ANTIHYPERLIPIDEMIC ACTIVITIES AND HEMATOLOGICAL PROPERTIES OF ETHANOL EXTRACT OF BLIGHIA SAPIDA KOENIG BARK IN ALLOXAN-INDUCED DIABETIC RATS

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ANTIHIPERLIPIDEMIJSKA AKTIVNOST I HEMATOLOŠKE KARAKTERISTIKE ETANOLNOG EKSTRAKTA BLIGHIA SAPIDA KOENIG BARK KOD PACOVA SA DIJABETESOM INDUKOVANIM ALOKSANOM

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ABSTRACT

Blighia sapida (BS) has been shown to be rich sources of antioxidant, thus, we evaluated effects of B. sapida Koenig stem bark ethanol extract (BSE) on lipid metabolism and hematological indices in diabetes rats.

Thirty male rats were divided into six groups of five rats each. Diabetes was elicited by intraperitoneal injection of alloxan (65 mg/kg body weight) once and orally administered with glibenclamide (5 mg/kg), B. sapida extract (50, 100 and 150 mg/kg body weight (bw) once daily for 21 days. Serum lipid profile, markers of hepato-renal toxicity and hematological indices were examined using automated analyzer. Data were analyzed using one-way ANOVA and $p < 0.05$ was considered to be statistically different.

Diabetic untreated animals showed considerably elevated total cholesterol $p < 0.05$, also, significant increase in AST, ALT, ALP, urea and creatinine compared to control. Triglycerides, LDL-c, VLDL-c, AI and CRI decreased with extract administration and HDL-c increased considerable compared to untreated diabetic rats. Furthermore, significant lower hemoglobin (Hb) levels, packed cell volume (PCV), red blood cells (RBCs) levels, white blood cells (WBCs) compared to normal animals was recorded in the untreated group. These changes were returned to normal after the administration of extract 50, 100 and 150 mg/kg body weight. Hence, these effects were most prominent in the animals treated with 150 mg/kg body weight of B. sapida bark.

This indicates that B. sapida stem bark possess anti-hyperlipidemic activity and improved the biochemical parameters within the hematological profile of diabetic rats.

Keyword: *Blighia sapida, antihyperlipidemic, hematological profile, diabetes*

SAŽETAK

Poznato je da je Blighia sapida (BS) bogati izvor antioksidanasa, stoga smo ispitivali efekte etanolnog ekstrakta B. sapida Koenig stem bark (BSE) na metabolizam lipida i hematološke indekse kod pacova sa dijabetesom.

Trideset pacova muškog pola bilo je podeljeno u šest grupa, po pet pacova u svakoj. Dijabetes je indukovan intraperitonealnom injekcijom aloksana (65 mg/kg telesne mase) tokom 21 dana i oralno je primenjivan glibenklamid (5mg/kg) i B. sapida ekstrakt (50, 100 i 150 mg/kg telesne mase (tm)) jednom dnevno tokom 21 dana. Serumski lipidni profil, markeri hepato-renalne toksičnosti i hematološki indeksi su odredjivani korišćenjem automatskog analizatora. Podaci su analizirani pomoću jednofaktorske analize varijanse (ANOVA) i $p<0.05$ se smatralo statistički značajnim.

Netretirane životinje sa dijabetesom su imale značajno povišen ukupni holesterol ($p < 0.05$) i takodje značajno povećanje AST, ALT, uree i kreatinina u poređenju sa kontrolom. Trigliceridi, LDL-c, VLDL-c, AI i CRI su se smanjili nakon administracije ekstrakta, a HDL-c se značajno povećao u odnosu na netretirane pacove sa dijabetesom. Osim toga, značajno niži nivo hemoglobina (Hb), hematokrit (PCV), broj eritrocita (RBCs), broj leukocita (WBCs) u poređenju sa zdravim životinjama je zabeležen u netretiranoj grupi. Ove vrednosti su se vratile u normalu nakon administracije 50, 100 i 150 mg/kg tm ekstrakta. Ovi efekti su bili najizraženiji kod životinja tretiranih sa 150 mg/kg tm B. sapida bark.

Ovo ukazuje da B. sapida stem bark poseduje antihiperlipidemijsku aktivnost i da poboljšava biohemijske parametre u okviru hematološkog profila kod dijabetičnih pacova.

