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ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *ROTULA AQUATICA* LOUR. ROOTS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT
Rotula aquatica (Lour.) family-Boraginaceae roots are used for the treatment of diabetes traditionally. The aim of the present study was undertaken to investigate the hypoglycaemic and antidiabetic effect of single and repeated oral administration of the aqueous extract in normal and streptozotocin induced diabetic rats respectively, serum lipid profile was also examined. The dose of 200, 300 and 500 mg/kg body weight p.o. of the aqueous extract were evaluated and the dose of 200 mg/kg was identified as the most effective which reduced blood glucose level upto 40.6% after 4 hr of administration in normal rats and a fall of 38% in blood glucose level within 1 hr during glucose tolerance test (GTT) in mild diabetic rats which has almost similar effect as that of standard drug glibenclamide (3 mg/kg bw). Severe diabetic rats were treated daily with 200 mg/kg bw for 14 days and a significant reduction of 56% was observed in fasting blood glucose level respectively, urine sugar level was decreasing with increasing in body weight and total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG) levels were decreased by 24, 59 and 41% respectively, in severely diabetic rats whereas, cardioprotective, high density lipoprotein (HDL) was increased by 26%. The data showed that aqueous extract has a remarkable hypoglycaemic, antidiabetic and hypolipidemic effect.
Keywords: *Rotula aquatica;* Streptozotocin; Glibenclamide; Hypoglycemic

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycaemia and relative insulin deficiency, resistance, or both. Till date, it has already affected more than 120 million people world-wide, and according to an estimate, 220 million people will be affected by the year 2020 [1]. Hence, it is imperative to intervene and look for new drugs to manage this metabolic disorder. India is a country with a vast reserve of natural resources with a rich history of traditional medicine [2]. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The available ethanobotanical information accounts for about 800 plants that may possess anti-diabetic potential [3]. Management of hyperglycemia or hyperlipidemia with minimal side effect in clinical experience, and relatively low cost is still a challenge to the medical system [4].

Rotula aquatica Lour. a member of the family Boraginaceae, is a small much branched shrub, 60-180 cm in height with numerous short lateral arrested branchlets often rooting [5]. The plant is scattered throughout peninsular and Western Ghats of India in the sandy and rocky beds of streams and rivers. The roots are bitter, astringent, cooling, diuretic and laxative, and useful in haemorrhoids, renal and vesical calculi, diabetes and venereal diseases [6]. Review of literature provides evidence for experimental evaluation of *R. aquatica* for its anti-inflammatory [7], crystal dissolution [8], antimitotic [9] and antilithiatic [10] nature. Although traditional literature documents its claim as antidiabetic, no scientific data are available to substantiate this fact. Therefore, the present study is aimed to investigate the hypoglycaemic, hypolipidemic and antihyperglycemic activity of aqueous extract of *R. aquatica*.

EXPERIMENTAL

General

Streptozotocin (STZ) was procured from Spectrochem Pvt. Ltd., Mumbai. Total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) cholesterol and triglycerides (TG) were estimated using standard kits of Transasia Bio-medicals Ltd., India. LDL cholesterol was calculated according to Friedwald et al. (1972). Body weight (bw) was determined gravimetrically, and urine sugar by reagent-based Pathozyme diagnostics Ltd. Mumbai.

Plant

Rotula aquatica Lour. plants and its roots were collected from Kumata, Karnataka, India. Plants were identified and authenticated by Dr. Harsha Hegde, Scientist, Regional Medical Research Centre, (ICMR), Belgaum, Karnataka, and deposited the voucher specimen in the Department of RMRC (Voucher No- 473).

Extraction

Roots were dried in shade, powdered and subjected to Soxhlet-extraction with petroleum ether, ethyl acetate, ethanol and aqueous using maceration method. The aqueous extract was evaporated in vacuo and the extractive yield was 5.62% w/w. Phytochemical screening of the extract revealed the presence of tannin, glycosides and reducing sugars [11]. The extract was suspended in water using Tween 80 as a suspending agent for the purpose of oral administration.

Experimental animals

Male albino Wistar rats of body weight 180-220 g were selected for the experiment. Animals were kept in animal house at an ambient temperature of 25–30^{0}C and 45–55% relative humidity with a 12 h light: dark cycle. Animals were fed pellet diet (Amrut pellets, Sangali) and water ad libitum. Study was carried out as per the ethical clearance KLE Belgaum/12/2008.

