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Hypoglycemic Activity of Red Kino Tree in Normal and Streptozotocin Induced Diabetic Rats

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Abstract: The aim of the present study was to evaluate the hypoglycemic activity of the methanolic extract from the bark of Red Kino Tree (RKT) (Fabaceae) at doses of 200 and 400 mg kg⁻¹ (p.o.) in streptozotocin induced diabetic rats. Screening for the hypoglycemic activity was assessed on normoglycemic, streptozotocin treated hyperglycemic rats and *in vitro* study on glucose utilization by isolated rat hemidiaphragm. The serum biochemical parameters were also studied. The extract was found to possess significant hypoglycemic activity when compared with the reference standard glibenclamide. The *in vitro* study on glucose utilization by isolated rat hemidiaphragm suggests that the methanolic extract may have direct insulin like activity which enhances the peripheral utilization of glucose and also have extra pancreatic effect. The methanolic extract (400 mg kg⁻¹) showed a significant decrease in triglycerides (TG) (p<0.01), Low Density Lipoprotein (LDL) (p<0.01), Very Low Density Lipoprotein (VLDL) (p<0.001) and a significant increase in High Density Lipoprotein (HDL) (p<0.05). The toxicity studies report safety usage of the plant extract. The present results clearly indicated that RKT, is potent for anti-diabetic effects in streptozotocin induced diabetic rats.

Key words: Red kino tree, hypoglycemic, streptozotocin, hemidiaphragm, *Pterocarpus marsupium*

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic, with a world wide incidence of 5% in the population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more than AIDS (Jarald *et al.*, 2008). India is one of the leading countries for the number of people with diabetes mellitus and it is estimated that the diabetes effects approximately 57 million people by the year 2025 in India (Pavana *et al.*, 2007) Chronic hyperglycemia causes many of the major complications of diabetes, including nephropathy, retinopathy, neuropathy and macro vascular and micro vascular damage (Brownlee, 2001). In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus (Sumana and Suryawashi, 2001). The treatment of Diabetes mellitus is based on oral hypoglycemic agents and insulin. However, in the indigenous Indian system of medicine good number of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and active principles were isolated (Grover *et al.*, 2002). WHO (1980) has also recommended the evaluation of the

effective use of plants where there are no safe modern drugs (Upadhayay and Pandey, 1984). The synthetic hypoglycemic agents used in clinical practices have serious side effects like hematological effects, coma, disturbances of liver and kidney. Compared with synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects (Momin, 1987). Therefore, the search for more effective and safer antidiabetic agents as become an area of active research. Keeping these facts in view, the present study was undertaken to create a scientific base of the traditional use of the bark extract of *Pterocarpus marsupium* as an anti-diabetic agent in diabetes associated complications. *Pterocarpus marsupium* Roxb. (Fabaceae), is a multipurpose deciduous forest tree. The plant is commonly known as Asanahm bijakah (Sanskrit), Red Kino tree (English) and Peddagi (Telugu). The trees are valued for its pharmaceutical properties as the gum Kino obtained from the tree is a powerful astringent used for treatments of dysentery, diarrhea, fever, stomachache, cholera, urinary complains and toothache (Tiwari *et al.*, 2004). The gum is locally applied in leucorrhoea and

passive haemorrhage (Pullaiah, 1999). An aqueous infusion of the wood is said to be of use in diabetes and water stored in the vessel made of the wood is reputed to have antidiabetic properties (Husain *et al.*, 2007). The antihyperglycemic activity of heartwood (Manisckam *et al.*, 1997) and antioxidant and analgesic activities of bark (Radhika *et al.*, 2008) has been reported. In the present study, hypoglycemic and antidiabetic activity of methanolic bark extract of red kino tree in normal and streptozotocin induced diabetic rats were evaluated.

MATERIALS AND METHODS

Materials: Streptozotocin was purchased from Sigma, St. Louis, MO, USA. Methanol was purchased from E. Merck Ltd., Mumbai, India. Glibenclamide pure substance was obtained from Cadila Health Care Ltd., Ahmedabad.

Plant material collection: The bark of Red Kino Tree (RKT) was collected from Kakatiya University campus in the month of July, 2008. Taxonomic identification of the specimen was performed and a voucher specimen is deposited in the herbarium of department of Biotechnology, Kakatiya University, Warangal.

