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Antidiabetic and hypolipidaemic effects of *Hippocratea africana* (Hippocrateaceae) in streptozotocin-induced diabetic rats

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Abstract
Evaluation of antidiabetic and hypolipidaemic activity of ethanolic root extract of *Hippocratea africana* in rats was carried out. Treatment of streptozotocin diabetic rats with the extract caused a significant (P<O.01) reduction in fasting Blood Glucose levels (BGL) of the diabetic rats both in acute study and prolonged treatment (2 weeks). The activity of the extract was comparable to that of the reference drug, glibenclamide. *H. africana* treatment showed considerable lowering of serum total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol and an increase in HDL cholesterol in the treated diabetic group. These results suggest that the root extract of *H. africana* possesses antidiabetic and hypolipidaemic effect on streptozotocin -induced diabetic rats

Keywords: Antidiabetic; Hyperglycaemia; Hypolipidaemic; *Hippocratea africana*

Introduction

Diabetes is a disease of disordered metabolism of carbohydrate, protein and fat which is caused by the complete or relative insufficiency of insulin secretion and /or insulin action (Balkau *et al.*, 2000). About 150 million people are diabetic and by the year 2025 the number is likely to double. Among the major factors ,besides hyperglycemia, which complicate diabetic state and result in death is hyperlipidaemia (Nabel, 2003; Nagappa *et al.*, 2003). Developing countries are the most affected because of expensive and inadequate treatments (Djrolo *et al.*,1998),coupled with the side effect associated with these drugs, thus the search for a new drug with low cost, more potentials and without adverse effects become inevitable. A great number of medicinal plants have been used in the treatment of diabetes in different parts of the world, some of which are without scientific scrutiny although World Health Organisation (WHO) had encouraged and recommended the use of plants as an alternative therapy for diabetes (WHO, 1980). Evaluation of the antidiabetic potentials of these plants becomes necessary to provide scientific proof and justify their use in ethnomedicine.

Hippocratea africana (Willd.) Loes. (Hippocrateaceae) is a green forest perennial climber without hairs (glabrous) and

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reproducing from seeds (Dalziel,1956).The plants is widely distributed in tropical Africa. The root of the plant is used traditionally by the *Ibibios* of the Niger Delta region of Nigeria in the treatment of various ailments such as fever, malaria, body pains and diarrhea (Okokon *et al.*, 2006).The plant (root) has been reported by Okokon *et al.* (2006) to possess in vivo antiplasmodial activity with LD_{50} of 2.45 g/kg. Analgesic and antiinflammatory activities of the root extract have also been reported (Okokon et al.,2008). Reports of scientific studies on *Hippocratea africana* are few and there is no information regarding the hypoglycaemic and hypolipidaemic activities of *H. africana* roots extract in rats.

The present study, therefore, was designed to establish if the roots of *H. africana* have any antidiabetic and hypolipidaemic effects on STZ induced diabetic rats.

Experimental

Plant materials. Fresh roots of *H. africana* were collected in November, 2006 at Nyan forest in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo. Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy Herbarium . The fresh stem bark (2kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder 100g was macerated in 95% ethanol (300ml) for 72 hours. The liquid filtrate obtained was concentrated *in vacuo* at 40^{o}C. The yield was 2.08% w/w. The extract was stored in a refrigerator at 4^{o}C until used for experiment reported in this study.

Animals. Albino Wistar rats (105 – 165g) and albino Swiss mice (21-28g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

Chemicals and drugs. Streptozotocin was purchased from sigma chemical co, St. Louis, MO. USA, Glibenclamide (Daonil) was obtained from Aventis, Germany. All the other chemicals used were of analytical grade. Randox kits for lipids assay was obtained from Randox laboratories Ltd, Co, Antrim, UK.

Induction of diabetes. The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of Streptozotocin (55mg/kg body weight) in ice cold 0.9 % NaCl saline solution. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with normal saline alone. After a week time for the development of diabetes, rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose level range above 200mg/dl) were considered as diabetic and used for the drug treatment. The root extract in aqueous solution was administered orally through gavage at a concentration of 200mg/kg body weight/rats/day for 14 days.

Experimental design. The animals were divided into two sets, one for the evaluation of antidiabetic activity and a second for the evaluation of hypolipidaemic potentials. Each set was further divided into five groups of 6 animals each as detailed below;

Group I: Diabetic rats administered *Hippocratea africana* extract (200mg/kg/rat/day) in aqueous solution orally for 14 days.

Group II: Diabetic rats given *H. africana* extract(400mg/kg/rat/day) in aqueous solution orally for 14 days.

