

**HYPOGLYCAEMIC ACTIVITY OF ROOTS OF *RUBIA CORDIFOLIA*
IN NORMAL AND DIABETIC RATS**

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Summary

The hypoglycaemic activity of the alcoholic extract of roots of *Rubia cordifolia* (RCAE, 50, 100 and 200 mg/kg, p.o.) was studied in normal, glucose fed and alloxan- induced diabetic rats. The normal rats were treated with single dose of RCAE (200 mg/kg, p.o.) reduced the blood glucose by 20.4 %. The rats pre-treated with RCAE (200 mg/kg, p.o.) improved oral glucose tolerance by 124.7 % at ½ h compared to glucose fed rats, exogenously injected insulin (1 IU/kg, s.c.) with RCAE (200 mg/kg, p.o.) caused 26% potentiation of hypoglycaemic effect at 6 h as compared with alone insulin treatment. A single oral dose of RCAE (200 mg/kg) to alloxan- induced diabetic rats showed 12.5 % reduction in blood glucose at 4 h.

After repeated oral administration of RCAE (200 mg/kg) in diabetic rats for two weeks, the blood glucose reduced by 27.1 %, serum total cholesterol by 19.8 % and serum triglyceride by 16.6 % and serum albumin concentration increased by 40.3 % as compared to diabetic control group. The hypoglycaemic activity of RCAE was compared with an oral hypoglycaemic agent, glibenclamide.

Key Words: *Rubia cordifolia*; hypoglycaemic; antihyperglycaemic; alloxan.

Introduction

Since ancient times, several medicinal plants, their extracts or different formulations have been given orally in the treatment of diabetes mellitus. The major merits of herbal medicines are their inherent efficacy, low incidence of side effects and low cost. Though many plant products have been proved to be useful in the control of diabetes mellitus, none of them as yet has emerged as a perfect remedy for this disease.

Indian Maddar (*Rubia cordifolia*) is commonly used herb in Ayurvedic system of medicine. It has a variety of uses such as blood purifier, immunomodulator, anti-inflammatory and anti-platelet activation [1]. The triterpenes isolated from *Rubia cordifolia* has anticonvulsant activity [2]. Rubiadin, a dihydroxy anthraquinone, isolated from alcoholic extract of *Rubia cordifolia*, possesses potent antioxidant property [3]. The protective effect of *Rubia cordifolia* on lipid peroxide formation in isolated rat liver homogenate is also reported [4]. Since oxygen free radicals are responsible for the severity and complications of diabetes [5], herbs having anti-peroxidative or anti-oxidant activity can be useful in the management of diabetes.

Rubia cordifolia L. (Rubiaceae) is a small plant [6] growing throughout India in hilly districts. Ethanolic extract of the aerial parts of the plant was found to possess hypoglycaemic activity in albino rats [7]. Since no scientific studies have been carried out on the roots, the present study was carried out to evaluate hypoglycaemic activity of the roots of the *Rubia cordifolia* in normal and diabetic rats.

Methods

Preparation of extracts

The roots of *Rubia cordifolia* were obtained from the local market and were taxonomically authenticated by Dr. A. K. Singhai, Division of the Pharmacognosy, Dept. of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar-470 003 (MP), India. A voucher specimen of the plant (AH23294) has been deposited in the Botanical Survey of India, Pune. The powdered roots were extracted with ethanol (95%) under reflux. The ethanolic extract (RCAE) of roots of *Rubia cordifolia* (1.76% w/w) was concentrated under reduced pressure. The preliminary phytochemical studies showed the presence of sterols, anthraquinone and saponins.

Experimental animals

Wistar rats weighing 200-250 g of either sex were used. Animals were housed in groups of five per cage at a temperature of $25^{\circ} \pm 1^{\circ}$ C and relative humidity of 45-55% and acclimatized for one week after their arrival. A 12:12 h dark: light cycle was followed during the experiments. Animals had free access to food and water except 18 h before and during the period of experiment. The standard diet was prepared by mixing wheat (60%), maize (20%), gram (10%), milk powder (2.5%), salt (2.5%), groundnut oil (2%) and sufficient tap water. The Institutional Animals Ethics Committee approved the protocol of the study.

