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Research Article

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Antidiabetic, antihyperlipidaemic and hepatoprotective activity of methanolic extract of *Ruellia tuberosa* Linn leaves in normal and alloxan induced diabetic rats

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ABSTRACT

Ruellia tuberosa Linn belongs to family acanthaceae is a large sized plant distributed throughout India, Srilanka and Nepal. The aim of the present study was to evaluate the anti diabetic potential of methanolic extract of Ruellia tuberosa linn leaves in normal and alloxan induced diabetic rats. The Preliminary phytochemical screening shows the presence of carbohydrates, glycosides, phytosteroids, flavonoids, tannins, fixed oils & fats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p).The methanol extract of Ruellia tuberosa linn leaves at a dose of 100 and 200mg/kg of body weight was administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of methanol extract of Ruellia tuberosa linn leaves on blood glucose, serum lipid profile [total cholesterol (TC), triglycerides (TG), high density lipoprotein – cholesterol (HDL-C), low density lipoprotein – cholesterol (LDL-C), very low density lipoprotein – cholesterol (VLDL-C) and serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT), alkaline phosphatase (ALP)], were measured in the diabetic rats. The methanol extract of Ruellia tuberosa linn leaves elicited significant reductions of blood glucose ($P<0.05$), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C at the dose of 200mg/kg was compared with the standard drug Glibenclamide (5gm/kg). From the above results, it is concluded that methanol extract of Ruellia tuberosa linn leaves possesses significant antidiabetic, antihyperlipidaemic and hepatoprotective effects in alloxan induced diabetic rats.

Key words: Antidiabetic, antihyperlipidaemic and hepatoprotective activity, MERT- Methanolic extract of *Ruellia tuberosa* linn leaves, Alloxan induced Diabetic rats.

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic, with a worldwide incidence of 5% in general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS [1]. Plants have been the major source of drugs for the treatment of diabetes mellitus (DM) in Indian medicine and other ancient systems in the world, and for a long time DM has been treated orally with herbal medicines or their extracts [2], because plant products are frequently considered to be less toxic and more free from side effects than synthetic ones [3]. Furthermore, after the recommendations made by the WHO on DM, investigations on hypoglycaemic agents from medicinal plants have become more important and the search for more effective and safer hypoglycaemic agents has continued to be an important area of active research. World ethnobotanical information about medicinal plants reports that almost 800 plants could be used to control DM. Many herbs and plants have been described as possessing hypoglycaemic activity when taken orally [2, 4]. *Ruellia tuberosa* Linn belongs to family acanthaceae is a large sized plant distributed throughout India, Srilanka and Nepal. The plant leaf contains a pigenin and luteolin. The seed oil yields myristic, capril and lauric acids. Flavonoids, glycosides, phenol, saponins and essential minerals with good nutritive value and secondary metabolites [5]. The plant used as Anthelmintic, joint pains, muscle strains, abortifacient.

The root part used against kidney diseases and whooping cough. Apart from this, plant parts used in gonorrhea, syphilis, bladder stones, bronchitis and cancer, heart ailments, colds, fever, hypertensions, and stomach problems [6]. The objective of the present work to evaluate the antidiabetic, antihyperlipidaemic and hepatoprotective activity of *Ruellia tuberosa* linn leaves by using animal models.

EXPERIMENTAL SECTION

Chemical

Alloxan were obtained from Sigma Chemical Co (St Louis, MO-USA). Bio-chemical kits and all other chemicals utilized were of analytical grade.

Plant Material

The plant *Ruellia tuberosa* linn leaves were collected from an open field around P.Punjaipuliampatti, Erode District, Tamilnadu. Mr.G.V.S Murthy carried out identification of the plant at the Botanical Survey of India, Coimbatore – 641 003.

Preparation of plant extract

The leaves of *Ruellia tuberosa* linn were first washed several times with distilled water and dried well. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with petroleum ether, chloroform, ethyl acetate, methanol using Soxhlet apparatus [7].

Preliminary phytochemical screening

One gram of the petroleum ether, chloroform, ethyl acetate, methanol extracts of *Ruellia tuberosa* linn leaves were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The extracts thus obtained were subjected to preliminary phytochemical screening [7-10].

Animals

The study was conducted on forty matured Wistar strain male albino rats; 3 months of age weighing about 150-200 g [11]. Animals were acclimated for a period of fifteen days in our laboratory conditions prior to the experiment. Rats were housed in tarsons cages (six rats per cage), at an ambient temperature of 25 ± 2^0C with 12 h light: 12 h dark cycle. Rats have free access to standard food and water *ad libitum.* The Principles of Laboratory Animal Care (NIH, 1985) were followed throughout the duration of experiment and instruction given by our institutional ethical committee was followed regarding injection and other treatment of the experiment. Normoglycemic animals were selected for this experiment having the fasting blood glucose level of 75±5 mg/dl.