Group III: Diabetic rats administered *H. africana* extract (600mg/kg/rat/day) in aqueous solution.

Group IV: Diabetic rats given Glibenclamide(10mg/kg/rat/day)for 14 days in aqueous solution orally for 14 days.

Group V: Diabetic control rats.

The body weight gain and fasting blood glucose levels(BGL) of all the rats were recorded at regular intervals during the experimental period. For acute study, the BGL was monitored after 1, 3, 5, and 7 hours of administration of a single dose of the extract and at the end of 1,3,5,7, and 14 days for prolonged treatments. The BGL was monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on the dextrostix reagent pad. This was inserted into microprocessor digital blood glucometer and the readings were noted (WHO, 1980).

Hypolipidaemic activity. After 14 days of treatments (24 hours after the last dose),the animals were anaesthetized with ethyl vapour and the blood collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuged at 1000rpm for 15 minutes to obtain the sera. Serum cholesterol, triglyceride and high density lipoprotein (HDL) levels were measured by enzymatic colorimetric methods using Randox diagnostic kits. All samples were analysed with a wine light Unicam spectrophotometer. The concentrations of low density lipoprotein (LDL) and very low density lipoproteins (VLDL) were calculated from the formula of Friedwald (1972).

Statistical analysis. All the group data were statistically analysed with Students' t –test and two-way ANOVA, followed by Tukey Kramer post test. Values of $P<0.05$ were considered significant.

Results

There were observable changes in body weight of treated and untreated rats. Significant weight loss was observed in the untreated diabetic rats. Treatment of diabetic rats with ethanolic root extract of *H. africana* or Glibenclamide improved the weight gain compared to untreated diabetic rats (Table1). Dose dependent reduction in BGL was observed in STZ induced diabetic rats treated with ethanolic root extract of *H. africana*. After a single dose of the extract on the streptozotocin diabetic rats, there was a significant ($P<0.05$) reduction in BGL of the diabetic rats within the period of acute study which was seven hours compared to the control. The effect was more significant than that of the standard drug, Glibenclamide (Table 2). During prolonged study (14 days), the extract produced a sustained significant ($P<0.01$) reduction in BGL of the diabetic rats compared to control (Table 3).Serum total cholesterol, triglycerides, LDL, and VLDL were significantly ($P<0.05$) elevated in the untreated diabetic rats as compared to the treated animals (Table4). All lipid parameters tested were reduced after the treatment with ethanolic root extract of *H. africana* and glibenclamide for 2 weeks except HDL which was significantly ($P<0.01$) elevated in the treated animals compared to control (Table 4).

Table 1: Effect of treatment with ethanolic root extract of *H. africana* on body weight of streptozotocin-induced diabetic rats.

Drug	Dose(mg/kg)	Average body weight (g)	
		Day 0	Day 15
Control		118.0 ±3.00	120 ±10.00
Extract	200	116.0 ± 11.0*	125.5 ± 5.50*
	400	121.0 ± 2.00*	129.5 ± 19.00*
	600	120.2 ±3.00*	133.3. ± 7.35*
Glibenclamide	10	119.0 ± 9.36*	115.0 ±5.63*

Values are expressed as mean + S.E.M,*$P<0.05$ (n=6) (Students' t – test)

Table 2: Effect of *Hippocratea africana* on blood glucose levels of streptozotocin diabetic rats after a single dose

DRUG	DOSE (mg/kg)	BLOOD GLUCOSE LEVEL (mg/dl)(Mean ± SD)					
		Initial	1 h	3 h	5 h	7 h	24 h
Control	-	242.2± 18.0	246.7 ± 3.88	257.6 ± 8.51	265.3±4.31	265.3 ±4.30	267.± 4.14
Extract	600	248.5 ±8.51	185.0 ± 5.50*	120.0 ± 6.00*	51.5± 1.50*	43.5±8.50*	40.0 ±0.50
	400	253.5 ± 7.50	140.0 ± 6.00*	76.0 ± 6.50*	59.5±6.50*	52.0±4.50*	40.5± 1.50
	200	246.8 ± 2.81	86.0 ± 11.20*	70.5± 12.50*	73.0± 9.50*	59.5± 3.41*	44.5±6.50
Glibenclamide	10	242.6 ±6.50	123.3 ± 2.15*	96.7 ± 5.61*	82.8±3.31*	70.4±3.4*	76.4 ±2.38

*p<0.01 when compared to control. F-11.75,12.08, df=4, 16(p<0.01), two-way ANOVA), n= 6 per group.

Table 3: Effect of *Hippocratea africana* on blood glucose levels of streptozotocin diabetic rats during prolonged treatment.