Chemicals

Alloxan (S D Fine- Chem, India), insulin (USV Ltd, India) and glibenclamide (Sun Pharma, India) were used in this study. Other chemicals used were of analytical grade, obtained from Qualigens, India.

Administration of RCAE and blood glucose estimation

RCAE was solubilized in saline and administered orally (50, 100 and 200 mg/kg in 0.1 ml) through a stainless steel canula fitted to a syringe. The reference standards glibenclamide (0.40 mg /kg) and insulin (1 IU/kg) were administered orally and subcutaneously, respectively.

For blood glucose determination, blood was obtained by snipping tail with sharp razor [8]. The blood glucose concentration was determined by using One Touch glucometer (Johnson and Johnson, India) at 2, 4 and 6 h after treatment.

Oral glucose tolerance test

After overnight fasting, all animals in control group received glucose (1.5 g/kg, p.o) and blood glucose was estimated at 0, ½, 1 and 2 h later using glucometer. The treatment group received glibenclamide (0.40 mg /kg) or RCAE (200 mg/kg) two hours prior to glucose (1.5 g/kg) and the blood glucose was determined.

Insulin co- administration

Soluble insulin (1 IU/kg) was injected subcutaneously to overnight fasted rats and blood samples were collected at 0, 2, 4 and 6 h after insulin. The blood glucose was estimated by using glucometer. A similar study was repeated with RCAE (200 mg/kg, p.o.), given two hour prior to the insulin [9].

Antihyperglycaemic activity

The overnight fasted rats were made diabetic by subcutaneous injection of alloxan monohydrate (120 mg/kg body weight). After 48 h diabetes was confirmed by measurement of fasting blood glucose. The animals having blood glucose below 250 mg % were discarded from the study. The diabetes was stabilized for a week and the rats were divided into different groups containing five each.

The blood glucose levels were determined from diabetic control group as well as RCAE (200 mg/kg) and glibenclamide (0.40 mg /kg) treated group at 0, 2,4 and 6 h.

Chronic treatment

Overnight fasted rats were injected with alloxan (120 mg/kg, s.c.) in the same manner described as above. Rats exhibiting blood glucose level > 250 mg %, 48 h after administration of alloxan were included in the study. After two weeks treatment with RCAE (200 mg/kg) and glibenclamide (0.40 mg /kg), blood glucose and biochemical parameters such as serum cholesterol, HDL-cholesterol, total protein, albumin, triglyceride and alkaline phosphatase were determined. All biochemical assays were carried out using diagnostic kits (Bio Labs, Mumbai).

Statistical analysis

All the data presented as mean ± SEM. One- way ANOVA followed by Dunnett's test was employed to calculate the significance. For dependent variables (before and after the treatment) Student's paired t- test was used. Statistical significance was tested at 5% level.

