

# Antidiabetic Activity of *Rotula aquatica* Lour Roots in Streptozotocin Induced Diabetic Rats

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## ABSTRACT

The study was designed to evaluate antidiabetic activity on methanolic extracts of *Rotula aquatica* Lour roots in Streptozotocin induced diabetic rats. The roots of *Rotula aquatica* Lour were extracted with methanol and evaluated for their antidiabetic activity in Streptozotocin induced diabetic rats at doses levels of 100, 200 and 400 mg/kg body weight. The lowering of blood sugar levels as well as percent blood glucose reduction were calculated and compared with the standard glibenclamide (0.45 mg/kg b.w.). Preliminary phytochemical screening was also conducted for occurrence of compounds. The oral administration of 400 mg/kg b.w. of methanolic extract of roots of *Rotula aquatica* showed more significant ( $P < 0.01$ ) decrease in blood glucose levels at 4 and 8<sup>th</sup> hrs and highly significant ( $P < 0.001$ ) decrease in blood glucose levels at 8<sup>th</sup> hr. The methanolic extracts of *Rotula aquatica* roots showed good activity at a dose of 400 mg/kg body weight. Phytochemical screening revealed presence of triterpenes and tannins in plant extracts.

**Keywords:** Antidiabetic activity, Glibenclamide, *Rotula aquatica*, Streptozotocin.

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by derangement in carbohydrate, fat and protein metabolism due to absolute or relative deficiency of insulin secretion and with varying degree of insulin resistance<sup>1,2</sup>. In diabetes, the deficiency of insulin leads into a complex series of reactions, which are clinically manifested as hyperglycemia<sup>3</sup> and is characterized by a loss of glucose

homeostasis<sup>4-8</sup>. The tribal and rural population of India are highly dependent on the medicinal plant therapy for meeting their health care needs. There is an urgent need to formulate some effective herbal medicinal preparations either with single plant or in combination with different plants after clinical trials for effective treatment and control of diabetes mellitus<sup>9</sup>.

*Rotula aquatica* (Family: Boraginaceae) is an important traditional medicine in the preparation of many ayurvedic formulations for the treatment of many diseases like coughs, heart diseases, dysuria, blood disorders, fever, poisonings, ulcers and uterine diseases. Traditionally it has been used in the treatment of diabetes by rural people. The plant has been reported to possess antidiabetic activity, as it is one of the component in a compound herbal drug formulation, cogentdb<sup>10</sup>. There is no scientific evidence upon the single plant extract for its antidiabetic action, Hence it was aimed to evaluate activity in Streptozotocin induced diabetic rats.

## MATERIALS AND METHODS

### Collection of Plant material

The plant, *Rotula aquatica*, was collected from travencore in September and was authenticated by Prof. M. Venkaiah, Department of Botany, Andhra University. The specimen was deposited in the herbarium with Voucher specimen number (RA/01).

### Preparation of plant extract

The freshly collected roots were shade dried and powdered in a Wiley mill. The root powder was extracted in a soxhlet apparatus for 6 hrs successively with petroleum ether, chloroform and methanol, which further concentrated to dryness under vacuum.

### Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by the addition of various reagents to find the presence of the active metabolites in methanolic extract such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, steroids, fixed oils and fats<sup>11,12</sup>. The flavonoids were tested by alkaline reagent and Shinoda test. The tannins were tested by ferric chloride and gelatin test.

The steroids and triterpenoids were tested by Salkowski test.

### Animals

Wistar albino rats of either sex (150-200 g) were maintained under controlled conditions for all sets of experiments. The rats were allowed to take standard laboratory feed and water *ad libitum*. The experimental protocol was approved by the institutional animal ethics committee of Andhra university, Vishakhapatnam, which was registered with Committee for the purpose of control and supervision of experiments on animal (CPCSEA), Govt. of India (registration no.516/01/A/CPCSEA).

### Design of experiment

In this experiment, 30 rats were used which were randomly divided into 5 groups of 6 animals each. The different doses of extracts were administered orally to the STZ induced diabetic rats. All the extracts were suspended in 1% sodium CMC suspension. In these 5 groups, one group served as untreated control as they received orally 1% Sodium CMC suspension only and one group received standard drug Glibenclamide (0.45mg /kg b.w). Methanolic extracts of selected plants were screened for antihyperglycemic activity in STZ induced diabetic rats at doses of 100, 200 and 400 mg/kg b.w. The drug treatment was given to the animals and was fasted for 12 hr before estimating the blood glucose level.

