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Subacute Toxicity of Aqueous Leaf Extract of Blighia sapida (K.D. Koenig) on Key Biochemical indices of Male Wistar Rats

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Article

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Abstract

The recent increase in the use of plants such as *Blighia sapida* for medicinal purposes has necessitated a thorough assessment of their toxicity. The present study assessed the effect of aqueous leaf extract of *Blighia sapida* leaves on selected biochemical indices in male Wistar rats. Twenty male Wistar rats (120–140 g) were randomly assigned to four groups. Group A received distilled water, whereas groups B, C, and D received 125, 250, and 500 mg/kg body weight of the extract, respectively, orally for 21 days. Animals were sacrificed by cervical dislocation. Blood was collected into EDTA and plain bottles for haematological analysis (using an automated analyzer) and serum preparation, respectively. The liver and kidney tissues were homogenized and analyzed spectrophotometrically. Data were analyzed using one-way analysis of variance using GraphPad Prism5. Compared to the control rats, administration of 250 and 500 mg/kg body weight of the extract led to a significant ($P < 0.05$) increase in serum ALP activity and a decrease in liver GSH. At 500 mg/kg body weight, there was a significant increase in serum bilirubin, $HCO3^-$, and liver protein carbonyl levels, along with a decrease in kidney GSH levels. Liver MDA concentration was significantly ($P < 0.05$) increased at 250 mg/kg body weight. There were dose-dependent increases in serum $PO43^-$, urea, cholesterol, and triglyceride levels and a significant ($P < 0.05$) rise in 8OHdG concentration. Subacute oral administration of aqueous leaf extract of *Blighia sapida* up to 500 mg/kg body weight was neither haematotoxic, hepatotoxic, nephrotoxic, nor atherogenic. However, it could cause cholestasis at a dose of 500 mg/kg body weight.

1.0 INTRODUCTION

The inseparable link between human health and the use of natural products, especially herbs, for millennia has made natural medicinal resources one of the oldest contributions of nature to the well-being of humans [1]. In recent decades, there has been a notable increase in the use and dependence of the human population on herbal medicines due to various factors, ranging from easy accessibility and affordability to fewer or absence of side effects compared to orthodox medicines. Contrary to popular belief, there are unfavorable outcomes associated with the use of herbs, especially because preparations and dosages are not standardized for many herbal preparations. Adverse effects such as hepatotoxicity, nephrotoxicity, and cardiotoxicity have been reported for various medicinal plants, including *Octomeles sumatrana*, *Syzygium polyanthum*, and *Hylocereus polyrhizus* [2], *Syzygium guineense* [3], and *Datra metel* [4].

Blighia sapida, a native of tropical African countries including Nigeria, is a member of the Sapindaceae family of plants. Commonly known as the akee apple, its local Nigerian names include Okpu (Igbo), Isin (Yoruba), and Ukpe (Edo). *B. sapida* tree grows to about 40 feet high with pear-shaped fruit that is green when young and immature and ripens to become yellow or red [5]. The leaves and bark of the plant are traditionally used for the management of diarrhea, dysentery, conjunctivitis, fever, internal hemorrhage, cutaneous infections, and bacterial infections [6] and the references cited therein. The antibacterial activities against K*lebsiella pneumonia* and *Staphylococcus aureus* [7], peripheral analgesic [8], antimalarial [6], antioxidant, anti-inflammatory [9], and hypoglycemic activities of various *B. sapida* leaf

extracts have been documented [10]. Despite the promising therapeutic benefits, there are concerns about the safety of continued consumption of plants to which many pharmacological effects have been attributed. For instance, Ajayi *et al.* [11] reported the neurotoxic (by increasing susceptibility to seizures and impairing locomotor and memory function) and hepatotoxic effects of the unripe fruit extract of *B. sapida*. *Clarias gariepinus* were reported to show irritation towards ethanol extract of *B. sapida* stem back [12]. Hepatocyte distortion was reported by Maigida *et al.* [13] when Wistar rats were exposed to the aril extract of *B. sapida* fruits. Attempts have been made to evaluate the toxicity of *B. sapida* leaves, with the lowest dose reported being 500 mg/kg body weight [14]. The present study reports the toxicity of aqueous leaf extract of *Blighia sapida* leaves at doses of 125, 250, and 500 mg/kg body weight.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant Materials

