Hypoglycemic Activity of *Ruellia tuberosa* Linn *(Acanthaceae)* in Normal and Alloxan-Induced Diabetic Rabbits

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Summary: The present study was carried out to determine the hypoglycemic activity of the methanol extract and solvent fractions (*n*-hexane and ethyl acetate) in normal and alloxan-induced diabetic rabbits. Optimum dose of *R. tuberosa* for hypoglycemic activity was determined by oral administration of methanol (ME) extract to normal and diabetic rabbits. Diabetes was induced by intra-peritoneal injection of alloxan monohydrate (150 mg/kg body weight). Hypoglycemic effect of *n*-hexane (HxF) and ethyl acetate (EaF) fractions was evaluated at a dose of 150 and 100 mg/kg respectively in normal and diabetic rabbits. Optimum dose (equivalent to 500 mg/kg) of *R. tuberosa* to normal and diabetic rabbits showed significant blood glucose lowering effect. EaF (100 mg/kg) showed the highest anti-diabetic activity with 34.31 ± 0.43 % ($P < 0.005$) decrease in glycemia while HxF (150 mg/kg) showed moderate anti-diabetic activity and lowered the blood glucose level around 15.17 ± 0.58 % ($P < 0.005$). The results were compared with the standard drug tolbutamide (100 mg/kg). Phytochemical investigation of EaF and HxF indicated flavonoids and triterpenoids in the extracts respectively. The results were in agreement with the folkloric use of *R. tuberosa* in the treatment of diabetes and related complications.

Key words: *Acanthaceae*, hypoglycemic activity, optimum dose, *R. tuberosa*, alloxan-monohydrate

Introduction

Ruellia tuberosa L. *(Acanthaceae)* is a tropical perennial plant. It is widely distributed in south East Asia including Thailand and Laos. In folk medicines, *R. tuberosa* has been used as diuretic, anti-diabetic, antipyretic, analgesic, antihypertensive, thirst quenching and antidotal agent. Recently, it has been introduced as a component of herbal drink in Taiwan [1, 2].

Diabetes mellitus (DM) is a major endocrine disorder and probably the world's largest growing metabolic disease which has been identified as a heterogeneous disease [3, 4]. Asia and Africa are the most viable areas where the disease is feared to raise 2-3 folds [5]. DM is affecting severely approximately 5 % of the world population. Worldwide projections suggest that more than 300 million people will have diabetes by the year 2025 [6]**.** According to World Ethnobotanical Information of medicinal plants almost 800 plants are used in the control of DM. However, only few of these plants have been studied [7]**.** Traditional medicines of plant origin play a vital role in the cure of DM [8].

World Health Organization (WHO) has also recommended the evaluation of traditional plant treatments for diabetes because they are effective, non-toxic, having less or no side effects and are considered good candidates for oral therapy [9]. In this regard several scientists carried out experimental and clinical trials on medicinal plants and found significant anti-diabetic activity [10–14]. Diabetic complications have been controlled worldwide by the use of traditional plant medicines. Drugs which are obtained from natural sources corresponded to 78 % of new drugs approved by Food and Drug Administration (FDA) between 1983 & 1994 [15]. Furthermore, the limitations of currently available pharmacological agents for control of hypoglycemia have stimulated research on novel anti-diabetic agents with different mechanism of action [16]. Literature data indicates that some of the flavonoids and triterpenes isolated from medicinal plants have significantly reduced the blood glucose level [17–20].

The objective of this study was to ascertain the scientific basis of the use of *R. tuberosa* in the treatment of diabetes. The present investigation reports the hypoglycemic activity of ME extract, HxF, EaF and WaF of the crude methanol extract of *R. tuberosa*. To the best of our knowledge this is the first report of the anti-diabetic activity of this plant.

