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Methanolic Root Extract of *Rauwolfia serpentina* Improves the Glucose Tolerance in *Wister* Mice

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ABSTRACT

In the present study, acute toxicity of methanolic root extract (MREt) of *Rauwolfia serpentina* (10 - 250 mg/kg) was observed in terms of hypoglycemic activity and behavior pattern including sedation and mortality rate in different groups of normoglycemic *wister* mice (6/group). Orally administrated MREt (10 to 60 mg/kg) was found non-sedative and showed hypoglycemic activity by reducing the mean blood glucose level from 80 to 62 mg/dL after 30 min of administration while the doses (100-250 mg/kg) of same extract showed sedation and mortality rate from 17 to 100% within 4 h. The value of median lethal dose (LD_{50}) of MREt of *R. serpentina* was 141.25 mg/kg (log LD_{50} = 2.15 mg/kg) from graph plotted between log-doses verses probits. In oral glucose tolerance test, MREt (10, 30 and 60 mg/kg) showed significant hypoglycemic activity at 0, 30 and 60 min by deceasing blood glucose level ranging from 31- 65%, 39 - 49% and 25 - 51% respectively compared to control and negative control (treated with 0.05% dimethylsulfoxide @ 1 mL/kg) groups. However, MREt (100 mg/kg), though hypoglycemic from 0 to 120 min, induced slight sedation and mortality in experimental mice. The results indicated that the MREt (10 - 60 mg/kg) was non-sedative and effective in lowering the blood glucose level in mice but showed lethal effect in terms of sedation and mortality at doses ≥ 100 mg/kg. Therefore, MREt of *R. serpentina* in small doses can improve the glucose tolerance either at pancreatic or extra-pancreatic level.

Key words: acute toxicity, hypoglycaemic activity, LD_{50}, oral glucose tolerance test, *Rauwolfia serpentina*

INTRODUCTION

Diabetes is one of the most common metabolic disorders and has become an epidemic of 21st century of both genders in developed and developing countries[1]. The overall prevalence of diabetes (type I & II) worldwide was found 2.8% in the year 2000 and expected to increase up to 4.4% by the year 2030[2]. Pakistan is also facing the same situation[3]. The conventional treatments of diabetes includes insulin injections, oral hypoglycemic drugs, exercise, diet or combination of these[4]. Oral hypoglycemic drugs, besides having effective and immediate therapeutic action, have adverse effects and are also costly[5]. Most of the people belong to low-paid countries like India, Bangladesh and Pakistan are used to taking extracts of medicinal plants for the treatment of various diseases including diabetes as prescribed by herbal healers or Hakims[6]. Ethno-botanical information indicates that more than 800 medicinal herbs and plants have been used as traditional remedies for the treatment of diabetes[7]. Therefore, the plant kingdom has become a target for drug companies and research institutes to discover new compounds that are potential anti-diabetic drugs with few or no side effects[8].

The *Rauwofia serpentina* Benth belongs to the family *Apocynaceae.* It is well-reported in the treatment of hypertension and many mental disorders in Ayurvedic and Western medicines[9,10]. Besides, previous researches of *R. serpentina* from 1950 - 1960 showed its brief and almost incomplete hypoglycaemic activity in diabetic patients[11], both normal & diabetic hypertensive patients[12] and in anesthetized cats[13]. However, a well-described proof related to its hypoglycaemic and hypolipidemic activities in alloxan-induced diabetic rats has been published in 2009 using a single dose of methanolic root extract (MREt) of this plant[14]. Therefore the present study was aimed to evaluate the dose-dependent hypoglycemic activity of MREt of *R. serpentina* in overnight fasted normoglycemic mice by conducting oral glucose tolerance test and determining its median lethal dose (LD_{50}). It was a therapeutic dose selection study which could be employed in the second phase to conduct long-term hypoglycemic activity in type I & II diabetic animal models.

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MATERIALS AND METHODS

I. *Animals*

Male *wister* albino mice (20 - 30 g) were purchased from the breading house of Dow University of Health Sciences (DUHS), Karachi and kept under usual management conditions in conventional animal house of the same University. They were given standard laboratory diet with free access to water *ad libitum*. During the course of experiment no physical stress was provided to animals. The present study was approved by the Institutional Ethical Review Board (IERB) of Dow University of Health Sciences (DUHS), Karachi in June 2010.