Fresh leaves of *B. sapida* were harvested within the premises of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso Oyo State (8° 8' 0" North, 4° 16' 0" East) with permission of the University Management. The leaf samples were authenticated by Prof. A. T. J. Ogunkunle of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Oyo State, Nigeria and voucher specimen (voucher number- LHO881) was deposited in the Herbarium Unit of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Nigeria. Furthermore, Prof. A. T. J. also gave the permission to collect plant name. On the issue of compliance with the International Union for Conservation of Nature (IUCN) Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora, *Blighia sapida* is currently assessed as Least Concern on the IUCN Red List of 2024 and is not listed under Convention on International Trade in Endangered Species (CITES). Rather, it is widely cultivated. In addition, the plant material, the leaves used in the current investigation was obtained from cultivated trees within the premises of LAUTECH and not from the wild protected population. So, no whole trees were felled and collection of a few leaves did not threaten local population. Ethical collection issues were upheld by obtaining adequate permission from necessary quarters and voucher specimen was deposited in the herbarium unit of the University.

2.1.2 Experimental animals

Twenty healthy male Wistar rats (130 ± 10 g) were obtained from a commercial breeder in Ogbomoso, Oyo State, Nigeria, and acclimatized for 14 days under standard laboratory conditions (temperature of 27–32°C and relative humidity of 49–56%, photoperiod of 12-hour natural light and 12-hour dark) with free access to feed (purchased from Glory Vet Ventures, Ogbomoso, Oyo State, Nigeria) and water *ad libitum* in plastic cages.

2.1.3 Chemicals and Reagents

Analytical kits for the determination of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activity were purchased from Randox Laboratories Ltd., County Antrim, United Kingdom, while those for bilirubin, sodium ion (Na^+), bicarbonate (HCO_3^-), magnesium ions (Mg^{2+}), phosphate ions (PO_4^3), creatinine, urea, and reduced glutathione (GSH) were purchased from Abcam Biotechnology Company, Cambridge, United Kingdom. Assay kits for cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were obtained from Fortress Diagnostic Limited, Northern Ireland, United Kingdom. A colorimetric TBARS microplate assay kit from Oxford Biomedical Research was used to evaluate the concentration of malondialdehyde (MDA). Assay kits for protein carbonyl and 8-hydroxyl deoxyguanosine (8OHdG) were purchased from ElabScience (Texas, USA). Other chemicals used were of analytical grade

2.2 Methods

2.2.1 Preparation of aqueous leave extract of *Blighia sapida*

Fresh leaves of *B. sapida* were rinsed under running tap water and air-dried to a constant weight. The dried leaves were pulverised using an electric blender. Five hundred grams (500 g) of the powdered sample was macerated in 2.5 litres of distilled water at 4°C for 72 h. Thereafter, the mixture was filtered using a Whatman No. 1 filter paper (Maidstone, United Kingdom). The filtrate was freeze-dried and stored at 4°C. The extract yield was 25.53 g, representing a 5.1% extraction yield.

2.2.2 Animal Grouping

Twenty male Wistar rats were randomly assigned to four groups of five animals each. Animals in group A received 1 ml of distilled water and served as the placebo control group, while those in groups B, C, and D were administered 125, 250, and 500 mg/kg body weight of the aqueous leaf extract of *B. sapida*, respectively, via the oral route using an oral cannula. Subacute administration lasted for 21 days. The experimental protocol was approved by the ethical committee of the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria (LBCH/2024/016).