Results

Hypoglycaemic activity

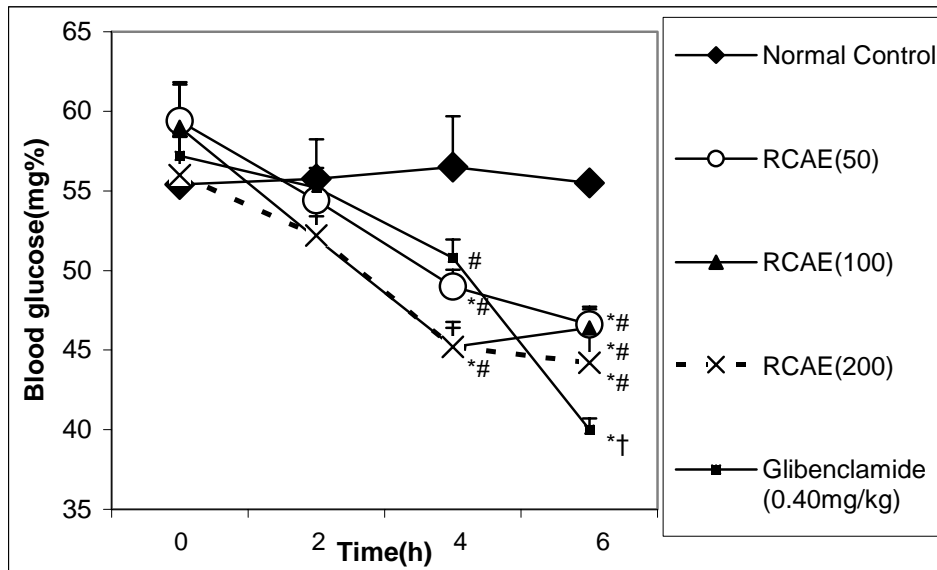
The preliminary phytochemical studies showed the presence of sterols, anthraquinone and saponins in RCAE. Figure 1 shows the effect of RCAE on blood glucose levels in normal fasted rats, which is aimed at selecting the most effective dose of plant extract. The maximum hypoglycaemic effect was seen in the group receiving 200 mg/kg, p.o. compared to the groups receiving 50 and 100 mg/kg, at 6 h sample. The respective percentage decrease in blood glucose levels was by 20.4 % and 29.90 % in RCAE (200 mg/kg) and glibenclamide (0.40 mg /kg) treated groups compared with control.

Oral glucose tolerance test

Results of the glucose tolerance test conducted on normal rats fed with RCAE (200 mg/kg, p.o.) shown in figure 2. Within ½ h of glucose load, blood glucose concentration increased to 145.75 from 56.75 mg % in glucose control group and hyperglycaemia maintained until 1 h and reduced to initial value at 2 h.

Administration of RCAE, two hours prior to glucose load showed improved glucose tolerance in normal rats. The rats pre-treated with RCAE and glibenclamide two hours before glucose load showed 124.7 % improved glucose tolerance from 56.4 to 87.0 mg % and 146.7 % improved glucose tolerance from 58.5 to 80.25 mg % at ½ h respectively.

Figure 1. Effect of RCAE on blood glucose in normal fasted rats



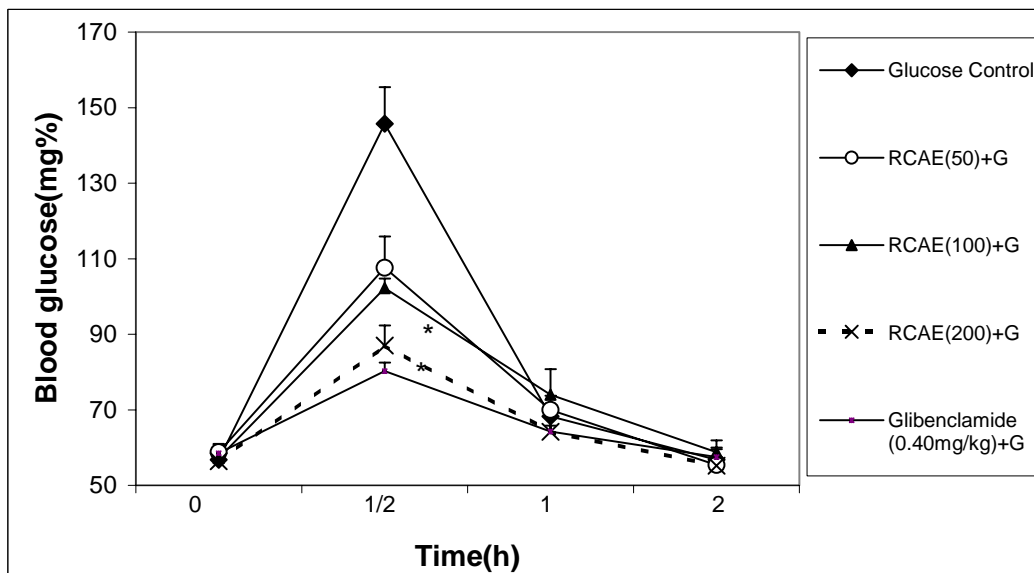
N = 5 in each group, values are mean±SEM

* p< 0.05 compared to control group (ANOVA followed by Dunnett's test)

p< 0.05 compared to initial value (0 hr) (paired t test)

