Evaluation of Anti-Diabetic Activity of Methanolic Extract from the Leaves of *Rotula Aquatica Lour* in Alloxan-Induced Diabetic Rats

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ABSTRACT

Objective: The objective of the present study was to evaluate the anti-diabetic activity of methanolic extract from the leaves of *Rotula aquatica lour* in Alloxan-induced diabetic rats. **Materials and Methods:** Diabetes was induced in rat by injection of Alloxan (120mg/kg, i.p.). Diabetic rats were divided into different groups and methanolic leaves extract of *Rotula aquatica lour* (RA-ME) was administered at dose ranges of 100–400mg/kg, p.o for 21 days. Control group received normal saline (0.9%) for 21 days. Glibenclamide (5mg/kg, p.o) was used as standard drug. Blood samples were collected from all the groups and analyzed for serum glucose and lipid levels such as total cholesterol (TC), triglyceride (TG), proteins (TP). RA-ME was also tested for oral glucose tolerance test (OGTT) in normal fasted rats. **Results:** RA-ME (400mg/kg, p.o) showed a significant ($P<0.01$) reduction of serum glucose level in Alloxan-induced diabetic mice as compared with diabetic control. RA-ME (200 and 400mg/kg) also showed a significant reduction in serum TC, TG, and TP levels in Alloxan-induced diabetic rats. RA-ME (200 and 400mg/kg, p.o) significantly ($P<0.01$) increased the glucose tolerance in OGTT. **Conclusion**: The results obtained from the present study revealed the potential anti-diabetic activity of methanolic extract from the leaves of *Rotula aquatica lour*.

Keywords: Alloxan, Anti-diabetic, Glibenclamide, *Rotula aquatica lour.*

INTRODUCTION

Diabetes mellitus is a leading metabolic disorder characterized by fasting and/or postprandial state hyperglycemia, resulting from defects in insulin secretion or action. It is well reported that diabetes mellitus is associated with a large number of macro vascular and micro vascular complications such as obesity, hypertension, hyperlipidaemia, nephropathy and neuropathy.[1,2] A growing body of research has suggested that diabetes mellitus is increasing in an epidemic proportion throughout the globe, especially in India. Moreover, the prevalence of diabetes is expected to increase by more than two-fold worldwide and approximately 57 million Indians would be affected by this disorder in the year 2025, illustrating the severity and impact of the disorder on the quality of life.[3,4] Despite the steady increase in the number of anti-diabetic agents, the prevalence of the disorder remains stable may be due to the inconsistent efficacy of currently available drugs. In addition, the currently available anti-diabetic drugs have a large number of adverse effects and high rates of secondary failure[5]. Therefore, this remains a grave need to develop and discover new therapy with a proper balance of risk to benefit that could be fruitful for the treatment of diabetes mellitus. In recent decades, many researchers have sought new plant products to treat diabetes mellitus, as they contain many bioactive substances with therapeutic potential.[4]

Rotula aquatica lour is species of aromatic flowering shrub belonging to family *Boraginaceae*. It is mostly present in aquatic region. It is a rare rheophyte native to India, where it is a member of the lotic ecosystem of streams.[6] *Rotula aquatic lour* is also called as pashanbed; it is widely distributed in India, Sri Lanka, tropical south-eastern Asia and Latin America. In India from kumaun to Assam and

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western to southern India. The plant contain Baunerol, steroid, alkaloid, and rhabdiol and allantoin. The medicinal values of plant lie in their component phytochemical such as alkaloids, flavonoid, phenolic compounds and other nutrients like as amino acid, proteins, which produce a definite physiological action on the human body. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research. It is Ayurvedic plant which is an important traditional medicine for kidney and bladder stones. In Ayurveda, plant used for cancer, piles, diabetes, venereal diseases, kidney and bladder stones, cough, heart problems, blood disorders, fever, poisonings, ulcers and uterine diseases. It is used as laxative, diuretic, antioxidant, anthelmintic, astringent, bitter etc. The different parts of plant extract have been reported to acquire above activities. But so far, there are no reports made on the comparison of antioxidant activity and phytochemical properties of stems of *Rotula aquatica lour* in various organic and aqueous extracts.[7]

MATERIALS AND METHOD

Animals

Wistar rats, of either sex, weighing 150–250g were used. They were housed under standard conditions of temperature (23±2°C), humidity and dark–light cycle (lights on from 6:00 am to 6:00 pm). Tap water was available at libitum. All the animals were carefully monitored and maintained in accordance with CPCSEA guidelines on control and supervision of experimental animals. The ethical clearance was obtained from the Institutional Animal Ethics Committee (Approval no. 651/02/c/CPCSEA) before the experiment.

