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**HYPOGLYCAEMIC AND ANTIDIABETIC EFFECT OF ROOT EXTRACTS OF
CEIBA PENTANDRA IN NORMAL AND DIABETIC RATS**

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Abstract

The effect of the root bark extract of *Ceiba pentandra* (Linn) in normal and streptozotocin-induced diabetic rats was studied. Blood glucose levels were determined after oral administration of graded doses of *C. pentandra* (40, 75, 150 and 300 mg/kg) in fasted normal and diabetic groups. In both groups, 40 and 75 mg/kg of the extract, significantly reduced blood glucose levels 8 h after administration, which was consistent and time-dependent. *C. pentandra* at the lower dose of 40mg/kg produced blood - glucose-lowering effect of 40.0% and 48.9%, in normal and diabetic rats respectively when compared with control rats. The higher doses of 150 and 300 mg/kg did not affect significantly the blood glucose levels. In multiple dose studies, the diabetic rats were treated orally by gavages, twice a day for 3 days. On day 3, *C. pentandra* (40 and 75mg/kg) significantly decreased blood and urine glucose levels as compared to initial values. The 14 h fasting blood glucose concentration was lowered by 59.8 % and 42.8% at the doses of 40 and 75 mg/kg and the corresponding urine glucose levels reductions were 95.7% and 63.6%, respectively. The results indicated that *C. pentandra* possessed hypoglycaemic effect. The plant extract was capable of ameliorating at lower doses, hyperglycaemia in streptozotocin-induced diabetic rats and could be a potential source for isolation of new orally active agent(s) for anti-diabetic therapy.

Key words: *Ceiba pentandra*, Plant extract, Diabetes mellitus, Hypoglycaemic.

Introduction

Diabetes mellitus is a group of disorders with different aetiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and / or insulin action (Balkau *et al.*, 2000). Approximately 140 million people worldwide suffer from diabetes (WHO, 1999). The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable (Djrolo *et al.*, 1998). Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed (WHO, 2002), because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries. Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease. *Ceiba pentandra* (L) Gaertner (Bombacaceae) known as silk cotton tree and locally as “dum” is widely reputed in the African traditional medicine (Ueda *et al.*, 2002). Various morphological parts of this plant have been reported to be useful as effective remedies against diabetes, hypertension, headache, dizziness, constipation, mental trouble, fever, peptic ulcer, rheumatism and leprosy. It is also used as a diuretic and to expel evil spirits (Noumi *et al.*, 1999; Ngounou *et al.*, 2000; Noumi and Dibako, 2000; Noumi and Tchakonang, 2001; Ueda *et al.*, 2002). The hypoglycaemic activity of the aqueous extract of the plant's stem bark at high doses has been reported (Olusola *et al.*, 2003). In the present study, the hypoglycaemic effect of the root bark methylene chloride / methanolic extract in normal and streptozotocin - induced diabetic rats was evaluated.

Materials and Methods

Animals

Male albino Wistar rat (175 - 225 g) raised in the Animal House of the Faculty of Science, University of Yaounde I were used. They were fed with standard chow and watered *ad libitum*. Before testing for blood glucose level or injection of streptozotocin to induce diabetes, the rats were fasted overnight (at least 12 h) but had free access to water. The study was approved by institutional animal ethical committee.

Preparation of the plant extract

Roots of *C. pentandra* were collected in Yaounde (Centre Province, Cameroon). Botanical identification was performed at the National Herbarium, Yaounde, voucher specimens HNC 43623. The barks were removed from roots, sliced into small pieces, air dried at ambient temperature and ground into powder form. A kilogram of the powdered of the plant was macerated in a 1:1 (volume /volume) mixture of methylene chloride / methanol for 2 days (with occasional stirring) at room temperature. The filtrate obtained after filtration were concentrated using a rotavapor at a temperature of 80°C to obtain 106g to yield an equivalent of 10.6% yield of the starting material.

Induction of diabetes

Streptozotocin (STZ), purchased from Sigma Chemical Co., (Saint Louis, MO), was dissolved in ice-cold 0.9% NaCl saline solutions immediately before use. The overnight fasted rats were anaesthetised with diethyl ether and received STZ (55mg/kg) through the dorsal vein of the penis. Fasted blood glucose levels were assessed 48 hours after STZ injection as well as glycosuria to confirm the diabetic states. The rats were kept for 15 days to stabilise the diabetic condition (Jyoty et al., 2002). Only rats with a fasting blood glucose level of at least 200 mg/dl and positive urine glucose were used in the experiment.

Treatment of animals

Two set of experiments were carried out: single and multiple dose studies.

Single dose studies

This experiment involved testing for hypoglycaemic effect of the plant in normal and diabetic rats after single oral administration. Six groups of normal and diabetic rats of 5 rats/group were used. The control and diabetic groups received 1.5% dimethyl sulphoxide in distilled water. Four groups each of normal and diabetic rats were given p.o. 40, 75, 150 and 300 mg/kg of the plant extracts respectively. Their positive control groups had 5 mg/kg glibenclamide (Daonil^R), a standard oral hypoglycaemic agent for comparison. Blood glucose levels were determined at time zero and subsequently at time 0.5, 1, 2, 3, 5 and 8 h.