† p<0.001 compared to initial value (0 hr) (paired t test)

Figure 2. Effect of RCAE on oral glucose tolerance test

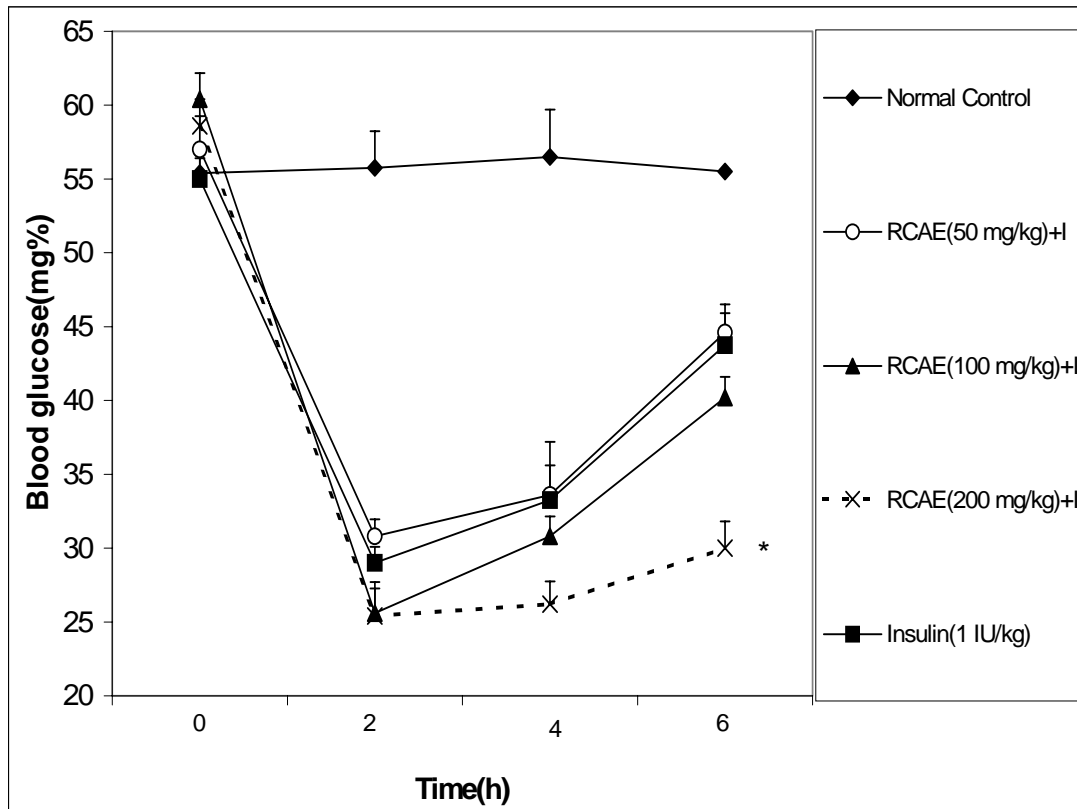


N = 5 in each group, values are mean±SEM

* p< 0.05 compared to Glucose control group (ANOVA followed by Dunnett's test)

Insulin co-administration

In the figure 3, pre-treatment with RCAE (200 mg/kg, p.o.) significantly ($p < 0.05$) increased the hypoglycaemic effect of insulin. Insulin (1 IU/kg, s.c.) produced fall in blood glucose from 55.0 (0 h) to 27.0, 33.25 and 43.75 mg % at the end of 2, 4 and 6 h respectively. After 6 h of RCAE treatment with insulin, the blood glucose reduced from 58.6 to 30.0 mg %. Thus, RCAE potentiated insulin effect by 26.0 % at 6 h.

