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Hypoglycaemic activity of aqueous extract of *Pentaclethra macrophylla* Benth. (Fabaceae) stem bark in streptozotocin-induced diabetic rats

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Abstract
The aqueous stem bark extract of *Pentaclethra macrophylla* Benth. (Fabaceae) was evaluated for hypoglycaemic activity in streptozotocin-induced diabetic rats. Diabetes was induced by streptozotocin, 50 mg/kg (i.p.) in fasted rats and the blood glucose levels were monitored at 0, 1, 2, 4, 8, 12 h and on the 14th day post treatment with the extract (250 and 500 mg/kg). Results showed that the extract (500 mg/kg) significantly reduced blood glucose in diabetic animals from the 4th h ($p<0.01$), 26.5% and was sustained throughout the entire period of the experiment. The maximum effect was seen at the 8th h ($p<0.001$), 38.6% and this was comparable to that of glibenclamide ($p<0.001$), 40.0%. The extract showed significant reduction in massive loss of body weights at 400 mg/kg ($p<0.05$) and 800 mg/kg ($p<0.05$) in diabetic rats. Phytochemical screening showed the presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides. Acute toxicity studies did not show any mortality at a dose of 10 g/kg. These results confirm the hypoglycaemic properties of the aqueous stem bark of *P. macrophylla* thus supporting the usage of the plant in traditional medicine in Eastern Nigeria in the treatment of diabetes mellitus

Keywords: Hypoglycemic, streptozotocin, *Pentaclethra macrophylla*

INTRODUCTION

Diabetes mellitus is a heterogeneous primary disorder of carbohydrate metabolism with multiple etiological factors generally involving absolute or relative insulin deficiency, or insulin resistance, or both (Yadav *et al*., 2008). Based on several epidemiological studies and clinical trials (Liv *et al*., 1993; Kleiv *et al*., 1994; Abaira *et al*., 1995), it has been strongly suggested that hyperglycemia is the principal cause of the complications. Although there have been enormous improvements in medication, strategy to cure diabetes mellitus completely has not been established so far. Therefore, numerous studies have been carried out to evaluate natural products, including plant materials, as alternative treatments for diabetes to be used in addition to conventional treatments.

There is an increased demand to use natural products with anti-diabetic activity due to the side effects associated with the use of insulin and oral hypoglycaemic agents (Holman and Turner, 1991). The available literature shows that there are more than 400 plant species showing hypoglycaemic activity (Mukherjee, 1981). Though some of these plants have great reputation in the indigenous system of medicine for their antidiabetic

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activities, many remain to be scientifically established.

Pentaclethra macrophylla Benth. is a tree of about 21m in height and about 60cm girth with characteristic low branching habit and an open crown, which allows substantial light under its canopy. Stem is straight stemmed and less buttressed which can pass for a good timber. Bark is grayish to dark-reddish-brown and patchy with irregular pieces flaking off. The leaves possess stout angular petiole. The compound leaves are usually about 20-45cm long and covered with rusty hairs. Some parts of the plant have medicinal values; the seeds when crushed and eaten with red ants can induce labour (Abbiw, 1990; Isawumi 1993) and can cause infertility, the anthelmintic bark is pounded and applied to leprosy sores, the stem bark decoction can also be used in the treatment of diarrhea, itching, lactogenecity and wound treatment (NFT, 1995; Zapfack *et al.,* 1999). It is also being used in the Eastern part of Nigeria to treat diabetes by boiling the stem bark and subsequent drinking, hence the need to investigate its hypoglycemic effects.