Group 1 : Vehicle control (1% Sodium CMC suspension)  
Group 2 : Standard (Glibenclamide 0.45mg/kg)  
Group 3 : Received extract of *Rotula aquatica* (100 mg/kg)  
Group 4 : Received extract of *Rotula aquatica* (200 mg/kg)  
Group 5 : Received extract of *Rotula aquatica* (400 mg/kg)

### Induction of Diabetes<sup>13-16</sup>

Diabetes was induced by a single intraperitoneal dose of 60 mg/kg of b. w of streptozotocin (STZ) (Sigma-Aldrich labs) dissolved in 0.1M fresh cold citrate buffer (pH 4.5) into 12 hr fasted rats.

### Collection of blood samples and serum glucose estimation

The blood samples (0.5ml) were collected for every time intervals of 0, 2, 4, 8, 12, 18, and 24<sup>th</sup> hr in 1ml Eppendorf's tubes. Serum was separated by centrifuging at 3000 rpm for 10 minutes. 30 µl of serum sample and 3 ml of working glucose reagent were taken in to a dry and clean test tube and incubated for 10 minutes at 37° C. The pink color developed was measured by using auto analyzer<sup>17-19</sup>.

### Statistical analysis

The values were expressed as mean±SEM. The data was subjected to the analysis of variance (one way ANOVA) to determine the significance of changes followed by students "t"-test<sup>20-22</sup>. The statistical significance of difference between two independent groups was calculated for the determination of blood glucose levels.

## RESULTS

Preliminary phytochemical screening of the plant extracts showed presence of terpenoids and tannins with various chemical tests used for their identification.

The mean blood glucose levels of control and extract treated groups after the oral administration of different dose (100, 200 and 400 mg/kg b.w) of methanolic extract of *Rotula aquatica* roots are shown in Table 1. The mean blood glucose levels at various time intervals were statistically evaluated in comparison with the initial blood glucose level. The results in table showed that blood glucose level of animals treated with 1% Sodium CMC suspension only did not differ

at any time interval significantly from zero hour level.

The rats treated with 100 mg/kg b. w of methanolic extract of roots of *Rotula aquatica* produced a significant (P<0.05) reduction in blood glucose levels at 8<sup>th</sup> hr only while the reduction was no significant (P>0.05) at remaining time intervals. The mean blood glucose levels were 339.7 ±12.4 and 302.37±4.75 mg/dl at 0 and 8<sup>th</sup> hr after the oral administration of 100 mg/kg b. w of methanolic extract of *Rotula aquatica* roots. The glucose levels of diabetic rats treated with 200 mg/kg b.w was decreased significantly (P<0.05) at 8 and 18<sup>th</sup> hr and more significantly (P<0.01) decreased at 12<sup>th</sup> hr. The mean blood glucose levels produced by 200 mg/kg b. w of methanolic extract of roots of *Rotula aquatica* were 343.24±11.42, 301.79±4.97, 282.16±7.44 and 310.42±5.68 mg/dl at 0, 8, 12 and 18<sup>th</sup> hr respectively. The oral administration of 400 mg/kg b.w of methanolic extract of roots of *Rotula aquatica* showed significant (P<0.05) decrease in blood glucose levels at 2<sup>nd</sup> and 18<sup>th</sup> hrs, more significant (P<0.01) decrease in blood glucose levels at 4 and 8<sup>th</sup> hrs and highly significant (P<0.001) decrease in blood glucose levels at 8<sup>th</sup> hr. The mean blood glucose levels after oral administration of 400 mg/kg b. w of methanolic extract of roots of *Rotula aquatica* were 355.29±8.09, 328.15±6.65, 295.04±9.15, 261.13±6.97, 291.59±5.50 and 318.50±5.13 mg/dl at 0, 2, 4, 8, 12 and 18<sup>th</sup> hrs respectively.

