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Research Report

Borassus aethiopum (Mart.) ethanol fruit extract reverses alloxan-treatment alterations in experimental animals

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Abstract.
BACKGROUND: *Borassus aethiopum* fruit is claimed to be used for the management of diabetes without scientific validation.
OBJECTIVE: This study seeks to evaluate the antihyperglycaemic activity of ethanol fruit extract of *Borassus aethiopum* in alloxan-induced diabetic rats.
METHODS: 36 rats were placed in six groups (i-vi) ($n = 6$). Animals in group i (standard) were given 0.4 mls of distilled water (d.w) whereas the ones assigned to group ii, iii, iv, v and vi which were induced into diabetes (by intake of 140 mg/kg body weight [b.w] of alloxan) were also respectively given d.w, 50 mg/kg b.w of metformin, 25, 50 and 100 mg/kg b.w of ethanol fruit extract of *Borassus aethiopum*, once daily for 14 days.
RESULTS: Flavonoid found in the extract (24.04 mg/ml) occurred the most with phenolic (0.35 mg/ml) being the least. While alloxan substantially *($p < 0.05$)* increased the levels of some biological molecules and enzyme activity, it lowered those of others. The extract however significantly *($p < 0.05$)* reversed all the alloxan-induced alterations, with the extract at 100 mg/kg b.w producing figures that compared *($p > 0.05$)* well with those of the d.w treated non-diabetic animals and metformin-treated diabetic animals. The extract also renewed the wholeness of histological damage in the pancreas.
CONCLUSION: The bioactive agents of *B. aethiopum* presented antihyperglycaemic property by preventing diabetes via reversal of alloxan-treatment alterations in the animals.

Keywords: Diabetes mellitus, *Borassus aethiopum*, insulin, Arecaceae

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1. Introduction

Diabetes mellitus, a clinically and genetically heterogenous metabolic disorder, is characterized by hyperglycaemia as well as protein and lipid metabolism due to deficiency in insulin release, insulin activity or both [1]. It is acknowledged as one among the five major mainsprings of mortality worldwide. It has been approximated that nearly 1.3% of the human race are inflicted by this disease [2]. In 2017, the prevalence of diabetes worldwide and Nigeria (20–79 years old) were 425 million and 1.70 million cases respectively and has been projected to 642 million and 3.40 million by 2040 [3]. This worldwide public health problem, currently affecting mankind, regardless of socioeconomic status and geographic location [4], may be brought about by lengthened breakdown of food rich in carbohydrate (polysaccharide), insufficient physical activity, raised body adiposity, elevated intake of saturated fatty acid as well as glandular system distortions like hyperthyroidic and polycystic ovary syndrome [5].

Diabetes can be classified into two groups, comprising type 1 diabetes mellitus (T1DM), evidenced by a relative inadequacy in insulin release and type 2 diabetes mellitus (T2DM) which presents with the conjugation of insulin resistance and insulin release issues [6]. Uncontrolled hyperglycaemia leads to progressive development of prolonged microvascular and macrovascular glucose-related inadequacies leading to death among sufferers [6]. Currently, insulin and glucose lowering drugs are about the only available diabetic regimens [7]. Although the available medications are effective for correcting initial onslaught of diabetic related disorders, severe-to-late onset diabetic complexities appear in many infected persons [8]. Therefore, on a solution finding adventure for diabetes mellitus, medicinal plants are deployed as panacea [86].

Currently, medicinal plants have been actively involved in the management and treatment of diabetes, mostly in developing countries where most persons may not have access to conventional and synthetic antihyperglycaemic agents [9]. In the developed world, the use of antihyperglycaemic agents made from herbs have dropped after the initiation of insulic-based and manufactured oral glucose-lowering medications in course of the 20th centenary. Contrarily, in the advanced territories, lately, there are being renewed attentiveness to natural products which carry glucose reducing action [10]. The renewed interest in herbal glucose-reducing medications in evolved territory is taken to have been activated by a few circumstances like damaging effect on organs of the biological system, heightened derivative non-performance level and expense for artificial glucose-lowering medication [11]. The World Health Organization has recommended the use of medicinal plants for the management of diabetes and further encouraged the expansion of the frontiers of scientific investigation of hypoglycaemic property of diverse plant species [12]. Consequently, estimates indicates that 425 – 642 million of the global population use traditional medicine for the management and alleviation of diabetes and its metabolic complications [13].

Borassus aethiopum Mart. (family: Arecaceae), a plant species of Borassus palm, is mostly known as African fan palm [14], *Muruchi* (Hausa, Northern Western Nigeria), *odo* (Igala, North Central, Nigeria) and *oku* (Tiv, North Central, Nigeria) [15]. A typical form of *B. aethiopum* is a solitary palm of 25 m in height and 1m in width towards the bottom [16]. It is widely cultivated in Nigeria, predominantly in Kaduna State and part of Nasarawa State, Benue, Adamawa and Niger States where they used as major food source, providing edible fruits and also a number of pharmacological uses [17]. Previously reported properties of *B. aethiopum* fruits include antioxidative and free radical-scavenging property [18], sensory [19], physicochemical [20], antiplasmodial [21], phytochemical [22], antimicrobial [23] and antidiabetic [24].

Until now however, despite a Ghanian study by [24] on the antidiabetic activity of aqueous fruit extract of *B. aethiopum* in alloxan-induced diabetic rats at 100, 250 and 500 mg/kg body weight, studies that have comprehensively validated the antidiabetic property of the *B. aethiopum* fruit in diabetic animals at the ethnobotanically derived doses of 25, 50 and 100 mg/kg body weight in Nigeria, is absent to the best of our knowledge in the open scientific literature. Sequel to this, the present work was designed to investigate, for the very first time in Nigeria, the anti-hyperglycemic activity of *B. aethiopum* fruit in diabetic rats.

Plate 1. *Borassus aethiopum* tree with fruits. **Credit:** Konstantin Konig.

