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ANTIDIABETIC EFFECT OF *Sarcocephalus latifolus AQUEOUS* ROOT EXTRACT IN EXPERIMENTAL RAT MODEL

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ABTRACT

Investigations were carried out to evaluate the blood sugar lowering activity of the aqueous extract of Sarcocephalus latifolus roots (SLA) in normal and streptozotocin-diabetic rats. The extract (250 mg/kg body weight; p.o) caused 76 % reduction in blood glucose within 6h in fasted diabetic rats. However, the extract at the same dose showed no effect in normorglycaemic rats. Acute toxicity studies of the extract in mice gave an oral LD_{50} value greater than 5000 mg/kg. Phytochemical tests revealed that SLA tested positive for alkaloids, tannins, saponins, terpenoids, reducing sugars, carbohydrates, sterols and glycosides. The results show that the aqueous root extract of S latifolus has blood glucose lowering effect, which is consistent with the use of the root in folklore diabetes management.

Keywords: *Sarcocephalus latifolus* roots, Streptozotocin, Antidiabetic

INTRODUCTION

Diabetes mellitus (DM), a very common and serious endocrine disorder which interferes with the metabolism of carbohydrates, lipids and proteins is caused by the complete or relative insufficiency of insulin secretion and / or resistance to insulin action (Balkau *et al*., 2000).

The key clinical manifestation of this disorder is chronic hyperglycemia (Scoppola *et al*., 2001) which causes glycation of body protein and so leads to secondary complications affecting the eyes, kidneys, nerves and arteries Kameswara *et al*., 1999) as well as micro/macro vascular complications and death (Nagappa *et al*., 2003). Though some non-insulin dependent diabetes mellitus (NIDDM) patients can be managed by diet alone, most require oral hypoglycemic agents and/or insulin therapy. Oral hypoglycemic agents and/or insulin therapy afford relatively effective glycaemic control, but they are not very ideal because of their numerous side effects (Rang and Dale, 1991).

Therefore, there is a great need for the development of newer alternative agents that meet the requirement of an ideal antidiabetic compound with little or no adverse side effects. In recent years research interests have shifted to the search for alternative and natural hypoglycemic agents, especially from plant sources (Krishna *et al*., 2004; Pepato *et al*., 2003).

Sarcocephalus latifolus belongs to the Rubiaceae family and is commonly called the 'pin cushion' tree. It is a shrub native to tropical Africa and Asia. Parts of the plant are commonly prescribed traditionally as a remedy for diabetes mellitus. The plant is also used for the treatment of such other diseases as malaria (Kokwaro, 1976; Akabue and Mittal, 1982; Boye, 1990), gastrointestinal tract disorders (Madubunyi, 1995) and hypertension; the stems of this plant are also recommended as a chewing stick (Asubiojo *et al*., 1982). The antihyperglycemic potential of the leaves has also been demonstrated (Gidado *et al*., 2005). The present study was undertaken to evaluate the hypoglycemic effect of aqueous extract of the roots of *S latifolus* in normal and streptozotocin induced diabetic rats. In Abakaliki community of Ebonyi State, Nigeria, a decoction of the roots of *S latifolus* is popularly used for the treatment of diabetes. Usually 1 glass of the decoction of the root of this plant is taken twice daily for the treatment of diabetes.

MATERIALS AND METHODS

Collection of Plant Materials: Fresh roots of *Sarcocephalus latifolia* were collected in December 2005 from Oba, Enugu State, Nigeria and authenticated by Mr. A. Ozioko of the Bioresources Development and Conservation Programme Centre (BDCP), Nsukka, Enugu State, Nigeria.

Plant Extract Preparation: The roots were cleaned, cut into smaller pieces dried under the shade at room temperature and ground into coarse powder using a mechanical grinder. About 500g of the powder was boiled in 2L of distilled water for 45min with two changes of solvent. The extract was then cooled, filtered and evaporated to at 40^{O}C, to obtain 57.62g of crude residue.