Multiple dose studies

The experiment consisted of two groups of 5 diabetic rats given orally, 40 and 75mg/kg the extract twice daily for 3 consecutive days. Normal and diabetic control groups received 1.5% DMSO/H₂O twice daily whereas the positive control rats took 2 IU of insulin once daily. Following 14 hours of fasting, blood and urine glucose levels were assessed before and at the end of the 3 day treatment period. Body weight, food and water intakes were also monitored.

Blood sampling and measurement of blood and urine glucose

Blood samples (20 µl) were obtained from the tail tip of fasted rats, and the blood glucose level was determined using a glucometer ACCUTREND GC (Boehringer, Mannheim, Germany) while the glucose in fresh urine was assessed using glucose indicator sticks (Boehringer Mannheim Germany) before and after treatment.

Statistical analysis

All the values in the test are presented as mean ± SEM (Standard Error of the

Mean). Statistical differences between the means of the various groups were evaluated by one-way analysis of variance (ANOVA) using the SPSS program followed by Students' *t*-test. P values of 0.05 or less were considered to be significant.

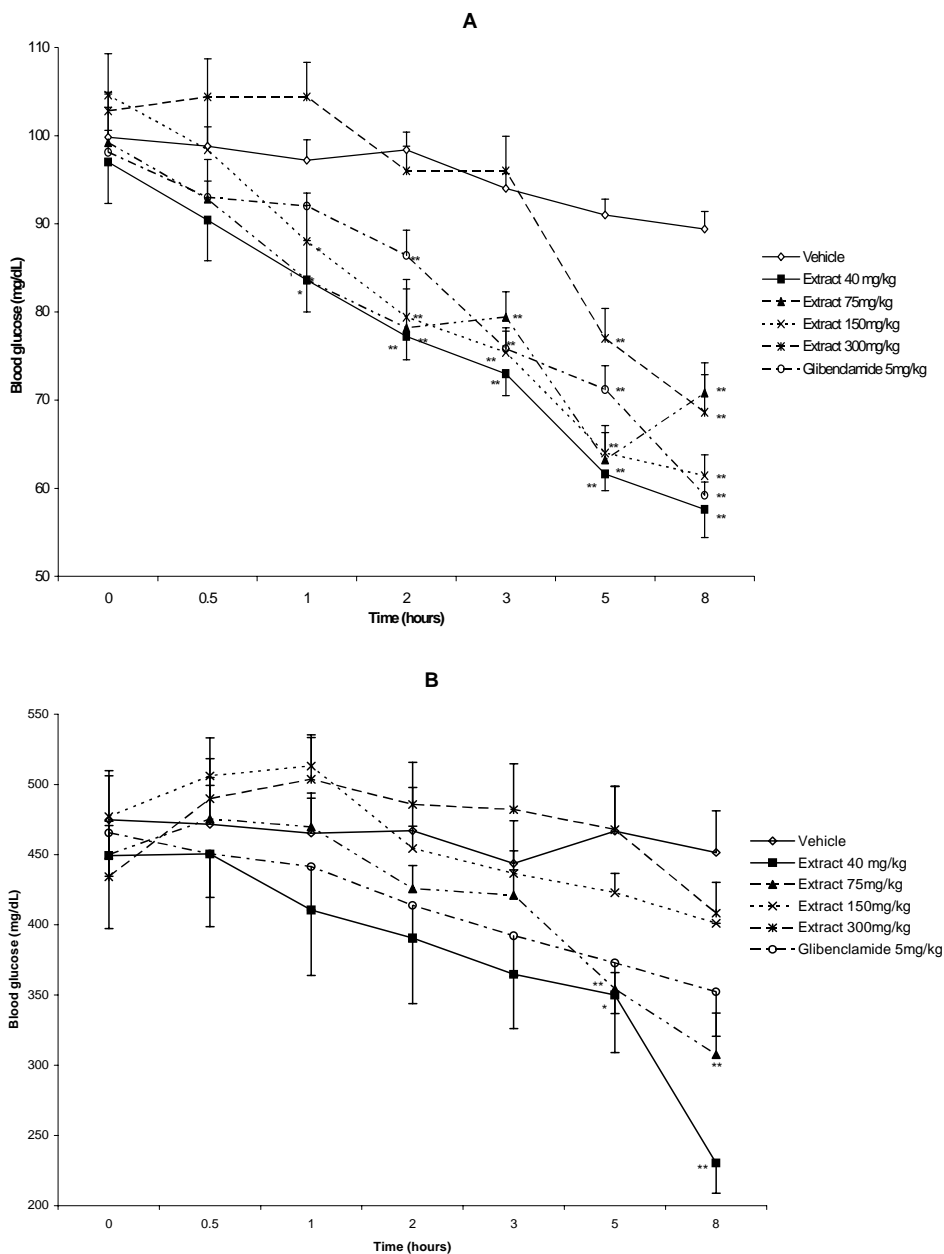


Figure 1: Upper Panel: Effect of single dose of methylene chloride/methanol extract of *Ceiba pentandra* on blood glucose levels in normal rats. Values expressed as mean \pm SEM; * P<0.05; ** P<0.01, with respect to initial values **Lower Panel:** Effect of single dose of organic solvent extract of *Ceiba pentandra* on blood glucose levels in diabetic rats. Values expressed as mean \pm SEM; * P<0.05; ** P<0.01, with respect to initial values.