Chemicals

All chemicals and solvents used were of analytical grade, from S.D Fine Chemicals Ltd, Mumbai, India. Alloxan was purchased from Sigma Chemical Co. (St Louis, MO, USA) and Glibenclamide (Aventis Pharma Limited, Verna, Goa). Diagnostic kits of total cholesterol, triglycerides, and total proteins (Agappe Diagnostics Ltd., Pattimattom, Ernakulum), and rest all other reagents and chemicals were of analytical grade.

Plant Material and Preparation of Plant Extract

The leaves of *Rotula aquatica lour* was collected from Sawantwadi, Maharashtra. The collected leaves were authenticated at botanical survey of India, Ministry of Environment and Forests, Government of India. The collected leaves of *Rotula aquatica lour* was dried under shade for 10 days and then made into a coarse powder. Initially, 400g of dried bark was defatted with petroleum ether (60–80°C) in soxhlet apparatus (continuous hot percolation process) and after complete extraction (46h), the solvent was removed by distillation under reduced pressure and resulting liquid was dried using heating plate at 50°C to get semisolid residue. After the extraction with petroleum ether, the same plant material was dried and further extracted with chloroform (36h) followed by methanol (75h) until the extraction was complete. The methanolic bark extract was concentrated under reduced pressure and dried using heating plate at 60°C to get semisolid residue or respective residue.

Acute oral toxicity studies

The acute oral toxicity studies[8] of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17 December 2001 received from CPCSEA, Ministry of Social Justice and Empowerment, Government of India. Administration of the stepwise doses of the methanolic extract of *Rotula aquatica lour* from 50mg/kg b. wt. up to a dose of 4000mg/kg b. wt. caused no considerable signs of toxicity in the tested animals. One-tenth of the upper limit dose was selected as the level for examination of Anti-Diabetic activity.

Oral glucose tolerance test

OGTT was performed in non-diabetic rats. The fasted rats were divided into 4 groups (n=6/group). Group I: glucose load control group. Group II, III and IV rats received RA-ME at a dose of 100, 200 & 400mg/kg body weight, respectively. The rats of treatment groups were loaded with glucose (2g/kg, p.o.) 30min after the administration of the RA-ME. Blood samples (100–200µL) were collected at 0min before the glucose load and 30, 60 and 120 min after the glucose load by retro-orbital vein plexus puncture under mild ether anaesthesia. The serum was separated and the glucose concentration was estimated by glucose oxidase-peroxidase (GOD-POD) method.[9]

Study of the *Rotula aquatica lour* leaves extract on the Alloxan-induced hyperglycemia

The cytoprotective effects of extract were studied in Alloxan-induced diabetic rats according to the method reported.[10] The diabetes was induced by administration of 120mg/kg Alloxan monohydrate (Sigma). The diabetic rats (glucose level >275mg/100ml) were divided into six groups of six rats each. Group I served as negative control and received distilled water. Groups II served as the

diabetic control, Group III, IV, V received the RA-ME at doses of 100, 200 and 400mg/kg as an aqueous solution, p.o. and Group-VI received the Glibenclamide 5mg/kg as standard drug. The administration of the extract was continued for 21 days, once daily. Blood samples were collected from the retro-orbital plexus on days 1, 15 and 21 of extract administration. The blood glucose levels, triglyceride level, total cholesterol and total proteins were determined for all the samples by the glucose oxidase method.

Blood collection

The blood samples (500–750µL) were collected by retro-orbital vein plexus puncture of anaesthetized mice. Blood samples were collected at the time of grouping of animals (basal reading) and at 1st, 15th, and 21st day of treatment. Blood was centrifuged at 3500 r.p.m. for 20 min and serum was separated for biochemical estimation.

Estimation of serum glucose

The glucose concentration was estimated by (GOD-POD) method[11] using commercially available kit. The absorbance and concentration of test and standard samples were noted against blank at 505nm with an auto-analyser.

Estimation of cholesterol

Serum total cholesterol was estimated by cholesterol oxidase-peroxidase (CHOD-POD) method[12] using commercially available kit.

Estimation of triglycerides

Serum triglyceride was estimated by glycerophosphate oxidase-peroxidase (GPO-PAP) method by the addition of enzyme present in reagent kit.[13] The absorbance and concentration of test and standard samples were noted against blank at 505nm with an autoanalyser.

Estimation of total proteins

Serum proteins were estimated by Biuret method using commercially available kit.[14] The absorbance and concentration of test and standard samples were noted against blank at 546 nm with an autoanalyser.

Statistical analysis

Data were expressed as the mean±S.E.M. The significance of the results was calculated using ANOVA and post hoc Dennett's t-test and the results were considered statistically significant when $P<0.05$.

