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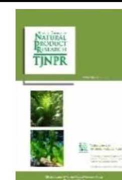

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Original Research Article

Toxicological Evaluation of the Aqueous Leaf Extract of *Blighia Sapida* K.D. Koenig (Sapindaceae) in Rodents

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ABSTRACT

The leaf of *Blighia sapida* K.D. Koenig (Sapindaceae) has been used traditionally to treat several conditions, such as diabetes mellitus and hypertension. However, there is still limited knowledge of its toxicity and safety. The study was aimed at investigating the sub-acute toxicological profile of the aqueous leaf extract of *Blighia sapida* in rodents. The leaf extract was obtained by cold maceration for 72 h. A 21-day sub-acute toxicity test was performed using doses of 500, 1000, and 2000 mg/kg orally. *In vitro,* antioxidant tests were performed using standard methods 2, 2-diphenyl-2-picryl-hydrazyl, ferric reducing antioxidant power, 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonate) and hydroxyl tests. Biochemical, hematological and histological studies were performed. The leaf extract demonstrated antioxidant activity. The spleen was significantly increased at 2000 mg/kg compared to the control. Marked increases in hemoglobin levels at all the doses, hematocrit levels at 500 mg/kg and platelet counts at 1000 mg/kg relative to the control were observed. Cholesterol and triglyceride levels were significantly decreased at 500 and 1000 mg/kg in relation to the control. Urea concentrations at 1000 and 2000 mg/kg were significantly increased relative to the control. A significant increase was observed in albumin levels at 500 and 2000 mg/kg compared to the control. Alkaline phosphatase was reduced at 2000 mg/kg compared to the control. Most organs showed normal histological features at 500 mg/kg. This study shows that repeated exposure of the extract to rats may be toxic at doses above 500 mg/kg and may be unsafe for traditional users at higher doses.

Keywords: *Blighia sapida*; Toxicity; Liver function tests; Lipid profile; Histology; Hematology

Introduction

Assessment of the toxicity profile of a novel drug and to be assured of its' safe use in humans is of a high premium. Toxicological studies are essential for the development of a new drug and to ensure that the existing drugs are still safe.[1] Toxicological evaluations involves the characterization of toxicity in animal models and the ability to correlate them with human situations.[1] To achieve this, conducting sub-acute toxicity tests becomes very important since it involves repeated dosing.[2] Although data from *in vitro* studies may indicate potential toxicity, *in vivo* animal studies are more indicative of toxicity and may be considered safety markers.[3]
In English, *Blighia sapida* is known as 'Ackee,' 'akee,' 'akee apple,' and 'breadfruit.' However, it is also known by different names in different countries and ethnic groups in West Africa.[4] *Blighia sapida* leaves and pulp are traditionally utilized to manage eye conjunctivitis.[5] The leaf extract is used in traditional medicine in treating diabetes mellitus and hypertension.[6]

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The bark pulp of *Blighia sapida* is used as an ointment for edema and to relieve people of pains arising from the intestines. The bark can also be formulated into powdery form with a mixture of capsicum and rubbed to stimulate the body.[7] *Blighia sapida* has also been used traditionally in managing dysentery, yellow fever, eye defects, burns, wounds, skin diseases and others.[8] Bioactive compounds such as chlorogenic acid, saponin, tannin, quercetin amongst others have been identified in the leaf extract.[9] Pharmacological studies have shown its anticancer, antioxidant, analgesic, antimicrobial, and hypoglycaemic activities.[9-13]
A limited number of toxicity studies have identified hypoglycins A and B in the plant. These toxic substances are found in unripe ackee fruit.[14,15] A report has shown that ackee fruit may be neurotoxic and hepatotoxic if the unripe fruit is eaten.[16] Urine samples taken from two toddlers with ackee fruit poisoning revealed significant levels of methylene-cyclopropyl acetic acid, including dicarboxylic acids 2-ethyl malonate, adipate, and glutarate.[17,18] However, these toxicity studies were conducted on the fruit of the plant.
Although the leaf of *Blighia sapida* is traditionally used for medicinal purposes, no study has reported on the toxicological evaluation of the leaves of *Blighia sapida* to demonstrate the safety relevance of its ethnomedicinal use. Therefore, this study evaluated the sub-acute toxicological profile of aqueous extract of *Blighia sapida* (AEBS) in rats to establish the safety use of *Blighia sapida* leaves in traditional medicine.