2. Materials and methods

2.1. Source of materials

2.1.1. Medicinal plant and verification

Fresh *Borassus aethiopum* fruits were collected from Jere, along Kaduna road, Kagarko Local Government Area in Southern Kaduna State, Nigeria. It was identified and authenticated by Mr. Lateef A. Akeem of the Herbarium and Ethnobotany Unit, Medicinal Plant Research and Traditional Medicine (MPR & TM) Department, National Centre for Research and Technological Development (NIPRD), Idu, F.C.T-Abuja, Nigeria where a Voucher Sample Identifier (NIPRD/H/7257) was placed at the Botanical garden for future use. The *Borassus aethiopum* tree with fruits is shown in Plate 1.

2.1.2. Animals

Albino rats of Wistar strain (147.28 ± 2.56 g) was obtained from the Zoological Garden of the National Institute for Veterinary Management, Jos, Nigeria. The animals which were kept in metallic compartment positioned in adequaely ventilated cage (Heat: 28–31°C; Light: 12 hours; Humidity: 50–55%) were allowed to feed on rat pellet (Excel Poultry Feeds, Shop 14, Powa Plaza, Asokoro, Abuja, Nigeria) and clean water.

2.1.3. Assay kits, chemicals and medications

The assay kit for serum total cholesterol were brands of Randox Laboratories Ltd., Co-Antrim, London. Glucose inducing compound (Alloxan) and metformin as well as other chemicals were products of Alloxan Daffodils Lab Private Limited Company; Uttar Pradesh, India; and product of Merck Sante, France respectively.

2.1.4. Glucose measuring device andtest strips

One Touch Glucose Examination Machine and One Touch Verio® Strips were products of LifeScan IP Holdings, LLC and SinoCare Company Ltd, Guyuan Road, Hitech Zone, Changsha, Hunan, China respectively.

2.2. Methods

2.2.1. Preparation of ethanolic fruit extract of Borassus aethiopum

Fresh fruits of *Borassus aethiopum* were air-dried under room condition till a constant weight was obtained. This was pulverized with an electric blender (Jiangmen Homemaster Electric Motors and Appliances Manufacturing Company Limited, Guangdong, China). A known weight of about 200 g of the powdered sample was extracted in 1200 mls of graded ethanol for a three-day period at room temperature. Filtration of the extract was done with a Whatman Number 1 porous paper with the corresponding mixture evaporated in a Rotary Evaporator (Model: BE-74E, Bioevopeak Co., Ltd, 17th Floor, Mingsheng Building, High-Teach Zone, Jinan City, China), with some ethanol recovered. The mixture was further transferred into steam Lab bath (Model: KL-9325S, Mobile BSL Laboratories, 4 Sunshine Blvd, Ormond Beach, FL32174, USA) where it was exposed to dryness yielding bluish-green residue (extract). The extract was thereafter mixed with a little quantity of water to generate the needed dose levels (25, 50 and 100 mg/kg body mass) used in this study.

The 25 and 50 mg/kg body weight of the extract correspond respectively, to a table spoon and a handful of the plant powder estimated to be consumed by an adult of 70 kg as a remedy for diabetes. The 100 mg/kg body weight of the extract which is quadruple-fold of the least dose was used to account for cases of 'abuse' by the users.

2.2.2. Qualitative and quantitative analysis of ethanol fruit extract of Borassus aethiopum

Qualitative investigation of secondary metabolite constituent to determine the presence of flavonoids/tannins, saponins, anthraquinones, alkaloids, phenolics, phlobatannins, cardiac glycosides, steroids and terpenoids were done using methods variously described by Trease and Evans, [25]; Odebiyi and Sofowora, [26]; Walls et al., [27]; Harborne, [28]; Ganesan and Bhatt, [29]; Awe and Sodipo, [30]; El-Olemy et al., [31]; Sofowora, [32]; Edeoga et al., [33] separately.

Quantitative evaluation of secondary metabolite component to estimate the quantity of flavonoids, saponins, anthraquinones, alkaloids, phenolics, phlobatannins and cardiac glycosides available were analyzed using the protocol variously described by Boham and Kocipai, [34]; Obadoni and Ochuko, [35]; El-Olemy et al., [31]; Adeniyi et al., [36]; Makkar et al., [37]; Swain, [38] and Van-Burden and Robinson, [39] individually.

2.2.3. Induction of diabetes mellitus and glucose determination

After an eight-hour period of fasting (without food intake, but water), glucose level of the animals was examined before the treatment of alloxan by drawing blood specimen (0.5 μL) from the pointly lacerated tail vein and dropping it on a test strip that had beeninsrted in a glucose measuring device. Afterwards, Alloxan (140 mg/kg body mass), in isotonic solution, was given intraperitoneally to induce diabetes in the experimental models [40]. Blood glucose was then determined after 48 hours of alloxan treatment. Moreover, 50 minutes after the injection of alloxan, the animals were made to have their food and 4% dextrose in a feeding bottle to overcome the initial hypoglycaemic phase [41]. Only rats with glucose range of more than 190 mg/dL were declared to have come down with diabetes and used for the antihyperglyaemic study [42].

2.2.4. Animal categorization and extract administration

Thirty-six Wistar rats were arranged in six groups (i-vi) of six as outlined below:

Category i: (Non-diabetic rats) + 0.4 mls of distilled water
Category ii: Diabetic rats + 0.4 mls of distilled water
Category iii: Diabetic rats + Metformin (50 mg/kg body weight)
Category iv: Diabetic rats + 25 mg/kg body weight of ethanol fruit extract of *B. aethiopum*
Category v: Diabetic rats + 50 mg/kg body weight of ethanol fruit extract of *B. aethiopum*
Category vi: Diabetic rats + 100 mg/kg body weight of ethanol fruit extract of *B. aethiopum*

The animals took daily oral gavage of the extract for 14 days when parameters includng blood glucose status, body weight, food and fluid consumption of the animals were estimated.

2.2.5. Measurement of weight, dietary and fluid intake of animals

The weight of the diet and quantity of water were estimated every hour they were to be administered to the rats. About 50 minutes after, the remaining food was measured and the quantity of water measured. The difference between the first and last weight of the food and quantity of water, calculated for per day were taken as the day's dietary intake and water intake respectively. Furthermore, the weight of the rats was taken separately prior to the start of the experiment at a space of 5 days for a 15-day investigative period to get the difference in body weight of the animals [43].