II. *Plant Material*

Roots of *R. serpentina* were purchased from Hamdard Dawakhana, Sadar, Karachi and identified by experts in Botany Department, University of Karachi, Karachi-75270, Pakistan (voucher specimen: KU/BCH/SAQ/02).

III. *Glibenclamide*

Glibenclamide (Daonil) was purchased from Sanofi-aventis Pakistan Ltd. and used as positive control in a dose of 5 mg/kg.

IV. *Dimethyl Sulphoxide (DMSO)*

DMSO of analytical reagent grade was purchased from Fisher Scientific (UK) and 0.05% in distilled water was used as vehicle for administering MREt of *R.serpentina* in experimental test mice.

V. *Preparation of Methanolic Root Extract (MREt)*

Forty grams of ground powder of roots of *R. serpentina* was extracted with methanol (1 L; 95%) overnight and filtered through Whatman No.1 filter paper twice. The filtrate was then concentrated at 40°C till dryness in a rotary vacuum evaporator (Eyela-NE) to obtain a brown residue that was referred as MREt[14]. This procedure yielded 3 - 4% (w/w) of the dry root. The MREt was stored in an airtight container in refrigerator below 10°C until used.

VI. *Acute Toxicity*

Mice fasted overnight were randomly divided into eight groups (6/group). A single dose of each of 10, 30, 60, 100, 150, 200 & 250 mg/kg of MREt of *R. serpentina* was separately administrated orally to the mice in each group, whereas six mice in control group were treated with distilled water (1 mL/kg) orally. The mice in both test and control groups were then allowed free access to food and water, and their activity were observed over a period of 12 h for behavior change (sedative or not) and mortality rate[15]. In addition, hypoglycemic activity was determined after 30 min in its respective group with the help of glucometer (*Optium Xceed*, Diabetes Monitoring system by Abbott).

VII. *Determination of Median Lethal Dose (LD_{50})*

LD_{50} of methanolic root extract of *R. serpentina* was determined by graphical method[16].

VIII. *Effect of MREt of R. serpentina on Glucose Tolerance in Mice*

Overnight fasted experimental mice were divided into different groups (6/group) according the treatments such as control group (distilled water @ 1 mL/kg), negative control (0.05% DMSO @ 1 mL/kg), positive control (glibenclamide @ 5 mg/kg) and test group. Test group was further subdivided into four groups according to doses of MREt of *R. serpentina* at 10, 30, 60 and 100 mg/kg in 1 mL 0.05% DMSO. Each group after receiving its respective treatment orally was immediately administered with glucose load at 2 g/kg body weight from the same route. Blood glucose was monitored from the tail vein of mice at 0, 30, 60, and 120 min from each group with the help of glucometer. Percent blood glucose change between control and test groups with different treatments was calculated using the following formula.

$$\% \text{ glycemic change} = (G_x - G_o) / G_o \times 100$$

Where Go = mean blood glucose levels of control group at different time intervals, Gx = mean blood glucose levels of groups treated with each of glibenclamide (positive control) and different doses of MREt of *R. serpentina* at different time intervals respective to control.

IX. *Statistical Analysis*

The results of present study are expressed as mean ± SD and analyzed by student's *t*-test (Graphpad Software, Quick Calcs Online calculator for Scientists). The differences were considered significant with $p < 0.01$, $p < 0.001$ & $p < 0.0001$.

RESULTS

I. *Acute Toxicity and Median Lethal Dose (LD_{50}) of MREt of R. serpentina*

The doses of MREt of *R. serpentina* from 10 to 60 mg/kg were found non-sedative and exerted hypoglycemic activity by reducing the mean blood glucose level from 80 to 62 mg/dL in overnight fasted normoglycemic mice as compared to the control group. The dose 100 mg/kg of MREt, though found hypoglycemic, gave 17% mortality within 4 h of oral administration. However, the doses of MREt at 150, 200 and 250 mg/kg showed acute toxicity by making animals extremely sedative and giving 50, 83 and 100% mortalities, respectively (Table 1). Therefore the value of median lethal dose (LD_{50}) of MREt of *R. serpentina* was determined as

141.25 mg/kg (log LD_{50} = 2.15 mg/kg) from graph plotted between log-doses verses probits.