Induction of diabetes in rats

Diabetes was induced by a single intra-peritoneal injection of freshly prepared streptozotocin (45 mg/kg bw) in 0.1 M citrate buffer (pH 4.5) to overnight fasted rats. After 3 days of STZ administration, rats were divided according to their fasting blood glucose (FBG) levels in two groups: mild diabetic rats (FBG 120-250 mg/dl) and severe diabetic (FBG > 250 mg/dl).

Acute toxicity studies

Acute toxicity studies were conducted on mice as per the Organization for Co-operation and Development (OECD) 423 guidelines. The aqueous extract of *R. aquatica* roots was

administered at different doses of 2000, 3000 and 5000 mg/kg bw to four groups of overnight fasting mice (n=6).

The animals were observed twice on the first day of extract administration, thereafter once daily for 14 days for mortality and gross change in their activity and behavioural pattern. Animals were also observed for the presence of tremors, convulsions, salivation, diarrhoea and lethargy.

Experimental design

Determination of hypoglycemic activity in normal healthy rats:

Twenty-four normal healthy male rats were fasted overnight. These fasted animals were divided into four groups of six rats, in each. Pretreatment FBG level for each group was evaluated. Group I served as untreated control and received vehicle (water only), whereas the other three groups, II, III and IV were given lyophilized aqueous extract suspended in distilled water orally in doses of 200, 300 and 500 mg/kg bw, respectively. Blood samples were collected from the tail vein after 2, 4 and 6 h, and the percentage change in blood glucose was determined.

Determination of antidiabetic activity on glucose tolerance in mild-diabetic rats:

The antidiabetic effect of aqueous extract was assessed in mild-diabetic rats using a glucose tolerance test (GTT). The overnight fasted rats were divided into five groups (V, VI, VII, VIII and IX) of six rats, in each. Pretreatment-FBG level of each group was evaluated. Group V (diabetic control) received vehicle (water only), while 200, 300 and 500 mg/kg bw of aqueous extract and a dose of 3 mg/kg bw of glibenclamide were given orally to groups VI, VII, VIII and IX, respectively. Blood glucose level (BGL) of each group was evaluated after 90 min of the treatment and considered as 0 h value. A known amount of 2 g/kg bw glucose solution was given to all the groups, and their BGL was estimated after 1 and 2 h of glucose administration.

Determination of antidiabetic activity on glucose tolerance in severely-diabetic rats:

The study was carried out on three groups (X, XI and XII) of six rats, in each. Group X served as normal control, while group XI as diabetic control and XII as diabetic treated group. Control rats (group X and XI) were given vehicle (distilled water) only, while group XII received orally a single dose of 200 mg/kg bw of aqueous extract suspended in distilled water daily for two weeks. Fasting blood glucose, total cholesterol, HDL and LDL cholesterol, urine sugar and triglycerides levels were measured before and after the treatment, weekly up to two weeks.

Statistical analysis

The data are expressed as mean ± S.E. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Dunnett multiple range test. The results are considered statistically significant if the *P* values are 0.05 or less.

RESULTS

Acute toxicity studies

Acute toxicity studies showed that orally administered *R. aquatica* extract was safe to the rats at all the doses tried, up to 5000 mg/kg bw. No mortality was observed during the observation period of 14 days.

Effect of extract on FBG in normoglycemic rats

Effect of *R. aquatica* aqueous extract on blood glucose level of normal rats is presented in Table 1. All the three doses of the extract produced significant hypoglycaemic effect after 4 h of oral administration. The animals treated with 300 and 500 mg/kg bw aqueous extract produced a reduction of 21 and 23.5% in blood glucose level, respectively to that of control after 4 h of administration. However, the reduction was maximal (40.6%) in animals treated with a dose of 200 mg/kg bw. The hypoglycaemic effect with all the three doses was moderate at 2 h of administration. However, increased BGL was observed after 6 h of treatment.

