multiple comparison post-test was used for statistical comparison between control and various treated groups. Fisher's *p* values were calculated to show the significance. Statistical significance was accepted at the $p < 0.05$ values.

3. Results

3.1. Determination of the effective dose of RCRE

3.1.1. Normal animals

The effect of RCRE on FBG of the normal rats is shown in Table 1. FBG decreased to 42 ± 3 and 36 ± 2 mg/dl at the 8th h from an initial value of 60 ± 8 mg/dl at the dose of 250 and 500 mg/kg b.wt. of the extract, respectively. A dose-dependent effect was observed on FBG up to a dose of 500 mg/kg b.wt. But higher doses up to 2000 mg/kg b.wt. did not show any dose-dependent effect. Tolbutamide caused a marked decrease in FBG, the maximum hypoglycemic effect being at 1st and 2nd h, when the FBG decreased to 28 ± 3 mg/dl (at both 1st and 2nd h), from an initial value of 61 ± 3 mg/dl.

3.1.2. Diabetic animals

The effect of RCRE on FBG of diabetic rats is shown in Table 2. A dose-dependent effect was observed in diabetic animals also up to a dose of 500 mg/kg. But, higher doses up to 2000 mg/kg b.wt. did not show any dose-dependent effect and a maximum decrease in FBG was observed at 8th h. 500 mg/kg b.wt. of RCRE decreased the FBG to 166 ± 19 from an initial level of 371 ± 21 mg/dl. Tolbutamide caused no significant lowering of FBG in the diabetic animals.

3.2. Treatment of diabetic rats with RCRE (for 20 days)

3.2.1. Effect on FBG

Administration of the extract for a long duration led to a significant diminution of FBG in the diabetic rats, while there was no significant alteration in the FBG of the control animals. RCRE significantly decreased the FBG of the diabetic rats from an initial level of 386 ± 41 mg/dl to 358 ± 33, 293 ± 28, 191 ± 25, 133 ± 29, 96 ± 20 and 79 ± 16 mg/dl on 2nd, 5th, 7th, 10th, 15th and 20th day, respectively. The FBG became normal by 20th day (Table 3).

3.2.2. Effect on total lipid profile

The effect of the administration of RCRE for 20 days on total lipid profile is shown in Table 4. Total cholesterol in the DC group was significantly higher (204 ± 18 mg/dl) compared to

Table 1
Effect of different doses of RCRE on FBG of normal animals

Groups	Dose (mg/kg b.wt.)	Blood glucose concentration (mg/dl)					
		0 h	1 h	2 h	4 h	6 h	8 h
Control	–	61 ± 1	65 ± 2	68 ± 5	64 ± 5	64 ± 4	65 ± 4
I	125	60 ± 6	61 ± 6	56 ± 3	54 ± 12	58 ± 5	65 ± 9
II	250	60 ± 8	60 ± 12	55 ± 10	44 ± 7	$43 \pm 5^{\alpha\alpha}$	$42 \pm 3^{\alpha\alpha}$
III	500	60 ± 8	57 ± 10	53 ± 6	43 ± 5	$36 \pm 4^{\alpha\alpha}$	$36 \pm 2^{\alpha\alpha}$
IV	750	61 ± 8	63 ± 8	59 ± 6	47 ± 3	$39 \pm 2^{\alpha\alpha}$	$40 \pm 3^{\alpha\alpha}$
V	1000	61 ± 10	61 ± 6	57 ± 2	47 ± 6	$50 \pm 4^{\alpha}$	51 ± 7
VI	2000	59 ± 11	61 ± 7	62 ± 7	56 ± 9	$46 \pm 9^{\alpha}$	56 ± 7
VII	Tol (200)	61 ± 3	$28 \pm 3^{\alpha\alpha\rho\rho}$	$28 \pm 3^{\alpha\alpha\rho\rho}$	$32 \pm 5^{\alpha\alpha\rho\rho}$	$32 \pm 4^{\alpha\alpha\rho\rho}$	$33 \pm 2^{\alpha\alpha\rho\rho}$

Each value represents mean ± S.E.M., $n = 5$, Tol = tolbutamide.
$^{\alpha}$Represent statistical significance vs control.
$^{\rho}$Represents statistical significance vs 0 h.
P values: $^{\alpha}<0.05$, $^{\alpha\alpha}<001$, $^{\rho\rho}<0.01$.