2.2.6. Collection of blood, composition of serum and tissue supernatant

The experimental animals were made unconscious in a glass jar that contains cotton fiber saturated in ethyl acetate. Blood (3 mls) was taken into bottles that contain Ethylene Diamine Tetraacetic Acid (EDTA) for the determination of haematology indices. Blood (2 mls) which were taken from rats into plain sample bottles were separated using a centrifuge (Farfly Energy Technology Co., Ltd, Model: ZJY-3, ShangHai, China) at 2000 gram for 10 minutes with serum obtained used for the estimation of biochemical analysis. Animals were dissected and the pancreas and liver were excised, measured, homogenized and centrifugation carried out (2500 gram at 20 minutes) while the filtrate obtained was refrigerated for 13 hours until it is being used for the estimation of some biomedical markers.

2.2.7. Assessment of haematological indices

Blood collected in EDTA containers were assessed for red blood cell (RBC) and their differentials which include: hemoglobin (Hb), and packed cell volume (PCV) as well as leucocytes (WBC) and their indices including: platelet (PLT), and lymphocytes (Lym) with the use of Auto Haematology Analyzer (Model RT-7200, Rayto Life & Analytical Sciences Co., Ltd, China).

2.2.8. Estimation of some serum biological molecules and hepatic enzymatic activities

The levels of selected biological molecules were ascertained using the protocol earlier handed down for serum creatininal level [44], serum ureal content [45], serum albumin concentration [46], serum cholesterol status [47] and pancreas insulinic range [48]. The enzymatic activity of liver hexokinase and glucose-6-phosphatase of the animals were measured following the protocol of Brandstrup et al. [49] and Koide and Oda [50].

2.2.9. Histological investigation of organ

The pancreas of the rats were fixed in 10% (v/v) formaldehyde, dried via ascending grades of ethanol (70%, 85% and 95% v/v), cleaned in xylol and immersed in paraffin wax (melting point 54°C) [51]. Segments of organs were then produced following templates of [52] and wet using haematoxylone/eosin (H&E). The histological slides were observed under a light Led camera magnifier (MIGHTEX, Model: EZ42, Pleasanton, CA 94566, USA). Pictures of the pancreas were captured at x200 via Canon! Microsoft Image Arrangement software (Model: Capture Strength A3600, Germany).

2.2.10. Data analysis

Results were presented as the Average ± S.E.M of six estimations. One Way ANOVA as well as Tukey's Multiple Range Test were used to determine the statistical significance of difference in indices within groups using SPSS version 23.0 Software. The significant level was taken at $p < 0.05$.

Table 1
Secondary molecular content of ethanol fruit extract of *Borassus aethiopum*

Secondary Molecule (mg/ml)	Observation	Mean ± SEM (mg/ml)
Flavonoids	Dark yellow precipitate with NH_3	24.04 ± 0.03
Alkaloids	Bluish coloration with Mayer's reagent	11.92 ± 0.01
	Greenish-brown with Wagner's reagent	
Cardiac glycosides	Brown violet circle at the middle	8.98 ± 0.01
Anthraquinones	Bright pink colouration with NH_3	6.75 ± 0.01
Saponins	Steady, constant froth with distilled water	3.35 ± 0.01
Phlobatannins	Bluish-brown colour with H_2SO_4	0.87 ± 0.02
Phenolics	Greenish precipitate with $FeCl_3$	0.35 ± 0.00
Tannins	Absence of greenish-brown precipitate	Not detected
Steroids	Absence of violet to black or green colour with H_2SO_4	Not detected
Terpenoids	Absence of bluish-red colour with H_2SO_4	Not detected

Data are Average ± SEM; $n = 3$; SEM = Standard Error of Mean.

Table 2
Glucose status (mg/dL) of alloxan-induced diabetic rats after oral gavage of ethanol fruit extract of *Borassus aethiopum*

			Treatment Days			
Treatment Group	Basal BG	BG Prior To Treatment	Day 4	Day 8	Day 12	Day 15
NR + DW	77.14 ± 0.14^a	76.24 ± 0.22^a	77.34 ± 0.68^a	78.62 ± 0.42^a	75.92 ± 0.01^a	76.24 ± 0.87^a
DR + DW	79.38 ± 1.26^a	253.87 ± 3.71^b	268.43 ± 3.72^b	281.76 ± 3.19^b	298.72 ± 3.48^b	305.22 ± 4.16^b
DR + Met.	77.39 ± 2.03^a	236.34 ± 4.25^c	206.72 ± 5.86^c	157.22 ± 2.70^c	92.44 ± 0.73^c	76.05 ± 0.39^a
DR + 25 mg/kg of extract	78.42 ± 1.27^a	258.34 ± 5.24^d	254.02 ± 4.28^d	142.08 ± 2.37^d	89.67 ± 0.94^d	79.08 ± 2.14^a
DR + 50 mg/kg of extract	77.65 ± 1.78^a	268.05 ± 6.27^e	250.78 ± 3.05^d	133.71 ± 1.52^e	90.28 ± 0.75^d	78.45 ± 0.23^a
DR + 100 mg/kg of extract	76.02 ± 1.32^a	265.74 ± 3.89^d	257.80 ± 4.39^e	118.24 ± 1.48^f	83.74 ± 0.54^e	76.39 ± 0.45^a

NR = Normal Rat; **DR** = Diabetic rats; **DW** = Distilled water; **Met.** = Metformin; **BG** = Blood Glucose. Results are mean ± SEM of six values. Test figures with superscript not the same with in relation to their individual control down the column for per period are significantly different ($p < 0.05$).

3. Results

The qualitative test of phytochemicals in ethanol fruit extract of *Borassus aethiopum* showed flavonoids, saponins, anthraquinones, alkaloids, phenolics, phlobatannins and cardiac glycosides while tannins, steroids and terpenoids were absent (Table 1). Quantitative evaluation of ethanol fruit extract of *B. aethiopum* revealed that flavonoids had the highest quantity (24.04 ± 0.03), saponins (3.35 ± 0.01), phlobatannins (0.87 ± 0.00), anthraquinones (6.75 ± 0.01), cardiac glycosides (8.98 ± 0.01), alkaloids (11.92 ± 0.02) and phenolics had the least amount (0.35 ± 0.00) (Table 1).