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Materials and Methods

Plant material

The leaves of *Blighia sapida* were obtained from Oke-Ureje in Ekiti State in January 2019. The plant name was checked with http://www.theplantlist.org. The latitude: 7.607463; longitude: 5.255823; accuracy: 2400 m were the GPS coordinates for the site for plant collection. The taxonomical identification and authentication of the plant were performed by Mr Omotayo Olorunfemi of the herbarium unit of Ekiti State University, Ado-Ekiti, Nigeria. A voucher specimen with identification number UHAE 2020092 was deposited in the herbarium section of Ekiti State University, and this was compared with the reference plant. Thereafter, the leaves were washed and air-dried for three weeks. The leaves were blended to a coarse powdery form using an electronic blender.

Extraction procedure

The blended leaves of *Blighia sapida* (1500 g) were extracted by cold maceration with distilled water (50L) for 72 h. The AEBS was concentrated using a water bath at 45℃ before it was dried in an oven for three days at 45℃. The dried AEBS was weighed and kept in airtight cans and preserved in a refrigerator until when it was used.

Chemicals and reagents

The chemicals and reagents used included formaldehyde (Griffin and George, Leics, England), biochemical assay kits (Randox laboratories, United Kingdom), phosphate buffer, 20% Tricyclic acid (Burgoyne Burbidges and Co., Mumbai, India), tris-KCl buffer (Hopkin and Williams Company, USA), and thiobarbituric acid (Guangdong Guanghua and Chemical Factory Co. Ltd.). All other organic solvents were bought from Rovet Chemicals and Reagents, Benin City, Nigeria. The maximum drug volume administered was 10 ml/kg (per os). The extracts were administered orally.

Fingerprint of AEBS

The fingerprint of AEBS using high performance liquid chromatography/photodiode array detector (HPLC/DAD) has been conducted in a previous study.[9]

Experimental animals

Forty Wistar rats (n=10) male and female, weighing 120-180 g were used for the study. The animals were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. All animals were housed in plastic cages in groups (males separated from females) under natural environmental conditions. The experimental rats were given food and water, and good hygiene was ensured. All experiments were conducted during the light period (between 09.00 and 17.00 daily).

Ethical issues

Experimental procedures and protocols used in this study conformed to the "Guide to the Care and Use of Animals in Research and Teaching" (NIH publications number 85-93 revised in 1985). Ethical approval (reference number EC/FP/020/13) was obtained from the Animal Ethics Committee of the Faculty of Pharmacy, University of Benin.

Test for In vitro antioxidant activity

The antioxidant activity of AEBS was assessed using four methods; 2, 2-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity, Ferric reducing antioxidant power (FRAP), hydroxyl (OH^-), and 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonate) ($ABTS^+$) free radical scavenging assay as follows:

2, 2-diphenyl-2-picryl-hydrazyl free radical scavenging activity

The DPPH test on AEBS was performed using the method of Chang.[19] Stock solution (1 mg/ml) AEBS was prepared. This was followed by administering serial dilutions of AEBS to get concentrations of 10, 20, 40, 60, and 80 µg/ml. The same quantity of the sample was mixed with 0.3 mM methanol solution of DPPH. The mixture was vortexed and thereafter stood in the dark for 30 min at 25C. After the incubation period, the absorbance was read against a blank at 518 nm using a double-beam Analykjena UV-VIS spectrophotometer (Model 205, Jena, Germany). The scavenging activity was calculated with the following formula:

$$\text{Inhibition percentage (I \%)} = \frac{ABS_{control} - ABS_{sample}}{ABS_{control}} \times 100$$

The $ABS_{control}$ refers to the absorbance of the control, and ABS_{sample} means the absorbance of the experimental sample. The IC_{50} value was obtained by plotting the percentage inhibition against the extract concentration. All determinations were performed in triplicate. Ascorbic acid was the standard drug.