2.2.3 Collection and preparation of serum and tissue supernatants

Twenty-four hours after the last dose of the extract was administered, the animals were weighed and sacrificed by cervical dislocation. Blood was collected into ethylenediaminetetraacetic acid (EDTA) and plain sample bottles for haematological analysis and serum preparation, respectively. The liver and kidney were quickly excised and rinsed in phosphate-buffered saline (0.1M, pH7.4, 4°C), weighed, and homogenized at a weight: volume ratio of 1:5 in the same buffer. The homogenates were centrifuged at 800 × g for 15 min at 4°C, and the supernatant was aspirated with a Pasteur pipette and kept at 4°C. Blood collected in plain bottles was left to clot for approximately 10 min and centrifuged at 2 000 x g for

10 min. The supernatant (serum) was aspirated using a Pasteur pipette and stored at $4^{\circ}C$. Both tissue supernatants and serum were used within 24 h of preparation [15].

2.2.4 Haematological and Biochemical Analyses

Packed cell volume (PVC) and hemoglobin (Hb) concentration. Red blood cell count (RBC), white blood cell count (WBC) and platelet count (PLT) of the experimental animals were analysed using the Sysmex Automated Haematology Diagnostic Machine (XP-300, Mundelein, USA)

The activity of serum ALT, AST [16], ALP [17], and the concentration of bilirubin [18], Na^{+}, HCO_3^{-}, Mg^{2+}, PO_4^{3-} [19], creatinine [20], urea [21], MDA [22], GSH, SOD [23] protein carbonyl [24], 8OHdG cholesterol [25], TG [26], HDL-C [27], and LDL-C were determined as outlined in the manufacturers' guidelines for each assay kit. Very low-density lipoprotein cholesterol (VLDL-C) was estimated as TG/5, and the atherogenic index (AI) was calculated as log (TG/HDL-C) [28]. The classification of AI as low, medium, or high risk of cardiovascular disease was described by [29].

2.2.5 Statistical Analysis of Experimental Data

Data {Mean ± S.E.M (n = 5)} obtained from this study were analyzed using a One-way Analysis of variance with Tukey's post-hoc test employed to determine the significance difference between groups at $P < 0.05$ using GraphPad Prism 17.

3.0 RESULTS

3.1 Effects of Aqueous Leaf Extract of *Blighia sapida* on Selected Hepatic Parameters of Experimental Animals

As presented in Table 1, administration of aqueous leaf extract of *B. sapida* at 125 mg/kg body weight led to a significant decrease in the relative liver weight of experimental animals, while 250 and 500 mg/kg body weight had no significant effect on the relative liver weight of male Wistar rats. There was a significant ($P < 0.05$) dose-dependent reduction in the serum activities of ALT and AST when compared with those of the placebo control animals. In comparison with the control rats, there was no significant alteration in the serum activity of ALP concentration of bilirubin; however, administration of 250 and 500 mg/kg body weight of the extract significantly increased the serum ALP activities, while serum bilirubin increased significantly only at 500 mg/kg body weight.

Table 1
Selected Hepatic parameters of male Wistar rats administered aqueous leaf extract of *B. sapida*

Groups	Relative Liver Weight (%)	Serum ALT (U/L)	Serum AST (U/L)	Serum ALP (U/L)	Serum Bilirubin (mg/dl)
A (Control)	4.40 ± 0.41^{a}	28.94 ± 0.52^{a}	103.53 ± 0.45^{a}	29.07 ± 0.13^{a}	22.19 ± 0.48^{a}
B (125 mg/kg body weight)	3.13 ± 0.12^{b}	28.88 ± 0.26^{b}	91.47 ± 0.68^{b}	26.08 ± 0.17^{b}	22.47 ± 0.28^{a}
C (250 mg/kg body weight)	3.97 ± 0.27^{ab}	25.15 ± 0.49^{c}	81.08 ± 0.39^{c}	61.40 ± 0.43^{c}	21.50 ± 0.33^{a}
D (500 mg/kg body weight)	4.93 ± 0.25^{a}	23.17 ± 0.29^{c}	75.80 ± 0.83^{d}	65.79 ± 0.30^{d}	28.99 ± 0.74^{b}

Values are mean ± S. E. M. (n = 5). Different alphabetic subscripts denote significant differences across each column ($P < 0.05$). ALT: alanine transaminase; AST: aspartate transaminase

There was no significant ($P < 0.05$) change in the liver SOD activity across all experimental groups. The GSH level was significantly ($P < 0.05$) increased in the liver of animals administered 125 mg/kg body weight of extract but decreased in animals administered 250 and 500 mg/kg body weight when compared to the placebo control. Liver MDA concentration was significantly ($P < 0.05$) increased in animals administered 250 mg/kg body weight but decreased in those administered 125 and 500 mg/kg body weight. Liver protein carbonyl level rose significantly in the 125 and 500 mg/kg body weight groups compared to the placebo control group, while 8-hydroxy- 2'- deoxyguanosine activity decreased significantly ($P < 0.05$) in a dose-dependent manner (Table 2).

