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Hypoglycaemic and Hypolipidemic Activities of *Rauwolfia serpentina* in Alloxan-Induced Diabetic Rats

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Abstract: The prevalence of diabetes is increasing worldwide. Changes in lipid metabolism are secondary to diabetes, which may become the cause of hypertension, atherosclerosis and other cardiovascular diseases. The present study was designed to investigate the effect of methanolic root extract of *Rauwolfia serpentina* (a known antihypertensive herb) on glucose, total cholesterol (TC), triglycerides (TG) and alamine aminotransferase (ALT). Alloxan-induced diabetic rats were divided in to 3 groups viz., group I: diabetic control treated with 5% dimethyl sulfoxide (DMSO) in distilled water (1 mL kg^{-1}), group II (positive control): diabetic rats treated with known anti-diabetic drug chlorpropamide (20 mg kg^{-1}) and group III (diabetic test): treated with methanolic root extract (30 mg kg^{-1}). Glucose was estimated from each group at 0, 1, 2 and 4 h after intra-peritoneal injection of each treatment by using glucometer. Rats were decapitated at 4 h, blood was collected to separate the serum that used to analyze TC, TG and ALT. There was a significant decrease ($p<0.0001$) found in glucose level from 0 to 4 h (94-106 mg dL^{-1}) in test rats as compared to diabetic control. Similarly, TG ($p<0.01$), TC and ALT ($p<0.05$) were also significantly decrease in test group. The methanolic root extract of *R. serpentina* was found hypoglycaemic, hypolipidemic and hepato-protective in alloxan-induced diabetic rats.

Key words: Alloxan, diabetes, hypoglycaemic, hypolipidemic, *Rauwolfia serpentina*

INTRODUCTION

Despite of the presence of oral hypoglycemic drugs in commercial market for the treatment of diabetes mellitus, researchers in all over the world still investigating anti-diabetic activity of many plants/herbal extracts to find out new active principle that must possess both hypoglycaemic and hypolipidemic properties with no side effects (Lu *et al*., 2009; Qureshi *et al*., 2009). This study is actually a consequence of encouragement paid by World Health Organization (WHO) to traditional plant treatment especially in developing countries (WHO Expert Committee on Diabetes Mellitus, 1980).

The *Rauwolfia serpentina* Benth (family: Apocynaceae) is a medicinally famous herb in the treatment of hypertension, insomnia, anxiety, excitement, schizophrenia, insanity, etc. in both Ayurvedic and Western medicines but currently its use become obscure in lowering blood pressure (Mashour *et al*., 1998; Salma *et al*., 2008). Several alkaloids have been isolated from root bark of this plant including reserpine, ajmaline, ajmalicine, yohimbine, etc. (Von Poser *et al*., 1990; Itoh *et al*., 2005; Srivastava *et al*., 2006). The root extract of this plant is very useful in disorders of gastro-intestinal tract viz., diarrhea, dysentery, cholera and colic (Ghani, 1998; Tona *et al*., 1999). *Rauwolfia* was also reported to have hypoglycemic activity in anaesthetized cats (Chatterjee *et al*., 1960) but its activity in chemically induced diabetes has not been published yet. In addition, most of the diabetic patients suffered from hypertension which is one of the serious complications of diabetic hyperlipidemia and the major causes of cardiovascular diseases and death in diabetic population of the world (Epstein and Sowers, 1992). Therefore, the present investigation was designed to evaluate the effect of methanolic root extract of known antihypertensive herb *R. serpentina* on glucose, Total Cholesterol (TC), triglycerides (TG) and alamine aminotransferase (ALT) in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Animals: Albino rats of both sexes, weighing from 150-250 g, were purchased from H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan and were kept under usual management conditions in conventional animal house of Department of Biochemistry, University of Karachi, Pakistan. Rats were given standard laboratory diet and free access to water *ad libitum*.

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Alloxan tetrahydrate (Sigma): Diabetes was induced in experimental rats by freshly prepared doses of alloxan tetrahydrate (120 mg kg^{-1}) for 3 consecutive days intra-peritoneally (Qureshi *et al*., 2009).

Chlorpropamide (Sigma): It was used as positive control (20 mg kg^{-1}) in present study (Cunha *et al*., 2008).

Reagent kits for biochemical analyses (Human and Randox): Glucose levels were analyzed by glucocard memory 2 (glucometer; Arkray, Inc. Japan). Other parameters viz., triglycerides (GPO-PAP method), total cholesterol (CHOD-PAP method) and alamine aminotransferase (Reitman-Frankel colourimetric method) were determined by commercially available reagent kits (Randox, United Kingdom and QCA, Spain, respectively).