RESULTS

Oral glucose tolerance test

The effects of RA-ME (100–400mg/kg, p.o) on OGTT are summarized in Table 1 & Figure 1. Maximum serum glucose level was found at 30 min. in all groups after glucose load. The control group had a significant elevation in serum glucose level throughout the total measurement period, i.e., for 120 min, with respect to RA-ME treatment group as shown in Figure 1. However, in the RA-ME extract treated groups, blood glucose level although it reached the peak level within 30 min of administration of glucose but it almost resettled to the normal level by 120 min. The glucose level significantly ($P<0.01$) resettled close to the normal value in RA-ME (200mg/kg and 400mg/kg) treated group. Moreover, at the doses of 200mg/kg & 400mg/kg, the glucose level was significantly ($P<0.01$) less as compared with glucose loaded control rats throughout at 120 min. However, no significant affect was observed at a dose of 100mg/kg.

Table 1. Effect of extract on oral glucose tolerance test in rats.

Groups	0 min	30 min	60 min	120 min
Vehicle control	78.33 ± 2.3	146.43 ± 1.7	122.87 ± 3.6	95.76 ± 1.9
Extract dose I	80.5 ± 1.8	141.56 ± 2.5 **	125.98 ± 1.4 ns	89.87 ± 3.1***
Extract dose II	81.42 ± 3.2	132.86 ± 1.6 ***	116.65 ± 2.7 **	86.75 ± 1.9 ***
Extract dose III	79.35 ± 2.7	125.86 ± 3.2 ***	108.72 ± 2.1 ***	82.73 ± 1.6 ***

Values are presented as mean ± SEM. (n=5), ANOVA followed by Dunnett test. *p<0.05, **p<0.01 when compared with Control group.

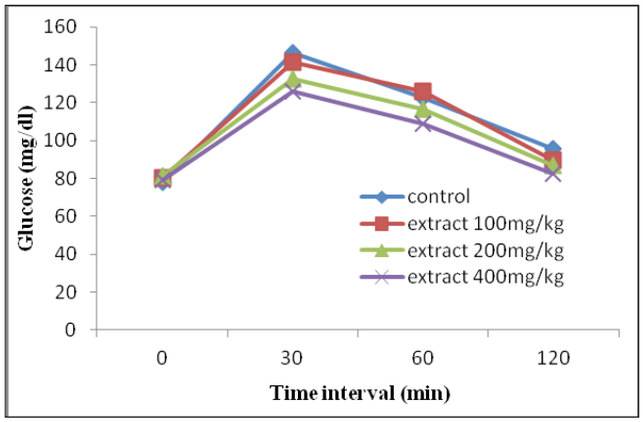

Figure 1. Effect of extract on blood glucose concentrations in fasting conditions at 0 min (pre-treatment) and 30, 60 and 120 min after oral glucose load in normal control rats, and rats treated with various doses of the methanolic leaves extract.

Alloxan-induced diabetic rats

Effect of RA-ME on serum glucose level

Table 2 summarizes the serum glucose levels in normal and diabetic rats. RA-ME at a dose range of 100–400mg/kg decreased serum glucose levels in diabetic rats. The significant ($P<0.01$) effect on serum glucose level was found at a dose of 400mg/kg body weight, observed on 15th and 21st day of treatment. Moreover, the most pronounced decrease in serum glucose was observed on Day 21st at a dose of 400mg/kg. In addition, the positive control Glibenclamide also significantly decreased the serum glucose level in diabetic rats as compared with diabetic control rats. However, RA-ME at a dose of 100mg/kg failed to reach the level of significance as compared with diabetic control rats.

Effect of RA-ME on serum triglyceride, cholesterol and protein levels

Table 3 illustrates the serum triglyceride, cholesterol and protein levels in normal and diabetic rats. Treatment of diabetic rats with RA-ME (200–400mg/kg) produced a significant reduction in serum levels of triglyceride, cholesterol. The effect observed in our study was dose dependent and time dependent. Moreover, Glibenclamide treatment also significantly decreased the serum levels of triglyceride, cholesterol.

DISCUSSION

Diabetes mellitus has been recognized as one of the most common metabolic disorders associated with common

Table 2. Effect of methanolic extract of *Rotula aquatica lour* on serum glucose levels in alloxan-induced diabetic rats.

Groups	Serum glucose (mg/dl)		
	1st day	15th day	21st day
Normal	89.41±5.42	91.15±3.89	85.12±5.71
Diabetic	308.8±5.27$^{\#\#}$	299.5±6.55$^{\#\#}$	302.64±8.54$^{\#\#}$
Glibenclamide (mg/kg)	299.74±8.9	159.67±7.87**	152.3±9.67**
Extract (100mg/kg)	297.1±6.13	287.98±11.56	288.56±6.78*
Extract (200mg/kg)	288.67±7.55	238.67±9.88**	231.43±5.87**
Extract (400mg/kg)	299.5±9.2	198.56±7.63**	186.67±9.82**

Values are presented as mean ± SEM. (n=5), ANOVA followed by Dunnett test. *p<0.05, **p<0.01 when compared with Control group. $^{\#\#}$p<0.01 when Diabetic control compared with Normal group.