Table 2
Liver Redox Status of rats administered aqueous leaf extract of *B. sapida*

Groups	SOD (U/ml)	GSH (mM)	MDA (µM)	Protein Carbonyl (x 10^{-1} nmol/mg)	8OHdG (ng/mol)
Placebo Control	1.158 ± 0.010^{a}	0.34 ± 0.019^{a}	2.04 ± 0.04^{a}	0.64 ± 0.03^{a}	78.12 ± 0.32^{a}
125 mg/kg body weight	1.213 ± 0.012^{a}	0.51 ± 0.004^{a}	1.57 ± 1.20^{c}	0.78 ± 0.04^{b}	52.43 ± 0.30^{b}
250 mg/kg body weight	1.372 ± 0.005^{ba}	0.24 ± 0.038^{b}	2.54 ± 0.08^{b}	0.64 ± 0.03^{a}	51.19 ± 0.20^{b}
500 mg/kg body weight	1.123 ± 0.092^{a}	0.13 ± 0.009^{c}	1.45 ± 0.06^{c}	0.80 ± 0.02^{c}	34.41 ± 0.42^{c}

Values are mean ± S. E. M. (n = 5). Different alphabet subscripts denote significant differences across groups at $P < 0.05$. SOD, Superoxide Dismutase; GSH, reduced glutathione; MDA - Malondialdehyde; 8OHdG – 8 -hydroxy- 2'- deoxy

3.2 Effect of Aqueous Leaf Extract of Blighia sapida on Selected Kidney Function Parameters of Male Wistar Rats

As presented in Table 3, there was no significant ($P < 0.05$) difference in the relative kidney weights of animals across the groups. Serum Na^{+} and creatinine concentrations were significantly ($P < 0.05$) decreased in rats administered 500 mg/kg body weight in a dose-dependent manner, while serum Mg^{2+} and HCO_3^{-} levels increased significantly ($P < 0.05$) only in those administered 500 mg/kg body weight compared to placebo control rats. However, there was a significant ($P < 0.05$) dose-dependent increase in serum PO_4^{3-} and urea levels in rats administered all three doses of the aqueous extract of *B. sapida* compared with the control group.

There was no significant ($P < 0.05$) change in the kidney SOD activity of animals across all experimental groups, while the GSH level decreased significantly ($P < 0.05$) in animals administered 500 mg/kg body weight. There was a significant ($P < 0.05$) decrease in MDA and protein carbonyl levels in all the test groups compared to the control group. The 8OHdG level increased significantly ($P < 0.05$) in a dose-dependent manner in the test groups compared to the control group (Table 4).

Table 3
Selected Kidney Function Parameters of Male Wistar Rats Administered Aqueous Leaf Extract of *B. sapida*

Groups	Relative Kidney Weight (%)	Serum Na^+ (mmol/L)	Serum Mg^{2+} (mmol/L)	Serum HCO_3^- (mmol/L)	Serum PO_4^{3-} (mmol/L)	Serum Creatinine (µmol/L)	Serum Urea (mg/dl)
A (Control)	0.99 ± 0.03^a	131.23 ± 0.81^a	1.17 ± 0.03^a	34.52 ± 0.61^a	1.30 ± 0.07^a	32.07 ± 0.11^a	35.49 ± 0.66^a
B (125 mg/kg body weight)	0.77 ± 0.03^a	130.18 ± 0.76^a	0.90 ± 0.03^b	33.60 ± 0.41^a	1.50 ± 0.03^b	33.42 ± 0.22^a	39.00 ± 0.53^b
C (250 mg/kg body weight)	0.98 ± 0.16^a	128.62 ± 0.55^a	0.98 ± 0.05^b	33.18 ± 0.71^a	1.65 ± 0.00^b	32.39 ± 0.72^a	39.28 ± 0.71^b
D (500 mg/kg body weight)	1.01 ± 0.14^a	124.43 ± 0.70^b	1.31 ± 0.01^a	59.30 ± 1.00^b	1.59 ± 0.03^b	24.82 ± 0.25^b	48.58 ± 0.54^c

