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Full Length Research Paper

Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of *Treculia africana* Decne and *Bryophyllum pinnatum* Lam. and their mixture on streptozotocin (STZ)-induced diabetic rats

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The plants *Treculia africana* and *Bryophyllum pinnatum* are ethnobotanically used in the treatment of various diseases including diabetes and heart diseases. Diabetes mellitus is a disease characterized by hyperglycaemia, and hyperlipidaemia which leads to an increased risk of atherosclerosis and other cardiovascular diseases. The effects of aqueous ethanol (80%) extracts of *T. africana* leaves and *B. pinnatum* plants and their mixture, in an equal proportion, were evaluated on postprandial glycaemic status. Three groups of normal rats were treated with the extracts and their mixture (1:1), at a dose of 500 mg/kg body weight and then charged with glucose (40%) at a dose of 1 ml/100 g body weight. Plasma sugar contents were analyzed from the blood collected from the tail vein at 30, 60 and 120 min intervals. Also glycaemic status and serum lipid profiles of normal and streptozotocin-induced diabetic rats were evaluated. Three groups of streptozotocin-induced diabetic (50 mg/kg ip) rats were treated with the extracts and the (1:1) mixture at a dose of 500 mg/kg, respectively for 21 days. A significant reduction ($p \leq 0.05$) in both postprandial and STZ-induced diabetes blood glucose levels, triglyceride levels, low density lipoprotein (LDL) level, and increase in high density lipoprotein (HDL) level were observed. This scientific finding supports the basis for the herbal use of *T. africana* and *B. pinnatum* in the management of diabetes and heart diseases.

Key words: *Treculia africana*, *Bryophyllum pinnatum*, hyperglycaemia, hyperlipidaemia, hypercholesterolaemia, streptozotocin, cardiovascular diseases.

INTRODUCTION

Diabetes is a major degenerative disease in the world today. Many deaths of diabetic subjects have been attributed to hyperglycaemia and its accompanied vascular diseases. Hyperglycaemia, in particular, is the primary clinical manifestation of diabetes (Nordestgaard et al., 1988) and is thought to contribute to diabetic complications by altering vascular cellular metabolism (Barrett-Connor et al., 1991) and vascular matrix molecules and circulating lipoproteins. Diabetes is, therefore, a major risk factor for the development of cardiovascular disease (CVD) (Chattopadhyay, 2004; Scott et al., 1999). Impaired carbohydrate utilization in the diabetic also leads to accelerated lipolysis, which results in elevated plasma triglycerides levels (hyperlipidaemia) (Granner et al., 1996; Horton et al., 1996). Consequently, the large amounts of fatty acids available to the liver in diabetic patients lead to excess acetylcoenzyme A (acetyl CoA), which is converted to form ketone bodies with subse-

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quent damage to the liver (Claudia et al., 2006). There is an elevation in plasma alanine aminotransferase (ALT) which is an indicator of distressed liver function and elevated creatinine level as a result of kidney damage. The increase in the availability of acetyl CoA from the β-oxidation of fatty acids is also responsible for the subsequent hypercholesterolaemia (Granner et al., 1996; Horton, 1996). To reduce the risk of vascular complications in diabetes mellitus, control not only of blood glucose levels but also lipid levels, blood pressure and weight are necessary (Williams, 1994).

Diabetes has been conventionally treated with orthodox medicines that function as hypoglycaemic agents, or insulin production modulators and/or lipoprotein lowering agents. Oral hypoglycaemic agents especially the sulphonylureas and biguanides have been commonly employed in the treatment and management of especially type II diabetes (Bunyapraphatsara et al., 1996). Sulphonylureas are the most widely used oral hypoglycaemic agents but may have some adverse effects such as exacerbating hyperinsulinaemia and causing weight gain in patients (Rastigo, 1977; Egwim, 2005). Biguanides are only weak hypoglycaemics and have limited clinical use (Rastigo, 1977). For these cogent reasons, therefore, there is a great need for a search for an acceptable, cheap and safe blood sugar lowering oral hypoglycaemic agents that would be effective in the treatment of diabetes and devoid of serious side effects of the currently used oral hypoglycaemic agents. Herbs and marine sources have been considered the best option.