Table 2
Effect of different doses of RCRE on FBG of diabetic animals

Groups	Dose (mg/kg b.wt.)	Blood glucose concentration (mg/dl)					
		0 h	1 h	2 h	4 h	6 h	8 h
Control	–	375 ± 25	380 ± 25	389 ± 28	356 ± 26	329 ± 27	330 ± 28
I	125	365 ± 20	333 ± 19	279 ± 19	282 ± 20	204 ± 24	221 ± 15
II	250	364 ± 23	$288 \pm 16^{\alpha}$	284 ± 14	$242 \pm 16^{\rho}$	204 ± 18	$197 \pm 17^{\alpha\rho}$
III	500	371 ± 21	$285 \pm 22^{\alpha}$	282 ± 22	$238 \pm 15^{\rho}$	183 ± 23	$166 \pm 19^{\alpha\alpha\rho\rho}$
IV	750	375 ± 25	347 ± 23	276 ± 18	268 ± 22	207 ± 30	189 ± 20
V	1000	373 ± 33	349 ± 34	287 ± 35	278 ± 49	205 ± 48	198 ± 53
VI	2000	372 ± 24	340 ± 18	245 ± 22	266 ± 30	212 ± 26	206 ± 24
VII	Tol (200)	366 ± 40	368 ± 44	391 ± 31	356 ± 34	342 ± 32	323 ± 37
VIII	Tol (500)	390 ± 38	367 ± 49	312 ± 36	232 ± 35	211 ± 39	208 ± 33

Each value represents mean ± S.E.M., $n = 5$, Tol = tolbutamide.
$^{\alpha}$Represents statistical significance vs control.
$^{\rho}$Represents statistical significance vs 0 h.
P values: $^{\alpha}<0.05$, $^{\alpha\alpha}<001$, $^{\rho}<0.05$, $^{\rho\rho}<0.01$.

Table 3
Effect of RCRE on FBG of diabetic animals

Day	0 day	2nd day	5th day	7th day	10th day	15th day	20th day
Normal control	75 ± 2	78 ± 2	75 ± 4	77 ± 3	74 ± 3	77 ± 2	77 ± 5
Diabetic control	360 ± 61**	370 ± 57**	369 ± 59**	369 ± 56**	366 ± 56**	372 ± 55**	373 ± 56**
Diabetic treated	386 ± 41	358 ± 33	293 ± 28	191 ± 25$^{\rho\rho\alpha}$	133 ± 29$^{\rho\rho\alpha\alpha}$	96 ± 20$^{\rho\rho\alpha\alpha}$	79 ± 16$^{\rho\rho\alpha\alpha}$

Treatment = 20 days, dose = 500 mg/kg b.wt.
Each value represents mean ± S.E.M., $n = 6$.
*Represents statistical significance vs normal control.
$^{\rho}$Represents statistical significance vs 0 day.
$^{\alpha}$Represents statistical significance vs diabetic control.
P values $^{**}<0.01$, $^{\rho\rho}<0.01$, $^{\alpha}<0.05$, $^{\alpha\alpha}<0.01$.

Table 4
Effect of RCRE on total lipid profile of animals

Assay	Day	Normal control	Diabetic control	Diabetic treated
TC (mg/dl)	0	102 ± 12	204 ± 18****	208 ± 2
	10th	103 ± 12	206 ± 18$^{\rho\rho****}$	142 ± 12$^{\rho\rho\rho\alpha}$
	20th	102 ± 11	210 ± 18$^{\rho\rho\rho\rho****}$	116 ± 7$^{\rho\rho\rho\alpha\alpha\alpha\alpha}$
HDL–Cholesterol (mg/dl)	0	56 ± 2	48 ± 2*	49 ± 2
	10th	56 ± 2	45 ± 1$^{\rho\rho****}$	60 ± 3$^{\rho\rho\rho\alpha\alpha\alpha}$
	20th	60 ± 3	42 ± 1$^{\rho\rho\rho\rho****}$	67 ± 3$^{\rho\rho\rho\rho\alpha\alpha}$
VLDL (mg/dl)	0	18.2 ± .9	28 ± 2****	28 ± 2
	10th	17.6 ± .7	30 ± 2****	22 ± 2$^{\rho\rho}$
	20th	18 ± .7	28 ± 2***	21 ± 4
LDL (mg/dl)	0	29 ± 10	129 ± 18****	131 ± 22
	10th	29 ± 11	132 ± 18**	60 ± 11$^{\rho\rho\rho\alpha\alpha}$
	20th	23 ± 9	140 ± 17$^{\rho\rho\rho****}$	28 ± 4$^{\rho\rho\rho\alpha\alpha\alpha\alpha}$
TG (mg/dl)	0	89 ± 5	139 ± 11**	140 ± 10
	10th	88 ± 4	143 ± 10****	111 ± 14$^{\rho\rho}$
	20th	91 ± 3	149 ± 13***	89 ± 9$^{\rho\rho\rho\rho\alpha\alpha}$

Treatment = 20 days, dose = 500 mg/kg b.wt.
Each value represents mean ± S.E.M., $n = 6$, TC = total cholesterol, HDL = high density lipoprotein, VLDL = very low density lipoprotein, LDL = low density lipoprotein, TG = triglycerides.
*Represents statistical significance vs normal control.
$^{\rho}$Represents statistical significance vs 0 day.
$^{\alpha}$Represents statistical significance vs diabetic control.
P values $^{**}<0.01$, $^{***}<0.005$, $^{****}<0.001$, $^{\rho}<0.05$, $^{\rho\rho}<0.01$, $^{\rho\rho\rho}<0.005$, $^{\rho\rho\rho\rho}<0.001$, $^{\alpha}<0.05$, $^{\alpha\alpha}<0.01$, $^{\alpha\alpha\alpha}<0.005$, $^{\alpha\alpha\alpha\alpha}<0.001$.