Figure 3. Effect of RCAE with insulin co-administration

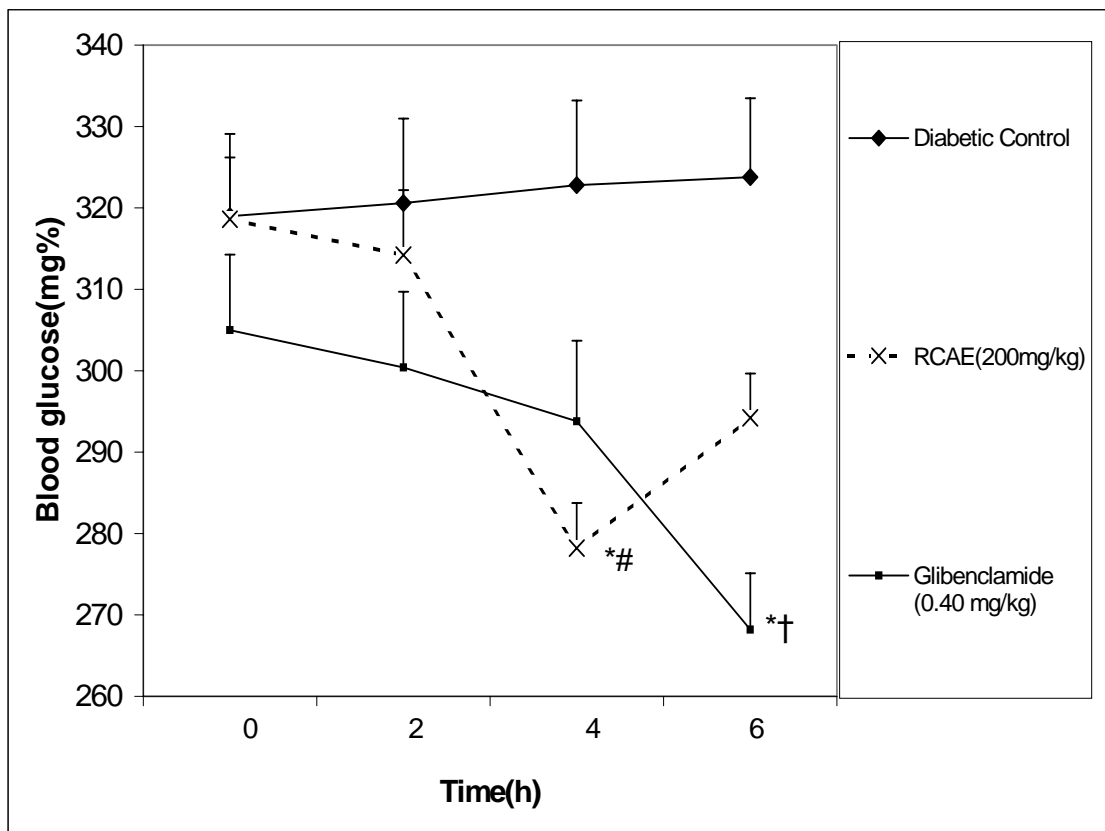
N = 5 in each group, values are mean \pm SEM

* $p < 0.05$ compared to Insulin control group (ANOVA followed by Dunnett's test)

Antihyperglycaemic activity

The effect of feeding 200 mg/kg, of RCAE on blood glucose level in alloxan- induced rats is shown in figure 4. In alloxan- induced diabetic rats the average fasting blood glucose was 295-315 mg %. The single oral administration of RCAE (200 mg/kg) caused 12.5 % reduction in blood glucose from 318.6 (0 h) to 278.2 mg % at 4 h sample. While glibenclamide caused 11.9 % reduction in blood glucose from 305.0 (at 0 h) to 268.2 \pm % at 6 h.

Fig 4: Effect of RCAE on blood glucose in alloxan- induced diabetic rats



N = 5 in each group, values are mean±SEM

* p< 0.05 compared to Diabetic control group (ANOVA followed by Dunnett's test)

p< 0.05 compared to initial value (0 hr) (paired t test)

† p<0.001 compared to initial value (0 hr) (paired t test)

Chronic treatment

After repeated oral administration of RCAE in diabetic rats for two weeks, the blood glucose significantly ($p < 0.05$) reduced. RCAE and glibenclamide reduced the blood glucose from 318.6 to 255.0 mg % (27.1 % decrease) and 305.0 to 227.7 mg % (25.0 % decrease) respectively (Table 1).