Preparation of extract: The dried bark was crushed to coarse powder. The powder was macerated with methanol for 48 h. Then the extract was filtered and dried using a rotary evaporator and stored at -4°C until further use.

Experimental animals: Wistar albino rats of either sex, weighing 230-250 g were procured from Mahaveer Enterprises, Hyderabad. The selected animals were housed five per each of acrylic cages at 25°C , 45-55% humidity and 12/12 h light/dark under controlled environment. Rats were fed with standard laboratory diet and water was given *ad libitum*. Animals were fasted for 18 h before commencing the experiment. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use. The experiment protocol was approved from the institutional animal ethical committee.

Acute toxicity studies: Wistar albino rats of either sex, weighing 230-250 g selected by random sampling technique were used in the study and allowed free access to water. The oral acute toxicity test of the plant extracts was performed according to method used by Sheth *et al.* (1972). The methanol extract, at concentration of 100, 500, 1000 and 2000 mg kg^{-1} were administered orally via a gastric catheter. After administration of test sample, the

animals were observed continuously for first 4 h for behavioral changes and at the end of 24 h for mortality if any. No death was observed till the end of the study. The test samples were found to be safe up to the dose of 2000 mg kg^{-1} and from the results, 200 and 400 mg kg^{-1} doses were chosen as the dose for further experimentation.

Screening for hypoglycemic activity: The method of Dash *et al.* (2001) was followed. The test samples were suspended in 25% Tween 20 in distilled water. Glibenclamide (2.5 mg kg^{-1}) was used as reference control during the study. All the test samples were administered through oral route.

In normoglycemic animals: The animals were fasted for 20 h, but were allowed free access to water before and throughout the experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tip of the tail of each rat under mild ether anaesthesia. Plasma was separated following centrifugation and the glucose was estimated by GOD/POD method using Glucose estimation kit from M/s. Excel Diagnostics Pvt. Ltd., Hyderabad, India. The normal rats were then divided into four groups of six animals each. Group I served as a solvent control, Group II and III received the test extract at a dose of 200 and 400 mg kg^{-1} , respectively, through oral route. Group IV received glibenclamide (2.5 mg kg^{-1} , p.o.) and served as reference control. All the test samples were administered in a similar manner. Blood glucose levels were examined after 1, 2, 4, 8 and 10 h of administration of test samples.

In streptozotocin induced diabetic animals: Rats of either sex weighing (200-250 g) were selected and fasted for 18 h prior to experiment and water supplied *ad libitum*. The rats were administered with Streptozotocin (65 mg kg^{-1} , i.v.) in citrate buffer (pH 4.4, 0.1 M). After 3 days, the blood samples were collected and analyzed for blood glucose levels. Rats with blood glucose levels more than 200 mg dL^{-1} were included in the experiment.

The diabetic animals were segregated into four groups of six rats in each. Group I served as solvent control and received only vehicle (2 mL kg^{-1} , p.o.) through oral route. Group II received glibenclamide (2.5 mg kg^{-1} , p.o.). Groups III and IV received the test extract at doses of 200 and 400 mg kg^{-1} , p.o. in a similar manner. Blood glucose level of each rat was estimated at 1, 2, 4, 8 and 10 h, respectively.

In vitro study on glucose utilization by isolated rat hemidiaphragm: The method of Chattopadhyay *et al.* (1992) was followed. The selected rats were killed by

decapitation and diaphragms were taken out quickly avoiding trauma and divided in to two halves. The hemidiaphragms were then placed in culture tubes containing 2 mL tyrode solution with 2 g% glucose and incubated for 30 min at 37°C in an atmosphere of 95% O₂ -5% CO₂ with shaking. Four sets of experiments were performed. The diaphragms were exposed to:

- Tyrode solution with 2 g% glucose which served as control
- Tyrode solution with 2 g% glucose + insulin (0.25 IU mL⁻¹)
- Tyrode solution with 2 g% glucose + extract (200 mg mL⁻¹)
- Tyrode solution with 2 g% glucose + insulin (0.25 IU mL⁻¹ + extract (200 mg mL⁻¹))

Following incubation, the hemidiaphragms were taken out and weighed. The glucose content of the incubated medium was measured. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubation medium.