102 ± 12 mg/dl in the healthy control, which remained high at the end of the experiment on 20th day. However, TC significantly decreased from 208 ± 2 mg/dl on 0 day to 142 ± 12 mg/dl and 116 ± 7 mg/dl on 10th and 20th day of treatment with the extract. Similarly LDL values in the DC group showed increase in 20 days. LDL increased to 140 ± 17 mg/dl on 20th day from an initial value of 129 ± 18 mg/dl on 0 day. In the RCRE treated group, LDL level was significantly decreased to 60 ± 11 mg/dl and 28 ± 4 mg/dl on the 10th and 20th day, respectively from an initial value of 131 ± 22 mg/dl. Likewise, the increased TG levels were also brought down close to the normal values by administration of the extract for 20 days. The value decreased to 111 ± 14 mg/dl on 10th day and to 89 ± 9 mg/dl on 20th day from initial value of 140 ± 10 mg/dl at the beginning of the experiment. Reduction in the VLDL level was also observed on 10th day in the extract treated group, while no such reduction was seen in the DC group. In contrast, HDL–cholesterol was significantly decreased in the DC group as compared to the healthy controls, and was further decreased to 45 ± 1 mg/dl and 42 ± 1 mg/dl on 10th and 20th day, respectively. But, treatment with the extract caused significant improvement and increased the level from 49 ± 2 mg/dl on 0 day to 60 ± 3 mg/dl and 67 ± 3 mg/dl on 10th and 20th day, respectively. No significant change occurred in the lipid profile of the animals in the normal control group during the experimental period.

3.2.3. Effect on liver and kidney functions

In the present study, ALKP levels were quite elevated in the DC group, but were significantly lowered on administration of the RCRE to 164 ± 13 U/L and 133 ± 5 U/L on 10th and 20th day, respectively from a level of 245 ± 16 U/L. The elevated levels of BIL in the DC group remained high, but were significantly lowered to 1.2 ± 0.15 mg/dl on 10th and 1.1 ± 0.09 mg/dl on 20th day of the treatment. There was an elevation of BUN and CRTN, in the untreated diabetic group of animals when compared to normal controls (Table 5). RCRE treatment significantly lowered CRTN to 0.78 ± 0.03 mg/dl and 0.55 ± 0.03 mg/dl on 10th and 20th day from an initial value of 1.4 ± 0.07 mg/dl on day 0. RCRE also significantly lowered the BUN level to 43 ± 2 mg/dl on 10th day and to 35 ± 2 mg/dl on 20th day from the initial value of 54 ± 2 mg/dl on 0 day. The SGOT and SGPT levels were significantly increased as compared to the normal animals in the diabetic animals. The increased SGOT levels were reduced to 58 ± 2 U/L and 50 ± 1 U/L after

Table 5
Effect of RCRE on other biochemical parameters

Assay	Day	Normal control	Diabetic control	Diabetic treated
ALKP (U/L)	0	100 ± 8	238 ± 21****	245 ± 16
	10th	98 ± 8	241 ± 21$^{\rho\rho\rho*****}$	164 ± 13$^{\rho\rho\rho\rho\rho\alpha}$
	20th	101 ± 7	246 ± 20$^{\rho\rho\rho*****}$	133 ± 5$^{\rho\rho\rho\rho\alpha\alpha\alpha\alpha}$
BIL (mg/dl)	0	0.96 ± 0.04	1.9 ± 0.08*****	1.9 ± 0.2
	10th	0.97 ± 0.02	2.1 ± 0.8****	1.2 ± 0.15$^{\rho\alpha\alpha}$
	20th	0.95 ± 0.02	2.1 ± 0.15*****	1.1 ± 0.09$^{\rho\rho\alpha\alpha\alpha\alpha}$
CRTN (mg/dl)	0	0.63 ± 0.02	1.5 ± 0.05*****	1.4 ± 0.07
	10th	0.6 ± 0.04	1.4 ± 0.12****	0.78 ± 0.03$^{\rho\rho\rho\rho\rho\alpha\alpha\alpha\alpha\alpha}$
	20th	0.62 ± 0.04	1.5 ± 0.09*****	0.55 ± 0.03$^{\rho\rho\rho\rho\rho\alpha\alpha\alpha\alpha\alpha\alpha}$
BUN (mg/dl)	0	25 ± 0.97	53 ± 3*****	54 ± 2
	10th	24 ± 0.56	56 ± 2$^{\rho\rho\rho\rho\rho\rho*****}$	43 ± 2$^{\rho\rho\rho\rho\alpha\alpha\alpha}$
	20th	25 ± 0.7	58 ± 2$^{\rho\rho\rho\rho\rho\rho*****}$	35 ± 2$^{\rho\rho\rho\rho\rho\alpha\alpha\alpha\alpha\alpha\alpha}$
SGOT (U/L)	0	46 ± 5	87 ± 5****	89 ± 4
	10th	44 ± 5	91 ± 4*****	58 ± 2$^{\rho\rho\rho\rho\alpha\alpha\alpha\alpha\alpha}$
	20th	44 ± 5	93 ± 5$^{\rho*****}$	50 ± 1$^{\rho\rho\rho\rho\rho\rho\alpha\alpha\alpha\alpha\alpha}$
SGPT (U/L)	0	17 ± 1	33 ± 2*****	34 ± 2
	10th	16 ± 1	35 ± 2*****	22 ± 3$^{\rho\rho\rho\alpha\alpha\alpha}$
	20th	16 ± 1	37 ± 2*****	19 ± 1$^{\rho\rho\rho\rho\rho\rho\alpha\alpha\alpha\alpha\alpha\alpha}$
TPR (g/dl)	0	6.9 ± 0.02	6.05 ± 0.10***	6.3 ± 0.19
	10th	6.7 ± 0.17	6.01 ± 0.06***	6.4 ± 0.21
	20th	6.8 ± 0.23	5.96 ± 0.10**	6.5 ± 0.20$^{\alpha}$