Ferric reducing antioxidant power assay

Benzie and Strain's[20] method was used to measure the FRAP. Ascorbic acid was the standard used. The crude drug was added to 3.8 ml of FRAP reagent (which consists of ten portions of 300 mM sodium acetate buffer at pH 3.6, one portion of 10.0 mM TPTZ solution, and one portion of 10.0 mM $FeCl_3$. $6H_2O$ solution) and the solution was incubated at for 30 min at 37℃. At 593 nm the increase in absorbance was read. For the calibration, the $FeSO_4$ was used. The antioxidant activity was obtained from the linear calibration curve and represented as mmol $FeSO_4$ equivalents/g. The ability of the sample to reduce ferric ions demonstrates its antioxidant function.

Hydroxyl radical scavenging assay

The AEBS hydroxyl radical scavenging activity was measured using the method described by Kunchandy and Rao[21]. Ascorbic acid was used as the standard. The reaction mixture (1 ml) consisted of 100 µl of 2-deoxy-D-ribose (2.8 mM in 20 mM KH_2PO_4– KOH buffer, pH 7.4), extract, 200 µl of EDTA (0.1 mM) and 200 µM of $FeCl_3$ (1:1 v/v), 100 µl of H_2O_2 (0.1 mM) and 100 µl ascorbic acid (0.1 mM). These were incubated for 1 h at 37ºC. A 1 ml of 1% thiobarbituric acid and 1 ml of 2.8% trichloroacetic acid were added and incubated at 100ºC for 20 min. When the solution is cool, an absorbance of 532 nm was measured against the blank sample.

ABTS radical scavenging assay

The ABTS assay free on AEBS was determined with the procedure of Stratil.[22] Ascorbic acid was used as the standard. The radical cation $ABTS^+$ was got by the persulfate oxidation of $ABTS^+$. A 1:1 v/v of 7.0 Mm of ABTS and 4.95 mM potassium persulfate was allowed to stay overnight at room temperature in the dark to form the radical cation $ABTS^+$. The working solution was diluted with phosphate buffer (pH 7.4) to absorbance values between 1.0 and 1.5 at 734 nm. A 0.1 ml aliquot of each sample was mixed with 3.9 ml of the working solution. A measurement of the reduction in absorbance was made at 734 nm after 10 min at 37°C with no light. A 3.9 ml aqueous phosphate buffer solution was used as the control.

Assessment of sub-acute toxicity in animals

Forty rats were utilized for the oral sub-acute toxicity test. The rats were alloted into four groups randomly. Group I was the control group and were administered distilled water (10 ml/kg) daily. Groups II, III and IV were the treatment groups and were administered 500, 1000, and 2000 mg/kg doses of the extract, respectively. Both extract and water were administered daily orally. Signs of toxicity and mortality were monitored daily for 21 the rats. Animals were sacrificed on the 22nd day after an overnight fast. Blood was withdrawn using a 5 ml syringe fitted with a 21G needle from the abdominal aorta of each rat. Portions of the blood were placed in plain bottles and bottles containing ethylene diamine tetra acetic acid (EDTA). Samples in EDTA bottles were used for hematological evaluation, while samples in plain tubes were used for biochemical analysis. The liver, kidneys, heart, spleen, lungs, and brains were harvested from each rat.