Values are mean ± S. E. M. (n = 4). Different alphabet superscripts denote significant differences across columns at $P < 0.05$.

Table 4
kidney Redox Status of rats administered aqueous leaf extract of *B. sapida*

Groups	SOD (U/ml)	GSH (mM)	MDA (µM)	Protein Carbonyl nmol/mg	8OHdG ng/ml
A (Control)	1.22 ± 0.01^a	0.22 ± 0.00^a	2.00 ± 0.07^a	0.10 ± 0.00^a	34.72 ± 0.19^a
B (125 mg/kg body weight)	1.22 ± 0.06^a	0.20 ± 0.00^a	1.45 ± 0.03^b	0.06 ± 0.00^b	58.03 ± 0.35^c
C (250 mg/kg body weight)	1.22 ± 0.04^a	0.22 ± 0.00^a	1.61 ± 0.18^b	0.06 ± 0.01^b	61.22 ± 0.26^d
D (500 mg/kg body weight)	1.34 ± 0.04^a	0.14 ± 0.03^b	1.80 ± 0.10^c	0.07 ± 0.02^b	51.00 ± 0.84^b

Values are mean ± S. E. M. (n = 4). Different alphabet subscripts denote significant differences across each column ($P < 0.05$). SOD, superoxide dismutase; GSH-Reduced glutathione; MDA- Malondialdehyde; 8OHdG – 8 -hydroxy- 2'- deoxyguanosine.

3.3 Effect of aqueous extract of *B. sapida* on serum lipid profile of male Wistar rats

As shown in Table 5, there was a significant dose-dependent ($P < 0.05$) increase in the level of serum cholesterol at 125, 250, and 500 mg/kg body weight of aqueous extract of *B. sapida* groups compared to the placebo control animals. There was no significant ($P < 0.05$) difference in the serum high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels, but serum triglycerides and very low-density lipoprotein levels increased significantly ($P < 0.05$) in rats administered 125 and 500 mg/kg body weight of aqueous when compared to the placebo control group.

Table 5
Serum lipid profile of male Wister rats administered aqueous leaf extract of *B. sapida*

Groups	Cholesterol (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL ($\times 10^{-1}$ mmol/L)	Artherogenic Index ($\times 10^{-1}$)
A (Control)	1.71 ± 0.01^{a}	0.43 ± 0.02^{a}	0.55 ± 0.045^{a}	1.08 ± 0.02^{a}	0.86 ± 0.01^{a}	-1.04 ± 0.09^{a}
B (125 mg/kg body weight)	1.80 ± 0.02^{b}	0.52 ± 0.01^{b}	0.55 ± 0.003^{a}	1.46 ± 0.26^{a}	1.04 ± 0.01^{b}	-0.26 ± 0.02^{b}
C (250 mg/kg body weight)	1.93 ± 0.01^{c}	0.44 ± 0.01^{a}	0.57 ± 0.004^{a}	1.28 ± 0.01^{a}	0.88 ± 0.01^{a}	-1.15 ± 0.03^{a}
D (500 mg/kg body weight)	2.27 ± 0.03^{d}	0.59 ± 0.01^{c}	0.59 ± 0.011^{a}	1.52 ± 0.02^{a}	1.19 ± 0.02^{c}	-0.03 ± 0.03^{b}

Values are expressed as mean ± S.E.M (n = 5). Significant ($P < 0.05$) differences are denoted by different alphabetical superscripts in each column. TG- Triglyceride; HDL- High-density lipoprotein; LDL- Low-density lipoprotein; VLDL- Very low-density lipoprotein; AI- Atherogenic index