Acute toxicity studies

The acute toxicity of methanolic extracts of *Ruellia tuberosa* linn leaves were determined by using female albino Wistar rats (150-200 g) which were maintained under the standard conditions. The animals (n=5 per dose) were fasted 12 h prior to the experiment, up and down procedures were adopted for toxicity studies. Animals were administered with single dose of extract of *Ruellia tuberosa* linn leaves at a dose of 2000 mg/kg and observed for their mortality during 2 and 7 days study period (short term) toxicity and the dose increased up to 5000 mg/kg and were observed up to 7 days for their behavioural, economical and neurological profiles except slight depression in their activity [12].

Induction of Diabetes Mellitus

Male Wistar strains of rats, each weighing 150-200 g were used for the study. They were housed in polypropylene cages lined with husk, renewed every 24 h under 12/12 h light/dark cycles at 25–30°C and at 45%–55% relative humidity. The animals were fed with a standard rat pellet diet and tap water was supplied *ad libitium.* A freshly prepared solution of alloxan monohydrate (120 mg/kg body weight), in sterile normal saline solution, was injected intraperitoneally to overnight fasted rats [13]. Blood glucose was measured after 72 hours of alloxanisation by one-touch glucometer, and it was confirmed by testing for glucosuria using glucose indicator sticks. Rats showing fasting blood glucose (FBG) levels > 250 mg/dL were selected as diabetic in this experiment.

Experimental design

Diabetes was induced in rats within 48 hours by the intra peritoneal administration of alloxan dissolved in distilled water (5%) in a dose of 100mg/kg body weight. The rats were divided into 5 groups of 6 animals each.

Group I: *(Untreated Control): Normal control received only saline (10ml/Kg),*
Groups II: *(Diabetic Control): Diabetic control, received alloxan and saline,*

Groups III and IV: *(Diabetic + MERT): Received alloxan and 48 hours later they were treated orally with methanolic leaves extract of Ruellia tuberosa linn at doses of 100 and 200mg/kg,*
Group V: *(Diabetic + glibenclimide): Was treated with glibenclimide (5mg/kg) [14] as standard.*
All the group of animals received the treatment by the above schedule for 14 days.

Antidiabetic activity
The fasting blood glucose levels (FBGL) of all the rats were recorded at regular intervals during the experimental period (0 day, 1st week and 2nd week). Blood samples were collected by tail vein and FBG level were measured by single touch glucometer [15-16].

Hypolipidaemic activity
After 14 days of treatments (24 hours after the last dose), the animals were anaesthetized with ethyl vapour and the blood collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuged at 1000rpm for 15 minutes to obtain the sera. Serum cholesterol, triglyceride and high density lipoprotein (HDL) levels were measured by enzymatic colorimetric methods using Randox diagnostic kits. All samples were analyzed with a wine light Unicam spectrophotometer. The concentrations of low density lipoprotein (LDL) and very low density lipoproteins (VLDL) were calculated from the formula of Friedwald [17].

Hepatoprotective activity
After 14 days of treatments (24 hours after the last dose), the animals were anaesthetized with ethyl vapour and the blood collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuged at 1000rpm for 15 minutes to obtain the sera. The total protein minus the albumin gives the globulin, Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel [18]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [19].

Statistical Analysis
Analysis of Variance (ANOVA) followed by Multiple comparison student two-tail‘t’ test was used for statistical analysis of collected data. Differences were considered significant at $p<0.05$. All the values were indicated in the tables and figures as Mean ± SEM.

RESULTS

Effect of ME of *Ruellia tuberosa* linn Phytochemical Study
The plant *Ruellia tuberosa* linn leaves was extracted with various solvent by using Soxhlet apparatus. The percentage yields were 1.24% in petroleum ether, 1.11% in chloroform, 2.1% in ethyl acetate and 15.3% in methanol in table 1.

The phytochemical screening was done by the all extracts of *Ruellia tuberosa* linn leaves showed the presence of carbohydrates, glycosides, phytosteroids, flavonoids, tannins, fixed oils & fats. Further, extracts of the aerial part showed the absence of alkaloids, proteins & amino acids, saponins in table 2.

Effect of ME of *Ruellia tuberosa* linn acute toxicity study

Acute toxicity studies revealed the non toxic nature of the ME of *Ruellia tuberosa* linn leaves. There was no lethality or toxic reaction found at any of the doses selected until the end of the study period. All the animals were alive, healthy and active during the observation period.