Results and Discussion

Hypoglycemic activity of the test samples (ME extract, HxF, EaF and WaF of *R. tuberosa*) was determined in rabbits according to Kato *et al* method. Samples of blood were collected at 0 h and 4 h after the extract or drug administration [21, 22]. Me

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extract of *R. tuberosa* was fractionated to obtain HxF, EaF and WaF in 26.44 %, 16.38 % and 55.12 % yields respectively. Phytochemical studies of HxF and EaF indicated the presence of triterpenoids and flavonoids respectively. WaF showed the presence of polyphenols.

Acute Toxicity Study

The ME extract of *R. tuberosa* was subjected to acute toxicity study up to a dose of 5000 mg/kg. which indicated non-toxic nature of the extract. No gross behavioral changes were observed at any of the doses selected until the end of the study period.

Hypoglycemic Study

Determination of Optimum Dose of R. tuberose

The effects of ME extract *R. tuberosa* on blood glucose level of fasting normal and alloxan-induced diabetic rabbits is shown in Table 1. Optimum activity was observed at 500 mg/kg level in normal animals. Minimum dose (125 mg/kg) did not show any blood glucose lowering activity. Fasting blood glucose level (FBGL) decreased to 73.7 ± 1.17 mg/dL ($P < 0.05$) after 4 h of the drug (500 mg/kg) administration from an initial value of 89.2 ± 0.64 mg/dL. A dose-dependent effect was observed on FBG upto a dose of 500 mg/kg. Tolbutamide (100 mg/kg) caused a marked decrease in FBG (glucose concentration from 94.5 ± 1.48 to 76.9 ± 1.18 mg/dL; 18.65 ± 1.73 % decrease) in the animals. A dose-dependent effect was also observed in diabetic animals upto a dose of 500 mg/kg. which decreased the glucose concentration to 226.0 ± 1.36 mg/dL from an initial level of 279.1 ± 1.56 mg/dL (19.03 ± 0.68 % reduction; $P < 0.005$). Tolbutamide showed significant anti-diabetic activity at a dose of 100 mg/kg in alloxan-induced diabetic rabbits.

Effect of R. tuberosa Fractions on Blood Glucose level

The effect of *R. tuberosa* solvent fractions on the blood glucose level of normal and alloxan-induced diabetic rabbits is shown in Table 2.

Normal control group showed no statistically significant hypoglycemic activity ($P > 0.2$ at 4 h). EaF and HxF of *R. tuberosa* produced a significant hypoglycemia in normal rabbits. EaF and HxF significantly decreased the blood glucose level at 4th h (16.70 ± 0.73 % and 7.58 ± 1.04 %) respectively in normal animals which is comparable with the standard drug tolbutamide.

The effect of *R. tuberosa* fractions on blood glucose of alloxan-induced diabetic rabbits is shown in Table 2. HxF showed moderate reduction of FBGL to 15.17 ± 0.58 % after 4th h in diabetic rabbits while the effect of EaF (100 mg/kg) is higher with reduction in blood glucose level of 34.31 ± 0.43 % after 4 h of drug administration. WaF showed no blood glucose lowering activity ($P > 0.1$) in normal as well as alloxan-induced diabetic animals.

The present study is a preliminary intent to validate the use of *R. tuberosa* to control DM. Our results revealed that *R. tuberosa* has significant blood glucose lowering effect in normal and alloxan-induced diabetic rabbits. Among the extract/fractions of *R. tuberosa*, ME extract (500 mg/kg), EaF (100 mg/kg) showed significant hypoglycemic effect, while HxF (150 mg/kg) exhibited moderate activity after 4 h of the administration. In the present work normal and alloxan-induced diabetic rabbits were used. HxF exhibited 7.58 ± 1.04 % reduction of blood glucose level in normal, while 15.17 ± 0.58 % reduction in diabetic animals. Similarly EaF showed 16.70 ± 0.73 and 28.64 ± 0.28 % blood glucose reduction in normal and alloxan-induced diabetic rabbits respectively. The results proved that HxF and EaF can effectively control diabetic conditions. Alloxan causes hyperglycemia by destroying pancreatic β-cells selectively, mediated by the generation of cytotoxic free radicals [23, 24].