Plant material: Roots of *R. serpentina* were purchased from Hamdard Dawakana, Sardar, Karachi and identified by experts in Botany Department, University of Karachi, Karachi-75270, Pakistan. The voucher specimen has been kept in our department (KU/BCH/SAQ/02).

Preparation of methanolic root extract: Grinded powder (40 g) 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 till dryness in a rotary vacuum evaporator (Eyela-NE) to obtain a brown residue that referred as methanolic root extract (Ju *et al*., 2008).

Experimental procedure: Experimental rats were divided into 3 groups (6 rats of both sexes/group) according to the treatments (Fig. 1). The rats were deprived of food but not water for over night (12-14 h) before starting the experiment. The rats were kept fasted during the whole experiment. After intra-peritoneal injection of each treatment, glucose was monitored at intervals of 0, 1, 2, and 4 h with the help of glucocard memory 2 (glucometer) by pricking the vein of their tails (Cunha *et al*., 2008).

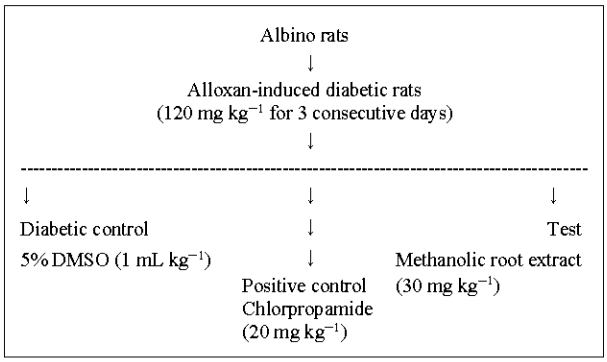

Fig. 1: Grouping of experimental rats according to treatments

Finally rats were decapitated at 4 h to collect the blood, serum was separated that used to analyze other biochemical parameters viz., TC, TG and ALT. Dimethyl sulfoxide (5% DMSO) was used as negative control and vehicle for methanolic root extract (Saleem *et al*., 1999). The whole experimental research from preparation of methanolic root extract to statistical analysis of biochemical parameters was conducted in Department of Biochemistry, University of Karachi from January to December 2008.

Statistical analysis: The data were analyzed by Student's t-test (Graphpad Software, Quick Calcs Online calculators for Scientists). Differences were considered significant with $p<0.0001$, $p<0.01$ and $p<0.05$. Values are expressed as Mean±Standard Error Mean (SEM).

RESULTS AND DISCUSSION

Diabetes and hypertension go together (Konzem *et al*., 2002). Researchers are interested to find out the cure of diabetes which also reduced the risk of chronic complications of this disease (Kim *et al*., 2006). Studies have already been published related to different medicinal plants/herbs in reducing the blood glucose levels in alloxan-induced diabetic rats (Pari and Maheswari, 1999; Kim *et al*., 2006; Qureshi *et al*., 2009). In the present study, methanolic root extract of *R. serpentina* (30 mg kg^{-1}) induced a significant decrease ($p<0.0001$) in serum glucose levels of alloxan-induced diabetic rats as compared to diabetic control group (Table 1). The acute reduction in glucose levels of alloxan-induced diabetic rats after single intra-peritoneal injection of extract showed in a time-dependent manner, which is as significant as decrease in glucose levels observed in chlorpropamide-treated diabetic rats. However, the effect of extract was persists up to 4 h interval of treatment as compared to chlorpropamide-treated group (Table 1). These findings are significant as compared to the previous hypoglycaemic results, reported that different fractions of *Rauwolfia* alkaloids first induced slight increase and then decrease in blood sugar levels of anesthetized cats (Chatterjee *et al*., 1960).

Diabetes was induced in experimental rats by alloxan, is known to destroy β-cells of pancreas and inhibit insulin production (Kamanyi *et al*., 1994). It has been confirmed by observing increase levels of glucose (289-298 mg dL^{-1}) in diabetic controls rats. On the contrary, it is also interesting to observe decrease glucose levels in chlorprapamide-treated diabetic rats, the drug known to decrease glucose level by stimulating β-cells (Rang *et al*., 2003). Therefore, it might possible that few β-cells were

Table 1: Effect of methanolic root extract of *R.serpentina* on serum glucose levels in alloxan-induced diabetic rats