Results

The effect of single dose administration of the extract on blood glucose level in fasting normal and diabetic rats are as shown in Figure 1a. The hypoglycaemic effects of the extract were significant ($P < 0.05$) one h. after dosing at 40, 75, and 150 mg/kg in the normal rats and only significant at 40 and 75 mg/kg in the diabetic rats (Figure 1b. At the highest dose tested (300 mg/kg), these effects were observed in normal rats 5 h post dosing. Glibenclamide had its significant effect 3 h post dosing. Twice daily administration for 3 days of the extract decreased the blood and urine glucose levels in diabetic rats (Table 1). The extract at 40 and 75 mg/kg significantly ($P < 0.001$), reduced blood glucose levels by 60.0% ($P < 0.001$) and 42.8%, compared to 27% produced by insulin ($P < 0.05$). These results confirmed the corresponding reduction ($P < 0.001$) of urine glucose levels by 91.0%, 64.0% at 40 and 75 mg/kg of the extract and 46.0% for insulin. There was no significant change in body weights, food and water intakes of the rats.

Discussion

Streptozotocin - induced hyperglycaemic has been described as a useful experimental model to study the activity of hypoglycaemic agents (Szkudelski, 2001). Streptozotocin selectively destroys the pancreatic insulin secreting β -cells, leaving less active cells and resulting in a diabetic state (Kamtchouing et al., 1998; Szkudelski, 2001). At a single dose of 40mg/kg, the root bark extract produced significant reduction ($P < 0.01$) in the blood glucose concentration of fasted normal and diabetic rats after 8 h. These results were similar to those of Olusola et al., (2003) who reported highest doses of the aqueous extract of the stem bark of *C. pentandra*. These observations may suggest that active principle (s) may be more liposoluble than hydrosoluble. A similar observation was reported by Kameswara et al. (2001) on the effect of bark extract of *Pterocarpus santalinus* on blood glucose in experimental animals.

Glibenclamide treatment (5 mg/kg) was not as effective in reducing blood glucose in STZ-diabetic rats as in normoglycaemic rats. It has been reported that glibenclamide was not effective when destruction of β -cells has occurred and hence more effective in moderate diabetic rats than in severe diabetic animals (Sharma et al., 1997; Cetto et al., 2000; Hosseinzadeh et al., 2002). The acute hypoglycaemic effect of glibenclamide results has been shown from the stimulation of insulin release from the residual β -cells and inhibition of glucagon secretion (Moller, 2001). The extract might possess insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. The phytochemical studies of *C. pentandra* revealed the presence of epicatechin isolated from other plants has been found to stimulate insulin secretion or possess an insulin-like effect (Marles and Farnsworth, 1995; Noreen et al., 1998; Kameswara et al., 2001). In this study, it was observed that administration of *C. pentandra* extract to diabetic rats reversed at lower doses their blood glucose which was also reflected in their urine sugar levels.

Table 1: Effect of *Ceiba pentandra* extract on blood and urine glucose levels after 3 days oral administration to streptozotocin – induced diabetic rats.

Groups	N	Dose (mg / kg)	Blood glucose (mg/dL)			Urine glucose (mg/dL)		
			Before treatment	After treatment	% reduction	Before treatment	After treatment	% reduction
Normal	5	-	99.0±1.3	98.8±1.9	-0.2%	0	0	0
Diabetic Control	5	-	343.0±13.6	347.4±11.2	+1.3%	4.8±0.2	4.8±0.4	+0.02%
Extract	5	40	330.0±39.6	132.6±18.6**	-59.8%	4.6±0.2	0.4±0.2**	-91.3%
Extract	5	75	339.2±14.4	192.2±12.3**	-43.3%	4.4±0.2	1.6±0.2**	-63.6%
Insulin	5	2 IU	379.2±24.2	277.4±35.2*	-26.9%	4.8±0.2	2.6±0.2**	-45.8

N: Number of rats used = 5 per dose; values expressed as mean ± SEM; * P<0.05; ** P<0.001, % reduction of blood and urine glucose was with respect to initial values.

Conclusion

These results indicated that the root bark of *C. pentandra* was effective in decreasing the blood glucose level in normal and induced diabetic animals. However the nature of the molecule(s) responsible for such an effect requires further investigation. The possible mode of action of the plant extract might be by potentiation of the insulin effect by increasing the pancreatic secretion of insulin from β -cells of islet of Langerhans or its release from the bound form

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