Animals: Ten to twelve weeks old outbred healthy Sprague-Dawley male albino rats (*Rattus novergicus*) were obtained from the animal facility of the Department of Biochemistry, University of Nigeria, Nsukka, Nigeria. Six to eight weeks old healthy outbred Swiss albino mice *Mus musculus* were obtained from the same source. All the animals were housed in an environmentally controlled room with a

12 h light/dark cycle and fed with standard Pfizer pellets and water *ad libitum*. The animals were acclimatized for 7 days.

Phytochemical Analysis: The extract was subjected to phytochemical analysis for constituent identification using standard procedures (Evans, 1989; Harborne, 1998).

Acute Toxicity Test: The acute toxicity (LD_{50}) of the extract was determined in mice by the method of Lorke (Lorke, 1983) using the oral route.

Experimental Design: The study was carried out on normoglycemic and streptozotocin-induced diabetic rats. The animals were fasted for 16h before each experiment, and blood samples were collected from the caudal vein of the rats.

Hypoglycemic Study: The initial fasting blood glucose concentration was estimated in fasted rats, after which, the extract (SLA) was administered orally via an intubator at a dose of 250 mg/kg. Blood glucose concentrations were then determined hourly for 6 h, using a One-Touch ultra glucometer (Lifescan Inc. U.S.A).

Induction of Diabetes/Antihyperglycemic Study: Diabetes was induced by intravenous injection of 60 mg/kg of streptozotocin (STZ) (Sigma Chemical Co, St Louis, Mo, USA), freshly dissolved in citrate buffer (0.01M, pH 4.5). Control rats received only the buffer. Seventy two hours after these injections the animals were fasted and their blood glucose levels tested; animals with glucose levels higher than 200 mg/dl were used for the study. The experimental animals were divided into three groups; group 1 received the extract (SLA), group II received a standard antidiabetic drug (glibenclamide) while group III was treated with distilled water After these treatments the blood glucose concentrations of the animals were determined at one hour interval for 6 h. Percentage glycemic change was then calculated as a time function using the formula: % induced glycemia = $G_X - G_O$ / $G_{O;}$ where G_O= the initial glucose level (Gupta *et al*., 1984), G_X= glucose level at 1, 2, 3, 4, 5 and 6 h respectively.

Statistical Analysis: Results were analyzed using one way analysis of variance (ANOVA) and expressed as Mean ± SEM. The Data were further subjected to Fischer LSD post hoc test and differences between means were regarded significant at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 showed that the yield of the crude aqueous extract (SLA) was 11.52 %. Phytochemical analysis of SLA extract was positive reactions for carbohydrates, glycosides, reducing sugar, alkaloids, saponins, tannins, terpenoids and sterols but showed negative for flavonoids and resins (Table 1).

This finding is in consonance with the findings of Hotellier *et al*. (1979), Abreu and Pereiera (2001) as well as Morah (1995), who reported independently that *S latifolus* contains alkaloids and terpenes respectively. The oral LD_{50} of the aqueous extract (SLA) estimated in mice, was > 5000 mg/kg which falls within acceptable safety limits.

Table 1: Phytochemical constituents of the extract

Constituent	SLA
Carbohydrates	+
Glycosides	+
Reducing sugars	+
Alkaloids	+
Flavonids	–
Saponins	+
Tannins	+
Terpenoids	+
Resins	–
Sterols	+

(Yield % = 11.52 of the starting material) Key; +=present; - = absent

The fasting blood glucose levels as well as the percentage change in blood glucose levels of normal rats at different time intervals after oral administration of 250 mg/kg *S. latifolus* root extracts, glibenclamide and water is shown in Table 2. The extract elicited a slight but insignificant increase ($p<0.05$) in blood glucose levels of the rats from the first hour of administration; while glibenclamide significantly lowered the blood glucose levels ($p<0.05$) by the first three hours. The reduction continued till the sixth hour although the reduction was not significant.