Ključne reči: *Blighia sapida, antihiperlipidemijska aktivnost, hematološki profil, dijabetes*

ABBREVIATIONS

ALT: alanine transaminase;
AST: aspartate transaminase;
ALP: alkaline transaminase;
DM: diabetes mellitus;
BS: Blighia sapida;
TG: Triglycerides;
TC: Total cholesterol;
LDL-c: low density lipoprotein cholesterol;
HDL-c: high density lipoprotein cholesterol;
VLDL-c: very low density lipoprotein;
Hb: hemoglobin;
MCHC: mean corpuscular hemoglobin concentration
MCV: mean corpuscular volume;
RBC: red blood cells,
PCV: packed cell volume;
WBC: white blood cells.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disturbance defined by hyperglycemia caused by lack of insulin or disruption of insulin signaling as a result of lack of hypoglycemic agent or insensitivity of insulin hormone. DM is related to aberrant metabolism of macromolecules [1, 2, 3]. The illness happens as a results of exocrine gland β-cells injury, resulting in decreased secretion of insulin. It might conjointly arise once the receptors are resilient to the roles of insulin [4]. Hyperglycemia reoccurrence during diabetes leads to body proteins being glycated, that successively results in complications affecting body organs and arteries [5]. Stiffening of red blood cells could be liable for and or related to, large vessel disease in diabetes state [6]. Management option for DM is predicated on insulin therapy and oral hypoglycemic agents that have several facet effects [7]. In diabetes state, the effects and locations of involvement in biochemical activity are varied and elevated serum total triglyceride level, increase level of transaminase, creatinine and urea are of concerned [6]. Additional major issue, that confounds diabetic state, leading to death is hyperlipidemia [8]. Different ways to this fashionable pharmacotherapy of diabetes mellitus are desperately required, attributed to the lack of accessible therapies to manage all the pathological basis of the ailment, in addition to the mammoth cost and poor accessibility for several populations in the developing world [6]. Therefore, plants use as medical aid for diabetes is inspired and acclaimed, though, a number of them are lacking scientific examination.

Blighia sapida K.D. Koenig, also known as 'Akee apple' in English, belongs to the plant family called Sapindaceae and it is noted for its highly characteristic reddish fruits. The species in this family include *B. sapida* K.D. Koenig, *B. unijugata* Baker, and *B. welwitschii* (Hiern) Radlk. It is recognized as 'isin' in Yoruba, 'gwanja kusa' in Hausa and 'okpu' in Igbo [9]. *B. sapida* K.D. Koenig is distributed throughout Nigeria [10]. *B. sapida* could be a therapeutic herbal plant ordinarily utilized by trado-medical healers in Nigeria, and highly appreciated in West Africa for the management of various diseases like diabetes mellitus [11]. The fruit has inhibitory effect against α-glucosidase and α-amylase as reported by Kazeem et al. [12]. *B. sapida* root extract has been shown to possess normoglycemic impact [13]. The stem bark has been shown to ameliorate pancreatic β-cell dysfunction in diabetic rats [11]. Therefore, this study was focused on analyzing the effects of *Blighia sapida* K.D. Koenig stem bark on antihyperlipidemic and hematological parameters in diabetic rats.

METHODS

Plant material

Fresh stem bark peelings of *B. sapida* Koenig was obtained at a farm in the suburbs of Abeokuta, Ogun State, Nigeria. The plant was identified and authenticated by a senior taxonomist at the university herbarium with herbarium approval number (UHAE/2016/020). All plant names in this manuscript are formatted according to the latest revision in "The Plant List" (www.theplantlist.org).

Plant extracts preparation

Stem bark was air-dried in the laboratory at temperature (25 ± 2°C), pulverized using an electrical blender and the powders obtained stored till further use. The small grained sample (100 g) was extracted with solvent combination of 70% ethanol for 48 h. The extract was filtered and thereafter evaporated to dryness using rotary evaporator [14]. The concentrated extract was stored at 4 °C until further analysis. The extraction yield is as follows.

$$\text{Percentage yield} = \frac{\text{Weight of the extract}}{\text{Weight of powdered stem bark}} \times 100\ \%\ [15]$$

The percentage yield of the extraction was 19.1 %.