Traditional medical practitioners have managed diabetes for decades with herbal remedies. The herbal drugs may be prepared from a single plant source or from a combination of two or more plant products. The latter practice being more frequent as the traditional medical practitioner believes that combination of many plant products will be more effective than use of a single plant product. Also the traditional medical practitioner tries to treat the whole person and not just the disease.

This study was aimed at the evaluation of the hypoglycaemic and hypolipidaemic effects of aqueous ethanol (80%) extracts of *T. africana* leaves, and *B. pinnatum* plant, and their (1:1) mixture on plasma glucose level, total triglycerides, HDL cholesterol, LDL cholesterol, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and plasma creatinine levels in streptozotocin (STZ)–induced diabetic albino rats (a model of type 2 diabetes). The use of the mixture is to investigate the synergistic effects of chemical constituents of the two plants in the treatment of diabetes

MATERIALS AND METHODS

Collection of plant materials

The leaves of *T. african* Decne.(Fam. Moraceae) and *B. pinnatum* Lam. (Fam, Crassulaceae) were collected in the month of January 2007, from Atani, a town located by the bank of River Niger in Anambra state of Nigeria and were identified and authenticated by Dr. O.A. Ugbogu at the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria.

Preparation of plants for extraction

The plant materials were respectively washed with copious tap water to remove foreign matters, spread on a mat to drain the water off and dried in an oven at about 50°C for a week. The dried plant materials were then milled to coarse particles and extracted as follows:

(a) 100 g each of *T. africana* powdered leaves and *B. pinnatum* of powdered plant respectively, was first defatted with 750 ml petroleum ether (60 - 80°C) for 2 – 3 h in a Soxhlet apparatus, the resulting marc was dried and extracted with 1 litre ethanol(80%) in Soxhlet apparatus for 5 h.
(b) 100 g of *T Africana* powdered leaves and *B. pinnatum* powdered plant in an equal proportion (1:1) was also defatted with 1 litre of petroleum ether (60 - 80°C) and the resulting marc extracted with 1 litre ethanol (80%) in Soxhlet apparatus.

The extracts were then concentrated in a rotary evapourator and dried in an oven at temperature of 40°C to constant weights. The dried flaky masses obtained were weighed to give percentage yields of 10.3, 18.6 and 17.2% respectively and were stored in the refrigerator until needed.

Laboratory animals

Forty five healthy Wistar strain albino rats weighing between 180 ±15 g were obtained from the Laboratory Animal Center of College of Medicine, University of Lagos, Idi -Araba, Lagos, Nigeria. The rats were housed in clean metallic cages and kept in a well-ventilated room and allowed to acclimatize to the laboratory condition for one week before being used. They were fed with standard animal pellet (Pfizer Feeds Plc., Nigeria) and had free access to water *ad libitum*. The animals were distributed randomly into four groups of five animals each for postprandial study and into five groups of five rats each for the streptozotocin-induced diabetic experiment.

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Postprandial test

This measures the body's ability to metabolize carbohydrates and produce insulin. Twenty albino rats of the above mentioned weight bracket were randomly assigned to four groups A, B, C and D consisting of five animals each. They were fasted for about 18 h with access to water only (Egwim, 2005). Blood was taken from the lateral veins of the tail and the blood sugar levels were initially monitored with a glucometer (ACCU-CHEK, Roche Diagnostics) for estimation blood sugar level. The extracts were respectively prepared by dispersing 2.0 g of the extract and in case of the mixture 1.0 g of each extract combined, with 5 ml Tween 80 (2%) solution transferred to a 10 ml volumetric flask and the volume made up to mark the Tween solution to give 20% solution. The animals were treated as follows:

Group A received A. *africana* extract (500 mg/kg bwt)
Group B received *B. pinnatum* (500 mg/kg bwt)
Group C received *T. africana* and *B. pinnatum* mixture(1:1)
Group D received 0.5 ml Tween 80 solution and served as control.