Flavonoids constitute one of the well-known and wide spread groups of phenolics in higher plants [25]. Several flavonoids; apigenin, luteolin, 3, 5-diglucoside, apigenin 7-O-glucuronoide, apigenin glucoside, apigenin rutinoside, luteolin glucoside, pedalitin, flavone glycoside, cirsimaritin, cirsimarin, cirsiliol 4′-glucoside, sorbifolin and pedalitin are reported in *R. tuberosa* [26, 27 and 2]. Among possible mechanisms to lower blood glucose level, *R. tuberosa* might act by quenching of free radicals in the body and to improve the metabolic disorder which can be attributed to the presence of flavonoids in the EaF. Thus, it can be proposed that the flavonoids of *R. tuberosa* play a vital role in the reduction of blood glucose level both in normal as well as in diabetic rabbits.

HxF of *R. tuberosa* exhibited reduction in blood glucose level after 4 h of the drug administration. Phytochemical analysis of the *n*-hexane fraction indicated the presence of sitosterols which has been reported to have anti-diabetic effect and work like sulphonyl urea medicine [28–30]. The hypoglycemic effect of the ME extract could possibly be related to its composition. Thus the significant anti-diabetic effect of *R. tuberosa* may be due to the presence of more than one hypoglycemic principle and their synergetic properties.

Table-1: Anti-diabetic effect produced by the oral administration of different doses of *R. tuberosa* methanol extract in normal and alloxan-induced diabetic rabbits

Study	Groups	Samples	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M. (mg/dL)		% reduction
				0 h (in fasting)	4 h	
Normal rabbits	I-N	Control	0.5 % CMC	84.9 ± 1.55	81.6 ± 2.75	3.85 ± 2.95
	II-N	ME Ext.	125	89.5 ± 1.48	88.2 ± 1.51	1.45 ± 1.86
	III-N	ME Ext.	250	86.5 ± 1.37	83.4* ± 1.60	3.62 ± 0.56
	IV-N	ME Ext.	500	89.2 ± 0.64	73.7*** ± 1.17	17.33 ± 1.53
	V-N	ME Ext.	750	86.6 ± 1.59	73.5 ± 1.52	15.14 ± 1.44
	VI-N	ME Ext.	1000	86.9 ± 1.48	73.8 ± 0.86	15.02 ± 0.97
	VII-N	ME Ext.	2000	93.1 ± 1.51	79.9 ± 1.41	14.10 ± 0.44
	VIII-N	Tolbutamide	100	94.5 ± 1.48	76.9 ± 1.18	18.65 ± 1.73
Diabetic rabbits	I-D	Control	0.5 % CMC	275.3 ± 1.96	270.4 ± 7.08	1.76 ± 2.50
	II-D	ME Ext.	125	269.4 ± 1.95	262.5** ± 3.32	2.52 ± 0.39
	III-D	ME Ext.	250	268.0 ± 2.33	258.5** ± 3.28	2.56 ± 0.60
	IV-D	ME Ext.	500	279.1 ± 1.56	226.0*** ± 1.36	19.03 ± 0.68
	V-D	ME Ext.	750	272.9 ± 2.27	226.6 ± 0.94	16.96 ± 0.52
	VI-D	ME Ext.	1000	276.7 ± 2.15	229.0 ± 1.24	17.21 ± 0.80
	VII-D	ME Ext.	2000	273.2 ± 1.32	230.6 ± 1.09	15.59 ± 0.29
	VIII-D	Tolbutamide	100	270.0 ± 0.81	214.5 ± 1.52	20.57 ± 0.56

n = 6; Values are mean ± S. E. M; ME Ext: Methanol extract.
* P < 0.05 significant from normal control.
** P < 0.025 significant from diabetic control.
*** Optimum dose (P < 0.005).

