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ANTIDIABETIC EFFECT OF *CEIBA PENTANDRA* EXTRACT ON STREPTOZO-TOCIN-INDUCED NON-INSULIN-DEPENDENT DIABETIC (NIDDM) RATS

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Abstract

The aim of this work was to investigate the effect of daily oral administration of root bark methylene chloride/methanol extract of *Ceiba pentandra* (Linn) in streptozotocin-induced type-2 diabetic rats, and the effect of this treatment on the physiological and metabolic parameters that are related in diabetic animals. The diabetic rats were separated into four groups and each given the following samples by gavage, daily for 28 days: vehicle (diabetic control), *Ceiba pentandra* extract at the dose of 40 mg/kg, *Ceiba pentandra* extract at the dose of 75 mg/kg and glibenclamide (5 mg/kg). All the parameters were also determined in healthy (non diabetic) rats for comparison. The methylene chloride/methanol extract of *Ceiba pentandra* treatment significantly reduced the intake of both food and water as well as the levels of blood glucose, serum cholesterol, triglyceride, creatinine and urea, in comparison with diabetic controls. The treatment also improves impaired glucose tolerance but no effect was observed in the level of hepatic glycogen. The effect of *Ceiba pentandra* (40 mg/kg) was more prominent when compared to glibenclamide in lowering blood glucose, with the added benefit of considerably reducing serum cholesterol and triglyceride concentrations. The results of this experimental animal study indicated that *Ceiba pentandra* possesses antidiabetic activity; and thus is capable of ameliorating hyperglycaemia in streptozotocin-induced type-2 diabetic rats and is a potential source for isolation of new orally active agent(s) for anti-diabetic therapy.

Key words: *Ceiba pentandra*, Plant extract, Type 2 diabetes, Streptozotocin.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration caused by insulin deficiency, often combined with insulin resistance. Diabetes mellitus is a major cause of disability and hospitalization and it results in significant financial burden (Vats et al., 2002). By the year 2010, the total number of people worldwide with diabetes mellitus is projected to reach 239 million. Region with greatest interest are Asia and Africa, where diabetes rates could rise to 2-3 folds than the present rates (ADA, 1997).

Many traditional plant treatments for diabetes mellitus are used throughout the world (Marles and Farnswort, 1995). Management of diabetes without any side effect is still a challenge to the medical system.

This has led to an increasing demand for natural products with antidiabetic activity and fewer side effects (Kameswara et al*.,* 1999). Many herbs and plant products have been shown to have hypoglycaemic action. *Ceiba pentandra* (L) Gaertner, known as silk Cotton tree and locally as " dum " belongs to the Bombacaceae family (Ueda et al., 2002). The plant is widely reputed in the African traditional medicine. 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 diuretic and to expel evil spirits (Ueda et al., 2002, Noumi et al., 1999, Ngounou et al., 2000, Noumi et al., 2000, Noumi et al., 2001).

The hypoglycaemic activity of stem bark aqueous extract of *Ceiba pentandra* at high doses (>800 mg/kg/day) on streptozotocin (STZ)-induced type 1 diabetes has already been reported by Olusola et al. (2003). To date, research on antidiabetic plants has been especially focused on streptozotocin-induced type-1 diabetic rats. It is assumed that herbal medicine can only be effective as an alternative to oral hypoglycaemic agents, in type-2 diabetes where pancreatic islets are not totally destroyed. This is why streptozotocin-induced type-2 diabetic rats were used in our study as well as glibenclamide, a known hypoglycaemic agent, for comparison. Since in a preliminary study, the aqueous extract was 15% less active than methylene chloride/methanol extract, the present study was undertaken to evaluate the antidiabetic activity of the root bark methylene chloride/ methanolic extract of *Ceiba pentandra* in STZ-induced type-2 diabetes in rats.