Estimation of biochemical parameters: Blood was collected through retro-orbital plexus of all the rats, under light ether anesthesia, using capillary tubes into eppendorf tubes. The serum was separated at 5000 rpm for 5 min using micro centrifuge. The biochemical parameter investigation was carried out to assess Total Cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and Triglycerides (TG) (Sood, 1999).

Statistical analysis: The results were presented as Mean±SD (n = 6). The statistical package Graph-Pad Prism, version 4 for Windows (San Diego, CA, USA) was used in the analysis.

RESULTS

The methanolic bark extract of Red Kino Tree (RKT) produced non toxic symptoms or mortality up to dose level 2000 mg kg⁻¹ b.wt. orally in rats and hence the drug was considered safe for further pharmacological screening. The methanolic extract of bark of RKT produced significant decrease in the blood glucose level when compared with the controls in normoglycemic, streptozotocin induced hyperglycemic rats at a dose level of 200 and 400 mg kg⁻¹ (Table 1, 2). Oral administration of RKT extract produced a significant reduction (p<0.05) in blood glucose levels at 8th and 4th hour with 200 and 400 mg kg⁻¹, respectively in the normal rats. The maximum reduction (p<0.001) in blood glucose level was seen at a dose of 400 mg kg⁻¹ of RKT extract administration. However, in streptozotocin induced diabetic rats, the methanolic extract of RKT (200 and 400 mg kg⁻¹) produced significant (p<0.01) antihyperglycemic effects at 4th hour (Table 2). These results of methanolic bark extract of RKT was comparable with standard drug Glibenclamide.

Table 3 shows that the diabetic rats showed an increased levels of Total Cholesterol (TC), Triglycerides (TG), LDL, VLDL enzyme activity, except HDL levels. Oral administration of methanolic extract of RKT had significant (p<0.05) effect in restoring the levels of serum lipids to near normal level with moderate increase in HDL cholesterol level.

The *in vitro* study on glucose utilization using isolated rat hemidiaphragm results are showed in Table 4. Incubation of rat hemidiaphragm with bark extract of RKT increased a significant uptake of glucose. This increase of glucose uptake by the rat hemidiaphragm was enhanced in presence of insulin.

Table 1: Effect of bark extract of Red Kino tree on blood glucose in normoglycemic rats

Groups	Dose (mg kg ⁻¹)	Blood glucose levels (mg dL ⁻¹)					
		0 h	1 h	2 h	4 h	8 h	10 h
Control	2	95.12±5.97	94.92±5.41	94.13±6.51	92.27±5.72	89.31±4.48	86.38±4.29
Glibenclamide	2.5	97.67±4.82	93.81±4.63	81.42±3.96	70.12±4.08**	65.45±3.39**	60.15±3.01***
Extract	200	97.52±5.45	96.12±5.74	93.13±4.81	88.38±4.25	80.12±3.85*	73.95±2.45**
Extract	400	96.87±6.25	93.15±6.06	89.45±5.39	83.03±3.99*	74.52±3.29**	62.63±2.64***

Values are expressed as Mean±SD (n = 6). *p<0.05, **p<0.01, ***p<0.001

Table 2: Effect of bark extract of Red Kino tree on blood glucose in streptozotocin induced diabetic rats

Groups	Dose (mg kg ⁻¹)	Blood glucose levels (mg dL ⁻¹)					
		0 h	1 h	2 h	4 h	8 h	10 h
Control	2	280.61±9.21	281.48±9.79	288.21±8.69	292.75±9.54	298.93±11.37	315.55±10.55
Glibenclamide	2.5	294.32±10.52	270.51±8.51	254.38±7.97**	220.43±8.15**	140.29±7.06***	92.12±6.54***
Extract	200	292.95±11.31	286.38±11.58	274.72±9.14	260.59±8.64**	195.49±8.15**	130.69±7.27**
Extract	400	295.49±10.85	288.19±8.84	277.91±8.11	240.42±7.78**	165.51±6.97***	100.37±6.62***

Values are expressed as Mean±SD (n = 6). *p<0.05, **p<0.01, ***p<0.001

Table 3: Effect of bark extract of Red Kino tree on serum biochemical parameters in streptozotocin induced rats