Treatment = 20 days, dose = 500 mg/kg b.wt.
Each value represents mean ± S.E.M., $n = 6$, ALKP = alkaline phosphatase, BIL = bilirubin, CRTN = creatinine, BUN = blood urea nitrogen, SGOT = serum glutamate oxaloacetate transaminases, SGPT = serum glutamate pyruvate transaminases, TPR = total protein.
*Represents statistical significance vs normal control.
$^{\rho}$Represents statistical significance vs 0 day.
$^{\alpha}$Represents statistical significance vs diabetic control.
P values, $^{**}<0.01$, $^{***}<0.005$, $^{****}<0.0005$, $^{*****}<0.0001$, $^{\rho}<0.05$, $^{\rho\rho}<0.01$, $^{\rho\rho\rho}<0.005$, $^{\rho\rho\rho\rho}<0.001$, $^{\rho\rho\rho\rho\rho}<0.0005$, $^{\rho\rho\rho\rho\rho\rho}<0.0001$, $^{\alpha}<0.05$, $^{\alpha\alpha}<0.01$, $^{\alpha\alpha\alpha}<0.005$, $^{\alpha\alpha\alpha\alpha}<0.001$, $^{\alpha\alpha\alpha\alpha\alpha}<0.0005$, $^{\alpha\alpha\alpha\alpha\alpha\alpha}<0.0001$.

treatment with the extract for 10 days and 20 days, respectively from an initial value of 89 ± 4 U/L. The initial level of 34 ± 2 U/L of SGPT was brought down to 22 ± 3 U/L and 19 ± 1 U/L on 10th day and 20th day of the treatment. TPR was significantly different in DC group as compared to NC group of animals and treatment with RCRE significantly increased TPR levels in DT group (Table 5).

3.2.4. Effect on urine sugar, body weight, liver and renal weight

Physiological parameters such as urine sugar level, body weight and tissue weight of animals in the control and treatment group are summarized in Table 6. Sugar was never detected in the urine of normal animals (−ve) and urine sugar of the untreated diabetic controls remained very high (+4) during the entire experimental period. The level of urine sugar decreased from +4 on day 0 to +1 on the 20th day with RCRE treatment.

There was no significant intra-group variation in the basal body weight on 0 day of the experiment. While the body weight of the animals in the NC group increased significantly from 177 ± 4 g on 0 day to 217 ± 5 g on 20th day, there was no appreciable increase in the diabetic controls. On the other hand, RCRE treated rats gained significant weight during the treatment period. Their body weight increased to 196 ± 4 g on 20th day from an initial weight of 178 ± 4 g at the beginning of the experiment.

Absolute weight of liver was not significantly different in any of the experimental group while the absolute weight of kidneys significantly increased to 1.7 ± 0.095 g in the diabetic control group. This increase was decreased to 1.5 ± 0.09 g on administration of the extract. When liver and two-kidney weights were expressed as percentage of body weight, there was significant increase in diabetic rats versus normal control rats. The liver weight increased to 4.08 ± 0.07 g as compared to 3.13 ± 0.02 g in the normal animals and the kidney weight increased to 0.93 ± 0.026 g as compared to 0.56 ± 0.03 g of the normal animals, and no such increase was observed in the extract treated group.