After 30 min, the animals were each treated with 40% (wt/v) glucose at a dose of 1 ml/100 g body wt given orally. Blood glucose levels were monitored from lateral tail veins at 30, 60, and 120 min

Table 1. Postprandial evaluation of the blood plasma sugar lowering effects of the aqueous ethanolic extracts of *T. africana* and B. pinnatum and their mixture (1:1) after 2 h period on rats.

Group	Glucose (mg/dl)			
	0	30 min	60 min	120 min
Group A	105.2± 1.5b	110.2± 2.2b	92.3 ±0.5b	100.02± 0.45 b
Group B	100.03± 0.7	104.03±1.2	99.1± 0.05b	114.06± 2.5
Group C	101.4± 2.2	103.01± 1.5a	103.5± 0.7b	93.5± 1.1b
Group D	95.05± 0.53a	103.1±0.6 a	110.8± 1.2a	118.02±0.2a

Means with the same letter in same column are not significantly different using Duncan Multiple Range Test (P≤0.05). Group A= *T. africana* extract, Group B = *B. pinnatum,* Group C = *T. africana* and *B. pinnatum* (mixture; 1:1), and Group D = No treatment (Control).

intervals after post glucose challenge. Percentage inhibition in post-prandial hyperglycaemia is considered as percent of hypoglycaemic activity (Rastogi et al., 2000). Blood glucose concentration was deter-mined by the Randox enzymatic Kit (Randox Lab. Ltd., UK). The intensity of the pink colour formed is proportional to the glucose concentration (Trinder, 1969; Barham and Trinder, 1972).

Streptozotocin-induced diabetic experiment

Diabetes was induced by intraperitoneal (ip) injection of streptozo-tocin (50 mg/kg body weight) dissolved in 0.0 1 M citrate buffer (pH 4.5). After 48 h blood was taken from the lateral veins of the tail and the blood sugar levels were monitored with a glucometer (ACCU-CHEK, Roche Diagnostics) for estimation blood sugar level. The animals with blood sugar level more than 200 mg/dl were consider-ed diabetic and included in the experiment.

The diabetic animals were randomly distributed into four groups of five animals each while the last group, the positive control, consists of five normal rats and were treated as follows:

Group 1: STZ- induced diabetic + *T. africana* extract (500 mg/kg bt)
Group 2: STZ- induced diabetic + *B. pinnatum* extract (500 mg/kg bt)
Group 3: STZ- induced diabetic + *T. africana* and *B. pinnatum* mixture (500 mg/kg bt)
Group 4: STZ- induced diabetic animals but untreated to serve as control negative.
Group 5: Normal rats (positive control).

Evaluation of hypoglycaemic and hypolipidaemic effects

The animals were treated daily for twenty one days. On the 21st day after treatment they were starved overnight, and on the 22nd day were anaesthetized with warm urethane and chloralose (25%:1%, v/v) at a dose of 5 ml/kg and blood obtained via cardiac puncture into heparinized container. The blood was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma which was analyzed for glucose level, total cholesterol, total triglyceride, HDL-cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan et al., 2001). LDL-cholesterol levels were calculated using Friedwald equation (Crook, 2006). Plasma was analyzed for alanine amino-transferase (ALT), aspartate aminotransferase (AST), and creatinines by standard enzymatic assay analysis and the plasma protein contents, plasma glucose contents were determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002).

Statistical analysis

Data is reported as Mean ± SEM. Statistical comparisons were determined by analysis of variance (ANOVA) and means were separated using Duncan's multiple range test (P ≤0.05).