3.4 Effects of aqueous extract of *B.* on the haematological parameters of male Wistar rats

Administration of all the doses of aqueous extract of *Biligha sapida* leaves did not significantly ($P > 0.05$) alter the levels of PCV, Hb, RBC, WBC and Platelet when compared with the distilled water treated placebo control (Table 6)

Table 6
Hematological parameters of male Wister rats administered aqueous leaf extract of *B. sapida*

Groups	PCV (%)	Hb (g/L)	RBC (10^{12}/L)	WBC (10^9/L)	PLT (10^9/L)
A (Control)	41.33 ± 0.71[a]	144.00 ± 1.83[a]	7.32 ± 0.23[a]	11.75 ± 2.21[a]	732.00 ± 34.94[a]
B (125 mg/kg body weight)	40.00 ± 1.73[a]	139.00 ± 6.11[a]	7.07 ± 0.11[a]	10.33 ± 1.20[a]	600.70 ± 16.37[a]
C (250 mg/kg body weight)	48.50 ± 3.96[b]	141.00 ± 5.86[a]	7.19 ± 0.23[a]	9.33 ± 1.33[a]	892.30 ± 16.93[a]
D (500 mg/kg body weight)	47.33 ± 0.33[b]	120.33 ± 13.11[a]	6.45 ± 0.61[a]	8.67 ± 0.33[a]	637.70 ± 11.90[a]

Values are expressed as mean ± S.E.M (n = 4). Significant (*P < 0.05)* differences are denoted by different alphabetical superscripts in each column. PCV: Packed cell volume; HB: Hemoglobin; RBC: Red blood cell; WBC: White blood cell.

4.0 DISCUSSION

The liver, being central to the metabolism of xenobiotics, is at risk of toxicity due to the substances being metabolized and/or their metabolites. Drug-induced hepatotoxicity is the leading cause of acute liver failure and associated mortality [30]. Serum indicators of liver function, including ALT, AST, ALP, and bilirubin, are used to assess the health status of the liver, where elevated levels may indicate hepatocellular damage [31]. The absence of hepatomegaly and dose-dependent reduction of the serum activities of the transaminases in the experimental animals could mean that the aqueous leaf extract of *B. sapida* does not cause hepatocellular injury when administered orally at doses up to 500 mg/kg body weight. However, elevated serum ALP and bilirubin levels suggest that there could be cholestatic injury [32], especially at 500 mg/kg body weight. Due to the complex nature of the crude aqueous extract, the actual causal phytochemical may not be identified, but some diterpenoids, lactones, and flavonoids have been implicated in causing hepatic cholestatic injury [33], and such classes of phytochemicals have been reported in the aqueous leaf extract of *B. sapida* [8]. The extract exhibited a significant antioxidant effect in the liver by inhibiting lipid peroxidation and oxidative DNA damage (as indicated by a significant reduction in liver MDA and 8OHdG content). The significant increase in protein carbonyl levels could have been due to a process unrelated to lipid peroxidation [34]. Moreover, the antioxidant activities of the ethanol extract from the stem bark of the plant have been variously reported [35, 36] due to the presence of phenols and flavonoids [8].

The kidneys are particularly vulnerable to toxic injury because of their high blood flow rate, large endothelial surface area, high metabolic activity, and involvement in the total body clearance of herbal

substances, which may exert their toxicity due to their inherent properties. Serum creatinine and urea are glomerular function biomarkers, and their elevated values may denote glomerulopenia, as occurs in chronic kidney disease, or abrupt, intrinsic, or reflex loss of glomerular function, which occurs in acute kidney injury [37]. Distorted serum electrolyte levels may indicate kidney injury as well as other conditions, including dehydration, malnutrition, lung disorders, gastrointestinal disorders, cardiac disorders, endocrine disorders, circulatory disorders, and acid-base imbalance [38]. In this study, relative kidney weight, serum creatinine, Na^+, and Mg^{2+} levels were not adversely affected by the administration of the aqueous leaves of *B. sapida*; however, the dose-dependent increase in serum urea, clinically referred to as uremia, and PO_4^{3-} and HCO_3^- raises suspicion of extra-renal involvement, since there is no concomitant increase in serum creatinine [39]. The SOD activity, MDA, and protein carbonyl levels suggest that the administration of aqueous extract does not initiate lipid peroxidation and protein carbonylation in the kidney. The reduction in reduced glutathione levels indicates that the extract (especially at 500 mg/kg body weight) may deplete GSH and dose-dependently cause oxidative damage to the genetic material of the kidney, as reflected by the increase in kidney 8OHdG levels.