Table 6
Effect of RCRE on urine sugar, body and organ-weight of animals

	Day	Normal control	Diabetic control	Diabetic treated
Urine sugar	0	−Ve	$+4^{**}$	+4
	10th	−Ve	$+4^{**}$	$+1^{\rho\rho}$
	20th	−Ve	$+4^{**}$	$+1^{\rho\rho}$
Body weight	0	177 ± 4	177 ± 4	178 ± 4
	10th	198 ± 5$^{\rho\rho\rho\rho\rho\rho}$	178 ± 5*	186 ± 4$^{\rho\rho\rho\rho\rho}$
	20th	217 ± 5$^{\rho\rho\rho\rho\rho\rho}$	179 ± 6***	196 ± 4$^{\rho\rho\rho\rho\rho\rho\alpha}$
Liver wt. (g)	20th	6.8 ± 0.16	7.3 ± 0.27	7.6 ± 0.14
Liver wt./100 g wt. g.wt.	20th	3.13 ± 0.02	4.08 ± 0.07*****	3.9 ± 0.12
Kidney wt. (g)	20th	1.2 ± 0.08	1.7 ± 0.095**	1.5 ± 0.09$^{\alpha}$
Kidney wt./100 g wt.	20th	0.56 ± 0.03	0.93 ± 0.026*****	0.93 ± 0.02

Treatment = 20 days, dose = 500 mg/kg b.wt.
Each value represents mean ± S.E.M., $n = 6$.
*Represents statistical significance vs normal control.
$^{\rho}$Represents statistical significance vs 0 day.
$^{\alpha}$Represents statistical significance vs diabetic control.
P values, $^{*}<0.05$, $^{**}<0.01$, $^{***}<0.005$, $^{*****}<0.0001$, $^{\rho\rho\rho\rho\rho}<0.0005$, $^{\rho\rho\rho\rho\rho\rho}<0.0001$, $^{\alpha}<0.05$.

Table 7
Effect of RCRE on insulin levels (μg/L) of animals

Day	Normal control	Diabetic control	Diabetic treated
0	5.34 ± 0.25	1.5 ± 0.08**	1.6 ± 0.075
7th	5.19 ± 0.17	1.3 ± 0.06****	1.8 ± 0.09$^{\alpha\alpha\alpha\alpha}$
15th	5.27 ± 0.21	0.9 ± 0.105$^{\rho\rho\rho\rho****}$	2.25 ± 0.08$^{\rho\rho\alpha\alpha\alpha\alpha}$
20th	4.94 ± 0.28	0.8 ± 0.09$^{\rho\rho\rho\rho****}$	2.95 ± 0.012$^{\rho\rho\rho\rho\alpha\alpha\alpha\alpha}$

Treatment = 20 days, Dose = 500 mg/kg b.wt.
Each value represents mean ± S.E.M., $n = 6$.
*Represents statistical significance vs normal control.
$^{\rho}$Represents statistical significance vs 0 day.
$^{\alpha}$Represents statistical significance vs diabetic control.
P values, $^{**}<0.01$, $^{****}<0.001$, $^{\rho\rho}<0.01$, $^{\rho\rho\rho\rho}<0.001$, $^{\alpha\alpha\alpha\alpha}<0.001$.

3.3. Effect on serum insulin levels

Treatment with RCRE for 20 days at a dose 500 mg/kg b. wt. significantly increased the serum insulin levels from 0 day (1.6 ± 0.075 μg/l) to 20th day (2.95 ± 0.12 μg/l) in contrast to untreated diabetic animals where there was a significant decrease

Table 8
Effect of different fractions of RCRE on FBG of diabetic animals

Groups	Different fractions	Blood glucose concentration (mg/dl)					
		0 h	1 h	2 h	4 h	6 h	8 h
Control		375 ± 25	380 ± 25	389 ± 28	356 ± 26	329 ± 27	330 ± 28
I	R1	365 ± 25	360 ± 28	368 ± 30	342 ± 31	334 ± 32	335 ± 33
II	R2	373 ± 24	378 ± 26	380 ± 29	361 ± 30	352 ± 31	350 ± 19
III	R3	366 ± 28	370 ± 30	369 ± 32	341 ± 31	334 ± 33	335 ± 34
IV	R4	375 ± 33	380 ± 40	378 ± 42	357 ± 39	340 ± 35	353 ± 39
V	R5	386 ± 40	379 ± 47	377 ± 45	360 ± 37	353 ± 42	350 ± 45
VI	R6	384 ± 43	393 ± 48	390 ± 45	366 ± 40	352 ± 39	355 ± 37
VII	R7	361 ± 34	365 ± 39	372 ± 42	350 ± 41	337 ± 32	336 ± 29
VIII	R8	379 ± 27	375 ± 30	382 ± 40	365 ± 37	357 ± 26	360 ± 38
IX	R9	375 ± 35	378 ± 42	373 ± 43	359 ± 38	351 ± 45	353 ± 34
X	R10	383 ± 48	381 ± 41	392 ± 47	379 ± 45	367 ± 42	364 ± 33
XI	R11	369 ± 53	352 ± 48	355 ± 45	340 ± 43	333 ± 39	335 ± 42
XII	R12	382 ± 35	378 ± 34	375 ± 31	353 ± 39	339 ± 20	340 ± 32
XIII	R13	376 ± 44	380 ± 41	383 ± 45	364 ± 42	357 ± 37	360 ± 32
XIV	R14	361 ± 78	324 ± 77	330 ± 77	293 ± 70	293 ± 68	306 ± 87
XV	R15	379 ± 98	339 ± 97	347 ± 97	282 ± 108	260 ± 116	256 ± 129
XVI	R16	369 ± 65	248 ± 98	272 ± 121	209 ± 123	238 ± 142	213 ± 58
XVII	R17	378 ± 103	397 ± 80	381 ± 62	330 ± 82	354 ± 76	336 ± 104
XVIII	R18	379 ± 72	294 ± 60$^{\alpha}$	284 ± 36$^{\alpha}$	184 ± 23$^{\alpha}$	182 ± 40$^{\alpha}$	149 ± 11$^{\alpha}$
XIX	R19	373 ± 74	411 ± 73	395 ± 88	310 ± 112	271 ± 114	243 ± 103
XX	R20	383 ± 55	330 ± 48	318 ± 71	229 ± 93	210 ± 105	218 ± 68