II. *Effect of MREt of R. serpentina on Glucose Tolerance in Mice*

MREt of *R. serpentina* at doses of 10, 30 and 60 mg/kg and glibenclamide exerted significant hypoglycaemic activity at 0, 30 and 60 min as compared with control and negative groups ($p < 0.01$, $p < 0.001$ & $p < 0.0001$). Doses of extract decreased blood glucose level in the ranges of 31 - 64.60%, 38.95 - 48.87% and 25 - 51.01%, respectively, from 0 to 60 min after oral glucose load (2 g/kg) as compared with control group. Almost same hypoglycemic activity was observed at 120 min after glucose load in control, negative & positive controls and test groups. However, MREt in a dose of 100 mg/kg was found hypoglycemic from 0 to 120 min after glucose load but induced slight sedation in experimental mice (Table 2).

DISCUSSION

Medicinal plants or herbs and their extracts from ancient times have been used for the treatment of diseases such as diabetes, hypertension, gastrointestinal and skin disorders, with or negligible side effects[17]. *R. serpentina* is a well-known anti-hypertensive medicinal herb but it induces

Table 1. Acute toxicity of methanolic root extract (MREt) of *R. serpentina*

Treatments	Hypoglycemic activity*	Sedative behavior	% Mortality rate
Control (distilled water 1ml/kg)	119.33 ± 12.56	-	0
MREt of *R.serpentina* (10mg/kg)	80 ± 2.56*** (- 33)	-	0
MREt of *R.serpentina* (30mg/kg)	65 ± 1.23*** (- 45)	-	0
MREt of *R.serpentina* (60mg/kg)	62 ± 2.83*** (- 48)	-	0
MREt of *R.serpentina* (100mg/kg)	55± 3.56*** (- 54)	+	17
MREt of *R.serpentina* (150mg/kg)	26 ± 0.82*** (- 78)	++	50
MREt of *R.serpentina* (200mg/kg)	-	+++	83
MREt of *R.serpentina* (250mg/kg)	-	+++	100

* Blood glucose level (Mean ± SD) after 30 minutes of oral administration of distilled water in control and each dose of extract in test groups. Values in parenthesis represent percent blood glucose decrease (-) compared to control group

*** = *p< 0.0001* when compared with control group

- = No sedation was observed

+ = slightly sedative and 17% mortality was observed within 4 hours of administration of dose

++ = moderately sedative and 50% mortality was observed within 4 hours of administration of dose while other mice regain normal behavior after providing diet and water

+++ = Acute toxicity (extremely sedative and 83-100 % mortality was observed)

Table 2. Effect of MREt of *R.serpentina* on glucose tolerance in mice

Groups	Treatments	Blood glucose level (mg/dL)			
		0 min	30 min	60min	120 min
Control	distilled water (1 mL/kg) + glucose load (2 g/kg)	150 ± 26.33	252.3 ± 25.8	185.6 ± 17.62	92 ± 23.30
Negative control	0.05% DMSO (1 mL/kg) + glucose load (2 g/kg)	140 ± 25.16	250 ± 5.57	178.6 ± 8.02	98.6 ± 10.60
Positive control	glibenclamide (5 mg/kg) + glucose load (2 g/kg)	96 ± 16.70** (-36)	110.6 ± 6.66*** (-56.16)	85.3 ± 18.04 *** (-54.04)	81 ± 10.44 (-11.95)
Test	MREt (10 mg/kg) + glucose load (2 g/kg)	102.3 ± 23.71* (-31.8)	89.3 ± 19.73*** (-64.60)	104 ± 15.72*** (-43.96)	94.3 ± 17.16 (+2.5)
	MREt (30 mg/kg) + glucose load (2 g/kg)	84 ± 16.09** (-44)	129 ± 0.22*** (-48.87)	113.3 ± 11.72*** (-38.95)	93.3 ± 8.02 (+1.41)
	MREt (60 mg/kg) + glucose load (2 g/kg)	112 ± 7.21* (-25)	123.6 ± 7.77*** (-51.01)	114.6 ± 9.07*** (-38.25)	100.6 ± 8.14 (+9.34)
	MREt (100 mg/kg) + glucose load (2 g/kg)	86.6 ± 14.15** (-42.2)	116.6 ± 40.38*** (-53.08)	109 ± 36.29** (-47.27)	106.3 ± 12.01 (+15.54)

*, ** & *** = *p< 0.01, p< 0.001* & *p<0.0001* when compared with respective control and negative control groups. All values are expressed as mean ± SD (n=6). Values in parenthesis represent percent blood glucose decrease (-) / increase (+) compared with respective time interval in control group.

sedation by depleting the catecholamine in central nervous system[9,10]. Some studies have reported the hypoglycemic activity of *R. serpentina* in hypertensive patients and in anesthetized cats but with less elaborative manner. Recently a single dose (30 mg/kg) of MREt of *R. serpentina* was proved to have hypoglycemic and hypolipidemic activities in alloxan-induced diabetic rats without affecting the serum level of liver-specific enzyme alanine aminotransferase[14].