Effect of ME of *Ruellia tuberosa* linn on fasting blood glucose level

The effect of oral administration of ME of *Ruellia tuberosa* linn leaves shows in table 3 in the level of fasting blood glucose level in normal and diabetic rats. There was a significant elevation in blood glucose in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of ME of *Ruellia tuberosa* linn leaves (Group III&IV) and glibenclamide (Group V) tends to bring the parameters significantly towards the normal.

Effect of ME of *Ruellia tuberosa* linn on hypolipidimic profile

The effect of oral administration of ME of *Ruellia tuberosa* linn leaves shows table 4 in the levels of TC, TG, HDL-C, LDL-C and VLDL-C in the serum of normal and diabetic rats. The diabetic rats had elevated levels of serum TC, TG, LDL-C, VLDL-C and decreased level of HDL-C as compared with normal control rats. Diabetic rats treated with ME of *Ruellia tuberosa* linn leaves extract and glibenclamide reversed serum lipid profiles to near normal levels.

Effect of ME of *Ruellia tuberosa* linn on hepatoprotective profile

The effect of oral administration of ME of *Ruellia tuberosa* linn leaves shows in table 5 in the levels of total protein, albumin, globulin, and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats**.** The diabetic rats (Group II) had decreased levels of serum total protein, albumin, globulin and elevated level of liver marker enzymes such as SGPT, SGOT and ALP when compared with normal control rats (Group I). After treatment with ME of *Ruellia tuberosa* linn leaves extract, glibenclamide, total protein, albumin, globulin, and liver marker enzymes were brought back to near normal levels (Group III & IV&V).

Table no 1: Extraction values of different extract of *Ruellia tuberosa* linn leaves

Extracts	% Yield (w/w)
Petroleum ether	1.24
Chloroform	1.11
Ethyl acetate	2.1
Ethanol	15.3

Table no 2: Qualitative phytochemical analysis of different extracts of *Ruellia tuberosa* linn leaves

Particulars	Alkaloids	Carbohydrates	Glycosides	Flavonoids	Proteins & Amino acids	Steroids	Tannins & Phenolic	Saponins	Fixed Oils
Petroleum ether extract	-	-	+	+	-	+	+	+	+
Chloroform extract	-	+	+	+	-	+	+	-	+
Ethylacetate extract	-	-	+	+	-	+	+	-	-
Ethanol extract	-	+	+	+	-	-	+	-	-

(+) = indicates present; (-) = indicates absent

DISCUSSION

The present study is assessment of antihyperglycemic of ME of *Ruellia tuberosa* linn leaves on male wistar rats. Alloxan causes a significant elevation in the level of blood glucose in rats. Administration of 100 and 200 mg/kg body weight of ME of *Ruellia tuberosa* linn leaves significantly decreased the blood glucose level after fourteen days of treatment in these rats suggesting that it has hypoglycemic properties and also the hypolipidimic and hepatoprotective profile were restored to control levels with the administration of the known drug glibenclamide and plant extracts *Ruellia tuberosa* linn. The result from the present study shows the significant changes in biochemical parameter during the experimentally inducted diabetes.

Table no: 3 Effect of methanol extract of *Ruellia tuberosa* linn leaves on blood glucose level of normal, diabetic induced and drug treated rats at different week intervals

Treatment	Blood glucose (mg/dl)		
	0 day	1st week (After 7 days)	2nd week (After 14 day)
Control	106.4 ± 3.72	105.0 ± 1.00	105.8 ± 1.36
Diabetic Control	439.8 ± 4.01*	401.0 ± 11.91*	408.0 ± 7.61*
Diabetic + MERT (100mg/kg)	393.4 ± 5.50*	165.2 ± 2.91*	160.8 ± 2.05*
Diabetic + MERT (200mg/kg)	406.8 ± 4.51*	136.4 ± 3.37*	131.4 ± 2.18*
Glibenclamide (5mg/kg)	397.6 ± 4.89*	127.2 ± 2.96*	119.4 ± 2.87*

*Values are given as mean ±SD for groups of six animals each. Values are statistically significant *P<0.05.*
Diabetic rats were compared with control rats; MERT diabetic rats were compared with diabetic rats; glibenclimide treated diabetic rats were compared with diabetic rats

Table no 4: Effect of methanol extract of *Ruellia tuberosa* linn leaves on the protein, albumin, globulin, SGPT, SGOT and ALP level of normal, diabetic induced and drug treated rats