Measurement of body weight

The initial body weights of the animals were recorded on the first day before drug administration (day 1) and the last day (day 21). Organs such as the liver, kidneys, heart, spleen, lungs, and brains were

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harvested and weighed using an electronic balance. The relative organ weights (ROW) of all rats were calculated using the formula:

$$ROW = \left(\frac{Absolute\ organ\ weight\ (g)}{Body\ weight\ (g) on\ the\ day\ of\ sacrifice}\right) x\ 100$$

Haematological assessment

The haematological indices measured were hematocrit levels (HCT), red blood cell count (RBC), haemoglobin concentration (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), white blood cell count (WBC), and the differentials: granulocyte (GRAN), monocyte (MONO), lymphocyte (LYM)), and platelet counts (PLT). These were measured using an automatic haematology analyzer (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan) (University of Benin Teaching Hospital haematology analyzer)

Biochemical assays

The blood samples in plain bottles were allowed to clot and then centrifuged at 5000 rpm to obtain serum. The serum samples were utilized for the biochemical assays:

Lipid function assays

Cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were estimated by the enzymatic colorimetric method.[23,24,25,26,27.]

Kidney function assay

The urease Berthelot method of Fawcett and Scott[28] were used to estimate the urea concentration. Creatinine (Cr) concentration was estimated using Jaffe's reaction method[29]. Electrolytes such as sodium ions (Na^+), potassium ions (K^+), bicarbonate ions (HCO_3^-), and chloride ions (Cl^-) were estimated.[30]

Liver function assays

Alkaline phosphatase (ALP) activity was determined by the method of Walter and Schutt.[31] Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the method of Reitman and Frankel.[32] Other proteins, such as total protein (TP) and globulin (GL) estimation, were done by the Biuret method.[33] The Jendrassik-Grof method was used to estimate total bilirubin (TB)[34] and conjugated bilirubin (CB).[35] The ALB was estimated using the methods of Doumas et al.[36] and Grant et al.[37] All assays were done using an auto-analyzer. (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan) (University of Benin Teaching Hospital haematology analyzer)

Histology of the organs

Two randomly selected hearts, livers, lungs, kidneys, and brains from the rats were rinsed in normal saline, blotted with Whatmann's No. 1 filter paper, and then checked for visible changes. After that, the organs were preserved in 10% formaldehyde in normal saline. Sections of 5 µm were made of each organ, and the sections were embedded in paraffin wax. Thereafter, the sections were stained with hematoxylins and eosin[38] Photomicrographs of the stained tissue sections were produced using a digital microscope (Olympus®) at ×400 magnification.

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM), and "n" stands for the number of animals per group. Excel software was used to analyze the absolute body weight of rats. One-Way Analysis of Variance (ANOVA) with Dunnett's was adopted to analyze the differences between the data being compared. Differences were considered significant at $p < 0.05$.

Results and Discussion

Antioxidant activities of aqueous extract of *Blighia sapida*

The study has shown that aqueous extract of *Blighia sapida* possesses *in vitro* antioxidant activity (Figure 1). In Figure 1A, aqueous extract of *Blighia sapida* demonstrated DPPH free radical scavenging activity in a non-concentration-dependent manner. The extract exhibited 69.99% free radical inhibition at a concentration of 80 µg/ml, whereas the standard, ascorbic acid, showed 97.49% free radical inhibition at the same concentration. The IC_{50} value of ascorbic acid (0.63 µg/ml) was higher than that of the extract (23.97 µg/ml).