Materials and method

Preparation of the plant extract

With the help of traditional healers, roots of *Ceiba 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, chopped into small pieces, air dried at ambient temperature and ground into powder form. A kilogram of the dried powder was macerated in a 1:1 (volume/volume) mixture of methylene chloride/methanol for 2 days (with occasional stirring) at room temperature. The solution obtained after filtration were concentrated using a rotavapor at a temperature of 50°C to obtain 106 g (10.6% yield) of solid methylene chloride/methanol extract of *Ceiba pentandra.*

NIDDM rat model and experimental protocol

Healthy albino Wistar rats were kept for breeding. To induce NIDDM, STZ (90 µg/g) freshly dissolved in 0.9% saline solution was administered intraperitoneally to a group of 2-day-old pups. Another group of pups received only saline. The pups were weaned after 30 days, and 3 months after the injection of STZ, the animals were checked for fasting glucose levels. The animals showing fasting glucose levels > 140 mg/dL and > 200 mg/dL 2 h after 5 mg/kg oral challenge of glucose were consider as diabetic. Pups that received saline were considered as control animals. The experimental animals were then divided into the following groups, (1) Control, (2) NIDDM control, (3) NIDDM treated with extract (40 mg/kg body weigh, p.o.), (4) NIDDM treated with extract (75 mg/kg body weigh, p.o.), (5) NIDDM treated with glibenclamide (5 mg/kg body weigh, p.o.). Treatment was given once daily for 4 weeks. The control group received an equal volume of the vehicle. Each group was made up of 5 animals. During the study, standard food and water were provided *ad libitum*. Changes in body weigh, food and water intakes and blood glucose levels were recorded every week. At the end of the treatment, rats were killed by decapitation after anaesthesia with diethyl ether. The free-running fasted blood was collected for the determination of serum levels of glucose, cholesterol, triglyceride, urea and creatinine. Liver was excised and sliced; fragments were rapidly frozen for later determination of glycogen. Hepatic glycogen was estimated following the method described by Murat et al., 1974. Serum triglyceride, cholesterol, urea, creatinine and glucose were measured spectrophotometrically, using enzymatic colorimetric assay kits (Human, Germany). The study was approved by institutional animal ethical committee.

Oral glucose tolerance test (OGTT)

At the end of the 4 weeks study, rats were subjected to an oral glucose tolerance test (OGTT). Glucose (5 g/kg) was administered to 14 hour fasted rats. Blood samples were collected from tail tip at 0, 30, 60 and 120 min after oral glucose administration. Serum was analysed for glucose using enzymatic colorimetric assay kits (Human, Germany).

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 Kruskal-Wallis test. P values of 0.05 or less were considered to be significant.

Results

Effect on general parameters

STZ induced NIDDM rats showed significant reductions in weight gain as compared to the control animals ($P < 0.01$). Treatment with *Ceiba pentandra* at 40 mg/kg caused significant increase in body weight gain of these animals (Figure 1A). Food and water intakes were significantly increased in diabetic animals (Figures 1B and 1C) but were significantly reversed by treatment (40 mg/kg) with *Ceiba pentandra.*

Effect on glucose levels and oral glucose tolerance test (OGTT)

The serum glucose level was higher in the untreated diabetic group than in the normal control. In treated diabetic rats, the fasting glucose levels were similar to that of the control group after 3 weeks of treatment (Figure 2). Figure 3 shows the changes in the levels of blood glucose in normal, diabetic control and experimental groups after oral administration of glucose (5 g/kg). The diabetic rats showed significant increase in the blood glucose at 30, 60 and 120 min. *Ceiba pentandra* treated animals tended to bring the glycaemic values to near normal. *Ceiba pentandra* at 40 mg/kg was more effective than glibenclamide (5 mg/kg).

Effect on serum lipids and kidney functions

In table 1, STZ-induced NIDDM rats showed significant cholesterolaemia as compared with control. This hypercholesterolaemia was associated with hypertriglyceridaemia. Treatment with *Ceiba pentandra* (40 mg/kg) significantly decreased cholesterol levels compared to diabetic ($P < 0.01$) rats and normal control ($P < 0.05$) rats. At the same dose, hypertriglyceridaemia was also significantly reduced ($P < 0.01$) compared to diabetic and not normal rats. NIDDM control rats showed a significant increase in urea ($P < 0.01$) and creatinine ($P < 0.01$) as compared with control animals. Treatment with *Ceiba pentandra* decreased these values.

Discussion

When rats are injected with streptozotocin during the neonatal period, they provide an animal model of non-insulin-dependent diabetes mellitus. In this model, mild hyperglycaemia appears between 1 and 2 month of life together with a partial deficiency in insulin (Berbera et al., 1997). In the present study, NIDDM control rats showed significantly higher levels of fasting and fed glucose levels, lower body weight, increase in food and water intakes as compared to normal control rats. This was consistent with early reports (Murali et al., 2002; Urmila and Goyal, 2003). Treatment with *Ceiba pentandra* significantly reduced body weight loss, food and water intakes. Those observations could be due to an improvement of elevated blood glucose and the effect of the plant extract on lipolysis.