Effect of extract on glucose tolerance in mild-diabetic rats

For the selection of optimum dose for the diabetic animals, different doses of aqueous extract (200, 300 and 500 mg/kg bw) were evaluated on glucose tolerance in mild diabetic rats along with the standard drug glibenclamide (3 mg/kg bw). Fig. 1 depicts the effect of different doses of *R. aquatica* aqueous extract and glibenclamide on glucose tolerance up to two hour in mild-diabetic rats. A reduction of 38, 25 and 30% in BGL was observed within an hour of GTT at doses of 200, 300 and 500 mg/kg bw of the extract. However, the identical doses decreased the BGL about 53, 46 and 44% after 2 h of glucose administration. A dose of 3 mg glibenclamide/kg bw reduced BGL by 21 and 46% at 1 and 2 h, respectively, during GTT. The drop produced by 200 mg aqueous extract/kg bw is more significant compared to that of 3 mg/kg bw of the standard drug glibenclamide. Since 200 mg/kg bw of the *R. aquatica* aqueous extract was found to be the most effective dose during GTT for STZ induced mild diabetic rats, it was selected for evaluation in severe diabetic rats. As in Fig:1

Effect of extract on FBG and lipid profile in severe diabetic rats

The effect of repeated oral administration of *R. aquatica* aqueous extract on severe diabetic rats is shown in Table 2**.** In normal control rats, the FBG level observed at 7 and 14 days after the treatment with vehicle was almost identical to that of pretreatment level. In diabetic control rats the FBG rises gradually during 14 days. However, administration of the most effective dose (200 mg/kg bw) of the extract produced a marked antihyperglycemic effect in treated diabetic rats. The FBG level decreased from 226 to 102.5 mg/dl after 2 weeks treatment. The post-treatment levels of total cholesterol, LDL cholesterol and triglyceride of the treated group were significantly lower than that of the pretreatment levels showing a reduction of 24% in total cholesterol, 59% in LDL cholesterol and 41% in triglyceride in severe diabetic rats after 14 days of treatment. There was also an increase of 26% in HDL cholesterol in the treated diabetic groups.

Effect of extract on urine sugar and body weight in severe diabetic rats

Table 3 depicts the effect of 200 mg/kg bw of *R. aquatica* aqueous extract on urine sugar level and body weight of severe diabetic rats. In treated diabetic groups, the level of urine sugar decreased by 75% after 14 days of treatment, while body weight increased continuously as compared to diabetic control.

DISCUSSION

The findings of this study indicate that the aqueous extract of *R. aquatica* has a significant hypoglycemic effect in normal rats, up to 4 h. The effective dose was 200 mg/kg bw, and the response decreased at higher doses of 300 and 500 mg/kg bw. Such a phenomenon of less hypoglycaemic response at higher doses is common with indigenous plants and has already been observed in *Psidium guajava* [12], *Trichosanthes*

dioica [13]. The GTT studies of the mild diabetic animals reveal a reduction of 38%, 25% and 30% in blood glucose level was observed within 1 h of glucose tolerance test by the doses of 200, 300 and 500 mg/kg bw of the aqueous extract. However, the same doses decreased the blood glucose level only about 53%, 46% and 44% after 2 h of glucose administration. A dose of 3 mg/kg bw of Glibenclamide reduced blood glucose level by 21% and 46% at 1 and 2 h, respectively, during glucose tolerance test. The GTT studies also confirm 200 mg/kg bw to be the most effective dose as found in the case of STZ induced mild diabetic rats. This dose was therefore, selected for further studies in the case of severely diabetic animals and a decrease of 16 and 54% was observed in FBG after 7 and 14 days of treatment, respectively. High levels of total cholesterol and more importantly LDL cholesterol are major coronary risk factors [14], whereas an increase in HDL cholesterol is associated with a decrease in coronary risk. Most of the drugs that decrease total cholesterol also decrease HDL cholesterol [15]. However, it is interesting to find out that in the present study the dose of 200 mg/kg bw of the aqueous extract not only lowered the total cholesterol, triglyceride and LDL levels by 24, 41 and 59%, respectively, but also enhanced the cardio protective lipid HDL by 26% after 14 days treatment. A decrease in body weight was registered in the case of STZ diabetic control group rats, while in the treated group the weight loss was reversed. The ability of the aqueous extract to protect body weight loss seems to be a result of its ability to reduce hyperglycemia. A decline of 75% in urine sugar was also observed after 14 days of treatment. Phytochemical investigation of *R. aquatica* reveals the presences of tannins and glycosides [7, 8]. These principles are known to be bioactive for the management of diabetes. It is well known that certain tannins exhibit hypoglycemic activity. Thus, the significant antidiabetic effect of aqueous extract of *R. aquatica* may be due to the presence of more than one antihyperglycemic principle and their synergistic properties. From this study we may conclusively state that *R. aquatica* aqueous extract has remarkable effect on blood glucose level, and marked improvement on hyperlipidemia due to diabetes. Its specific effect on HDL has additional advantage in checking coronary risks. The extract seems to have no toxicity as no death is reported upto 10 times of effective dose. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of the antidiabetic and hypolipidemic effect of *Rotula aquatica*.