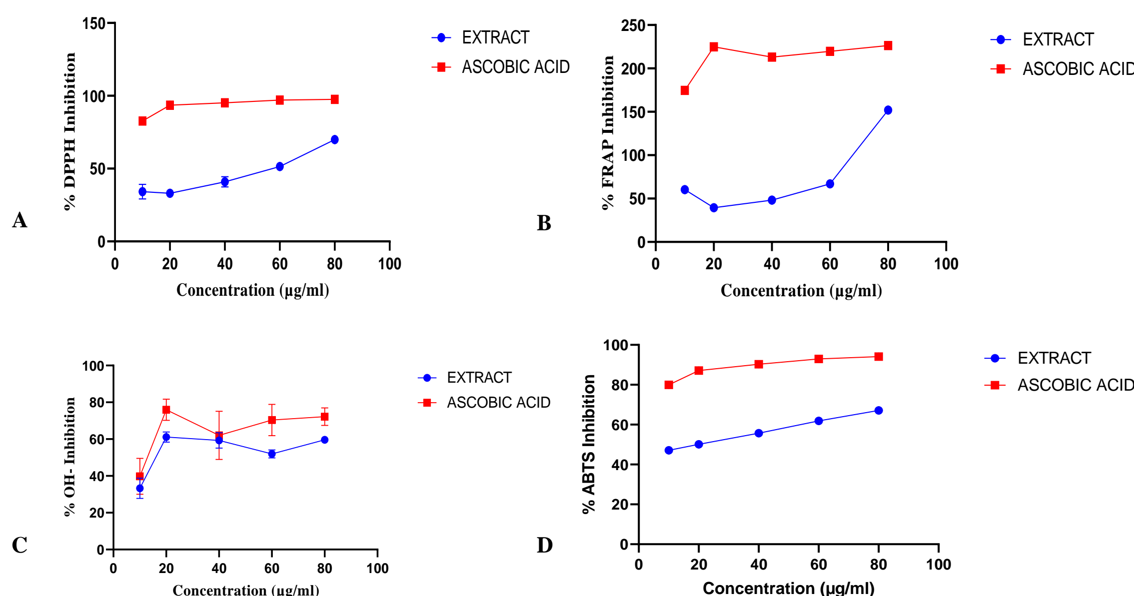

Figure 1: Antioxidant activity of AEBS in rats: Data were expressed as mean ± SEM, *n=3 replicates. (A) 2,2-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity; (B) Ferric reducing antioxidant power (FRAP); (C) Hydroxyl ion free radical scavenging activity; and (D) 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonate) (ABTS) scavenging activity.

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Table 1: Effects of daily administration of AEBS for 21 days on relative organ weight in rats

Organ weight (g)	Control (mg/kg)	500 (mg/kg)	1000 (mg/kg)	2000 (mg/kg)
Liver	7.138 ± 0.23	8.461 ± 0.53	7.370 ± 0.68	7.558 ± 0.45
Kidney	0.532 ± 0.02	0.608 ± 0.02	0.581 ± 0.08	0.537 ± 0.03
Spleen	0.656 ± 0.05	0.700 ± 0.06	0.599 ± 0.03	0.944 ± 0.09[a]
Lungs	1.588 ±0.09	1.885 ± 0.09	1.467 ± 0.10	1.537 ± 0.10
Brain	1.442 ± 0.07	1.545 ± 0.05	1.444 ± 0.11	1.430 ± 0.07
Heart	0.681 ± 0.02	0.669 ± 0.02	0.644 ± 0.03	0.637 ± 0.04

Values are expressed as mean ± SEM. *n= 10. [a] $p < 0.01$ *vs* control rats.

Table 2: Effects of daily administration of aqueous extract of *Blighia sapida* for 21 days on hematological indices in rats

Hematological parameters	Control (mg/kg)	500 (mg/kg)	1000 (mg/kg)	2000 (mg/kg)
WBC (x103/µl)	7.117 ± 1.12	7.257 ± 0.94	6.933 ± 0.94	6.700 ± 0.85
LYM (%)	89.380±1.26	88.960 ± 0.70	88.720 ± 1.29	90.770 ± 0.49
MONO (%)	5.600 ± 0. 81	5.943 ± 0.48	5.317 ± 0.19	5.183 ± 0.32
GRAN (%)	5.017 ± 0.47	5.100 ± 0.28	4.550 ± 0.35	4.086 ± 0.28
RBC (x106/µl)	4.938 ± 0.23	5.456 ± 0.09	5.512 ± 0.15	5.401 ± 0.17
HGB (g/dl)	11.680 ± 0.62	14.060 ± 0.29[c]	13.850 ± 0.21[c]	13.610 ± 0.37[b]
HCT (%)	37.270 ± 1.70	41.310 ± 1.14[b]	40.600 ± 0.89	40.710 ± 0.69
MCV (fl)	74.820 ± 1.50	74.040 ± 0.54	73.880 ± 1.76	75.810 ± 1.98
MCH (pg)	24.330 ± 0.73	24.960 ± 0.48	25.030 ± 0.48	25.200 ± 0.49
PLT (x103/µl)	675.0 ± 46.03	770.6 ± 50.60	941.3 ± 88.50[a]	750.9±68.18