The mean percent decrease in blood glucose levels produced by different doses of methanolic extract of *Rotula aquatica* roots at various time intervals compared with vehicle treated group are shown in Table 2. The mean percent blood glucose levels after oral administration of 100 mg/kg b.w of methanolic extract of roots of *Rotula aquatica* was found to be 11.90±3.44 % at 8<sup>th</sup> hr which was statistically significant compared with the vehicle treated groups at identical times. Oral

administration of 200 mg/kg b. w of methanolic extract of roots of *Rotula aquatica* produced more significant ( $P < 0.01$ ) percent reduction in blood glucose levels at 12<sup>th</sup> hr. The mean percent decrease in blood glucose levels produced by 200 mg/kg b. w of methanolic extract of roots of *Rotula aquatica* were  $10.85 \pm 2.73$  %,  $16.58 \pm 3.47$  % and  $8.30 \pm 2.91$  % at 8, 12 and 18<sup>th</sup> hr respectively. Oral administration of 400 mg/kg b.w of methanolic extract of roots of *Rotula aquatica* produced highly significant ( $P < 0.001$ ) percent reduction in blood glucose levels at 4,8,12 and 18<sup>th</sup> hrs compared to the control group at identical times. The mean percent decrease in blood glucose levels produced by 400 mg/kg b. w of methanolic extract of roots of *Rotula aquatica* were  $8.40 \pm 2.94$ %,  $17.86 \pm 1.78$ %,  $27.26 \pm 1.58$ %,  $18.74 \pm 2.16$ % and  $11.26 \pm 0.85$ % at 2, 4, 8, 12, and 18<sup>th</sup> hr respectively.

## DISCUSSION

It has been discussed that most of the medicinal plant extracts exhibits hypoglycemic activity as glibenclimide by the reactivation of destructed pancreatic  $\beta$ -cells and potentiate them to secrete insulin<sup>23</sup>. Methanolic extract is a source for the availability of tannins and triterpenoids. Recent studies patedified about the plants containing tannins also showed antidiabetic activity<sup>24</sup>. It also showed hypoglycemic activity in dose dependent manner in STZ induced diabetic rats. The maximum percent reduction in blood glucose level was observed at 8<sup>th</sup> hr after oral administration of 400 mg/kg. However, the significant antihyperglycemic activity was quick acting and sustained up to 18<sup>th</sup> hr.

## CONCLUSION

Phytochemical screening of *Rotula aquatica* disclosed the existence of bioactive metabolites like triterpenoids and tannins.

Hence, the antidiabetic activity of this plant is probably due to the presence of above bioactive compounds and seems to have a promising value for the development of potent phytomedicine for diabetes<sup>25</sup>.

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**Table 1.** Effect of methanol extract of *Rotula aquatica* roots on blood glucose levels (mg/dl) in STZ induced diabetic rats

Group (n=6)	Treatment mg/kg b.w.	Time in hours						
		0	2	4	8	12	18	24
1	Control	352± 14	343± 12	343± 14	341± 12	335± 11	341±11	339±10
2	Glibenclamide 0.45	353 ±12	261±8	201± 5*	274± 15	302 ± 3	323±5	333±7
3	RAM 100	339± 12	333± 7	321± 5	302± 4	304±6	309±4	313 ±5
4	RAM 200	343±11	333± 9	324± 4	301± 4	282 ± 7	310±5	324±10
5	RAM 400	355 ± 8	328± 6	295± 9	261± 6*	291±5	318±5	344±3

Each value is expressed as mean±S.E.M.(n = 6). \* ( $P < 0.001$ ) highly significant decrease as compared to zero hr.

**Table 2.** Effect of Methanol extract of *Rotula aquatica* roots on percent decrease blood glucose levels in STZ induced diabetic rats

Group (n=6)	Treatment mg/kg b.w.	Time in hours					
		2	4	8	12	18	24
1	Control	2.30±2.02	2.48±2.83	2.90±2.61	4.54±2.92	2.91±2.55	3.29±2.34
2	Glibenclamide 0.45	25.47±4.23	42.41±2.94*	21.65±5.62	13.81±3.41	7.59±4.60	4.87±4.18
3	RAM 100	2.73±3.79	6.13±4.15	11.90±3.44	10.99±4.46	9.66±4.02	8.51±4.08
4	RAM 200	1.63±2.78	4.32±2.56	10.85±2.73	16.58±3.47	8.30±2.91	4.18±4.37
5	RAM 400	8.40±2.94	17.86±1.78*	27.26±1.58*	18.74±2.16*	11.26±0.85*	3.91±1.87

Each value is expressed as mean±S.E.M.(n = 6).

\* (P< 0.001) highly significant decrease as compared to control.