	Treatments (h)			
Groups	0	1	2	4
Diabetic control+5% DMSO	298.0±2.7	290.5±4.9	289.0±0.0	297.5±21.07
Diabetic+chlorpropamide (PC)	287.2±4.5*	115.2±5.4***	100.3±4.7***	134.6±2.9***
Diabetic+meth. root extract (DT)	260.5±2.6***	100.0±15.9***	94.0±16.7***	106.5±3.7***

Values are expressed as Mean±SEM (n = 6). Diabetic Test (DT) and Positive Control (PC) compared with their respective diabetic control (***p<0.0001 and *p<0.05)

Table 2: Serum Total cholesterol (TC), triglycerides (TG) and alanine aminotransferase (ALT) in alloxan-induced diabetic rats. Rats were decapitated after 4 h of intra-peritoneal injection of each treatment (n = 6)

	TC	TG	ALT
Groups	----------(mg dL^{-1})--------		($U L^{-1}$)
Diabetic control+5% DMSO	112.5±11.8	220.5±14.1	57.5±10.3
Diabetic+Chlorpropamide (PC)	100.2±1.8	135.5±2.8**	48.7±1.4
Diabetic+Meth. root extract (DT)	83.07±2.3*	160.7±1.9**	29.7±2.3*

Diabetic test (DT) and Positive control (PC) compared with their respective diabetic control (*p<0.05 and **p<0.01)

remained alive and their function were improved by chlorpropamide, the work is still in progress to prove this claim. The following mechanisms have been proposed for hypoglycemic effect of extract in test rats viz., to inhibit hepatic glucose production, to inhibit intestinal glucose absorption, to enhance glucose absorption in muscles and adipose tissues or to correct insulin resistance (Kamanyi *et al*., 1994; Ju *et al*., 2008; Qureshi *et al*., 2009). The possibility of β-cells regeneration has not been ruled out as many medicinal plants were reported to regenerate β-cells leading to an increased insulin production and secretion (Daisy *et al*., 2004).

Alloxan-induced hyperglycaemia is also accompanied with hypertriglyceridemia and hypercholesterolemia (Ju *et al*., 2008). In the present study, TC ($p<0.05$) and TG ($p<0.01$) were significantly decreased in test rats by methanolic extract as compared to diabetic controls (Table 2). While a significant decrease ($p<0.01$) was only observed in serum TG level of chlorpropamide-treated rats (Table 2). The significant effect of methanolic extract on diabetic hypertriglyceridemia could be due to its effect on glycemic control. It has also been confirmed by observing the decrease in TG levels of chlorpropamide-treated rats. The improved reduction in glucose levels by sulfonylureas accompanied with decrease serum VLDL and TG levels has already been reported by Roa *et al*. (1999). On the other hand, the reduction in cholesterol level in test rats may be due the inhibitory effect of methanolic extract on 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG CoA reductase), the rate-regulatory enzyme of cholesterol biosynthesis (Sharma *et al*., 2003; Ju *et al*., 2008) or by stimulating effect on glucose utilization by peripheral tissues (Kamanyi *et al*., 1994).

Rauwolfia is used to treat numbers of brain disorders where it known to depletes catecholamines from nerves in central nervous system (Craig *et al*., 1999). Catecholamines are not only hypertensive but also hyperglycaemic in action by enhancing glycogenolysis in liver and muscles, gluconeogenesis, lipolysis in adipose tissues, stimulating glucogen secretion while inhibiting hepatic glycogen formation and insulin secretion (Rang *et al*., 2003). Therefore, it might be possible that methanolic root extract has also one indirect effect in reducing glucose and lipid contents in alloxan-induced diabetic rats. No depression or sedation was observed in test rats during the experiment. Alanine aminotransferase (ALT) is the liver-specific enzyme and its high concentration in blood represents the damage of hepatocytes (Bishop *et al*., 2005). Chronic mild elevation of aminotransferases is frequently found in type 2 diabetic patients (Harris, 2005). In the present study, methanolic root extract-treated rats were showed normal or decreased levels of ALT as compared to diabetic control and chlorpropamide-treated rats ($p<0.05$).

In conclusion, the present investigation proved that the methanolic root extract of *R. serpentina* was found to have hypoglycaemic and hypolipidemic effects (both hypotriglyceridemic and hypocholesterolemic) in alloxan-induced diabetic rats. In addition, the extract has hepato-protective effect by observing normal serum levels of ALT. Due to the significant effect of methanolic extract on alloxan-induced diabetic hyperglycaemia and hyperlipidemia, work is still going on in lab to investigate the same effects in different fractions of methanolic extract in order to isolate an active principle having same activity with no side effects followed by establishing the most probable mechanism of action of reducing serum glucose and lipids.

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