RESULTS AND DISCUSSION

The postprandial test results showed that the plants extracts and their (1:1) mixture exerted some hypo-glycaemic effects on the blood glucose level in the fasting normal rats. The critical test for diabetes does not lie in hyperglycemia or hyperlipidaemia but in blood-sugar tolerance. After ingesting sugar, both normal and diabetic individuals will show an increase in the blood sugar level as it happens after a meal, but the increase remains high in the diabetic, whereas in the normal individual the excess glucose is rapidly converted into glycogen. The mixture C (*T. africana* and *B. pinnatum*; 1:1) was observ-ed to be most active in lowering the postprandial the blood glucose level (Table 1 and Figure 1). This may be due to synergistic effects of the chemical constituents of the two plants. It shows a great promise as an oral hypoglycaemic agent.

The effects of the extracts and their mixture (1:1) on the blood sugar and biochemical parameters of the streptozotocin–induced diabetic rats are summarized in Table 2. Streptozotocin-induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents (Szkudelski, 2001). Plasma glucose concentration in excess of 200 mg/dl confirms the diabetic state of the rats (Henry et al., 1974; Ellenberg and Rifkin, 1983, Nimenibo–Uadia, 2003). The results of this study clearly indicated that the aqueous ethanolic extracts of *T. africana* leaves and *B. pinnatum* plants and their mixture (1:1) not only had hypoglycaemic effects but also hypolipidaemic effects in streptozotocin-induced diabetic rats.

A significant reduction (P ≤0.05) in the blood glucose levels of all the treated groups was observed. Though the mixture demonstrated to have the most postprandial activity but had least sugar lowering activity in long term treatment compared to individual components. This observation suggests that the action of the mixture might not have been additive.

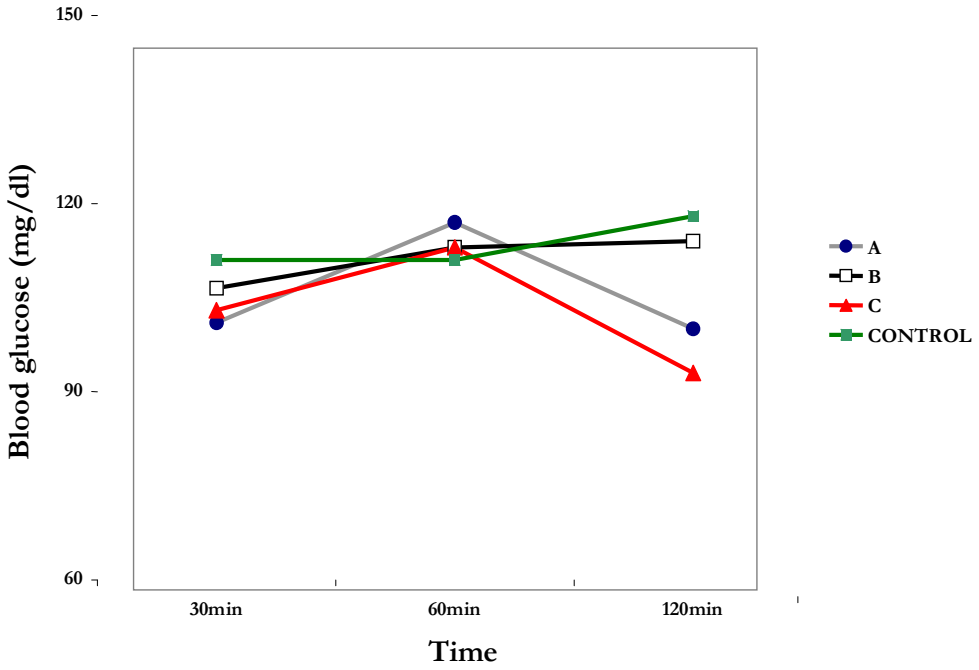

Figure 1. Postprandial evaluation of the blood sugar lowering effects of the aqueous ethanolic extracts of *T. africana* and *B. pinnatum* and their mixture (1:1) after 2 h on rats. **A =** *T.africana* extract, **B =** *B. pinnatum,* **C =** *T. africana* and *B. pinnatum* (mixture; 1:1) **Control =** No treatment.

Table 2. Effect of *T. africana* land *B. pinnatum* extracts and their mixture (1:1) on glucose level and on serum lipids levels in the normal and streptozotocin-induced diabetic rats.