Table-2: Effects of ME extract, HxF, EaF and WaF of *R. tuberosa* on blood glucose level in normal and alloxan-induced diabetic rabbits

Test Samples	Dose (mg/kg)	Mean blood glucose concentration ± S.E.M. (mg/dL) in normal animals			Mean blood glucose concentration ± S.E.M. (mg/dL) in diabetic animals		
		0 h	4 h	% reduction	0 h	4 h	% reduction
Control	0.5 % CMC	94.3 ± 0.60	93.8 ± 0.49	0.57 ± 0.53	277.3 ± 1.38	273.8 ± 1.58	1.23 ± 0.90
ME Ext.	500	89.2 ± 0.64	73.7 ± 1.17*	17.33 ± 1.53	279.1 ± 1.56	226.0 ± 1.36**	19.03 ± 0.68
HxF	150***	88.3 ± 0.88	81.5 ± 0.38*	7.58 ± 1.04	274.0 ± 1.71	232.4 ±2.08**	15.17 ± 0.58
EaF	100***	105.1 ± 0.98	87.5 ± 0.84*	16.70 ± 0.73	289.8 ± 2.20	190.4 ± 1.60**	34.31 ± 0.43
WaF	275***	89.9 ± 1.34	90.2 ± 0.34	1.04 ± 0.96	269.8 ± 2.70	266.5 ± 1.63	1.20 ± 0.41
Tol	100	100.7 ± 4.12	68.3 ± 1.93*	31.70 ± 2.92	258.7 ± 2.01	166.7±11.87**	35.52 ± 4.61

n = 6; Values are mean ± S. E. M; ME Ext: methanol extract; HxF: *n*-hexane fraction, EaF: ethyl acetate fraction, WaF: water fraction, Tol: Tolbutamide. The doses selected for various fractions are based on their relative amounts present in 500 mg ME extract (500 mg/kg of ME extract is the optimum dose).
* P < 0.005 significant from normal control.
** P < 0.005 significant from diabetic control.
*** ≈ 500 mg/kg of ME Extract.

Experimental

Plant Material

The fresh whole plants of *R. tuberosa* were collected from University of Sargodha, Sargodha campus. The plant was identified at the Department of Botany GC University Lahore where a voucher specimen was deposited (Voucher No. GC.Herb.Bot. 987).

Preparation and Fractionation of Methanol Extract

The plant was cleaned, dried under shade and powdered plant material was soaked in methanol for 7 days at room temperature. The extract was filtered and evaporated using a rotary evaporator (Laborta 4000-Hedolph). The dried crude extract was dissolved in water and fractionated with *n*-hexane and ethyl acetate. The fractions and remaining aqueous layer were concentrated under reduced pressure. The amounts of solvent fractions for oral administration were calculated from optimum dose (500 mg ME extract/kg).

Preparation of Test Samples

A weighed portion of each fraction (equivalent to 500 mg ME extract) was suspended in 0.5 % aqueous carboxymethyl cellulose (CMC) solution in distilled water prior to oral administration to animals (5 ml/kg). Animals in control group received only 0.5 % CMC suspension in distilled water. Tolbutamide (100 mg/kg) was used as reference drug.

Animals

Adult normal male rabbits (O*ryctolagus cuniculus)* obtained from the animal house, University College of Pharmacy, University of the Punjab (UCP-PU) Allama Iqbal Campus Lahore were used for the study. All the rabbits were kept in individual metal cages located in the UCP-PU. The rabbits were allowed for one week to acclimatize in

animal house of Pharmacology Department (UCP-PU). The animal experiments were preceded following the internationally accepted ethical guidelines for the care of laboratory animals. The animal house facility was maintained at standard conditions: temperature (28 ± 2 ˚C), relative humidity (50 ± 5 %) and a 12 h light dark cycle. They were fed with standard diet and water *ad libitum*. Prior the experiments, they were fasted overnight but allowed free access to water.

Collection and Processing of Blood for Estimation of Glucose

Blood glucose concentration (mg/dL) of overnight fasted animals was determined using standard kit (Randox UK) based on glucose oxidase method using a UV-visible spectrophotometer at 546 nm. Blood samples were collected from the marginal ear vein just prior to and 4 h after dosing. Serum was separated by centrifuging the samples at 5000 rpm for 10 min [22].

