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Antidiabetic and Hypolipidemic Effects of *Mammea africana* (Guttiferae) in Streptozotocin Induced Diabetic Rats

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Abstract: Evaluation of antidiabetic and hypolipidaemic activities of ethanolic stembark extract of *Mammea africana* in rats was carried out. Treatment of streptozotocin diabetic rats with the extract caused a significant ($p<0.01$) 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. *M. 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. This results suggest that the stembark extract of *M. africana* possesses antidiabetic and hypolipidaemic effect on streptozotocin induced diabetic rats.

Key words: Antidiabetic, hypolipidaemic activity, rats, streptozotocin, *Mammea africana*

INTRODUCTION

Diabetes mellitus 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). According to Zimmet (2000), there are about 150 million diabetic patients world wide and the number is likely to double by the year 2025. Besides hyperglycaemia, several other factors such as hyperlipidaemia contribute to the development of cardiovascular complications related to diabetes which are the major causes of death (Nabel, 2003; Nagappa *et al*., 2003). The disease constitutes a major health problem in the developing countries because of expensive and inadequate treatments (Djrolo *et al*., 1998), coupled with the side effects associated with these drugs, hence search for a new drug with low cost, more potentials and without adverse effects is being pursued in several laboratories around the world (Kumar *et al*., 2006). 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 or medical scrutiny although World Health Organisation (WHO) has recommended and encouraged the use of plants as an alternative therapy for diabetes (WHO, 1980). Evaluation of the antidiabetic potentials of these plants is therefore necessary to provide scientific proof and justify their use in ethnomedicine.

Mammea africana sabine (Guttiferae) (syn. *Ochrocarpus africana* Oliv.) is a large forest tree of 50 to 100 feet high with bark often yellow with pale scales and resinous yellow sap (Daziel, 1956).

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The plant is widely distributed in tropical Africa. The stem bark of the plant is used traditionally by the Ibibios of Niger Delta region of Nigeria in the treatment of malaria related fever, internal heat and microbial infections. The chloroformic and ether stembark extract are reported to posses cytotoxic activity on cell culture (Chapuis *et al*., 1988). Ouahouo *et al*. (2004) reported cytotoxic coumarins with antimicrobial activity against *Staphylococcus aureus* from the plant stembark. Methanolic fractions of the stem bark have been reported to contain compounds that are potent urease inhibitor (Rahman and Choudry, 2001). Also, Okokon *et al*. (2006) reported of the antiplasmodial activity of the stembark. The stembark has been reported to contain 5-7-dihydroxy-8- (12-methyl-butryl)-4-N-Pentyl coumarins (Carpenter *et al*., 1971; Crichton and Waterman, 1978; Carpenter *et al*., 1970), Mesuxanthone B (Carpenter *et al*., 1971). Alkaloids have been reported to be absent in the entire plant parts (Gartlans *et al*., 1980). Although reports of scientific studies on *Mammea africana* have been widely published, there is no information regarding the hypoglycaemic and hypolipidaemic activity of the stembark extract in rats.

The present study, therefore, was to establish if the stembark of *M. africana* has any antidiabetic and hypolipidaemic effects in strepotozotcin induced diabetic rats.

MATERIALS AND METHODS

Plant Materials

Fresh stembark of *M. africana* were collected in November, 2005 at Anwa 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. Hebarium specimen was deposited at Faculty of Pharmacy Hebarium with voucher No. FPHUU 381. The fresh stembark (2 kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder 100 g was macerated in 95% ethanol (300 mL) for 72 h. The liquid filtrate obtained was concentrated in vacuo at 40°C. The yield was 2.08% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

Animals

Albino wistar rats (105-165 g) and albino swiss mice (21-28 g) 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 gotten 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 (55 mg kg^{-1} 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 glysuria and hyperglycemia (blood glucose level range above 200 mg dL^{-1}) were considered as diabetic and used for the drug treatment. The stem bark extract in acqueous solution was administered orally through a gavage at a concentration of 200 mg kg^{-1} body weight rats day for 14 days.

Experimental Design

The animals were divided into two sets, one for the evaluationof 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 *Mammea africana* extract (30 mg/kg/rat/day) in acqueous solution orally for 14 days.
Group II: Diabetic rats given *M. africana* extract (60 mg/kg/rat/day) in acqueous solution orally for 14 days.
Group III: Diabetic rats administered *M. africana* extract (90 mg/kg/rat/day) in acqueous solution.
Group IV: Diabetic rats given Glibenclamide (10 mg/kg/rat/day) for 14 days in aqeous 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 h 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 h 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 1000 rpm for 15 min to obtain the sera. Serum cholesterol, triglyceride and High Density Lipoprotein (HDL) levels were measured by enzymatic calorimetric methods using Randox diagnostic kits. All samples were analyzed 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 Friedewald *et al*. (1972).