Each value represents mean ± S.E.M., $n = 5$.
$^{\alpha}$Represents statistical significance vs control.
P values: $^{\alpha}<0.5$.

Table 9
Effect of different doses of RCRE on different biochemical parameters of normal animals

Group	Assay	ALKP (U/L)		BIL (mg/dl)		CRTN (mg/dl)		SGOT (U/L)		SGPT (U/L)		TPR (g/dl)	
	Dose (mg/kg b.wt.)	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
Control	–	106 ± 22	122 ± 6	.99 ± .002	1.00 ± 0.00	0.63 ± 0.09	0.64 ± 0.08	45 ± 4	36 ± 3	17 ± 3	17 ± 3	6.8 ± 0.5	6.0 ± 0.45
1	2.5	100 ± 15	100 ± 32	1.00 ± .00	1.00 ± 0.00	0.71 ± 0.09	0.75 ± 0.03	43 ± 3	46 ± 2	19 ± 3	17 ± 3	7.8 ± 0.2	5.9 ± 0.54
2	5	101 ± 24	135 ± 35	.99 ± .002	1.00 ± 0.00	0.63 ± 0.04	0.56 ± 0.09	36 ± 2	38 ± 4	16 ± 3	13 ± 2	7.3 ± 0.2	6.2 ± 0.96
3	7.5	98 ± 17	104 ± 22	1.00 ± .00	1.00 ± 0.00	0.79 ± 0.10	0.63 ± 0.01	44 ± 7	37 ± 2	16 ± 5	13 ± 2	6.9 ± 0.6	6.7 ± 0.67
4	10	114 ± 32	128 ± 27	1.00 ± .00	1.00 ± 0.00	0.87 ± 0.05	0.73 ± 0.05	47 ± 3	38 ± 1	22 ± 5	15 ± 0.9	7.3 ± 0.3	6.9 ± 0.61

Each value represents mean ± S.E.M., $n = 4$, ALKP = alkaline phosphatase, BIL = bilirubin, CRTN = creatinine, SGOT = serum glutamate oxaloacetate transaminase, SGPT = serum glutamate pyruvate transaminase, TPR = total protein.

i.e. from 0 day (1.5 ± 0.08 μg/l) to 20th day (0.8 ± 0.09 μg/l). No significant change was observed in normal healthy control animals in the treatment period (Table 7).

3.4. Phytochemical investigation of RCRE

Preliminary phytochemical screening of the root extract in the present study revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, anthrones and saponins while the terpenoids, sterols, proteins and amino acids were absent.

3.5. Purification of RCRE

Purification of RCRE was done using silica gel column chromatography. The column was eluted using solvents of increasing polarity which yielded twenty different fractions i.e. R1–R20.

3.6. Effect of different fractions of RCRE on diabetic animals

Out of the twenty column-purified fractions of RCRE tested for antihyperglycemic activity in diabetic rats, only one fraction (R18) showed a significant decrease in FBG of the diabetic rats as shown in Table 8. Fraction R18 decreased the FBG to 294 ± 60 (22.4% decrease), 284 ± 36 (25% decrease) 184 ± 23 mg/dl (51.4% decrease), 182 ± 40 mg/dl (51.9%decrease) and 149 ± 11 mg/dl (60.6%decrease) at 1st, 2nd, 4th, 6th and 8th h, respectively from an initial value of 379 ± 72 mg/dl at the beginning of the experiment.

3.7. Acute toxicity study

In order to observe any acute toxic effects of RCRE if present, the effect of increasing doses of RCRE were observed on different parameters such as ALKP, BIL, CRTN, SGOT, SGPT, and TPR and the results are summarized in Table 9. No significant difference in any of the biochemical parameters tested was observed after the administration of the extract at any of the doses at 24 and 72 h. The behavior of the rats appeared to be normal immediately after administration of the extract, as well as up to 72 h and also when observed once daily up to a week.