DRUG	DOSE (mg/kg)	BLOOD GLUCOSE LEVEL (mg/dl)(Mean ± SD)				
		Initial l	3rd day	5th day	7th day	15th day
Control	-	242.2± 18.0	270.4± 2.34	272.0 ±4.50*	275.9±6.33	277.8 ±1.96
Extract	600	248.5 ±8.51	177.5±5.50*	166.0±13.50*	72.5±7.50*	50.50 ±8.50*
	400	253.5 ± 7.50	148.5 ±6.50*	136.5±6.50*	78.0±6.00*	51.5 ±0.50*
	200	246.8 ± 2.81	154.0±2.50*	127.5 ±2.05*	85.5±5.50*	57.0 ±1.50*
Glibenclamide	10	242.6 ±6.50	78.8±1.42*	71.3±3.87*	68.9± 3.73*	64.4±2.65*

*p<0.01 when compared to control, F=9.20, 11.16, d.f.=20,5 P(<0.01) (Two-way ANOVA) n=6 per group.

Table 4: Effect of ethanolic stem bark extract of *Hippocratea africana* on serum total cholesterol, triglycerides, HDL – cholesterol, LDL-cholesterol and VLDL-chol of streptozotocin diabetic rats.

Drug	Dose Mg/kg	Total Cholesterol	Average Serum lipids profile (mmol/L)			
			Triglyce-Rides	HDL Cholesterol	LDL Cholesterol	VLDL Chol
Control		4.15±0.14	2.83±0.19	0.85±0.07	2.74±0.51	0.56±0.07
Extract	200	3.83±0.38*	2.55±0.23*	1.66±0.13*	1.66±0.58*	0.51±0.21*
	400	.3.77 ±0.71*	2.49±0.37*	1.68±0.22*	1.60±0.51*	0.49±0.12*
	600	3.65 ±0.13*	2.20±0.81*	1.72±0.13*	1.49±0.28*	0.49±0.48*
Glibenclamide	10	2.61 ±0.30*	1.31±0.14*	1.61±0.13*	0.74±0.09*	0.26±0.18*

Values are expressed as mean ± SEM, *P<0.05 (n=6) (Student's t-test)

Discussion

Evaluation of antidiabetic activity using streptozotocin induced hyperglycaemia model has been described by Szkudelski (2001) to be very useful. Streptozotocin selectively destroys the pancreatic insulin secreting beta cells, leaving the less active cells and resulting in a diabetic state (Kamtchouing *et al.*, 1998; Szkudelski., 2001). Glibenclamide is often used as a standard drug to compare the efficacy of the hypoglycaemic agents in STZ- induced diabetes. In this study, acute and prolonged treatment of STZ-induced diabetic rats with various doses of the *H. africana* extract produced a significant (P<0.05) reduction in BGL of the rats in a manner comparable to that of the standard drug. The treatment also caused a significant increase in weight of the animals which is attributable to the extracts' hypoglycaemic activity .This hypoglycaemic effect of the extract is linked to the presence of flavonoids and terpenes in the extract (Okokon *et al.*, 2006).These compounds have been implicated in the antidiabetic activities of many plants (Schimizu *et al*., 1984; Reher *et al.*, 1991, Ivorra *et al.*, 1989). The hypoglycaemic action of this extract maybe by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin (Pari and Armanath, 2004). Serum lipids and free radicals generation are known to be elevated

during diabetes and have been implicated in the development of atherosclerosis (Mironava *et al.*, 2000; Kaplan, 1989). Serum lipids levels of untreated diabetic rats were found to be elevated, while that of the treated diabetic rats were reduced significantly after 2 weeks of treatment with the extract. Diabetes induced hyperlipidaemia is attributable to excess mobilization of fats from adipose tissue due to the under utilization of glucose (Krishanakumar *et al.*, 2000). Lowering of cholesterol levels in rats has been reported to be due to the antioxidant activity of phytochemicals like polyphenols-flavonoids and coumarins (Igarashi and Onhuruma, 1995; Amic *et al.*, 2003). Flavonoids have also been reported to possess free radical scavenging ability (Amic *et al.*, 2003). The regression of diabetic state due to *H. africana* root extract administration coupled with the antioxidant and free radical scavenging ability of its polyphenols phytochemicals may have increased the utilization of glucose, thereby depressing the mobilization of fats.

In conclusion, the present study shows that the ethanolic stem bark extract of *H. africana* has potential hypoglycaemic action in STZ –induced diabetic rats and the effect was found to be comparable to glibenclamide. Further study to isolate and identify the active principle as well as elucidation of its mode of action is necessary (Szkudelski, 2001).

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