The results indicated that all the rats that took with 150 mg/kg body weight of alloxan came down with diabetes 72 hours after with blood glucose status spanning through 236.34 ± 4.25 to 268.05 ± 6.27 (Table 2). The blood glucose range of the rats that took alloxan and distilled water substantively *($p < 0.05$)* rose from 79.38 ± 1.26 to 305.22 ± 4.16 mg/dL. Treatment with ethanol fruit extract of *Borassus aethiopum* at the dosage levels of 25, 50 and 100 mg/kg body mass exceptionally *($p < 0.05$)* lowered the sugar level slowly till the end of the observative treatment duration. Treatment of alloxan-induced diabetic animals at all dosal rate of the extract (25, 50 and 100 mg/kg body mass) notably *($p < 0.05$)* lessened the sugar range in a pattern liken with the un-diabetic control

Table 3
Body weight of alloxan-induced diabetic rats at the end of oral gavage of ethanol fruit extract of *Borassus aethiopum*

		Body Weight (g)			
Treatment Group	Initial Weight	Day 1	Day 5	Day 10	Day 15
NR + DW	162.34 ± 2.98^{a}	165.73 ± 1.72^{a}	172.48 ± 2.45^{a}	189.48 ± 3.01^{a}	193.44 ± 3.93^{a}
DR + DW	157.82 ± 1.96^{b}	154.72 ± 1.03^{b}	146.30 ± 0.77^{b}	137.47 ± 0.02^{b}	129.78 ± 1.49^{b}
DR + Met.	153.79 ± 1.56^{c}	157.92 ± 4.33^{c}	161.92 ± 2.28^{c}	169.44 ± 2.79^{c}	175.21 ± 2.85^{c}
DR + 25 mg/kg of extract	147.23 ± 1.75^{d}	150.89 ± 1.08^{d}	158.49 ± 1.44^{d}	162.69 ± 2.85^{d}	166.23 ± 1.59^{d}
DR + 50 mg/kg of extract	148.18 ± 1.48^{d}	160.34 ± 1.74^{e}	164.34 ± 2.65^{c}	168.63 ± 1.35^{c}	172.41 ± 2.23^{c}
DR + 100 mg/kg of extract	146.32 ± 1.89^{d}	149.69 ± 1.35^{d}	153.45 ± 1.82^{e}	159.32 ± 2.73^{e}	168.74 ± 1.46^{d}

Values are Average ± SEM of six parameters. Data having subheading varying with their respective control to the bottom of the column each day are essentially non-identical ($p < 0.05$).

Table 4
Impact of ethanolic fruit extract of *Borassus aethiopum* on a few biological molecules and hepatic carbohydrate metabolizing enzyme activity of alloxan-induced diabetic rats

Treatment Group	Mass of Pancreas (g)	Pancreas Insulin (μIU/ml)	Urea (mg/100 ml)	Creatinine (mg/100 ml)	Albumin (g/L)	Total Chol. (mmol/L)	Hexokinase (units/g protein)	Glucose-6-Phosphatase (U/mg protein)
NR + DW	0.72 ± 0.23^{a}	11.72 ± 0.24^{a}	114.34 ± 4.23^{a}	$4.6.87 \pm 0.03^{a}$	15.37 ± 0.04^{a}	10.84 ± 0.06^{a}	19.42 ± 0.02^{a}	5.67 ± 0.03^{a}
DR + DW	0.45 ± 0.42^{b}	3.05 ± 0.03^{b}	195.65 ± 1.47^{b}	12.52 ± 1.25^{b}	32.14 ± 0.85^{b}	22.14 ± 0.03^{b}	7.24 ± 0.04^{b}	18.45 ± 0.68^{b}
DR + Met.	0.63 ± 0.03^{a}	9.36 ± 0.30^{a}	116.92 ± 4.76^{a}	5.26 ± 0.14^{a}	15.85 ± 0.21^{a}	12.55 ± 0.02^{a}	20.54 ± 0.13^{a}	5.16 ± 0.12^{a}
DR + 25 mg/kg of extract	0.60 ± 0.01^{a}	11.67 ± 0.45^{a}	128.34 ± 2.14^{c}	5.62 ± 0.05^{a}	15.53 ± 0.06^{a}	11.74 ± 0.26^{a}	19.26 ± 0.05^{a}	5.25 ± 0.04^{a}
DR + 50 mg/kg of extract	0.78 ± 0.04^{a}	13.75 ± 0.35^{a}	136.18 ± 1.48^{d}	5.33 ± 0.25^{a}	14.69 ± 0.05^{a}	12.54 ± 0.05^{a}	19.56 ± 0.71^{a}	4.97 ± 0.01^{a}
DR + 100 mg/kg of extract	0.76 ± 0.02^{a}	14.37 ± 0.29^{c}	115.85 ± 0.96^{a}	5.82 ± 0.62^{a}	16.02 ± 0.35^{a}	12.30 ± 0.16^{a}	20.14 ± 0.03^{a}	5.08 ± 0.02^{a}

Figures are mean ± SEM of six indices. Results having subtitle that differ from their individual control down the column are statistically not the same ($p < 0.05$). **Chol.** = Cholesterol.

animals and the diabetic rats that took metformin (Table 2). On day 15, there was no statistical difference in blood glucose between the three dose levels and their respectively basal BG, although the 100 mg/kg body weight of the extract produced the best glucose reducing ability (Table 2).

A gradual decrease in body weight of distilled water treated diabetic animals, throughout the 15 days investigation period, when matched with the non-diabetic control untreated rats (Table 3). In variance however, the extract at all dose levels (50, 100, 200 mg/kg body weight) substantively *($p < 0.05$)* elevated the body weight of the rats when placed side to side with the distilled water treated diabetic rats (Table 3).

When compared with the non-diabetic control rats, the levels of serum albumin, urea, creatinine and total cholesterol statistically *($p < 0.05$)* rose in the diabetic-induced animals (Table 4). On the contrary, treatment with ethanol fruit extract of *B. aethiopum* at the three dose levels meaningfully *($p < 0.05$)* brought down these biological substances in a manner that matched *($p > 0.05$)* well with those of the non-diabetic control rats as well as the diabetic rats that took metformin (Table 4).