Experimental Animals

Six-week-old male Wistar rats with an initial mean body weight of 150 ± 50 g were obtained from Animal house Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. The animals were divided into six groups of five each and adapted to investigational condition for two weeks. The animals were housed in clean metabolic cages that provided free access to water and rat pellets (Ladokun Feeds, Nigeria). The principles of Animal Care (Public Health Services, 1986) was monitored through the duration (for twenty-one days) of the experiment. The standards of National Institute of Health (NIH publication 85-23, 1985) for experimental maintenance and handling of animals was conformed to [16]. The ethical committee of the Afe Babalola University approved this study with approval number (ABUAD/ACA/121). The rats in this study followed the rules of the established Animal Ethical Committee of Afe Babalola University.

Animal grouping

The animals were randomly divided into six groups of 5 animals namely;

Normal control group (distilled water),
Diabetic untreated group (65 mg/kg Alloxan, intraperitoneal)
Diabetic + 5 mg/kg bw
Diabetic + 50 mg/kg bw BSE, oral gavage
Diabetic + 100 mg/kg bw BSE, oral gavage
Diabetic + 150 mg/kg bw BSE, oral gavage

Dose dependent study was previously carried out in our laboratory with three different doses of BSE (50, 100 and 150 mg/kg body weight) based on ethnobotanical survey conducted by [11].

Induction of diabetes

In induction of diabetes, alloxan (65 mg/kg body weight) was dissolved in sterile physiological saline and intraperitoneal injected (5.6 mL) into the animals in the diabetic control, diabetic + glibenclamide (5 mg/kg body weight), diabetic + 50 mg/kg body weight of BSE (2.5 mL), diabetic + 100 mg/kg body weight of BSE (5.5 mL), diabetic + 150 mg/kg body weight of BSE (8.5 mL) groups to induce β-cell dysfunction. This was done in morning after the rats have been fasted overnight, while the animals in normal control group received a similar volume of distilled water. Forty-eight hours after induction, fasting blood glucose (FBG) of all rats were measured by collecting blood from the tail vein using a portable glucometer. Animals with a FBG level ≥ 200 mg/dl were considered as diabetic while animals with a FBG level < 200 mg/dl were disqualified [17].

Collection of blood

Under di ethyl anaesthesia, the neck area of the rats was quickly cleared to expose the jugular veins. Blood samples from each animal was collected after 21 days through their jugular vein and preserved until further processing. The blood sample was collected into a dry tube and allowed to clot for 30 min before centrifuging at 3500 rpm for 10 min to collect the serum for further study [18].

Biochemical parameters

Serum lipid concentrations, in additional to aspartate and alanine aminotransferases and alkaline phosphatase as well as urea and creatinine concentrations were assayed using commercially available kits (DRG Diagnostics, USA) according to manufacturer's protocol.

Low density lipoprotein-cholesterol was estimated according to equation as shown below:

LDL-cholesterol (mg/dl) = TC- HDL- (TG/5)

Non-HDL-cholesterol (mg/dl) = TC – HDL-cholesterol

Whereas, TG/5 is equivalent to the concentration of very low density lipoprotein. VLDL means very low density lipoprotein, TG means triacylglycerol

Atherogenic index (AI) was calculated and expressed as:

$$\text{Atherogenic index (AI)} = \frac{\text{TC-HDL-cholesterol}}{\text{HDL-cholesterol}}$$

Coronary artery risk index (CRI) was deduced using the formula below.

$$\text{Coronary artery risk index (CRI)} = \frac{\text{TC (mg/dl)}}{\text{HDL-cholesterol (mg/dl)}}$$

Whereas, HDL means high density lipoprotein-cholesterol, TC means total cholesterol

Hematological analysis

The hematological parameters like packed cell volume (PCV), hemoglobin (Hb), WBC count and WBC percentage composition, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were analysed by means of an automated analyzer (Sysmex K-2 IN, Japan). Total white blood cell counts (WBC) was analysed by the hemocytometer method, whereas smears were ready and marked by using Leishman technique and numbered by the longitudinal counting method to resolve differential count. Packed cell volume (PCV) was analyzed by the micro-haematocrit method while hemoglobin (Hb) levels was assessed by the cyano methemoglobin method [19].