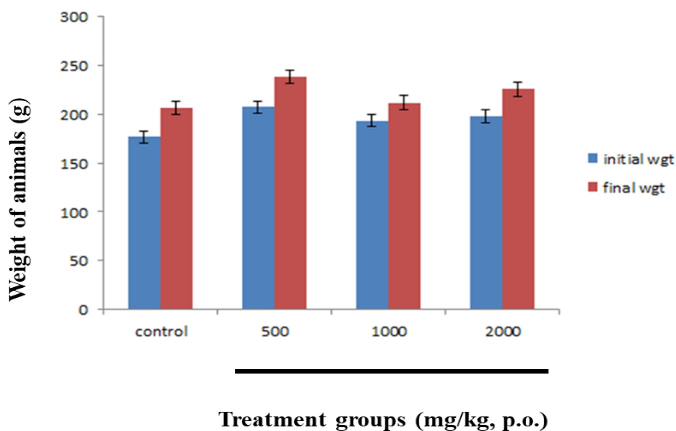

Figure 2: Effect of daily administration of aqueous extract of *Blighia sapida* for 21 days on absolute body weight of rats. Values are expressed as mean ± SEM. *n=10 rats.

The extract also demonstrated ferric-reducing antioxidant activity in a concentration-dependent manner (10-80 µg/ml). However, the reducing power of aqueous extract of *Blighia sapida* was significantly lower ($p < 0.05$) in comparison to the standard ascorbic acid. The IC_{50} value of ascorbic acid (6.13 µg/ml) was higher than that of aqueous extract of *Blighia sapida* (61.67 µg/ml) (Figure 1B).
At the same concentration of 20 µg/ml, aqueous extract of *Blighia sapida* and ascorbic acid demonstrated OH^- scavenging activity of 61.10% and 75.92%, respectively (Figure 1C). At this concentration, both aqueous extract of *Blighia sapida* and ascorbic acid exhibited the highest level of inhibition. The IC_{50} value of aqueous extract of *Blighia sapida* (9.6 µg/ml) was slightly higher than that of ascorbic acid (11.03 µg/ml).
Figure 1D shows that aqueous extract of *Blighia sapida* demonstrated $ABTS^+$ scavenging activity in a concentration-dependent manner (10-80 µg/ml). Although, ascorbic acid had a higher percentage (94.12%) of $ABTS^+$ scavenging activity compared to aqueous extract of *Blighia sapida* (67.16%). The IC_{50} value of ascorbic acid (6 µg/ml) was comparable with that of aqueous extract of *Blighia sapida* (7.23 µg/ml).