When compared with the non-diabetic control rats, the administration of alloxan substantively *($p < 0.05$)* reduced the concentration of insulin in the pancreas of the animals. However, intake of the extract at 25 and 50 mg/kg body weight sufficiently *($p < 0.05$)* heightened the pancreatic insulin level in a way that had no statistically significant *($p > 0.05$)* change occurring in the distilled water treated diabetic rats and diabetic-induced rats

Table 5
Haematological indices as well as food and water intake of alloxan-treated diabetic rats after oral gavage of ethanolic fruit extract of *Borassus aethiopum*

Parameter	NR + DW	DR + DW	DR + Met.	mg/kg body weight of the extract		
				DR + 25 mg/kg of extract	DR + 50 mg/kg of extract	DR + 100 mg/kg of extract
RBC ($x10^6/\mu L$)	0.23 ± 0.03^a	0.05 ± 0.01^b	0.22 ± 0.01^a	0.34 ± 0.02^c	0.33 ± 0.01^c	0.19 ± 0.01^d
PCV (%)	62.75 ± 0.02^a	44.30 ± 0.03^b	61.56 ± 0.02^a	63.05 ± 0.03^a	61.97 ± 0.41^a	62.70 ± 0.02^a
Hb (g/dL)	17.81 ± 0.05^c	7.36 ± 0.35^b	16.96 ± 0.03^a	21.48 ± 0.04^c	22.95 ± 0.02^c	20.88 ± 0.03^c
WBC ($x10^3/\mu L$)	32.92 ± 0.34^a	8.27 ± 0.30^b	13.96 ± 0.28^c	17.32 ± 0.12^d	17.82 ± 1.22^d	22.04 ± 0.42^e
PLT ($x10^3/\mu L$)	422.35 ± 1.92^a	56.23 ± 0.25^b	27.41 ± 0.55^c	134.11 ± 0.34^d	327.04 ± 0.82^e	164.45 ± 0.78^f
Lym ($x10^3/\mu L$)	9.62 ± 0.75^a	2.45 ± 0.02^b	6.55 ± 0.03^c	13.72 ± 0.15^d	22.62 ± 0.04^e	17.11 ± 0.33^f
Feed Intake (g)	46.50 ± 0.30^a	126.24 ± 1.25^b	45.60 ± 1.63^a	63.35 ± 1.73^c	47.03 ± 0.53^a	46.36 ± 0.56^a
Water Intake (ml)	84.16 ± 0.23^a	214.50 ± 2.05^b	85.46 ± 1.45^a	90.62 ± 0.67^c	135.63 ± 1.63^d	96.55 ± 0.76^e

Results are mean ± SEM of six estimations. Figures depicting superscript in disparity with those of control across the row are substantively different ($p < 0.05$). **RBC** = Erythrocytes; **PCV** = Packed cell volume; **Hb** = Hemoglobin; **WBC** = Leucocytes; **PLT** = Platelet; **Lym** = Lymphocytes.

treated with metformin. Although the 100 mg/kg body mass of the extract increased pancreatic insulin level, but did not compare *($p > 0.05$)* well with the distilled water treated diabetic rats and animals that took metformin (Table 4).

The administration of alloxan glaringly *($p < 0.05$)* lowered the weight of pancreas in the distilled water treated diabetic animals when juxtaposed with the non-diabetic control rats. The weight of pancreas in alloxan-treated diabetic rats that took 25, 50 and 100 mg/kg body weight of the extract did not affect *($p > 0.05$)* those of both the non-diabetic control rats and the metformin treated diabetic rats (Table 4).

The activity of hexokinase in the liver of the animals was remarkably *($p < 0.05$)* lowered just after alloxan intake in the diabetes-distilled water treated rats when weighed with the non-diabetic control rats. However, extract administration at all dosal levels markedly *($p < 0.05$)* heightened the liver hexokinase activity of the animals in way that matched up *($p > 0.05$)* adequately with the non-diabetic control rats and the diabetic animals that took metformin (Table 4).

Alloxan administration exceptionally *($p < 0.05$)* elevated the hepatic glucose-6-phosphatase activity in the diabetic-distilled water treated rats when weighed with the non-diabetic control animals. Treatment of the diabetic rats with all extract doses notably *($p < 0.05$)* lowered hepatic glucose-6-phosphatase action in a manner that did not affect *($p > 0.05$)* the non-diabetic control animals and the diabetic rats that received metformin (Table 4).

The results obtained in RBC, Hb and PCV in the diabetes-distilled water treated rats were appreciably *($p < 0.05$)* lowered when stacked up against the non-diabetic control rats. In variance however, treatment with the ethanol fruit extract of *B. aethiopum* at 25, 50 and 100 doses substantially *($p < 0.05$)* increased the levels of RBC, Hb and PCV when juxtaposed with diabetes-distilled water treated rats. Treatment of diabetic animals with metformin did not significantly *($p > 0.05$)* affect the outcomes of RBC Hb and PCV when measured up with the non-diabetic control rats (Table 5).

Alloxan administration remarkably *($p < 0.05$)* lowered the levels of white blood cells, platelets and lymphocytes in the distilled water treated diabetic animals in comparison with the non-diabetic control rats. Extract administration significantly *($p < 0.05$)* elevated the level of white blood cells and their differentials when juxtaposed with the distilled water treated diabetic animals. Treatment of diabetic-induced rats with metformin glaringly *($p < 0.05$)* raised the level of white blood cells and lymphocytes, while it decreased platelet level, when stacked up with the distilled water treated diabetic rats (Table 5).