Changes in fasting blood glucose levels and percentage glycaemic change of diabetic rats after administration of SLA, glibenclamide or water are shown in Table 3. When compared with control rats treated with distilled water, the aqueous extract SLA (250 mg/kg) significantly lowered blood glucose levels of streptozotocin diabetic rats ($P < 0.05$). The aqueous extract of the roots of *S latifolus* caused a 29.26% decrease in blood glucose levels of the diabetic rats within 1h of administration. A maximal reduction of 76.2 % was attained at 6h, this compared with the maximal reduction for glibenclamide at the 5 h. Treatment of non diabetic rats with the same dosage, for the same duration, did not however, show the same hypoglycemic activity. The hypoglycemic activity was not similar with that of glibenclamide, an oral hypoglycemic agent that lower blood glucose in both normal and diabetic animals (Gupta *et al*., 1984; Subramanian *et al*., 1996; Prince *et al*., 1999 and Okyar *et al*., 2001).

In conclusion, the aqueous root extract of *S latifolus* was found to exhibit a hypoglycaemic activity in streptozotocin diabetic rats and the activity was consistent up to the sixth hour. However, studies are in progress in our laboratory to elucidate the possible mechanism of action of this extract as well as the hypoglycaemic principle(s) of the plant.

Table 2: **Effect of the extract on blood glucose levels of normoglycaemic rats (h)**

Treatment	Dose	Fasting Blood glucose at time after treatment						
		0 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**
S. latifolus **Extract SLA**	250 mg/kg	55.75 ± 4.30	66.50 ± 1.55 (+19.6)	62.75 ± 2.25 (+12.5)	65.50 ± 3.6 (+17.85)	68.50 ± 2.92 (+23.21)	65.25 ± 2.92 (+16.07)	60.5 ± 13.39 (+8.92)
Gliben clainde	2 mg/kg	68.50 ± 4.87	62 ± 5.02 (-10.14)	48.75 ± 3.22 (-28.98)	41.25 ± 1.75***** (-40.57)	44.25 ± 1.49***** (-36.23)	50.25 ± 1.75 (-27-53)	53.50 ± 2.95 (-18-84)
Control Distilled water	5 mg/kg	62.20 ± 4.18	63.0 ± 3.50 (+2.25)	58.80 ± 3.78 (-5.46)	57.60 ± 3.58 (-7.39)	65.00 ± 3.27 (+4.50)	63.40 ± 3.65 (+1.92)	61.2 ± 3.15 (-1.61)

*Values are means ± SD; n=5 (One way ANOVA, Fischer LSD Post Hoc test) ±SEM; n = 4 -5 *P<0.05 compared to control values in parenthesis represent % glycaemic change.*

Table 3: Effect of the extract on fasting blood glucose levels of streptozotocin – diabetic rats

Treatment	Dose	Fasting Blood glucose (mg/dl)at time(h) after treatment						
		0 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**
S. latifolus **extract**	250mg/kg	311.00 ± 4.26	220.00 ± 3.36* (-29.26)	175.00 ± 24.95* (-43.72)	120.8 ± 7.02* (-61.09)	98.60 ± 10.07* (-68.16)	92.20 ± 9.27* (-70.41)	73.60 ± 2.73* (-76.20)
Gliben clainde	2mg/kg	247.25 ±5.64	161.75 ± 8.07* (-34.41)	123.60 ± 8.68* (-49.72)	84.75 ± 6.15* (-65.99)	80.50 ± 6.65* (-70.04)	66.00 ± 10.80* (-73.27)	85.50 ± 2.39* (-65.18)]
Control Distilled water	5mg/kg	293.60 ± 4.73	300.20 ± 3.85 (+2.24)	289.60 ± 25.24 (-1.36)	285.60 ± 6.07 (-2.72)	295.20 ± 3.61 (+0.34)	280.40 ± 6.75 (-4.76)	276.60 ± 25.27 (+1.02)

*Values are means ± SEM; n = 4-5; *P<0.05 compared with control (One way ANOVA, Fischer LSD Post Hoc Test) Values in parenthesis represent % glycaemic change.*

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