Groups	Dose (mg kg ⁻¹)	Biochemical parameters				
		TC	TG	HDL	LDL	VLDL
Control	2	229.64±4.50	203.15±3.57	29.87±1.08	152.42±2.97	39.49±0.71
Glibenclamide	2.5	138.10±2.35*	79.62±2.02**	47.24±0.92*	76.81±1.74***	15.49±0.65***
Extract	200	204.42±3.15	107.45±3.14*	35.18±1.10	139.24±1.62*	22.63±0.49**
Extract	400	180.67±2.91	85.24±2.60**	45.48±0.85*	114.08±1.47**	17.94±0.42***

Values are expressed as Mean±SD (n = 6). *p<0.05, **p<0.01, ***p<0.001

Table 4: Effect of bark extract of Red Kino tree on glucose uptake using isolated rat hemidiaphragm

Incubation medium	Glucose uptake (mg/g/30 min)
Tyrode solution with glucose (2 g %)	5.26±0.18
Tyrode solution with glucose (2 g %) + insulin (0.25 IU mL ⁻¹)	9.14±0.14*
Tyrode solution with glucose (2 g%) + bark extract (200 mg mL ⁻¹)	7.54±0.85*
Tyrode solution with glucose (2 g%) + insulin (0.25 IU mL ⁻¹) +bark extract (200 mg mL ⁻¹)	12.31±0.54*

Values are expressed as Mean±SD (n = 6). *p<0.001

DISCUSSION

Medicinal plants are widely used by the populations of developing countries as alternative therapy. In India, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately only a few of such Indian medicinal plants have received scientific scrutiny. The present work was therefore designed to study the hypoglycemic effect of RKT bark extract against Streptozotocin-diabetic rats. Streptozotocin-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents (Szkudelski, 2001). Streptozotocin selectively destroyed the pancreatic insulin secreting β-cells, leaving less active cell resulting in a diabetic state (Szkudelski, 2001).

In the present study, methanolic extract of bark of RKT produced a significant decrease in the blood glucose level at a dose level of 200 and 400 mg kg⁻¹ in normoglycaemic and hyperglycemic rats. The significant decrease in the levels of blood glucose in diabetic rats treated with the RKT bark may be by stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose (Erah *et al.*, 1996).

From the results it is assumed that the bark extract could be responsible for stimulation of insulin release and the observed restoration of metabolic activities. A number of other plants have also been shown to exert hypoglycemic activity through stimulation of insulin release (Prince and Menon, 2000). Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide; they reduce blood glucose in normoglycemic animals (Davis and Granner, 1996). Glibenclamide is reported to enhance the activity of β cells of pancreas resulting in secretion of larger amounts of insulin which in turn brings down blood glucose level (Andrew, 2000).

Diabetes is associated with hyperlipidemia (De Sereday *et al.*, 2004). Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk for coronary heart disease. A reduction in serum lipids, particularly of the LDL and VLDL fraction and triglycerides, should be considered as being beneficial for the long-term prognosis of these patients (Chattopadhyay and Bandyopadhyay, 2005). Lowering of blood glucose and plasma lipid levels through dietary modification and drug therapy seems to be associated with a decrease in the risk of vascular disease.

Hypercholestraemia and hypertriglyceremia in STZ induced diabetic rats are well documented. Insulin deficiency leads to increased serum lipids because of increased lipolysis (Ohaeri, 2001). The methanolic extract of RKT had significant effect in restoring the levels of serum lipids to near normal level with moderate increase in HDL cholesterol level. The extract reduced the VLDL, TC and TG, it may be presumed that the extract is responsible for the enhancement of the transcription of lipoprotein lipase similar to that of insulin, since in the untreated or under treated diabetes patients the level of triglycerides and cholesterol increases due to increased production of VLDL and unavailability of protein lipase which hydrolyses the triglycerides to VLDL because of insulin deficiency.

The enhanced glucose utilization of hemidiaphragm in presence of bark extract revealed that the glucose uptake is similar to that of insulin. These findings suggest that the methanolic extract of bark may have direct insulin like activity which enhances the peripheral utilization of glucose and have extra pancreatic effect (Chattopadhyay *et al.*, 1992).

In conclusion, the present study findings indicated that the methanolic bark extract of the Red Kino tree is not toxic and as potent as the standard drug in the management of diabetes.

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