4. Discussion

RCRE had shown significant hypoglycemic activity in normal animals and antihyperglycemic activity in diabetic animals in our initial screening studies. Therefore, it was considered worthwhile to perform a systematic investigation of antidiabetic activity of RCRE. The result of the present study indicates that RCRE reduces the glucose level in normal as well as diabetic rats. The maximum decrease in FBG was observed at a dose of 500 mg/kg b.wt. and no dose-dependent effect was observed on increasing the dose further. Such a phenomenon of low hypoglycemic response at higher dose is common with indigenous plants and has been observed earlier with many plants like *Aegle marmelos* (Sharma et al., 1996a), *Murraya koenigii* (Kesari et al., 2005) and *Cinnamomum tamala* (Sharma et al., 1996b), *Eugenia jambolana* (Sharma et al., 2003) *Terminalia pallida* fruit (Rao et al., 2003), *Psacalium decompositum* (Alarcon-Aguilar et al., 2000). It has also been reported that high concentrations of *Syzygium cumini* seed extract may auto inhibit its hypoglycemic action (Prince et al., 1998). The decreased activity at a higher dose of the extract could be due to reduced or no effect of components present in the extract at higher doses (Prince et al., 1999; Rao et al., 2001a,b). It is also likely that the higher doses could not produce the expected higher hypoglycemic effect because of the presence of some other substances in RCRE, which interfere with the hypoglycemic effect. In this context, it is worth noting that Murthy et al. (2003) have reported the presence of some hyperglycemic compounds also along with hypoglycemic compounds in three plants, *Trigonella foenum graecum* (fenugreek) seeds, *Ficus bengalensis* (banyan tree) bark and *Momordica charantia* (bitter gourds). So, higher doses of the extract might have higher doses of hyperglycemic compounds. Thus, 500 mg/kg b.wt. was found to be the effective dose of RCRE on FBG of normal as well as diabetic animals.

Maximum hypoglycemic effect was produced at 8th h, after administration of RCRE which indicates that it takes about 8 h or more for the active ingredient(s) of the extract or its metabolites to enter into circulation and their target tissues to bring about hypoglycemic effect. Unlike the sulfonylureas and biguanides, the onset of action of RCRE is slow and persists for a longer duration of time. Tolbutamide caused marked decrease in FBG in normal animals, but no decrease in FBG was observed in diabetic animals. Absence of significant falls in glucose level in diabetic animals even at a dose of 500 mg/kg b.wt. of tolbutamide has been observed in earlier studies (Jafri et al., 2000; Diatewa et al., 2004). Sulfonylureas have been reported not to decrease the blood glucose level in alloxan diabetic animals (Goth, 1985; Goodman and Gilman, 2001). Alloxan is a beta cytotoxin and causes diabetes by destruction of the insulin secreting cells (Rerup, 1970). It is well established that tolbutamide (a sulfonylurea) lowers the blood glucose level by stimulating β-cells to release insulin and requires functional pancreas for its therapeutic effect (Yallow et al., 1960; Grodsky et al., 1971) and ineffectiveness of tolbutamide in the present study might be because of the absence of sufficient functional pancreatic β-cells.

It has been reported earlier that to obtain maximum effect, therapy with plant products should be continued for longer duration (Grover et al., 2000). To obtain the maximum effect of RCRE on the diabetic rats, the extract was administered daily once at a dose of 500 mg/kg b.wt. for 20 days, the period which produced a significant reduction in FBG of diabetic animals and this effect was more potent after repeated oral administration than after single dose administration. These results confirm the previous findings that the effectiveness of the drugs depends, probably, on the cumulative effect of active principles (Obatomi et al., 1994; Peu-

ngvicha et al., 1998). Thus, RCRE effectively controls hyperglycemia and maintains normoglycemia which may prevent the microvascular complications in diabetes.

Diabetes mellitus is usually associated with abnormal levels of serum lipids and such an increase causes the risk factor for coronary heart diseases (Davidson, 1981; Al-Shamaony et al., 1994). The marked increase in blood glucose and associated lipid levels that characterize the uncontrolled diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat deposits (Goodman and Gilman, 1985). Lowering of serum lipid concentration through drug therapy or dietary measures seems to decrease the risk of vascular diseases (Rhoads et al., 1976). Increase in lipids, TG and TC levels in alloxan diabetic rats observed in the present study may be a result of increased breakdown of lipids and mobilization of free fatty acids from the peripheral depots. Regular administration of RCRE for 20 days normalized lipid profile in diabetic animals. The dose of 500 mg/kg b.wt. not only lowered the TC, TG and LDLC but also enhanced the HDL–cholesterol which is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed 'reverse cholesterol transport', and is considered to be a cardio protective lipid. A highly negative correlation between serum HDL–cholesterol level and incidences of atherosclerosis (Levy, 1978) is also known to exist. Thus RCRE has significant impact in improving the imbalance in lipoprotein metabolism.