Table 1: Metabolic parameters of type 2 diabetic rats after 4 weeks of treatment.

Groups	Dose	Serum cholesterol (mg/dL)	Serum triglyceride (mg/dL)	Serum urea (mg/dL)	Serum creatinine (mg/dL)	Hepatic glycogen (mg/100g of liver)
Normal control		76.2 ±6.3	109.1 ± 2.3	52.2 ± 2.3	26.9 ± 1.3	59.2 ± 2.2
Diabetic control		117.1 ± 6.7**	177.5 ± 8.7**	90.1 ± 4.8**	48.4 ± 2.7**	68.0 ± 2.6*
Extract	40 mg/kg	52.7 ± 1.9*$^{\$\$}$	99.0 ± 4.7$^{\$\$}$	59.2 ± 2.9$^{\$\$}$	30.1 ± 2.2$^{\$\$}$	61.8 ± 2.5
Extract	75 mg/kg	67.0 ± 6.1$^{\$\$}$	102.7 ± 5.6$^{\$\$}$	63.1 ± 7.3$^{\$}$	34.6 ± 1.4**$^{\$\$}$	65.6 ± 0.8*
Glibenclamide	5 mg/kg	80.7 ± 9.2$^{\$}$	113.0 ± 3.7$^{\$\$}$	65.8 ± 5.1*$^{\$}$	35.1 ± 3.0*$^{\$}$	63.4 ± 4.6

Values are given as mean ± SEM for five rats in each group.
*, $P < 0.05$; **, $P < 0.01$, with respect to normal control group.
$^{\$}$, $P < 0.05$; $^{\$\$}$, $P < 0.01$, with respect to diabetic control group.