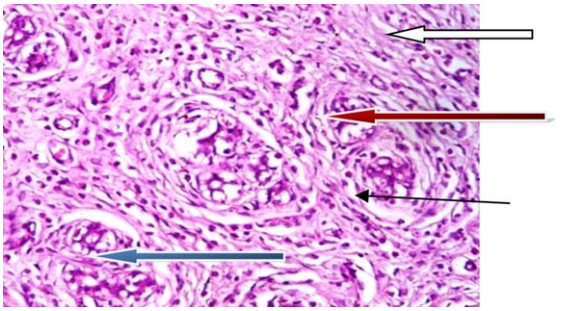

Plate 2. Picture of pancreas of rat that took distilled water (x200; H & E). Normal anatomy, the medullary of the pancreas shows normal submucosal acinose and zymogenic organs (Slender indicator), the interlobular connective tissues appear severely thickened and fibrotic (Blue pointer). There are broad septa noted as well as thickened and necrotized duct (Red arrow). There is intact package. Langerhans islets (White indicator) consisting circular to ovoid arrangement of gonadal cells.

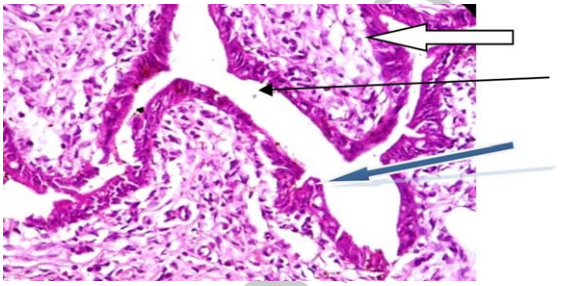

Plate 3. Cross section of the pancreas of diabetic animal that was given distilled water (x200; H & E). Disintegrated structure, the lobule of the pancreas showing disrupted submucosal acinose and zymogenic tissues (Slender indicator) consisting little granular eosinophilic cytosol, deranged interlobular joining tissues (Blue arrow) and septa (Red signal) are noticed. There are variously bad Langerhans islets compartment (White pointer) consisting of spherical to oval array of exocrine tissues.

Administration of alloxan notably $(p < 0.05)$ elevated the feed and water intake in the distilled water treated diabetic rats when compared with the non-diabetic control rats. Contrarily, treatment of diabetic rats with the extract at all the dosal levels meaningfully $(p < 0.05)$ decreased food and water ingestion. Treatment of diabetic animals with metformin although reduced feed and water intake, produced figures that did not compare $(p > 0.05)$ well with those of the distilled water treated control animals (Table 5).

Histological investigation indicated necrotic beta tissues of the Langerhans islets without patterns of endocrine cells of the pancreas in the diabetic-induced rats that took distilled water (Plate 2), when placed side by side with that of the non-diabetic control animals (Plate 2). It also showed degenerated tissues of the pancreas with damaged submucosal acinose and zymogenic organ segments having a few granular eosinophilic cytoplasm as well as damaged interlobular connective tissues (Plate 3). Treatment of diabetic-induced rats with all dosal levels of the ethanol fruit extract of *B. aethiopum* at 25, 50 and 100 mg/kg body weight showed normal compartment of beta cells of the Langerhans islets. Furthermore, intact mesenchyme pancreas, unaffected submucosal acinose as well as zymogenic tissues were observed (Plates 5, 6, and 7). The pancreas of metformin treated diabetic rats showed patterns that matched well with those of the diabetic rats that took the extract (Plate 4).

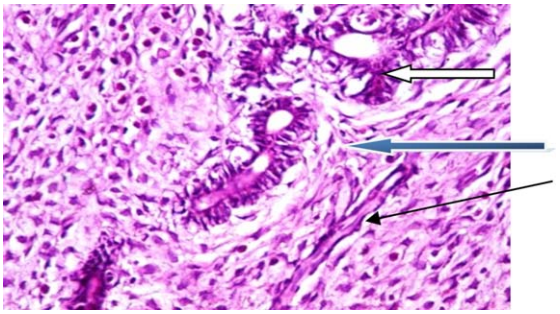

Plate 4. Photomicrograph of pancreas of diabetic animal that took metformin (x200; H & E). Normal anatomy, the epithelium of the pancreas Displaying standard submucosal acinose and zymogenic organs (Slender indicator), orderly interlobular conjoining organs (Blue arrow) and septa (Blue pointer) is seen. There are regular packaged Langerhans islets (White arrow) containing of disc-shaped to egg-shaped assemblage of holocrine tissues.

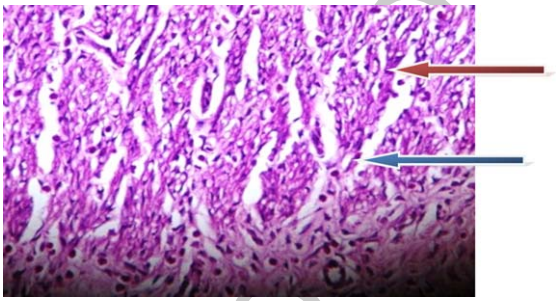

Plate 5. Micrograph of pancreas of diabetic rat that received 25 mg/kg body weight of extract (x200; H & E). Regular anatomy within the vasculature of the pancreas, there are stock submucosal acinose and zymogenic organs, typical interlobular conjoining organs (Blue pointer). There are routine compact of Langerhans islets (Red indicator) containing of spherical to ovoid arrangements of merocrine tissues.

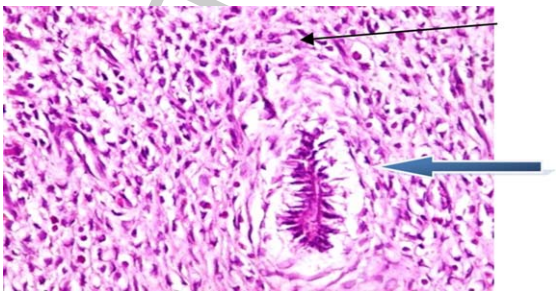

Plate 6. Pictoral arrangement of pancreas of diabetic animals that took 50 mg/kg body weight of the extract (x200; H & E). Standard anatomy, the stroma of the pancreatic organ revealing normal submucosal acinose and zymogenic cells (Slender indicator) containing plenty granular eosinophilic cytosol, unaffected interlobular connective organs (Blue bolt). There are good packaged Langerhans islets (White pointer) comprising of circular to ovoid packages of hepatobiliary organs.