Toxicological evaluations
In a previous study, the acute test of aqueous extract of *Blighia sapida* was assessed, and was safe at an oral LD_{50} value above 5000 mg/kg.[9] In the current research, a 21-day sub-acute test was conducted and the effect of aqueous extract of *Blighia sapida* on absolute body weight showed no significant difference observed in the initial weight and the final weight between the treatment groups and the control (Figure 2). However, the effect of aqueous extract of *Blighia sapida* on organ weight showed a marked increase in the weight of the spleen at the dose of 2000 mg/kg/day compared to the control group (Table 1). This weight gain in the spleen might not reflect any histopathological alterations, since the histology of the spleen in this study (Figure 8) shows a normal histomorphology at all the treatment doses. A previous study had stated that a change in organ weight may be due to inter-animal variability, stress, and physiologic factors, which may not be associated with the treatment options.[39]
No significant differences between the control and treatment groups in the total WBC and the differentials (LYM, MONO, GRAN), RBC, MCV, and MCH concentration were observed in the study (Table 2). However, marked differences were seen between the control group and treatment groups in the HGB concentration at 500 mg/kg/day, 1000 mg/kg/day, and 2000 mg/kg/day; HCT at 500 mg/kg/day dose, and PLT platelet counts at 1000 mg/kg/day dose (Table 2). This shows that the treatment did not affect the total RBC, WBC, MONO, LYM, GRAN MCV, and MCH. The lack-of-effect observed on the LYM levels may indicate that the extract did not induce any immuno-stimulatory response in the rats since these cells are usually elevated in response to foreign agents and are known to produce antibodies that allow the destruction of infectious cells.[40] The significant increases in the HGB and HCT levels are risk factors for cardiovascular disease development.[41,42] Platelets maintain blood hemostasis.[43,44] The increased PLT count may account for hemostatic disorders or

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thrombocytes.[43,44] A report has shown that the normal ranges for HGB and HCT levels, including PLT counts in rats are between 11-19 g/dl, 35-57%, and 200-1500 ×103/µl, respectively.[45] Although the haematological values (HCG, PLT and HCT) obtained from this study fall within these normal ranges in rats, they don't appear sufficient enough to infer that the crude drug was non-toxic to the blood at those doses. Hence, the significant increases in the HGB, HCT and PLT counts observed in the study may be suggestive of some underlying haematological disorder or cardiovascular diseases. However, further studies may be necessary to elucidate these findings.

The study has shown no statistically significant differences in the values for serum lipids (LDL and HDL) in the treatment groups compared to the control group (Table 3). However, a statistically significant decrease in serum lipid profile of CHOL at 500 mg/kg and 1000 mg/kg/day dose compared to the control was observed. Also, TRIG levels were significantly decreased at 500 mg/kg/day and 1000 mg/kg/day doses in the test groups compared to the control group (Table 3). These Findings are consistent with a previous study, were the leaf extract of *Blighia sapida* attenuated diabetes-mediated changes in the breakdown of lipids.[13] In like manner, the present study has shown that aqueous extract of *Blighia sapida* can regulate lipid metabolism. The reduced CHOL and TRIG levels may be cardioprotective. The ability of aqueous extract of *Blighia sapida* to regulate lipid metabolism may be due to the presence of saponins, chlorogenic acid, and quercetin in aqueous extract of *Blighia sapida* (9), and which have been noted for their hypocholesterolemic effect, ability to reduce the cytotoxic effects of LDL, reduce plasma concentrations of HDL, and lower triglyceride levels in rats.[46-51] These bioactive compounds have strong antioxidant potentials, which aqueous extract of *Blighia sapida* has demonstrated in this study (Figure 1).

The concentrations of Na^+, K^+, HCO_3^-, Cl^-, and creatinine in the treatment groups were not significantly different from the control (Table 4). However, a significant increase in urea concentration was observed between the treatment groups at 1000 and 2000 mg/kg/day doses of aqueous extract of *Blighia sapida* and the control group (Table 4). Urea, Cr, and electrolyte levels are important markers of renal function that are used routinely. An increase in blood urea nitrogen may often be related to renal disease, kidney stones, cardiac failure, and shock.[52,53] Accordingly, the histology of the kidneys has revealed the presence of active interstitial congestion and interstitial lymphocyte, which are indicators of acute kidney injury following an ischemic or toxic insult.[54] Given these findings, the increase in urea concentration may suggest signs of toxicity to the kidneys by the crude drug at higher doses (1000 and 2000 mg/kg).