Statistical Analysis

All the group data were statistically analyzed with Students' t-test and two-way ANOVA, followed by Tukey Krammer post test. Values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

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 stembark extract of *M. 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 stembark extract of *M. 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 (Table 4). All lipid parameters tested were reduced after the treatment with ethanolic stem bark extract of *M. 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 stembark extract of *M africana* on body weight of streptozotocin induced diabetic rats

		Average body weight (g)	
Drug	Dose (mg kg^{-1})	Day 0	Day 15
Control		239.0±3.10	203.9±2.91
Extract	30	113.8±2.41*	117.0±5.00*
	60	100.5±1.50*	122.5±4.50*
	90	118.0±3.00*	141.5±3.50*
Glibenclamide	10	229.0±0.18*	190.0±2.66*

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

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

		Blood glucose level (mg dL^{-1}) (Mean±SD)				
Drugs	Dose (mg kg^{-1})	Initial	1 h	3 h	5 h	7 h
Control		243.5±10.5	246.0±9.60	257.6±8.50	262.5±8.10	265.3±4.30
Extract	30	247.5±38.5	141.6±3.60*	70.2±0.60*	62.5±7.50*	56.5±2.50*
	60	244.5±37.5	120.5±3.70*	59.3±7.00*	47.5±5.5*	49.5±2.50*
	90	246.8±12.8	91.5±2.90*	52.6±4.00*	48.5±3.50*	43.3±2.0*
Glibenclamide	10	247.5±0.50	123.3±3.90*	96.7±5.60*	82.9±3.30*	70.4±3.4*

*p<0.01 when compared to control. F-11.59, 12.91, df = 4, 16 (p<0.01), two-way ANOVA), n = 6 per group

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

		Blood glucose level (mg dL^{-1}) Mean±SD)					
Drugs	Dose (mg kg^{-1})	Initial	1st day	3rd day	5th day	7th day	15th day
Control		243.5±10.5	267.7±4.10	270.4±2.30	272.0±4.50*	275.9±6.33	277.8±1.96
Extract	30	247.5±38.5	49.2±2.00*	73.0±2.50*	69.0±1.10*	64.5±4.28*	65.4±2.33*
	60	244.5±37.5	48.5±9.50*	65.5±1.40*	51.0±1.00*	58.0±3.24*	61.5±6.54*
	90	246.8±12.8	50.5±8.50*	51.5±7.50*	58.5±4.50*	57.5±6.78*	62.0±2.69*
Glibenclamide	10	247.5±0.50	76.1±2.4*	78.8±1.42*	71.3±3.90*	68.9±3.73*	64.4±2.65*

*p<0.01 when compared to control, F = 5.98, 29.16, d.f = 20, 5 p<0.01 (Two-way ANOVA) n = 6 per group

Table 4: Effect of ethanolic stembark extract of *Mammea africana* on serum total cholesterol, triglycerides, hdl-cholesterol, ldl-cholesterol and VLDL-CHoL of alloxan diabetic rats

			Average Serum lipids profile (mmol L^{-1})			
Drugs	Dose (mg kg^{-1})	Total cholesterol	Triglycerides	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	30	3.31±0.30*	2.12±0.73*	1.81±0.33*	1.08±0.48*	0.42±0.18*
	60	2.93±0.11*	2.03±0.18*	1.89±0.43*	0.63±0.37*	0.41±0.06*
	90	2.80±0.51*	1.95±0.61	2.15±0.76*	0.26±0.14*	0.39±0.22*
Glibenclamide	10	2.61±0.30*	2.31±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

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 *M. 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 (coumarins) and terpenes in the extract (Carpenter *et al*., 1970, 1971; Crichton and Waterman, 1978). These compounds have been implicated in the antidiabetic activities of many plants (Shimizu *et al*., 1984; Reher *et al*., 1991; Ivorra *et al*., 1989). The hypoglycaemic action of this extract maybe by potententiating 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 artherosclerosis (Mironova *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 (Krishnakumar *et al*., 2000). Lowering of cholesterol levels in rats have been reported to be due to the antioxidant activity of phytochemicals like polyphenols-flavonoids and coumarins (Igarasi and Ohmuma, 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 *M. africana* stembark extract administration coupled with the antioxidant and free radical scavenging ability of its polyphenol phytochemicals may have increased the utilization of glucose, thereby depressing the mobilization of fats.

In conclusion, the present study shows that the ethanolic stembark extract of *M. africana* has potential hypoglycaemic action in STZ-induced diabetic rats and the effect was found to be comparable to glibenclamide. Further studies to isolate and identify the active principle as well as elucidation of its mode of action is necessary.

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