Table 3. Effect of methanolic extract of *Rotula aquatica lour* on Triglyceride, cholesterol and protein levels in alloxan-induced diabetic rats.

Groups	Days	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	Total proteins (g/dl)
Normal group	0	83.17 ± 4.63	79.17 ± 4.62	6.33 ± 0.21
	15	89.69 ± 5.31	82.31 ± 5.19	6.38 ± 0.32
	21	87.71 ± 5.42	81.45 ± 3.72	6.22 ± 0.21
Diabetic control	0	84.75 ± 6.69	75.63 ± 4.56	6.45 ± 0.32
	15	175.67 ± 7.76$^{\#\#}$	177.45 ± 7.75$^{\#\#}$	5.33 ± 0.29$^{\#\#}$
	21	187.51 ± 8.46$^{\#\#}$	183.66 ± 5.77$^{\#\#}$	5.12 ± 0.29$^{\#\#}$
Glibenclamide	0	83.43 ± 5.87	74.89 ± 6.73	5.78 ± 0.67
mg/kg	15	138.56 ± 6.75**	143.64 ± 6.39**	5.72 ± 0.64
	21	133.59 ± 5.37**	136.45 ± 6.91**	6.3 ± 0.43**
Extract	0	84.74 ± 8.15	75.98 ± 5.38	6.38 ± 0.58
(100mg/kg)	15	167.55 ± 7.6	162.41 ± 7.71**	5.42 ± 0.55
	21	174.01 ± 8.43*	158.91 ± 5.78**	5.36 ± 0.3
Extract	0	85.02 ± 5.84	75.51 ± 6.71	6.23 ± 0.49
(200mg/kg)	15	159.55 ± 5.88**	163.06 ± 6.9**	5.46 ± 0.41
	21	156.63 ± 7.51**	151.11 ± 6.8**	5.58 ± 0.56
Extract	0	84.35 ± 6.54	74.88 ± 5.95	6.15 ± 0.6
(400mg/kg)	15	151.17 ± 6.13**	149.66 ± 6.67**	5.59 ± 0.52
	21	145.67 ± 5.84**	145.86 ± 7.53**	5.89 ± 0.47*

Values are presented as mean ± SEM. (n=5), ANOVA followed by Dunnett test. *p<0.05, **p<0.01 when compared with Control group. $^{\#\#}$p<0.01 when Diabetic control compared with Normal group.

features such as hyperglycemia and hyperlipidaemia. Alloxan is a β-cytotoxin diabetogenic agent, which induces diabetes by destructing the β-cells of the islets of pancreas, leading to a decreased insulin release and increased blood glucose level.[15] In accordance with the previous findings, the present study reports the significant increase in serum glucose level in Alloxan-induced diabetic rats.

The chronic administration (21 days) of the RA-ME produced a decrease in serum glucose levels of diabetic rats. This effect may be due to regeneration of the β-cell following destruction by Alloxan. The growing body of data suggested that to achieve maximum effect, therapy with plant products should be continued for a longer duration.[16] Considering this, RA-ME was administered daily for 21 days, the period which may be produced a significant reduction in all the diabetic markers, and this effect was potent as compared to acute dosing.

In the present study we also investigated glucose tolerance test in normal rats. The RA-ME significantly decreased the serum glucose levels in glucose loaded rats, and this information could be endorsed to the potentiating of the insulin effect of blood by increasing the pancreatic secretion of insulin from existing β-cells or its release from bound insulin.[17] In this context, a number of other plants have been observed to have similar pattern of hypoglycemic effects. Results on the insulin release from pancreas directly indicate that the anti-diabetic activity of *Rotula aquatica lour* may be through the release of insulin from the pancreas.

CONCLUSION

Herbal hypoglycemic agents can provide better option to avoid harmful side effects caused by prolong intake of synthetic ones. From present preclinical studies, *Rotula aquatica lour* proved to be hypoglycemic in action. But one can speculate that in clinical trials, the drug may act as safe and effective hypoglycemic agent. The remarkable hypoglycemic potential of *Rotula aquatica lour* was quite competent with standard drug. Although the test drug could not correct deranged levels of serum metabolites, it can be used in polyherbal formulations. Further studies are necessary to elucidate details of active phytochemical and their mechanism of hypoglycemic action. Isolation and study of active principles are under process.

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