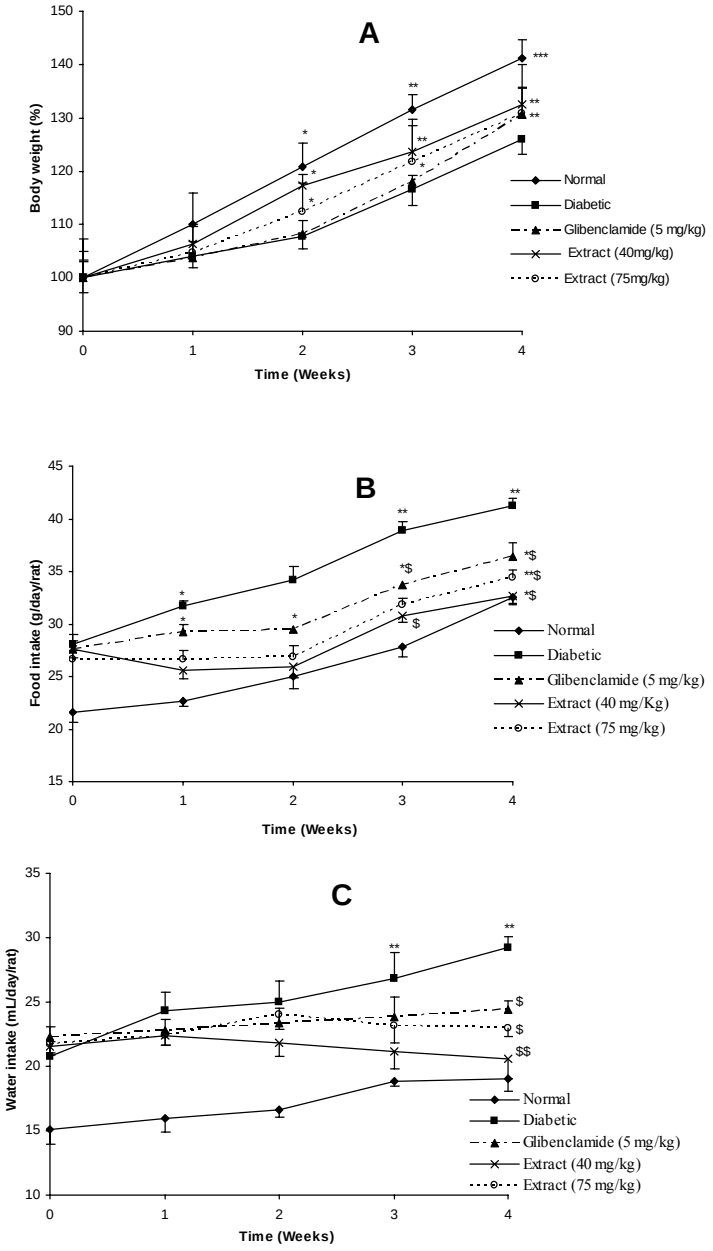

Figure 1: Effect of *C. pentandra* on general parameters. A: Body weight, B: Food intake, C: Water intake. Values are given as mean ± SEM for five rats in each group.*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ with respect to initial value. $^{\$}$, $P < 0.05$; $^{\$\$}$, $P< 0.01$ with respect to diabetic control group. 0: represent the beginning of the treatment.

Treatment with *Ceiba pentandra* significantly reduced blood glucose levels of treated rats ($P < 0.001$) compared to diabetic control, to values similar to those of healthy control rats. *Ceiba pentandra* also prevented the rise of blood glucose after oral administration of glucose. This could be due to an improvement of insulin response to glucose levels because the main cause of hyperglycaemia in neonatal STZ-induced diabetic rats appeared to be the lack of response of beta-cells to a glucose stimulus (Berbera et al., 1997). The hepatic glycogen was found to be increased in both treated and untreated diabetic rats. These results were in line with those of Berbera et al 1997; Murali et al*.,* 2002 and Urmila and Goyal, 2003, which showed that hepatic glycogen was increased in neonatal STZ-induced diabetic rats. In the present study, hepatic glycogen of treated rats was not significantly different from diabetic control. These observations suggested that the drop in glycaemia in diabetic rats did not involve a reduction in glycogenolysis and/or increase in glycogenesis. Similar results were obtained with rats treated with *Bohinia fortificata* (Pepato et al., 2002). The effect of *Ceiba pentandra* could be related to a stimulation of remaining β cells or regeneration of β cells. It had been reported that β cells regeneration occurred through both increasing the replication of pre-existing β cells and neogenesis from the precursor cells located in or by the pancreatic duct (Lei et al., 2004). Phytochemical study of *Ceiba pentandra* revealed the presence of epicatechin (Noreen et al., 1998) and flavonoids (Noreen et al., 1998; Ngounou et al., 2000). Epicatechin, isolated from other plants had been found to stimulate β cells regeneration, increased insulin secretion or possessed an insulin-like effect (Marles and Farnsworth, 1995; Kameswara et al., 2001). Some flavonoids were reported to possess hypoglycaemic activity (Lamba et al., 2000; Cetto et al., 2000).

Insulin deficiency is associated with hypercholesterolaemia and hypertriglyceridaemia. STZ-induced diabetes showed increased plasma levels of cholesterol, triglyceride, free fatty acid and phospholipids (Murali et al., 2002). Insulin deficiency or insulin resistance could be responsible for dyslipidaemia because insulin increases fatty acid as well as triglyceride synthesis in adipose tissue and liver. It inhibits lipolysis, partly via dephosphorylation (and hence inactivation) of lipases (Rang et al., 2003). Insulin deficiency leads to fall in lipoprotein lipase activity. In our study, NIDDM rats showed hypercholesterolaemia and hypertriglyceridaemia and the treatment with *Ceiba pentandra* significantly decreased both cholesterol and triglyceride levels. These findings supported the hypothesis that *Ceiba pentandra* improved insulin synthesis and/or sensitivity.

In the present investigation, diabetic rats showed a significant elevation in serum urea and creatinine levels as compared with controls, indicating impaired renal function of diabetic animals. These results were consistent with those reported by Urmila and Goyal, (2003). Treatment with *Ceiba pentandra* produced considerable reduction in the intensity of these changes. Improvement in the kidney function associated with diabetes with *Ceiba pentandra* treatment could be attributed to its antidiabetic action resulting in alleviation of the altered metabolic status of diabetic rats.

The effects of the extract were not dose-dependent. This could be due to antagonism. The extract contained many molecules, some of which could be antagonistic. Therefore, at low doses, the concentration of this antagonistic molecule(s) were low, thus, offering no hindrance to the hypoglycaemic causative substance(s). A similar observation was reported by Kameswara et al. (2002) when they worked on the effect of bark extract of *Pterocarpus santalinus* on blood glucose in streptozotocin-induced diabetic rats.