The kidneys remove metabolic wastes such as urea, uric acid, creatinine and ions and thus optimum chemical composition of body fluids is maintained. But the concentrations of these metabolites increase in blood during renal diseases or renal damage associated with uncontrolled diabetes mellitus. Blood urea nitrogen and creatinine are considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). In the present study, there was an elevation in BUN and CRTN in the untreated diabetic group of animals indicating renal damage, while significant decrease in both these parameters was observed in the animals of the treated group. Lowering of BIL in the treatment group indicated improvement of the liver damage by the extract. The increased levels of ALKP, SGOT and SGPT in the diabetic rats reflected the increased hepatic damage in alloxan-induced diabetes. The elevated levels of all the above biochemical parameters were significantly decreased on treatment with RCRE for 20 days. This indicates that RCRE treatment not only increased the uptake of blood glucose, but also reversed the damage of liver and kidneys seen in diabetic animals.

Decrease in urine sugar level and significant increase in body weight of treated rats were observed as compared to the control animals. The ability of a plant extract to protect weight loss seems to be a result of its antidiabetic activity and a similar action may be attributed to RCRE. Similar kind of body weight gain has been observed previously with other plants, such as *F. bengalensis*, *T. foenum graecum*, *Ocimum sanctum* etc., which are well known for their antidiabetic activity (Solomon et al., 1999; Sheeja and Augusti, 1995; Thakran et al., 2004; Vats et al., 2004). No significant changes were observed in the body weight of the diabetic untreated animals probably because the duration of the experiment was only 20 days. Renal hypertrophy (Sun et al., 2002; Meyer et al., 1998) or increase in the kidney weight, measured as the ratio of kidney weight per 100 g b.wt was observed in the DC group in the present study, which might be due to glucose over utilization and subsequent enhancement in glycogen synthesis, lipogenesis and protein synthesis.

Flavonoids are reported to have hypoglycemic activity (Lamba et al., 2000) and can regenerate the damaged beta cells in the alloxan diabetic rats (Chakravarthy et al., 1980). The beneficial effects of RCRE in the present study possibly due to the flavonoids present in *R. communis* (Khogali et al., 1992; Kang et al., 1985).

In the present study, diabetes was induced in the rats using alloxan. Alloxan produces hyperglycemia by selective cytotoxic effect on pancreatic β-cells and one of the intracellular phenomenons for its cytotoxicity is through generation of free radicals, which has been demonstrated both in vivo and in vitro (Grandy et al., 1982; Papaccio et al., 1986). Free radicals and associated reactive species have been implicated in diabetes and its complications (Baynes and Thorpe, 1999). In view of above facts, it has been suggested that a variety of antioxidants that scavenge reactive oxygen species may improve hyperglycemia and prevent diabetic complications. Fifty percent of ethanolic extract of *R. communis* roots is known to possess free radical scavenging activity (Ilavarason et al., 2006). In view of above, it can be proposed that mechanism of antidiabetic activity of RCRE may be its protective role in quenching free radicals (which destroy pancreatic cells) and thus a beneficial role in survival of pancreatic beta cells.

RCRE caused significant increase in the serum insulin levels in diabetic treated group compared to the untreated diabetic animals. Enhanced serum insulin levels in the RCRE treated animals can be a direct evidence for the regeneration or the survival of pancreatic β-cells. Increased levels of serum insulin in diabetic animals treated with plant products is not an uncommon phenomenon as this effect has been reported with some other medicinal plants like *Terminalia chebula* and *E. jambolana* (Murali et al., 2007; Sharma et al., 2003).

Out of the twenty fractions obtained from purification of RCRE, only one fraction (R18) showed significant blood glucose lowering activity in diabetic animals, though the precise active component/s of the antidiabetic effect of RCRE still remain/s to be determined. RCRE showed no toxicity in the acute toxicity study. Most interesting observation was that there was no death even at 10 g/kg b.wt. which is twenty times of the effective dose of root extract. LD_{50} of RCRE is more than twenty times of its effective dose and thus it appears to have a high margin of safety.

In conclusion, the results of the present study show that RCRE showed potent blood glucose lowering activity, both in the normal as well as alloxan diabetic rats. The effective dose of RCRE was found to be 500 mg/kg b.wt. Administration of RCRE to the diabetic rats for 20 days not only significantly lowered the FBG of the diabetic animals to almost normal level, but also increased the insulin levels and caused improvement in lipid profile and body weight of the diabetic animals. RCRE also caused reversal of the damage of liver and kidneys seen in diabetic animals. RCRE was found to have a high margin of safety and thus *R. communis* seems to have a promising value for the development of a potent phytomedicine for diabetes, though further comprehensive pharmacological investigations are needed to elucidate the exact mechanism of action of the *R. communis* root extract.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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