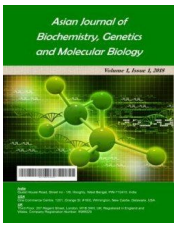

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Hypoglycaemic and Hypolipidemic Effects of *Treculia africana* Aqueous Leaves Extract in Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author IAK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GAP managed the analyses of the study. Author MA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the hypoglycaemic and hypolipidaemic effects of *Treculia africana* plant used in Nigeria as medicinal plant. Diabetes mellitus was induced by a single dose intraperitoneal injection of alloxan 150 mg/kg body weight. Twenty five (25) male albino rats were divided into five groups, five (5) rats per group; normal control, diabetic control and diabetic groups treated with aqueous leaves extract of 200,400 and 800 mg/Kg body weight respectively for 21 days orally. The effects of the extract on some biochemical parameters were evaluated; fasting blood glucose level was assayed using glucose oxidase method, total cholesterol and HDL –cholesterol were assayed using enzymatic method while LDL- cholesterol was determined by Friedewald equation. The results showed that, extract significantly ($p<0.05$) decrease the elevated fasting blood glucose

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levels, total cholesterol, triglyceride and LDL- cholesterol when compared with the diabetic control rats. The extract also caused significant ($p<0.05$) increased in HDL –cholesterol and body weight when compared with diabetic control rats. Aqueous leave extract of *Treculia africana* possess hypoglycemic effect and the most effective dose was 800 mg/Kg body weight in amelioration of hyperglycaemia and most all toxicity effects of alloxan on lipid profile.

Keywords: Hypoglycaemic; hypolipidaemic; Treculia africana plant.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by a persistent elevation of fasting blood glucose level (FBGL) above 200 mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action [1]. It is characterized by persistent hyperglycemia and disturbances in the metabolism of fuel compounds [2]. It occurs when pancreas cannot produce insulin (type 1 DM) or cannot produce enough of insulin or the body cannot effectively use the produced insulin for effective uptake of glucose to the target cells (type II DM) [3]. Lipid abnormalities, anemia, alteration of liver and kidney functional indices has been implemented as major risk factors to the progression of both microvascular and macrovascular diabetes complications [4]. Globally, as of 2013, an estimated 382 million people have diabetes worldwide, with type 2 diabetes making up about 90% of the cases. This is equal to 3.3% of the population, with equal rates in both women and men [5]. Ethnomedicinal plants might provide new oral hypoglycemic compounds, which can counter the high cost of the current hypoglycemic medicines for many rural populations in developing countries [6].

African breadfruit is a large, slow-growing, evergreen tree with a dense, spreading crown; usually growing 15 - 30 metres tall but with some specimens up to 50 metres [7]. *Treculia africana* (African breadfruit) is a genus of the evergreen flowering plant and belongs to the family of moracae. There are three (3) varieties of *Treculia africana: Treculia africana var. africana, Treculia africana var. inverssa and Treculia african mollis* [8]. It is a common forest tree called by various names among different tribes in Nigeria, such as “Ukwa” (Igbo), “afon” (Yoruba), “barafuta” (Hausa), “Ize” (Benin), “eyo” (Igala) and “edikang” (Efik) [9]. *Treculia africana* are highly nutritious and constitute a cheap source of vitamins, minerals, proteins, carbohydrate and fats [10]. It also contained appreciable amounts of flavonoid, polyphenols, anthraquinones, saponins and cardiac glycosides. These secondary metabolites are known to have potential ethno-medicinal effects as well as other physiological activity [11]. Survey among herbalist and number of patients attending Diabetic clinics in the University College hospital Ibadan revealed the ethno-medicinal hypoglycemic effect of *Trecuia Africana* [12]. In Ghana, the root decoction is used as an anthelmintic and febrifuge. Ethnomedically, it is used as a verbrifuge, vermifuge, galactogogue and laxative [13]. The stem and bark of *Treculia africana* are employed in the treatment of helminthiasis, malaria, leprosy and rheumatism [14]. It also used for the treatment of inflammation, diabetes mellitus, diarrhea, and tapeworm infection [15]. Herbal medicine is in used by about 60% of the world population both in the developing and in the developed countries where modern medicines are predominantly used [16]. Ethno-medicinal anti-diabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines for many rural populations in developing countries [6].