In conclusion, our data suggested that *Ceiba pentandra* methylene chloride/methanol extract at the dose of 40 mg/kg had a potential antidiabetic activity. In addition to decreasing serum glucose and lipid levels, it could also improve impaired glucose tolerance and preserve kidney dysfunction in NIDDM rats.

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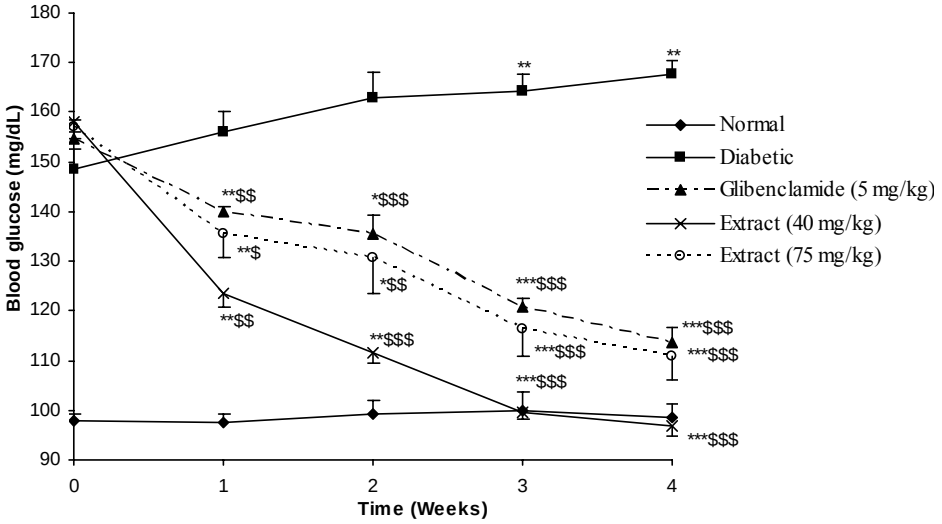

Figure 2: Effect of *C. pentandra* extract on blood glucose levels in STZ-induced type 2 diabetic rats. Values are given as mean ± SEM for five rats in each group. *, P < 0.05; **, P < 0.01; ***, P < 0.001, with respect to initial value. $, P < 0.05; $$, P< 0.01; $$$, P< 0.001, with respect to diabetic control group. 0: represent the beginning of the treatment.

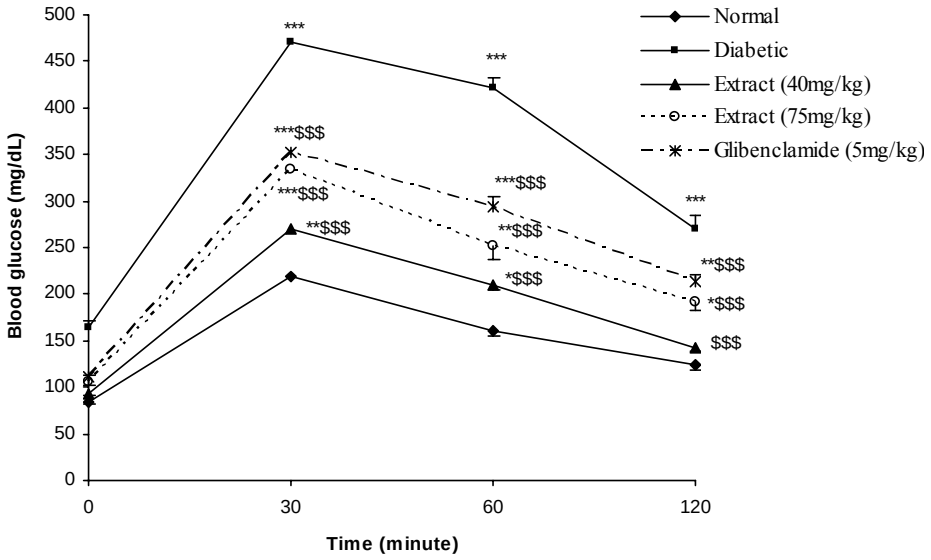

Figure 3: Oral glucose tolerance test of experimental rats after 4 weeks of treatment.
Values are given as mean ± SEM for five rats in each group. *, P < 0.05; **, P < 0.01; ***, P < 0.001, with respect to normal control group. $$$, P< 0.001, with respect to diabetic control group.

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