Data analysis

Data are presented as the mean ± SD (n=5). The data were analyzed by one-way analysis of variance via a statistical software package (SPSS, Version 20.0, IBM Corporation, NY, USA) one-way ANOVA using Duncan multiple range *post-hoc* test (DMRT). Values were considered to be significantly different at $p < 0.05$.

RESULTS

The effect of *Blighia sapida* stem bark ethanol extract on fasting blood glucose level of alloxan-induced diabetic rats is presented in Table 1. Fasting blood glucose levels in all animals induced with alloxan was significantly increased, compared to the normal control group, $p < 0.05$. The fasting blood glucose levels were however, reduced considerably in diabetic + 50 mg/kg body weight of BSE, diabetic + 100 mg/kg body weight of BSE and diabetic + 150 mg/kg body weight of BSE groups in addition to glibenclamide treated animals.

Table 1: Effects of ethanol extract *Blighia sapida* stem bark on the fasting blood glucose level of alloxan-induced diabetic rats

Groups	Initial fasting blood glucose level (mg/dl)	Fasting blood glucose at 48 h after induction (mg/dl)	Final fasting blood glucose at 21 days after induction (mg/dl)
Control	86.10 ± 0.14^{a}	85.65 ± 0.10^{a}	87.20 ± 1.14^{a}
Diabetic control	86.48 ± 1.01^{a}	287.10 ± 1.01^{b}	364.10 ± 2.10^{d}
Diabetic + glibenclamide	84.02 ± 1.96^{a}	244.20 ± 1.45^{e}	88.40 ± 1.0^{a}
Diabetic + 50 mg/kg B.S	85.46 ± 1.42^{a}	287.10 ± 1.12^{e}	102.10 ± 1.10^{c}
Diabetic + 100 mg/kg B.S	84.49 ± 1.20^{a}	306.46 ± 1.24^{d}	96.96 ± 2.10^{b}
Diabetic + 150 mg/kg B.S	86.10 ± 1.32^{a}	386.12 ± 2.12^{d}	88.201 ± 1.10^{a}

Data are presented as the mean ± SEM of 5 animals. Values with different superscript letters (a-e) along a column for a given parameter are significantly different from each other between groups at P < 0.05.

Table 2: Effect of administration of ethanol extract of *Blighia sapida* stem bark on Serum lipid profiles, atherogenic and coronary risk indices of alloxan-induced diabetic rat

Groups	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Non-HDL (mg/dl)	HDL (mg/dl)	AI	CRI
Control	56.28±1.04^{a}	40.21±0.02^{a}	19.39±0.01^{a}	8.04±0.01^{a}	27.44±0.98^{a}	28.84±0.01^{a}	0.95±0.02^{a}	1.95±0.01^{a}
DC	95.84±0.12^{e}	104.26±0.14^{d}	64.91±0.21^{d}	20.85±0.12^{d}	85.76±1.22^{e}	10.08±0.02^{e}	8.51±1.01^{d}	9.51±1.22^{d}
DC + gliben-clamide	62.20±0.14^{d}	53.12±1.12^{c}	33.39±1.11^{c}	10.62±0.04^{c}	44.01±0.67^{c}	18.19±0.30^{d}	2.42±0.33^{c}	3.42±0.31^{c}
Diabetic rats + 50mg/kg BSE	64.32±1.20^{c}	54.36±1.18^{c}	33.33±1.08^{c}	10.87±0.38^{c}	44.20±0.01^{c}	20.12±0.14^{c}	2.20±0.41^{c}	3.19±0.58^{c}
Diabetic rats + 100mg/kg BSE	69.92±1.10^{b}	44.10±0.06^{b}	34.98±0.04^{b}	8.82±0.25^{a}	44.80±0.45^{b}	25.12±0.06^{b}	1.74±0.30^{b}	2.78±0.28^{b}
Diabetic rats + 150mg/kg BSE	55.89±1.48^{a}	40.82±1.06^{a}	18.74±0.05^{a}	8.16±0.01^{a}	26.91±1.12^{a}	28.98±1.01^{a}	0.93±0.01^{a}	1.93±0.02^{a}

Data are presented as the mean ± SEM of 5 animals. Values with different superscript letters (a-e) along a column for a given parameter are significantly ($P < 0.05$) different from each other. TC, Total cholesterol; TG, Triglyceride; LDL-cholesterol, Low density lipoprotein-cholesterol; HDL-cholesterol; High density lipoprotein-cholesterol, AI; atherogenic index, CRI; coronary artery index.