2. METHODOLOGY

2.1 Collection and Identification of Plant Materials

Treculia africana leaves was obtained from Umuidi community of Anambara State of Nigeria. The sample was identified and authenticated by the Department of Science Laboratory Technology, Federal Polytechnic Kaura Namoda, Zamfara State. Nigeria.

2.2 Preparation of Samples

The samples were thoroughly washed to remove sand and the drained parts were air dried later. The samples were grounded using wooden mortar and pestle until powder was obtained to ensure homogeneity. 100 g of the plant powder

was soaked in 1000 ml of boiled distilled water and agitated intermittently for 24 hours. The solution was then filtered using filter paper to obtained the aqueous extract which was then be allowed to dried in an oven dryer at 100°C to obtained the crude extract [17]. The extract was stored in an air tight container for further work, the required doses of 200 mg, 400 mg and 800 mg/kg body weight was obtained by reconstituting the stored extract using distilled water and administered [18].

2.3 Experimental Animals

Twenty five (25) apparently healthy young male Wister albino rats weighing between 100 – 200 g were used for this study. The rats were kept at animals' house under normal environmental conditions and maintained with free access to pelletized growers feed, and access to water *ad libitum.* The animals were allowed to acclimatize for two weeks (14 days). All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health as well as the guidelines of the Animal Welfare Act. Experiments were carried out with prior permission from the Institutional animal Ethical committee, Federal Polytechnic Kaura Namoda, Zamfara State, Nigeria.

2.4 Induction of Diabetes

All rats, except the Normal Control Group were intraperitoneally injected with 150 mg/kg body weight of the prepared alloxan. Seventy two hours (three days) after alloxan administration, the animals were fasted overnight and diabetes was confirmed from the rats by measuring their fasting blood glucose level with the aid of a single touch glucometer. Rats that have fasting blood glucose level >7.0 mmol/l (126 mg/dl) were considered diabetic and included in the study [19].

2.5 Experimental Design

By the end of the seven days acclimatization period, the animals were randomly assigned into five different groups of five rats each, designate as group A-E. Group A received water and feed only and serve as positive control. Diabetes was induced in group B, C, D, and E. Group B serve as negative control while group C to E corresponding to 200, 400 and 800 mg/kg doses of the aqueous leave extract of *Treculia africana* were administered orally.

2.5.1 Collection of blood sample

After four weeks of treatment all albino rats were fasted overnight, the rats where anesthetized by placing them in a seal cotton wool soaked in diethyl ether inhalation jar, the animals were sacrificed by decapitation at the end of three weeks of treatment and blood samples were obtained and centrifuged at 4000 ×g for 10 min at 4°C and supernatant kept at 37°C for further biochemical measurements.Different biochemical parameters were analyzed including estimation of fasting blood sugar level, Lipid profile, Heamatolgical and liver function indices.

2.5.2 Determination of biochemical parameters

The use of biochemical parameter test result in diagnostic, treatment and decision making is an integral part of enthonomedicinal plant research.

2.5.3 Estimation of serum glucose level

Serum glucose was estimated by glucose oxidase/ peroxidase method using Randox kit [20].

Reagent composition (Randox Kit)

Contents	Concentration in the test
R1. Buffer	
Pipes	100 mmol/l, pH 7.6
ATP	4 mmol/l
NAD^+	3 mmol/l
Magnesium ions	15 mmol/l
R2. Enzyme Reagent	
Hexokinase	≥ 0.5 U/ml
G-6-PDH	≥ 1.5 U/ml
R3. PGI	
Phosphoglucose Isomerase	≥ 6.8 U/ml
CAL Standard Glucose	

2.5.4 Procedure

Test tubes were set up in triplicates and labeled blank, test and standard. 10µl of serum, standard (5. 5 mmol/L) and distilled water were respectively pipetted in to the test tubes. Each test tube is then followed by 1000 µl of the reagent. The tubes were mixed properly, incubated at 37°C for 10 minutes and the absorbance of standard and tests read against the blank at 500 nm using spectrophotometer.

Calculation: The glucose concentration was calculated using the relation:

Serum glucose (mmol/L) =

$$\frac{Absorbance\ \ of\ \ Test}{Absorbance\ \ of\ \ \text{Standard}} \times conc\ of standard$$

2.5.5 Estimation of serum total cholesterol

Serum total cholesterol (TC) was estimated by enzymatic method using Enzopak kit [21].