Lipid profile (measurement of cholesterol, lipoproteins, and triglycerides) has clinical relevance in coronary artery diseases [40]. Despite the dose-dependent increase in serum cholesterol and triglyceride, the atherogenic index suggests that oral consumption of aqueous extract of *B. sapida* does not predispose individuals to atherosclerotic cardiovascular diseases. This is because the atherogenic index (AI) of none of the groups was more than 0.1 (AI of − 0.3 to 0.1, 0.1 to 0.24 and > 0.24 indicate a low, medium and high risk of cardiovascular diseases respectively [41. Similar non-atherogenic findings have been reported for both aqueous and ethanol extracts of *B. sapida* leaves at doses of 100 mg/kg body weight [42]. The increase in cholesterol and triglyceride levels in the serum could be linked to cholestatic liver injury [43], indicated by a rise in serum ALP and bilirubin levels, as discussed above.

Haematological parameters are assessed to appraise the physiological and pathological changes induced by toxic stress in organisms [44]. In this study, administration of aqueous leaf extract of *Blighia sapida* had no adverse effect on the PCV, RBC, and Hb, indicating that the extract at 125, 250, and 500 mg/kg body weight does not cause anemia, while the absence of significant influence on WBC and platelet count may indicate no inflammation and thrombocytopenia. The aqueous extract of *B. sapida* leaves has no hematotoxicity when administered up to 500 mg/kg body weight. These findings are in agreement with those of [45], who reported that the ethanol stem bark extract of *B. sapida* does not have any adverse effects on the haematological indices of rats when administered at doses up to 750 mg/kg body weight.

CONCLUSION

Subacute oral administration of aqueous leaf extract of *Blighia sapida* for 21 days did not cause hepatocellular injury but could lead to cholestatic liver injury at 500 mg/kg body weight. The extract did not produce nephrotoxicity while preserving the haematological indices. The atherogenic index indicates a low risk of cardiovascular disease. The lipid profile was consistent with cholestasis, which could be

reversed by discontinuation of the extract administration. It is recommended that aqueous leaf extract of *Blighia sapida* should be used at dosages lower than 500 mg/kg body weight to avoid toxicity.

ABBREVATIONS

Abbreviations

8OHdG: 8-hydroxyl deoxyguanosine

AI: atherogenic index

ALP: alkaline phosphatase

ALT: alanine transaminase

AST: aspartate transaminase

EDTA: Ethylenediaminetetraacetic acid

GSH: reduced glutathione

Hb: Haemogligin concentration

HDL-C: high-density lipoprotein-cholesterol

LDL-C: low-density lipoprotein-cholesterol

MDA malondialdehyde

PLT: platelet count

PVC: The packed cell volume

RBC: Red blood cell count RBC

SOD: superoxide dismutase

TBARS: thiobarbituric acid reactive substances

TG: triglycerides

VLDL-C: very low-density lipoprotein-cholesterol

WBC: white blood cell count

Declarations

Ethics approval

The animals were handled according to the international guidelines issued by The Basel Declaration issued on November 30, 2010.

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Author Contribution

M.O.A and T.D.O. conducted experimental investigation, collected the data, performed the laboratory analyses, and prepared the initial draft of the manuscript. M.O.A and M.T.Y. contributed to the study conceptualization, methodology, supervision, data interpretation, and critical revision of the manuscript. M.O.A. contributed to the study design, statistical analysis, interpretation of the findings, manuscript writing, editing, and preparation of the final version for submission. All authors reviewed and approved the final manuscript.

Data Availability

All data generated or analysed during this study are included within the manuscript.

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