According to literatures, root bark of *R. serpentina* are rich in alkaloids such as reserpine, ajmaline, etc[18,19]. Hence it is necessary to devise the effective dose of root extract of this plant with respect to hypoglycemic effect because of its sedative (antihypertensive) effect which could make it lethal in higher doses. Therefore the present study for the first time determined the median lethal dose (LD_{50}) of MREt of *R. serpentina* and demonstrated the hypoglycemic activity of various doses (10 - 250 mg/kg) of same extract by conducting oral glucose tolerance test in overnight fasted normoglycemic mice. The experiment of acute toxicity proved that doses of MREt at 10, 30 & 60 mg/kg were safe and hypoglycemic by reducing the mean blood glucose level from 33 to 48% after 30 min administration as compared to control group while doses of same extract from 100 to 250 mg/kg induced severe hypoglycemia with sedation and mortality rate from 17% to 100% in mice within 4 h of administration.

The oral glucose tolerance test (OGTT) is designed to evaluate the person's ability to tolerate orally administrated glucose and is well-known in diagnosis of pre-diabetic and diabetic conditions[20]. In addition, it is also used to diagnose a malabsorption syndrome in which sugar is not properly absorbed through intestines into the blood stream[4]. In the present study, doses of MREt extract of *R. serpentina* at 10, 30 and 60 mg/kg were found effective in reducing blood glucose level after 0, 30 and 60 min of treatment (MREt + glucose load) without being sedative in their respective test groups. Three of these doses significantly decreased blood glucose level by 25 - 65% at 0, 30 and 60 min after oral administration in their respective test group as compared to control and negative control groups. This decrease in blood glucose level might be due to the extra-pancreatic action of extract either by inhibiting glucose absorption in intestine or by increasing glucose tolerance in mice by enhancing the glucose uptake in tissues such as muscles and liver and thereby stimulating glycolysis and hepatic glycogenesis[14]. Interestingly, the hypothetical mechanism of enhancing glucose tolerance in mice has been proven by comparing the values of blood glucose at 0 min in each of the group treated with different concentrations (10, 30 and 60 mg/kg) of MREt along with glucose load (2 g/kg) with respective control at the same time which was only treated with distilled water and glucose. However, almost the same levels of blood glucose were found in all groups including control, negative & positive controls and test groups (MREt @10, 30, 60 mg/kg) after 120 min of their respective treatment with glucose load (2 g/kg). The MREt (100 mg/kg) induced sedation and 17% mortality beside being hypoglycemic up to 120 min after its administration along with glucose while the rest of the live mice of this group regain normal behavior after feeding with normal diet and water. The oral hypoglycemic agent glibenclamide (positive control) was also found significant in increasing glucose tolerance in mice as compared to control and negative control groups from 0 to 60 min ($p < 0.001$ & $p < 0.0001$).

The glibenclamide, a second generation sulfonylureas (known antidiabetic drug) which increases the release of insulin from functional β-cells of pancreas[21]. This give another possible mechanism of action of MREt of *R. serpentina* in reducing the blood glucose level by acting at pancreatic level and enhancing the release of insulin which in turn decrease blood glucose level by stimulating all the anabolic effects[22]. Some medicinal plants were reported to enhance the release of insulin from beta-cells of pancrease such as *Gymnema sylvestre, Ocimum sanctum*, *Phyllanthus species*[23-26]. MREt from 10 to 60 mg/kg did not severely deplete the concentration of catecholamines in central nervous system as no sedation was found in their respective test groups. Therefore, MREt from 10 to 60 mg/kg could be chosen for studying the long-term hypoglycemic activity in type I and II diabetic animal models which will not only help in elucidating the exact mechanism of action of the extract in reducing blood glucose level but also in finding a new bioactive compound with hypoglycemic activity.

CONCLUSIONS

The methanolic root extract of *R. serpentina* was effective in lowering the blood glucose level in doses from 10 - 60 mg/kg and induced no sedation in experimental mice but showed lethal effect by inducing sedation and mortality at doses from 100 - 250 mg/kg. Therefore 10, 30 & 60 mg/kg doses have been selected to conduct a study concerning long-term hypoglycemic activity in type I and II diabetic animal models.

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