Serum lipid concentrations, calculated atherogenic index (AI) and coronary risk index (CRI) scores are displayed in Table 2. High serum concentrations of TC, TG, LDL-cholesterol and non-HDL-cholesterol levels in addition to analyzed AI and CRI scores with progressive decrease in serum HDL-cholesterol were observed within diabetic untreated group compared to the normal control. Administration with *B. sapida* stem bark ethanol extract to diabetic animals considerably and dose-dependently abridged TC, TG and LDL-cholesterol levels, AI and CRI in 50 ,100 and 150 mg/kg bw of BSE and glibenclamide groups compared with the diabetic untreated. Although considerably increase in serum HDL-cholesterol level was observed in the *B. sapida* K.D. Koenig stem bark ethanol extract treated groups compared to the diabetic untreated.

Serum ALT, AST, ALP, urea and creatinine levels are presented in Table 3. Concentrations of serum ALT, AST, ALP, urea and creatinine levels were considerably increased within the diabetic untreated compared to the normal control. On the other hand, administration of *B. sapida* stem bark ethanol extract to diabetic animals progressively ameliorated these changes in 50, 100 and 150 mg/kg bw of BSE and glibenclamide treated groups.

Hemoglobin (Hb) levels, packed cell volume (PCV) count, red blood cells (RBCs), white blood cells (WBCs), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) are displayed in Table 4. Hemoglobin (Hb) levels, packed cell volume (PCV), red blood cells (RBCs) levels, white blood cells (WBCs), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) were evidently reduced in diabetic untreated compared to normal control. Though, administration of 50-150 mg/kg bw of BSE groups demonstrated a considerably increase on the biochemical parameters mentioned above. Glibenclamide treatment conjointly considerably increase these biochemical parameters.

DISCUSSION

In this study, we examined the effect of oral administration of *B. sapida* Koenig stem bark ethanol extract in diabetic rats through twenty-one days' post-treatment period. The typical characteristics of diabetic dyslipidemia include high serum cholesterol, triglyceride (hypertriglycerolemia), LDL-cholesterol concentrations and low HDL-

Table 3: Effect of administration of ethanol extract of *Blighia sapida* stem bark on selected biomolecules of alloxan-induced diabetic rat

Groups	AST (µ/l)	ALT (µ/l)	Urea (mg/dl)	Creatinine (mg/dl)	ALP (µ/l)	Total Bilirubin (mg/dl)
Control	96.48±0.14^{a}	62.20±1.02^{a}	26.11±1.16^{a}	4.00±1.22^{a}	164.14±2.10^{a}	6.10±1.10^{a}
Diabetic Control	180.20±2.12^{e}	100.20±1.42^{e}	56.43±2.43^{e}	10.39±2.08^{e}	482.01±4.07^{e}	18.14±2.10^{d}
Diabetic rats + Glibenclamide	140.40±1.20^{d}	84.20±2.04^{d}	42.12±3.46^{d}	6.04±1.12^{d}	240.11±3.17^{d}	10.42±1.19^{c}
Diabetic rats + 50mg/kg BS	110.20±1.05^{c}	76.40±1.04^{c}	38.36±4.69^{c}	6.47±1.12^{c}	210.14±1.64+c	8.14±2.48^{b}
Diabetic rats + 100mg/kg BS	102.34±34^{b}	72.49±1.80^{b}	30.48±2.04^{b}	5.29±1.15^{b}	180.13±3.01^{b}	8.04±1.10^{b}
Diabetic rats + 150mg/kg BS	96.84±1.04^{a}	61.84±1.42^{a}	26.92±3.11^{a}	4.13±1.06^{a}	168.89±3.19^{a}	6.32±2.10^{a}

Data are presented as mean ± SEM of 5 animals. $^{a-e}$ Values with different superscript letters along a column for a given parameter are significantly different from each other group of animals. BS, *Blighia sapida*; ALT, Alanine transaminase; AST, Alanine transaminase; ALP, Alkaline phosphate.