Reagent composition (Enzopak)

Reagent composition Active ingredients	Initial concentration
R1. Enzyme Reagent 1	
Cholesterol oxidase	≥500U/l
Cholesterol Esterase	$\geq 600U/l$
Peroxidase	$\geq 6000U/l$
4-Amino Antipyrine	0.5 mmol/l
R2. Enzyme Reagent 2	
Buffer	100 mmol/l
Detergent	15 mmol/l
Phenol	20 mmol/l
Surfactant	20 mmol/l
pH 7.00±0.1 at 25°C	
Cholesterol standard (200 mg/dl)	
Also contains non-reactive filters and stabilizers	

Three test tubes were set up and labeled blank, test and standard. In to test tubes labelled test, standard and blank, 10 µl of serum, standard (200 mg/dl) and distilled water were respectively pipetted in to the test tubes. Each test tube is then followed by 1000 µl of the reagent as shown above. The test tubes were mixed, incubated at 37°C for 5 minutes and the absorbance of the standard and test were read against the blank at 500 nm against the reagent blank.

Calculation: Cholesterol concentration was obtained using the relation:

Serum total cholesterol (mg/dl) =

$$\frac{Absorbance\ \ of\ Test}{Absorbance\ \ of\ \text{Standard}} \text{ x Conc of Standard}$$

2.5.6 Estimation of serum HDL – C

This was done by enzymatic method of [22] using Randox Kit. Into centrifuge tubes, 200 µl of serum and 500 µl of precipitant (0.55 mmol/L phosphotungastic acid and 25 mmol/l Magnesium Chloride) were added, mixed and allowed to stand for 10 minutes at room temperature. The tubes were centrifuged for 10 minutes at 4000 rpm. The supernatant was collected and used for the analysis. Three test tubes were set up and labeled blank, test and standard. In to test tubes labeled test, standard and blank, 10 µl of serum, standard and distilled water were respectively pipetted in to the test tubes. Each test tube is then followed by 1000 µl of the reagent. The test tubes were mixed, incubated for 5 minutes at 37°C and the absorbance of the samples and standard were measured against the reagent blank at 500 nm.

Reagent composition (Randox Kits)

Contents	Initial concentration
R1. Enzyme Reagent 1	
N,N-Bis(2-hydroxyethyl)- 2-aminoethanesulfonic acid	100 mM, pH 6.6 (+25°C)
N-(2-hydroxy-3-Sulfopropyl)- 3,5-dimethoxyaniline, sodium salt (HDAOS)	0.7 mM
Cholesterol Esterase [E.C.3.1.1.13. Microorganism]	≥800 U/L
Cholesterol Oxidase [E.C.1.1.3.6. *Streptomyces* sp]	≥500 U/L
Catalase [E.C.1.11.1.6. Microbial]	≥300 U/L
Ascorbate oxidase [EC.1.10.3.3. *Acremonium* sp.]	≥3000 U/L
R2. Enzyme Reagent 2	
N,N-Bis(2-hydroxyethyl)- 2-aminoethanesulfonic acid	100 mM, pH 7.0 (+25°C)
4-Aminoantipyrine	4.0 mM
Peroxidase [E.C.1.11.1.7, Horse Radish, +25°C]	≥3500 U/L
Sodium Azide	0.05 w/v %
Surfactants	1.4 % w/v %

Calculation: The HDL-C concentration was obtained from the relation:

$$\text{Serum HDL-C (mg/dl)} = \frac{Absorbance\ of\ Test}{Absorbance\ of\ \text{Standard}} \times \text{Conc. of Standard}$$

2.5.7 Estimation of serum triglyceride

This was assayed by the method of [23] using Randox Kit.

Reagent composition (Randox Kit)

Contents	Concentration in the test
RIa. Buffer	
Pipes Buffer	40 mmol/l, pH 7.6
4-Chloro-phenol	5.5 mmol/l
Magnesium- ions	17.5 mmol/l
Rib. Enzyme Reagent	
4-aminophenazone	0.5 mmol/l
ATP	1.0 mmol/l
Lipase	≥150 U/ml
Glycerol-kinase	≥ 0.4 U/ml
Glycerol-3-phosphate oxidase	≥ 1.5 U/ml
Peroxidase	≥ 0.5 U/ml
CAL Standard	11.95 mmol/l

The tubes were mixed and incubated at 37°C for 5 minutes and the absorbance of the standard and tests were read at 500 nm against the blank.