The biochemical estimation after two weeks administration of RCAE (200 mg/kg) and glibenclamide (40 µg/kg) significantly ($p < 0.05$) reduced the cholesterol by 19.8 and 25.6 % and triglycerides by 16.0 and 11.4 % respectively compared to diabetic group. While RCAE and glibenclamide significantly ($p < 0.05$) increased serum albumin by 40.3 and 32.5 % respectively (Table 1).

Table 1: Effect of two weeks treatment with RCAE on different blood parameters in alloxan-induced diabetic rats

Sr. No.	Treatment	Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Triglyceride (mg/dl)	ALP (IU/L)	BGL (mg %)
1.	Normal	116.18 ± 6.91	38.94 ±1.39	7.11 ± 0.14	5.13 ± 0.39	153.53 ± 7.20	105.33 ± 7.57	57.00 ± 1.08
2.	Diabetic	163.95 ± 3.33*	34.0 ± 0.8*	6.30 ± 0.15*	3.57 ± 0.39*	185.46 ± 4.38*	140.45 ± 10.78*	326.25 ± 7.05*
3.	Diabetic + RCAE(200)	131.30 ± 7.35@	36.97 ± 0.70	6.77 ± 0.20	4.88 ± 0.10@	154.95 ± 5.04@	113.59 ± 6.59	255.00 ± 7.08@
4.	Diabetic + Glibenclamide(0.40mg)	121.64 ± 5.11@	37.55 ± 0.71	6.75 ± 0.27	4.61 ± 0.17@	163.93 ± 5.78@	113.59 ± 12.68	227.75 ± 13.09@

N = 5 in each group, values are mean ± SEM

* p< 0.05 compared to Normal group (ANOVA followed by Dunnett's test)

@ p< 0.05 compared to Diabetic group (ANOVA followed by Dunnett's test)

Discussion

The alcoholic extract of *Rubia cordifolia* exerted significant hypoglycaemic effect in normal fasted, glucose fed and alloxan- induced diabetic rats. It potentiated the effect of exogenously- administered insulin. The biochemical investigations after daily administration of RCAE for two weeks showed significant decrease in serum cholesterol and triglyceride and increase in serum albumin compared to diabetic control group.

In normal fasted rats, though the onset of hypoglycaemic effect was seen in a single dose, the maximum effect continued to increase with duration. Grover *et al.*, [10] reported that to obtain maximum effect, therapy with plant products be continued for longer duration. In glucose tolerance test, the oral administration of RCAE suppressed the increase in glucose level induced by glucose loading. It suggests that RCAE plays role in glucose homeostasis. This might be due to increased peripheral utilization of glucose. This was strongly supported by the potentiating effects of RCAE to exogenously injected insulin.

The biochemical studies in diabetic animals revealed the changes in the carbohydrate, protein and lipid metabolism. A single injection of alloxan monohydrate produces diabetes within 48 h. The animals become

progressively hyperglycemic, hyperlipidaemic and hypoalbuminemic. It is known that the factor influencing glucose metabolism under various physiological conditions do influence lipid metabolism as well [11]. It has also been revealed that triglyceride accumulation increase considerably in diabetes mellitus [12]. Pandey et al., [4] found the protective effect of *Rubia cordifolia* on lipid peroxide formation in isolated rat liver homogenate. Excessive breakdown of body protein in conjunction with either inadequate supply or defective utilization of glucose observed in uncontrolled diabetes may be accompanied by hypoalbuminemia [13]. A marked increase in serum triglycerides and free fatty acids and some fluctuations in serum cholesterol were observed in uncontrolled diabetes rats. RCAE decreased the level of serum lipids and increased serum albumin significantly in diabetic rats.

Thus, it is concluded that, alcoholic extract of *R. cordifolia* reduced blood glucose in normal and diabetic rats, improved glucose tolerance and potentiated insulin effect. This suggests that the extract may stimulate the β - cells of the pancreas to release the insulin, which is similar to that of sulfonylureas. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of the antidiabetic effect of *R. cordifolia* root extracts.

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