Induction of Diabetes

Diabetes was induced in rabbits by intraperitonial injection of alloxan monohydrate at a dose of 150 mg/kg. dissolved in normal saline [31]. Seven days after, rabbits were confirmed as diabetic rabbits with blood glucose ≥ 250 mg/dL were selected for the experiments. The results are presented in Table 1.

Determination of Optimum Dose

To find out the optimum dose of methanol extract of *R. tuberosa*, eight groups (I-N to VIII-N; N: normal) comprising of six rabbits each were used. The animals were fasted for 8 hours before the experiments. Group I-N animals received 0.5 % CMC in distilled water and served as control. After collecting the blood samples at zero h, various doses (125, 250, 500, 750, 1000 and 2000 mg/kg) of the ME extract of *R. tuberosa* were administered orally to the animals of group II-N to VII-N respectively. Blood samples were collected for the estimation of glucose concentration from the marginal ear vein of the rabbits after 4 h of the drug (ME extract) administration [32-34]. Results were compared with tolbutamide which was administered orally to animals of group VIII-N (reference) at a dose of 100 mg/kg.

Diabetic animals were similarly divided into eight groups (I-D to VIII-D; D: diabetic) of six animals each. Different doses (125, 250, 500, 750, 1000 and 2000 mg/kg) of ME extract were administered to animals of group II-D to VII-D while group I-D served as control. Tolbutamide was administered to group VIII-D at a dose of 100 mg/kg.

Hypoglycemic Effect of Fractions of R. tuberose

Fasted normal rabbits were divided into five groups (IX-N, X-N, XI-N, XII-N and XIII-N; N: normal; six animals in each group). The IX-N group (normal control) received only 0.5 % CMC (10 mL/kg) and served as control. Various fractions (HxF, EaF and WaF) suspended in CMC were administered orally at the dose of 150, 100 and 275 mg/kg (The doses selected for various fractions are based on their relative amounts present in 500 mg methanol extract; 500 mg/kg of ME extract is the optimum dose) orally in a volume of 10 mL/kg to the animals of group X-N, XI-N and XII-N respectively. Group XIII-N received tolbutamide (100 mg/kg) as reference drug suspended in 0.5 % CMC

Similarly, the overnight fasted alloxan-induced diabetic rabbits were divided into five groups (IX-D, X-D, XI-D, XII-D and XII-D; D: diabetic) of six rabbits each. Group IX-D (diabetic control) received vehicle (0.5 % CMC). Doses of 150, 100 and 275 mg/kg of HxF, EaF and WaF respectively were given orally to group X-D, XI-D and XII-D. Group XIII-D received tolbutamide as reference drug (100 mg/kg) suspended in vehicle (10 mL/kg).

Toxicity Evaluation in Rabbits

The methanol extract was tested for its acute toxicity (if any) in rabbits. To study the acute toxicity, six groups of animals (3 animals in each group) were taken. Different doses (100, 500, 1000, 3000, and 5000 mg/kg) of the extract were orally administered to the over night starved rabbits of group T1─ T5. The control group (T6) was given only CMC. After the administration of extract, the rabbits were observed for gross behavioral, neurological, autonomic and toxic effects continuously for 2 h and then at 6 h interval upto three days and daily upto two weeks.

Phytochemical Studies

Phytochemical study of the various solvent fractions was carried out to determine the presence of alkaloids, flavonoids, triterpenoids, sterols and phenolics.

Statistical Analysis

All the data was expressed as mean ± SEM for six rabbits in each group. The significance of blood glucose reduction produced by the ME extract

and its various fractions was determined by applying student's unpaired't' test [22].

Conclusion

In conclusion, we have demonstrated that the methanol extract and solvent fractions (ethyl acetate and *n*-hexane) of *R. tuberosa* possess significant blood glucose lowering effect both in normoglycemic and in alloxan-induced diabetic rabbits. The present results suggest the validity of the clinical use of *R. tuberosa* in the control of diabetes mellitus. However, further comprehensive chemical and pharmacological investigations are required to isolate and evaluate the hypoglycemic effects of the active components from *R. tuberosa*.

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