Treatment	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGOT (u/l)	SGPT (u/l)	ALP (u/l)
Control	6.67 ± 0.10	3.14 ± 0.12	2.76 ± 0.08	63.33 ± 6.56	44.33 ± 6.98	168.0 ± 27.15
Diabetic control	5.49 ± 0.24*	2.12 ± 0.10*	2.36 ± 0.50*	122.67 ± 3.92*	72.00 ± 5.86*	287.0 ± 10.21*
Diabetic +MERT (100 mg/kg)	5.82 ± 0.10*	2.96 ± 0.02*	2.62 ± 0.08*	81.33 ± 6.89*	61.67 ± 6.44*	211.6 ± 52.46*
Diabetic +MERT (200mg/kg)	6.05 ± 0.42*	3.10 ± 0.09*	2.70 ± 0.04*	71.67 ± 8.81*	45.00 ± 5.77*	199.3 ± 29.45*
Glibenclamide (5mg/kg)	6.35 ± 0.25*	3.11 ± 0.18*	2.72 ±0.04*	68.00 ± 8.50*	42.67 ± 3.93*	172.6 ± 19.20*

*Values are given as mean ±SD for groups of six animals each. Values are statistically significant *P<0.05.*
Diabetic rats were compared with control rats; MERT diabetic rats were compared with diabetic rats; glibenclimide treated diabetic rats were compared with diabetic rats

Table 5: Effect of methanol extract of *Ruellia tuberosa* linn leaves on the TC, TG, HDL-C, LDL-C and VLDL-C in the plasma of normal, diabetic induced and drug treated rats

Treatment	Total Cholesterol	TGL (mg/dl)	HDL (u/l)	LDL (u/l)	VLDL (u/l)
Control	67.33 ± 4.63	98.66 ± 2.33	63.33 ± 4.56	44.33 ± 8.98	22.08 ± 7.15
Diabetic control	178.67 ± 4.67*	185.67 ± 12.45*	32.67 ± 3.92*	92.00 ± 6.86*	47.00 ± 10.21*
Diabetic + MERT (100 mg/kg)	91.33 ± 6.89*	113.67 ± 4.70*	41.33 ± 6.89*	31.67 ± 4.44*	19.06 ± 9.46*
Diabetic + MERT (200mg/kg)	77.67 ± 1.45*	110.33 ± 7.31*	53.67 ± 6.81*	40.00 ± 5.77*	20.75 ± 8.45*
Glibenclamide (5mg/kg)	70.67 ± 3.48*	109.33 ± 10.97*	61.00 ± 7.50*	42.67 ± 4.73*	21.60 ± 9.20*

*Values are given as mean ±SD for groups of six animals each. Values are statistically significant *P<0.05.*

A significant reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (Group II), when compared to control (Group I) and glibenclamide treated rats (Group V). On administration of methanol extract of *Ruellia tuberosa* linn leaves to the diabetic rats, protein, albumin and globulin levels were found to be restored in normal. These results were in accordance with the effect of *Wattakaka volubilis* leaf in diabetic rats [20].

The animals treated with alloxan developed hepatic damage which was evident from the increase in the enzyme activities. Pretreatment with methanol extract of *Ruellia tuberosa* linn leaves and glibenclamide resulted in a decrease of transaminase activities in alloxan treated rats. The serum AST and ALT levels increases as a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes [21]. Similarly in the present study, it was observed that the levels of SGPT and SGOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan [22]. AST and ALT were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats [23]. In this study, the methanol extracts of *Ruellia tuberosa* linn leaves regulated the activity of SGPT, SGOT and ALP in liver of rats intoxicated with alloxan. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study [24].

Alloxan induced diabetic rats showed significantly increased serum lipid profiles except HDL-C, when compared with normal rats. The glibenclamide and methanol extract of *Ruellia tuberosa* linn leaves treated rats showed a significant decrease in the content of lipid profiles, when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats when compared with normal rats. On administration of methanolic extract of *Ruellia tuberosa* linn leaves and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. The level of serum lipid profiles are usually raised in diabetic rats in the present study and such elevation represents a risk factor for coronary heart diseases [25]. Lowering the serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease [26].

It is concluded that, medicinal plants have been reported to possess antihyperglycemic activity; *Ruellia tuberosa* linn leaves is gaining much importance in diabetic control as it has been used as a traditional medicine for diabetes; since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, glycosides, phytosteroids, tannins and phenols. Several authors reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive antidiabetic principles [27, 28]. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues [29, 30]. Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intraluminal physicochemical reaction. Hence, it has been reported to have hypocholesterolemic effect and thus may aid a lessening metabolic burden that would have been placed in the liver [31].

In the present study, the phytochemical analysis of methanol extract of *Ruellia tuberosa* linn leaves clearly points out the presence of above said active principles. The preliminary investigation on the antidiabetic efficacy of methanol extract of *Ruellia tuberosa* linn leaves will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity.

Diabetic rats were compared with control rats; MERT diabetic rats were compared with diabetic rats; glibenclimide treated diabetic rats were compared with diabetic rats

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