Table 4: Effect of administration of ethanol extract of *Blighia sapida* stem bark on haematological parameters of alloxan-induced diabetic rat

Parameters	Control	Diabetic control	DC + Glibenclamide	Diabetic rats + 50 mg/kg BSE	Diabetic rats + 100 mg/kg BSE	Diabetic rats + 150 mg/kg BSE
PCV (%)	55.10±1.01^{a}	27.10±1.01^{e}	42.00±0.10^{d}	48.10±1.01^{c}	51.20±1.04^{b}	55.84±2.02^{a}
Hb (g/dl)	11.98±0.50^{a}	6.14±0.02^{e}	8.20±0.20^{d}	9.58±0.32^{c}	10.62±0.04^{b}	11.64±0.52^{a}
WBC (x w^{3}/µl)	2.42±0.22^{a}	0.45±0.14^{e}	1.26±0.28^{d}	1.69±0.46^{c}	2.00±0.07^{b}	2.44±0.84^{a}
N (%)	49.20±1.12^{a}	26.20±2.10^{e}	36.21±0.14^{d}	39.40±1.10^{c}	46.24±0.12^{b}	49.62±0.45^{a}
L (%)	30.11±0.01^{a}	20.20±0.12^{d}	24.03±0.12^{c}	27.20±1.12^{b}	28.20±1.02^{b}	30.44±1.20^{a}
M (%)	8.01±0.14^{a}	4.28±0.18^{d}	6.01±0.19^{c}	6.99±0.14^{c}	7.46±0.12^{b}	8.46±0.26^{a}
E (%)	3.01±0.11^{a}	0.92±0.13^{d}	1.49±0.16^{c}	1.94±0.08^{c}	2.46±0.02^{b}	3.68±0.04^{a}
RBC (xw^{11}/l)	3.20±0.31^{a}	0.80±0.04^{d}	1.45±0.02^{c}	1.64±0.42^{c}	2.48±0.12^{b}	3.48±0.06^{a}
MCHC (g/dl)	34.10±0.06^{a}	22.10±0.04^{d}	28.41±1.10^{c}	29.10±0.12^{c}	32.41±0.02^{b}	34.46±0.12^{a}
MCV (fl)	79.01±1.10^{a}	54.12±0.16^{e}	63.49±0.10^{d}	69.10±0.52^{c}	75.96±0.48^{b}	79.60±0.09^{a}
MCH (pg)	35.11±1.20^{a}	24.01±0.06^{e}	28.42±0.04^{d}	30.20±0.44^{c}	32.69±0.16^{b}	35.64±1.10^{a}

Data are presented as mean ± SEM of 5 animals. Values with different superscript letters (a-e) along a column for a given parameter are significantly ($P < 0.05$) different from each other group of animals. Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), white blood cells (WBCs), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E).

cholesterol (hypercholesterolemia) contents [20]. Likewise, the increase levels of LDL-C and atherogenic index by the alloxan indicates predisposition to cardiovascular risk [21]. The significant elevation in cholesterol, triacylglycerol and LDL-cholesterol concentrations in the diabetic rats treated with distilled water may be owing to improved mobilization of free fatty acids from the peripheral fat depots. This obvious hyperlipidemia that describe the diabetic state may hence be considered as a consequence of uninhibited actions of lipolytic hormones on the fat depots [22]. Aberrant lipid metabolism, resulting in amassing of LDL-C, VLDL and total cholesterol in addition to reduced HDL-cholesterol, is often related to diabetes mellitus [23]. Elevated levels of LDL, VLDL, atherogenic index, coronary artery index and total cholesterol are thought of as main menace for cardiovascular disease (CVD). On the contrary, elevated HDL-cholesterol that functions in the transport of cholesterol from the periphery to the liver reduces the chance of CVD [24]. Oral intervention of *B. sapida* stem bark ethanol extract and the standard drug both averts dyslipidemia, decreases the chance of developing atherogenesis and coronary artery disease and amplified serum HDL-cholesterol level. This is in accordance with newly published studies [8, 25, 26, 27].