EXPERIMENTAL

Plant material and extraction. The stem bark of *Pentaclethra macrophylla* Benth. was collected from Umuoshi village in Alor Town, Anambra state in the month of March 2011. The plant was identified by Dr Ayinde of the Department of Pharmacognosy, Faculty of pharmacy, University of Benin, Benin City. The stem bark was cut into several pieces and dried under the sun to a constant weight. The dried bark was then powdered using a mechanical grinder and stored in an airtight container. 400g of the powdered bark was boiled with 2 L of water for 30min. After cooling, the material was then filtered and the aqueous extract was then concentrated in vacuum using a rotary evaporator (Buchi and Swiss) at a temperature of 68^{O}C in an oven (Baird and Tatlock, London). The dried extract (yield=31%) was then stored in clean glass containers in the refrigerator at 40 ^{0}C until required.

Phytochemical screening. The aqueous extract of *Pentaclethra macrophylla* Benth was subjected to various tests in order to determine the classes of the various chemical constituents present, by using standard methods (Evans, 1989).

Animals. All experiments were performed using male Wistar rats (180-220 g) and mice (17-23 g) of either sex. All the animals were obtained from the Laboratory Animal Centre of the College of Medicine, Lagos Teaching Hospital (LUTH), Idiaraba, Lagos state. The animals were maintained under standard environmental conditions and had free access to standard rodent cubes (Lakodun Feeds, Ibadan) and water *ad libitum.* All animals were handled according to protocols approved by the Faculty of Pharmacy Committee on animal ethics, University of Benin.

Acute toxicity studies. Acute toxicity studies were carried out on the extract as described by the Organization of Economic Co-Operation and Development (OECD) as per 423 guidelines (OECD Guidelines for the Testing of Chemicals, 2010). Swiss albino mice (19-23g) of either sex were divided into four groups of five mice each. Each group was administered via an orogastric syringe, the aqueous extract of *Pentaclethra macrophylla* (Benth) at doses of 1, 5 and 10 g/kg respectively. The control group received 3 ml/kg of distilled water. Mortality and symptoms of toxicity within 24 h period were observed. Animals were observed for further signs of delayed toxicity two weeks thereafter (Miller and Tainter, 1944).

Determination of hypoglycemic activity

Induction of diabetes. Diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma Chemical Co. USA), 50 mg/kg in citrate buffer (0.1M, pH 4.5) to

the overnight fasted rats. Blood glucose was measured after 48 h and animals having fasting blood glucose level above 200 mg/dl were considered diabetic and were used for the experiment (Igbe *et al*., 2009; Rastogi *et al*., 1997).

Streptozotocin-induced diabetic rats. Diabetic male albino Wistar rats (200-240g) fasted overnight was divided into four groups (A to D) of five rats each. Fasting blood glucose levels were determined at 0 h for each group before commencement of the experiment. The extract (250, 500mg/kg) was administered orally via an orogastric tube to groups A and B respectively. The reference drug, glibenclamide (5 mg/kg) suspended in 5% gum Acacia was administered to animals in group C. Animals in group D served as the negative control were administered 3 ml/kg of 5% gum Acacia. The blood glucose level was monitored at time intervals of 0, 1, 2, 4, 8, 12 h and on the 14^{th} day after administration of single daily doses of the extract.

Determination of blood level. Blood samples for glucose determination were obtained from the tail tip of the rats by using a sterile blade to make an incision on the tail tip of the fasted animals. Blood glucose level was determined using a glucometer (ACCU-CHEK ® active Roche, USA). The percentage glycaemic change was then calculated using the formula:

$$\% \text{ Glycaemic change} = \frac{\text{Glucose concn. at t} - \text{Fasting blood glucose} \times 100}{\text{Fasting blood glucose}}$$

Where t = 1, 2, 4, 8, 12 h or 14^{th} day

(Gidado *et al*., 2005)

Determination of change in body weights. Animals were weighed on the first day and then on 14^{th} day of the experiment. Changes in body weights in each animal was then determined.

Statistical analysis. Data were expressed as the mean $\pm$ SEM. The data were analyzed using one way analysis of variance (ANOVA) followed by Turkey's test. Differences between two means were detected using the student's t-test. Data were considered different at significance level of $p>0.05$.