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Table 1: Hypoglycemic effect of graded doses of aqueous extract of *Rotula aquatica* Lour. on normal rats (mean ± S.E.)

Experimental animals	Treatment (extracts, mg/kg)	Blood glucose level (mg/dl)			
		Pretreatment level	Post-treatment		
		FBG	2 h	4 h	6 h
Control	Distilled water	78.0±1.0	79.0±3.0	75.5±2.5	74.0±4.5
Treated	200mg/kg	80.0±5.5	72.0±3.0	47.5±0.5*	60.0±5.0*
Treated	300mg/kg	83.5±1.4	64.0±1.0	66.0±6.0*	66.0±1.1*
Treated	500mg/kg	68.0±1.0	58.0±6.5	52.0±1.3*	70.0±1.5

*$P < 0.05$ as compared to pretreatment level

Table 2: Effect of oral administration of the aqueous extract of *Rotula aquatica* Lour. on fasting blood glucose (FBG) and serum lipid profile in severe diabetic (SD) rats (means ± S.E).

Experimental animals	Treatment (aqueous exract)	Pretreatment level	Post-treatment level	
			7 day	14 day
FBG (mg/dl)				
Normal (control)	Distilled water	71.0±2.0	72.5±5.5*	71.5±2.5*
SD (Control)	Distilled water	291.5±6.5	295±3.2*	306.0±5.5*
SD (treated)	200 mg/kg bw	226.0±2.4	190±6.0*	102.5±7.5*
Total Cholesterol (mg/dl)				
Normal (control)	Distilled water	67.5±2.5	76.0±6.0*	71.5±3.5*
SD (Control)	Distilled water	120.5±5.5	126.0±7.0*	139.0±9.0*
SD (treated)	200 mg/kg bw	114.0±2.0	102.5±0.5*	86±2.0*
HDL Cholesterol (mg/dl)				
Normal (control)	Distilled water	34.0±3.0	39.5±4.5**	40.0±4.6**
SD (Control)	Distilled water	26.0±6.5	20.5±3.2**	19.0±5.5**
SD (treated)	200 mg/kg bw	35.0±2.2	40.0±7.6**	47.0±1.5**
Triglycerides (mg/dl)				
Normal (control)	Distilled water	72.0±6.0	86.0±3.3*	77.0±1.5*
SD (Control)	Distilled water	141.5±3.5	151.5±3.5*	160.0±8.0*
SD (treated)	200 mg/kg bw	157.5±3.5	109.0±7.0*	93.0±2.5*
LDL Cholesterol (mg/dl)				
Normal (control)	Distilled water	18.5±3.5	18.5±6.5*	16.5±2.5*
SD (Control)	Distilled water	66.0±7.0	75.0±5.0*	87.0±6.5*
SD (treated)	200 mg/kg bw	47.5±7.5	40.0±3.0*	19.5±4.5*

*P< 0.001 as compared to that of pretreatment level, **P<0.05 as compared to that of pretreatment level

Table 3: Effect of most effective dose of *Rotula aquatica* Lour. aqueous extract on urine sugar and body weight in severe diabetic rats (mean ± S.E).

Experimental animals	Treatment (aqueous exract)	Pretreatment level	Post-treatment levels	
			7 day	14 day
Urine sugar				
Normal (control)	Distilled water	Nil	Nil	Nil
SD (Control)	Distilled water	+++	+++	+++
SD (treated)	200 mg/kg bw	+++	++	+
Body weight (g)				
Normal (control)	Distilled water	202.0±8.0	207.0±5.0	220.0±3.5
SD (Control)	Distilled water	238.0±5.2	223.0±5.5	174.0±4.0
SD (treated)	200 mg/kg bw	222.0±2.7	230.0±2.9	144.0±2.5

Fig. 1. Effect of aqueous extract of *Rotula aquatica* roots on glucose tolerance test in mild diabetic rats.