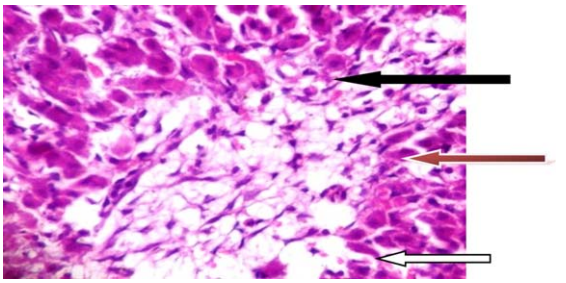

Plate 7. Cross section of pancreas of diabetic-induced rats given 100 mg/kg body weight of extract (x200; H & E). Normal antomical make-up, the epithelium of the pancreas showing orderly submucosal acinose and zymogenic tissues (Red shaft), good interlobular conjoining tissues (Black indicator). There are robust compact Langerhans islets (White pointer) consisting of disc-shaped to ovoid segments of luteal tissues.

4. Discussion

The present study has scientifically proven the Nigerian local claim in the usage of *B. aethiopum* fruit as a therapeutic regimen against diabetes by expressing their glucose lowering property via stimulation of pancreatic insulin secretion, improved glucose take-up and reversal of alloxan-treatment alterations in the diabetic rats.

The elevated sugar level of the distilled water treated diabetic animals may be attributed to massive reduction in insulinic release out of the fragmental beta cells of the Langerhans islets of the pancreas, thus causing diabetes. The reduction of elevated blood glucose level by the ethanol fruit extract of *B. aethiopum* suggest antihyperglycaemic property of the plant extract. Although, the other doses of the extract also produced glucose-reducing effect, the most effective was at the highest dose of 100 mg/kg body weight. The extract showed an insulin-like action by lowering the increased blood glucose concentration, stimulating glucose uptake by peripheral organs or increasing insulin generation by the pancreas from regenerated β-cells and/or by reversal of alloxan-treatment changes in the animals.

The weight loss in distilled water treated diabetic animals may be adduced to low glycaemic control leading to muscle wasting [53]. The excessive degradation of dietary protein to yield glucogenic amino acid for gluconeogenesis during insulin insufficiency leads to muscle wasting and weight loss in distilled water treated diabetic rats [53]. The improvement in body weight by the extract, in this investigation, may be attributed to the ability of the extract to regulate muscle atrophy [54].

Biological molecules like cholesterolic lipid, ureal range, creatininal level, albumin status are markers to diabetes-related complications [55]. The elevation of these biological molecules, in the present study, in the serum of the distilled water treated diabetic rats may be adduced to insulin deficiency following induction of hyperglycaemia by alloxan. The reduction in the levels of these biological molecules in the exract treated diabetic animals suggests the extract's insulin stimulatory activity evidenced by its ability to normalize some complications usually connected with diabetes consequently reducing the levels of these biological molecules [56].

The reduced pancreatic weight in diabetic rats, in this study, could be ascribed to a reduction in quantity of secretory granules of the Leydig tissues of the pancreas [57]. The increased pancreatic weight after treatment of diabetic rats with the extract may have occurred because of its ability to restore the normal architecture of the secretory granules by producing values that matched well with those of the non-diabetic distilled water control rats as well as those of the metformin-treated diabetic rats. Also, the extract controlled the onset of pancreatic degeneration, a distinctive feature of diabetes.

Diabetes mellitus is connected with increased concentration of blood cholesterol and its associated lipids consequently accounting for hypertension and other metabolic disorders of cholesterol metabolism [58].

The increase in serum cholesterol in diabetics is linked with hypercholesterolemia owing to its inhibitory activity on lipoprotein lipase to degrade lipids [59]. Therefore, the elevated serum total cholesterol in the diabetic animals, in this investigation, may be adduced to deactivation of lipoprotein lipase to catabolize lipids leading to increased serum cholesterol [60]. The reduced level in serum total cholesterol by the extract may also be attributed to its ability to regulate complications concerned with diabetes by amending abnormalities of lipid metabolism [59].

Insulin is a peptide hormone released by the beta cells of pancreas in response to elevated blood sugar level [61]. Insulin enhances body tissues to use-up glucose from the blood for their daily energy needs. When insulin is absent or low, glucose utilization from the blood by body organs is inhibited, consequently facilitating accumulation of blood glucose predisposing to hyperglycemia [57]. In this investigation, the reduced pancreatic insulin concentration by alloxan may be adduced to selective cytotoxic effect of the substance on pancreatic β-cells, consequently affecting endogenous insulin secretion hence elevated blood sugar level [62]. The increased pancreatic insulin level observed in this work may be ascribed to the ability of the extract to increase insulin secretion by the pancreas possibly from the regenerated β-cells [61]. The reduction in elevated levels of biological molecules after extract administration to diabetic rats may be adduced to the ability of the extract to normalize a some of the metabolic complexities mostly associated with diabetes mellitus.

Insulin increases liver glycolytic processes by accelerating the action and quantity of many major enzymes including hexokinase. Hexokinase is notably found in all cell types [63], where it catalyzes the phosphorylation of glucose to glucose-6-phosphate by ensuring glucose balance, storage and excretion [64]. The decrease in hepatic hexokinase activity in the diabetic-distilled water treated rats, in this investigation, may be adduced to insulin insufficiency, owing that insulin facilitates and activates hexokinase activity as well as the deceleration of liver glycolytic procedure, which appropriately hamper glucose usage/take-up, subjecting the animals to diabetes [65]. Treatment of diabetic rats with the extract, which elevated liver hexokinase activity, might be ascribed to the extract ability to enhance insulin release, which would trigger hexokinase, hence elevating hepatic glucose uptake for energy propagation, and duly diminishing hepatic glucose volume [66]. Glucose-6-phosphatase is a key member of the foremost catalyst for balancing gluconeogenesis [67]. Scientific investigation has observed increased glucose-6-phosphatase activity in diabetics [67]. In this work, the increased liver activity of glucose-6-phosphatase in the distilled water treated diabetic rats might be attributed to insulin inadequacy. Insulin reduces gluconeogenesis by reducing the activity of the key enzyme, glucose-6-phosphatase [69]. The increase may also imply activation or elevated biosynthesis of the enzyme mainly involved in boosting glucose generation during diabetes in the liver of the animals. In contrast, the reduced enzyme activity following treatment of diabetic rats with the extract may be due to elevated insulin secretion, which is obligated for the suppression of the gluconeogenic crucial enzymes [67]. The observed balance of the action of the liver enzyme by the extract implies that the extract displayed glucose lowering tendency by enhancing blood glucose uptake thereby correcting impaired liver glycolysis and by slowing down its gluconeogenic formation [70].