Aminotransaminses are significant and key enzymes involved in the breakdown of amino acids into α-keto acid, which are directed for complete metabolism via the Krebs cycle and electron transport chain. They are considered exact biomarkers for liver damage [28]. In hepatocyte injury, there is impairment in the biomembrane of liver cells which leads to permeability of cytoplasmic enzymes such as AST and ALT which leak into the circulatory system and result in significant elevation in their activities in serum. Moreover, modification of membrane bound alkaline phosphatase (ALP) affects membrane permeability and produces imbalance in the transport of metabolites [29]. Elevated serum transaminase, alanine aminotransferase (ALT), aspartate aminotransaminse (AST), alkaline phosphatase (ALP), urea and creatinine levels are thought of as biomarkers of hepato-renal damage, related to liver disease and hyperglycaemia [30, 31]. Treatment with *B. sapida* stem bark ethanol extract at 50 - 150 mg/kg bw significantly reduced ($p < 0.05$) ALT, AST, ALP, urea and creatinine levels, indicating that *B. sapida* bark might ameliorates alloxan-induced injury in diabetic rats. The considerably lower serum creatinine and urea levels in the *B. sapida* stem bark ethanol extract treated groups compared with diabetic untreated, indicates its doable impact on the betterment of diabetes induced kidney injury [32, 33].

Vital reductions in haemoglobin (Hb) levels, packed cell volume (PCV), red blood cells (RBCs), white blood cells (WBCs), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) were observed ($p < 0.05$) in the diabetic untreated rats. Substantial significant ($p < 0.05$) increase in haematological parameters occurred upon administration of the *B. sapida* stem bark ethanol extracts at 50 - 150 mg/kg doses compared to the diabetic untreated rats. Constant trend was observed in the levels of parameters mention above for glibenclamide treated rats. Though these effect were more pronounced in the 150 mg/kg bw of BSE group [6, 34]. This may be as a result of the presence of metabolites like saponins, phenols and alkaloids [35 36, 37]. Blood examination could be a great means of evaluating the well-being standing of animals because it performs significant physiological and biological roles in organisms [38]. However, in diabetic state, the extra glucose present reacts with haemoglobin to produce glycated haemoglobin. This therefore shown that diabetic untreated rats displayed aberrations in the hematological profiles. A number of these aberrations may result in destruction of developed red blood cells resulting in the small Hb counts accompanied with the drop in the RBC and PCV [39, 40]. Extremely low values

of RBC, Hb and hematocrit might specify anemia [34, 39]. Furthermore, modulatory effect and confined toxicity might ensue as noted within the lymphocytes and neutrophils of the diabetic untreated animals. Administration of the *B. sapida* stem bark ethanol extract stimulates positive changes in the hematological profile of the diabetic rats. Hence, upsurge in RBC by the extract is a sign of its restorative impact on alloxan-induced anemia whereas the alteration in level of lymphocytes by the extract could specify an anti-infection activity [36, 40, 41].

CONCLUSION

The results from this study indicates that *B. sapida* ethanol stem bark extract possesses robust anti-diabetic activity via improving dyslipidemia and ameliorate anemic condition in diabetic rats. Hence, ethanol extract of *B. sapida* K.D. Koenig stem bark could be a possible anti-diabetic natural product with no significant side effects. Further studies to isolate the bioactive principles using HPLC, complete safety assessment as well as activities on metabolizing enzymes and pro-inflammatory biomarkers should be carried out *in vivo*.

STATEMENT

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Statement of Ethics

The handling of animals was conformed to the standards of National Institute of Health (NIH publication 85-23, 1985) for experimental animal maintenance. The ethical committee of the Afe Babalola University approved this study with approval number (12/SCI03/015). The rats in this study followed the rules of the established Animal Ethical Committee of Afe Babalola University.

Disclosure Statement

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Authors Contribution

OAO design the study, ODI carried out the study, ABO wrote the manuscript, ABO and ODI carry out analysis and interpretation of data, BOA and BEO assisted with and supervised the manuscript writing, BEO did the first proof reading and BOA and BEO did the second proof reading. OAO, BOA and BEO supported the manuscript preparation, made conceptual contributions on data analysis, manuscript drafting and critically revised the manuscript. The authors have read and approved the final manuscript.

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