Treatment	Glucose	Total chol.	HDL	LDL	Triglycerides	AST	ALT	Creatinine
A	65.75±3.88a	78.5±9.44	16.25±2.9b	52.25±8.80	49.75±5.71ab	102.75±5.02	20.00±1.78	1.05±0.31
B	68.25±4.63a	65.75±7.28	13.00±0.71b	42.00±7.38	52.50±3.77ab	97.50±7.23	22.00±2.97	0.78±0.42
C	85.00±6.66a	68.67±4.06	10.00±1.15b	47.67±5.24	55.00±10.58ab	104.67±5.93	27.33±2.19	1.13±0.45
+ve control	145.67±34.53	55.67±7.22	7.67±1.20	35.00±5.51	67.33±5.78	109.00±6.66	27.00±4.58	0.93±0.03
-ve control	70.33±5.21	86.33±13.17	17.67±2.40	60.33±11.46	40.00±7.00	74.67±8.69	21.33±2.33	0.77±0.27

Values are mean of five observations ± standard error of mean (n=5). Means with the same letter in same column are not significantly different using Duncan Multiple Range Test (P≤0.05). A = *T. Africana,* B = *B. pinnatum* and C = *T. africana* and *B. pinnatum* mixture (1:1). HDL = high density lipoproteins; LDL = low density lipoproteins; AST = aspartate aminotransferase; and ALT = alanine aminotransferase.

The low plasma total cholesterol (TC) concentration observed in the diabetic animals treated with the extract and the mixture clearly demonstrated the presence hypolipidemic agents in the extract. There was also a significant decrease in both triglyceride (TG) and LDL-cholesterol levels while significant increase in HDL-cholesterol levels were observed. It could be interpreted that the extract had some beneficial effects on cardio-vascular risk factors which contribute to death of a diabetic patient (Barnett and O'Gara, 2003). This observation supported the local use of the *T. africana and B. pinnatum* and their mixture as hypoglycaemic agents. An increment in HDL cholesterol and a reduction in LDL and total cholesterol could be considered beneficial in the long-term prognosis of diabetic subjects. The treated animals showed significant increase in the levels of HDL-cholesterol compared to the negative control. The observed abnormalities of triglyceride and HDL metabolism are in accordance with reports on early manifestation of insulin resistance, the precursor to diabetes (Frederickson and Lee, 1965; Lyons, 1992; Otvos et al., 2002).

The liver releases alanine aminotransferase (ALT) and an elevation in plasma concentrations are an indicator of liver damage. The liver and heart release aspartate aminotransferase (AST) and ALT, and an elevation in their plasma concentrations are an indicator of liver and heart damage (Wasan et al., 2001; Crook, 2006). There was a significant increase ($p<0.05$) in the plasma AST especially in the animals treated with the mixture and ALT levels did not show any significant changes in all the groups. This clearly indicates that long term consumption of the drugs and their mixture may not provoke some

harmful heart effects to the animals. There was no significant change observed in the creatinine levels of the diabetic treated with the extracts and the mixture compared with positive control. An increase in plasma creatinine levels may be a sign of impaired renal function for the animals affected. The elevation in the plasma creatinine concentration indirectly suggests kidney damage specifically the renal filtration mechanism (Wasan et al., 2001).

Preliminary phytochemical screening (result not tabulated) indicated the presence of steroidal saponins, anthraquinones and polyphenols. Steroid containing drugs have been found to lower blood cholesterol and lipids levels in animals and they decrease LDL-and VLDL–cholesterol and increases HDL-cholesterol/total cholesterol ratio (Jean, 1999). Polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits, which include lowering of blood lipids. Furthermore, recent reports have suggested that several plant sterols reduce serum cholesterol by the inhibition of intestinal cholesterol absorption (Sushruta et al., 2006). Thus, it can be suggested that the synergistic interaction of polyphenols and tannins contents in the extract may impart hypolipidemic property to the extract, hence the local use of the plants as antidiabetic agents.

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