Calculation: The TG levels were calculated using the relation:

$$\text{Serum TG (mg/dl)} = \frac{Absorbance\ of\ Test}{Absorbance\ of\ \text{Standard}} \times \text{Conc. of Standard}$$

2.5.8 Estimation of serum LDL – C

This was calculated using Friedewald formula [23].

$$\text{LDL - C (mg/dl)} = TC - (HDL - C) + \left(\frac{TG}{5}\right)$$

2.5.9 Estimation of serum VLDL – C

This was calculated using Friedewald formula [24]

$$\text{VLDL - C (mg/dl)} = \frac{TG}{5}$$

3. RESULTS AND DISCUSSION

As shown in Table 1, after induction of diabetes mellitus by alloxan, the serum glucose level raised to about 268.33 mg/dl with significant increase levels of serum triacylglyceride (TAG), cholesterol, and LDL- Cholesterol with significant decrease in HDL- Cholestrol level in all diabetes induced groups.

Fasting blood glucose concentration which is the most routine biochemical marker in the diagnosis of diabetes mellitus in clinical and experimental settings [25] was measured in this study. Table 1 revealed significant increased ($PC<0.05$) in fasting blood glucose concentration, triacylglyceride (TAG), total cholesterol and low density lipoprotein (LDL) cholesterol with significant ($P<0.05$) decreased in HDL-cholesterol in all alloxan induced diabetes albino rats compared to non-diabetic control rats. Elevated value of fasting blood glucose concentration (268.33 mg/dl) observed in diabetic rat is due to the toxic effect of alloxan on islet beta cells of the pancreas [26] through its ability to induce reactive oxygen species (ROS) formation, resulting in the necrosis of the pancreas [27] and loss of capacity of the pancreas to secrete insulin [28] leading to hyperglycemia. Chronic exposure to hyperglycemia is the primary casual factor in the pathogenesis of diabetic complications and cause changes in vascular tissue that promote atherosclerosis [29]. The elevated values for lipid profile parameters such as triglyceride, LDL-cholesterol, and total cholesterol observed in the alloxan induced diabetic rats could be partly due to increased intestinal biosynthesis of cholesterol [17] because diabetes shifted the major site of cholesterogenesis from the liver to the small intestine [30] leading to hypercholesterolemia. Severe diabetes mellitus due to insulin deficiency might be accompanied with a reduced LDL-receptor [31] resulting to high concentration of serum LDL cholesterol in diabetic subject.

Table 2, Revealed that oral administration of high dose of aqueous leaves extract of *Treculia africana* 800 mg/kg body weight for twenty one (21) days to the diabetic rats significantly ($p<0.05$) decreased the elevated fasting blood sugar concentration compared with the diabetic control rats. Our result was in line with the finding of Ogbonnia et al. [32] which stated that, aqueous ethanolic extracts of

Table 1. Effect of alloxan on fasting blood sugar levels and lipid profile in alloxan induced diabetic albino rats

	Fasting blood sugar (FBS) (mg/dl)	Serum triglyceride (TAG) (mg/dl)	Cholesterol (mg/dl)	High density lipoprotein (HDL)(mg/dl)	Low density lipoprotein (LDL) (mg/dl)
Non diabetes control	61.33±2.9a	1.58 ± 0.3a	3.11±0.6a	1.75±0.4a	1.04±0.2a
Diabetes control	268.84±0.3b	4.86±0.3b	5.98±0.3b	1.37±0.3b	3.31±0.1b

Values are expressed as mean ± SD, n= 5 for each group, Mean values having different superscript letter in the same column are significantly different at (p<0.05)

Table 2. Effect of aqueous leaves extract of *Treculia africana* on fasting blood sugar levels in alloxan induced diabetic albino rats

Experimental groups	Fasting Blood Glucose levels (mg/dl)
Diabetic control	268.33 ± 16.8 ^{bcd}
Non-Diabetic control	61.43 ± 8.73^{acd}
Alloxan + 200 mg/kg b.w of the *T. africana*	73.66 ± 5.77 ^{abd}
Alloxan + 400 mg/kg b.w of the *T. africana*	66.01 ± 5.00 ^{abc}
Alloxan + 800 mg/kg b.w of the *T. africana*	53.33 ± 5.50 ^{abcd}