RESULTS

Phytochemical screening. Phytochemical screening of aqueous stem bark extract of *P. macrophylla* revealed the presence of alkaloids, saponins, tannins, steroidal components flavonoids, cardiac glycosides and anthracene derivatives.

Acute toxicity studies. The acute toxicity studies showed that all the doses (1, 5, 10 g/kg) of *P. macrophylla* extract used for the study were found to be non-toxic. There was no mortality even at a maximum dose of 10 g/kg of the extract.

Hypoglycaemic activity. The hypoglycaemic activity of the extract at different time points is shown in Figure 1. The aqueous extract at 500 mg/kg showed significant percentage decrease in blood glucose levels from the 4^{th} h ($p<0.01$), 26.5% up to the 12^{th} h ($p<0.05$), 45.7% compared to the control and the effect was comparable to that of glibenclamide which also produced a significant reduction from the 4^{th} h ($p<0.01$), 30.2% up to the 12^{th} h ($p<0.01$), 52.9%. However, both doses of the extract (250 and 500 mg/kg) significantly reduce blood glucose on the 14^{th} day by 47.2 and 38.7 % respectively.

Effect of extract on body weights. In the streptozotocin-induced diabetic rats there was a significant reduction in the loss in body weights (table 1) on daily administration of *P. macrophylla* extract at doses of 250 mg/kg ($p<0.05$), 500 mg/kg ($p<0.05$) and with glibenclamide ($p<0.05$) at the end of a 14day period when compared to the control.

Table1. Effect of the aqueous seed extract on body weight in streptozotocin-induced diabetic rats

Groups	Dose mg/kg/day	Day 0	Day 14	Change in body weight (g)
Control	-	210.1 ± 4.55	181.0 ± 4.14	-28.5 ± 5.31
Glibenclamide	5	206.0 ± 9.81	195.0 ± 10.10	-11.0 ± 2.30*
P. macrophylla	250	198.3 ± 11.37	187.5 ± 2.66	-10.4 ± 8.21*
	500	196.5 ± 6.33	181.2 ± 9.51	-15.0 ± 4.57*

There was a significant reduction in the loss in body weights with aqueous extract at doses of 250 mg/kg/day ($p<0.05$), 500 mg/kg/day ($p<0.05$) and with glibenclamide ($p<0.05$) at the end of a 14 day period when compared to the control. (*$P<0.05$ as compared to the control, $n = 5$ per group)