The reduced level of RBC, Hb and PCV in diabetic rats represent an anaemic condition [71]. The early stage of anaemia in diabetes had since been severally reported to increase non-enzymatic glycosylation of erythrocyte membrane proteins which corresponds to diabetes [72]. The oxidation of these glycosylated membrane proteins and hyperglycaemia in diabetes mellitus leads to elevation of RBC and PCV by the extract, a process responsible for elevated rate of erythropoiesis. The increased Hb level by the extract may be adduced to increased oxygen-carrying capacity of the blood and amount of oxygen released to the tissues. The amelioration of elevated RBC, Hb and PCV levels suggests that the extract have the capacity to normalize haematological abnormalities mostly associated with diabetes.

Alloxan, a urea derivative with carcinogen and cytotoxic glucose analog, chemically known as 5,5-dihydroxyl pyrimidine-2,4,6-trione, has been reported to be an organic compound for suppressing the internal defence mechanism by damaging WBC and other related body tissues [73]. Alloxan administration which reduced WBC count, in this study, may be ascribed to inhibition of leukocytosis from the bone marrow which may account for poor internal defense system against infection. Consequentially, they might have effect on the immune system and phagocytic activity of the experimental animals [74]. The reduction in WBC count following extract

administration might be adduced to improved defensive architecture against microorganism invasion resulting in increased body protection against microbial agents as well as a boost in the immune system. The increased platelet level by the extract may imply better blood clotting capacity and stimulatory effect on thrombopoietin [74]. The increased lymphocyte level by all extract doses suggests stimulatory effect on the immunoregulatory tissues of the white corpuscles, since lymphocytes are the key stimulatory organs of the immune system. The elevation of reduced WBC indices by the extract suggests its stimulatory activity on effector cells of the immune system in diabetic rats.

In the present work, the elevation of feed intake (polyphagia) in diabetic animals, by alloxan, may be attributed to stimulation of the release of ghrelin, the shunger hormone. Ghrelin is a peptide hormone released when the stomach is empty, thereby elevating appetite and excessive eating tendency in diabetics [75, 76]. The reduction in polyphagia by the extract may be ascribed to secretion of leptin, a hormone which decreases hunger thereby reducing feed intake in the diabetic treated animals [77]. The minimization in dietary ingestion seen in the existing investigation is indicative of the extract's capacity to regulate appetite via leptin secretion.

The characteristic rise in water consumption (polydipsia) of the diabetic animals, observed in this study, may be due to incresed secretion of antidiuretic hormone which stimulates thirst, thereby increasing water intake in the animals. The secretion of antidiuretic hormone from the pitituray gland to the kidney leads to failure of water to be absorbed back into the blood thereby elevating polydipsia and water intake in the diabetic animals [78]. The reduced water intake by the extract might be adduced to regulation in the release of antidiuretic hormone consequently reducing excessive thirst and water consumption in the animals [79].

The induction of diabetes by alloxan into rats have been variously reported to produce severe cytotoxic effect on the pancreas [80]. The β-cell cytotoxic action of alloxan is thought to be mediated by the inhibition of free radical scavenger-enzyme, superoxide dismutase. Consequently, this leads to diabetes mellitus and diabetes is associated with the generation of ROS causing to oxidative damage [81]. Therefore, the damage to histoarchitecture of the pancreatic β-cells by alloxan in the distilled water treated diabetic animals, in the present investigation, may imply low levels of the antioxidant enzyme superoxide dismutase (SOD), weakening the antioxidant defence mechanism and overwhelmed by redox imbalance resulting from over production of ROS [80]. Administration of all extract doses to diabetic animals repaired the damages caused to the pancreas by alloxan. The extract may have therefore acted by protecting the pancreatic β-cells through reduced oxidative stress or preservation of the pancreatic β-cells integrity [82].

The presence of bioactive agents in natural products have been reported in the management of diabetes [83]. Saponins may have a glucagon decreasing effect and may enhance glucose utilization as well as stimulate insulin release from the pancreas [84], probably by decreased degradation of glucagon-like peptides. Phenolics and flavonoids are validated to possess insulin secretive, free radical scavenging and insulinonematic activity [85].

Alkaloids may induce hypoglycaemia via inhibition of intestinal glucose uptake by inhibiting α-amylase and α-glucosidase activities [86]. Alkaloids have also been reported to enhance high glucose uptake in pancreatic β-cells of diabetics [87]. Therefore, the antidiabetic activity of the ethanol fruit extract of *B. aethiopum* may be attributed to the presence of phytoconstituents including alkaloids, phenolics, flavonoids and saponins which could perform their work either singly or synergistically as seen from the stimulation of glucose take-up and utilization of peripheral tissues or increasing insulin production by the pancreas from regenerated β-cells or reversal of alloxan-induced alterations in animals.

5. Conclusion

This investigation has scientifically validated the local use of *B. aethiopum* fruit in the management of diabetes by its phytomolecules which may have expressed their glucose lowering effect by stimulating pancreatic insulin secretion, glucose take-up/usage and reversing alloxan-treatment alterations in the diabetic rats. The bioactive principle in the fruit responsible for the antidiabetic property should be identified.

Ethic statements

This study was carried out after an Ethical Approval from the University of Abuja Ethical Review Committee by a letter referenced with protocol number UAECAU/2020/0013 and dated 15th September, 2021.

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Declaration of competing interest

The authors declare that they have no competing interests.

Researchers' participation

Origination and Blueprint: MDA.
Accession of Result: MDA.
Investigation and Elucidation of Figures: MDA and EDE.
Framing the Manuscript: MDA and EDE.
Correcting for Academic Satisfaction: MDA.
Last Acceptance of the Concluded Write-Up: MDA and EDE.

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