Values are expressed as mean ± SD, n= 5 for each group, Mean values having different superscript letter are significantly different at (p<0.05)

Table 3. Effect of aqueous leaves extract of *Treculia africana* on serum lipid profile in alloxan induced diabetic albino rats

Experimental groups	Total cholesterol (mg/dl)	TAG (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)
Diabetic control	5.86 ± 0.15^{a}	4.86 ± 0.15^{b}	1.37± 0.06^{c}	3.31± 0.72^{d}
Non- Diabetic control	3.11± 0.05^{a}	1.58± 0.50^{b}	1.75 ± 0.55^{c}	1.04 ± 0.55^{d}
Alloxan + 200 mg/kg b.w. of the *T. africana*	3.18 ± 0.65^{b}	1.55 ± 0.66^{c}	2.2 ± 0.70^{d}	0.67 ± 0.65^{e}
Alloxan + 400 mg/kg b.w. of the *T. africana*	3.02 ± 0.60 ^{ab}	1.56 ± 0.51^{bc}	1.95 ± 0.61^{cd}	0.84 ± 0.55^{db}
Alloxan + 800 mg/kg b.w. of the *T. africana*	3.13 ± 0.55 ^{ac}	1.4 ± 0.62 ^{bd}	1.9 ± 0.90 ^{ad}	0.84 ± 0.66 ^{dc}

Values are expressed as mean ± SD, n= 5 for each group, Mean values having different superscript letter in the same column are significantly different at (p<0.05)

Treculia africana Decne was the most powerful in amelioration hyperglycemia. According to researchers at the Departments of Physiology and Biochemistry, College of Medical Sciences, University of Calabar, who conducted a study using a *Treculia africana* (breadfruit) seed diet on rats, found that it significantly lowered blood lipid levels and blood glucose levels in diabetic rats compared to rats fed on normal diet [33]. Oral administration of high dose of aqueous ethanol extract of *Treculia africana* root bark (500mg/kg for 21 days) to the diabetic rats ameliorated the diabetic complications by declined the glucose levels [34] reflecting a restoration of the pancreatic β-cells activity [35]. Phytochemical screening revealed that, *Treculia africana* was found to contain polyphenol and tannins [34,11]. Phenolic compounds might be useful medicinal food components and could contribute to manage both hyperglycemia and proper cellular redox status [36]. Quercetin-3-0- glucoside, quercetin-3-0 (-6''- malonyl glucoside) and kaempferol-3-0 (-6''-malonyl glucoside) which are polyphenol compounds were reported to be responsible for hypoglyceamic activity [37] due to their inhibitory activity on α- glucosidase enzyme [38,39].

Table 3 revealed that oral administration of aqueous extract of *Treculia africana* at a doses of 200, 400 800 mg/kg body weight for 21 days to diabetic rats resulted in significant ($p<0.05$) reduction in total cholesterol, low density lipoprotein and triglyceride. These finding of the present study was in agreement with the finding of [32,34], this reduction in total plasma cholesterol, low density lipoprotein and triglyceride confirming the hypolipidaemic properties of *Treculia africana.* This could be due to the presence of saponins that can inhibit taurocholate and deoxycholate absorption in a dose-dependent manner [40]. Phytochemical screening revealed that, *Treculia africana* was found to contain saponin [32]. Oral administration of *Treculia africana* leave extract to the diabetic rats ameliorated the diabetic complications by declined the LDL-Cholesterol, total cholesterol and triglyceride levels reflecting a restoration of the pancreatic β-cells activity [35] to secrete insulin. Insulin is a portent activator of lipoprotein lipase and inhibits VLDL production by the liver as well as to promote the reduction of LDL-cholestreol concentration [41]. Thus, the effect of *Treculia africana* at reducing the LDL-cholesterol level of the diabetic rats may suggest the ability of the extract to increase the number of LDL receptors, with consequent reduction in LDL-cholesterol [42], this confirming the hypoglycemic and hypolipidaemic properties of *Treculia africana* leaves.

4. CONCLUSION

Aqueous leave extract of *Treculia africana* possess hypoglycemic effect and the most effective dose was 800 mg/Kg body weight in amelioration of hyperglycaemia and most all toxicity effects of alloxan on lipid profile and some liver biomarker enzymes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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