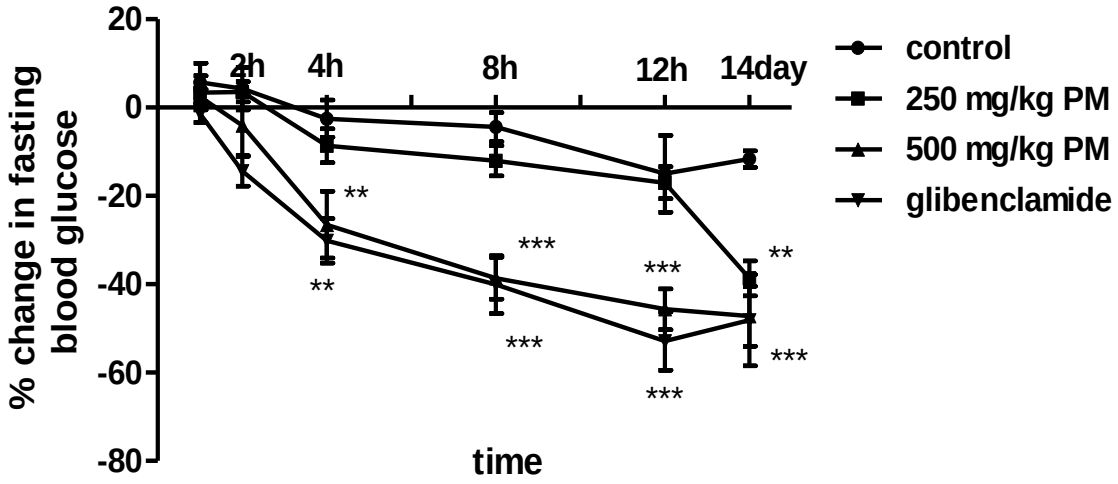

Fig 1. Percentage change in fasting blood glucose after oral administration of single daily doses of extract in streptozotocin-induced diabetic rats (**P<0.01, ***P<0.001 as compared to the control. $n = 5$ per group) PM = *P. macrophylla*

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder affecting a major population worldwide. A sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications (Kim *et al*., 2006). The conventional therapies for diabetes have many disadvantages such as unwanted side effects and high incidence of secondary failure. On the other hand, herbal extracts are expected to have similar efficacy without side effects like that of the conventional drugs. The present investigation reports the hypoglycaemic effect of the aqueous extract of the stem bark of *Pentaclethra macrophylla* Benth. in streptozotocin-induced diabetic rats.

The acute toxicity tests on the plant extract showed that there was no mortality at a dose of 10 g/kg after 24 h and the absence of any toxicity symptoms suggests that means that the extract when administered orally is considered practically non-toxic (Loomis, 1978).

Streptozotocin-induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents (Szkudelski, 2001). However, in this study, at the dose of streptozotocin (STZ) used, the model involved Type 2 diabetes mellitus because the animals develop blood sugar levels 3-4 times higher than normal, survive without insulin supplementation and do not develop ketosis (Igbe *et al*., 2009; Rastogi *et al*., 1997). The present study

showed that administration of the fasted diabetic rats with the aqueous extract at 500 mg/kg produced a significant hypoglycemic effect which started at the 4th h and was sustained all throughout the period of the experiment. The maximum effect of the extract was seen in the 8th h. Both doses of the extract (250 and 500 mg/kg) reduced blood glucose on the 14th day indicating that the extract may be active on acute as well as prolonged (sub-acute) administration and the effect is dose related. The hypoglycemic effect of the extract at different time points was similar to that of the standard drug, glibenclamide. Though the reduction in blood glucose by glibenclamide was higher than the plant extract at different time points of the experiment, the hypoglycemic effect of the extract was comparable to that of glibenclamide. Glibenclamide is a sulphonylurea which acts mainly by stimulating the β-cells of the pancreas to release insulin which in turn lowers blood glucose (Moller, 2001). The results in the present study suggests that since the pattern of blood glucose reduction by the extract was similar to that of glibenclamide, hence there is a probability that extract maybe acting via the same mechanism as glibenclamide i.e. stimulating the residual β-cells in pancreas of the diabetic rats to secrete insulin. The significant reduction in the loss in body weights of the diabetic animals administered the plant extract (250 and 500mg/kg) compared to the control, showed that the plant extract had effect on degradation of fat depot which is a common problem in diabetes mellitus. This reduction in the degradation of fat depot by the plant extract may be due to the glycaemic control in the diabetic animals as this will prevent the breakdown of adipose tissue (lipolysis) required as an alternative source of energy in the absence of glucose.

A preliminary phytochemical analysis of the extract revealed that it contains alkaloids, saponins, tannins, steroidal components, flavonoids, cardiac glycosides and anthracene derivatives. The hypoglycemic activities of the extract may be attributed to one or more of the identified or as-yet-unidentified compounds (Carini *et al*., 2001; Czinner *et al*., 2000).

In conclusion, the aqueous extract of the stem bark of *P. macrophylla* Benth exhibited strong hypoglycemic action in diabetic animals. This has clinical implications in that the relatively nontoxic extract, if used as a hypoglycemic agent, may prevent the complications which are very prevalent in diabetic patients. Our results suggest that *P. macrophylla* has the potential to be a suitable candidate for further investigations as an anti-diabetic agent in humans.

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