No significant differences in the levels of liver enzymes - ALT and AST when compared with the control group (Figures 3) were observed. But, a significant decline in the level of ALP at 2000 mg/kg/day dose of the aqueous extract of *Blighia sapida* compared to the control group (Figure 3) was observed. The liver is an organ at risk of toxicity. The liver enzymes: AST, ALT, and ALP are used routinely as important liver function biomarkers[55,56] Cellular injury to the liver is the main trigger for releasing these enzymes into circulation. High levels of AST and ALT are indicators of hepatocellular disease, whereas increased levels of ALP may indicate cholestasis.[57] The reduction in the activity of ALP at 2000 mg/kg doses may suggest an underlying perturbation in some metabolic functions in the cells. Moreover, considering the comparable levels of ALT and AST with the control in the study, suggesting that this effect may be hepatoprotective. This hepatoprotective function could be attributed to the extract's free radical scavenging effect demonstrated in the study (Figure 1) and the presence of polyphenols (e.g., tannins, quercetin, gallic acid) and phenolic acids (caffeic acid and chlorogenic acid), that have been previously identified in the leaf extract.[9]

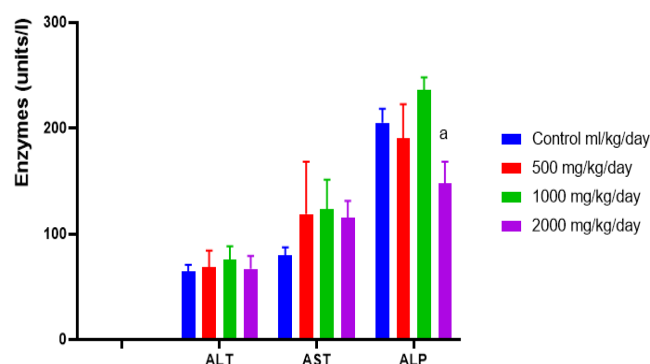

Figure 3: Effect of AEBS on liver enzymes. Values are represented as mean ± SEM. *n = 10 rats. ALT: Serum alanine transferase levels in rats; AST: Serum aspartate aminotransferase levels in rats; ALP: Serum alkaline phosphatase levels in rats. [a] $p < 0.001$ *vs* control.

Table 3: Effect of daily administration of aqueous extract of *Blighia sapida* for 21 days on serum lipid profile in rats

Lipids (mg/dl)	Control (mg/kg)	500 (mg/kg)	1000 (mg/kg)	2000 (mg/kg)
HDL	30.17 ± 1.66	31.57 ± 0.75	33.83 ± 1.19	27.00 ± 1.93
LDL	30.67 ± 2.79	40.14 ± 1.28	40.83 ± 0.41	30.71 ± 4.38
CHOL	75.50 ± 3.13	52.50 ± 7.14[a]	45.00 ± 7.05[b]	68.50 ± 3.03
TRIG	71.17 ± 3.18	48.50 ± 9.29[a]	36.33 ± 3.16[c]	73.83 ± 3.72

Values were expressed as mean ± SEM, *n=10 rats. [a] $p < 0.01$ *vs* control, [b] $p < 0.001$ *vs* control, [c] $p < 0.0001$ *vs* control. CHOL: cholesterol; TRIG: triglyceride; LDL: low-density lipoprotein; HDL: high-density lipoprotein

Table 4: Effect of aqueous extract of *Blighia sapida* on some electrolytes, urea, and creatinine levels in rats

Electrolytes	Control (mg/kg)	500 (mg/kg)	1000 (mg/kg)	2000 (mg/kg)
Urea conc	22.67 ± 1.17	27.86 ± 1.59	37.67 ± 3.68[a]	33.14 ± 3.11[a]
Na^+	131.70 ± 1.02	134.30 ± 0.94	132.30 ± 1.38	135.10 ± 0.96
K^+	5.48 ± 0.56	5.91 ± 0.32	5.68 ± 0.27	5.64 ± 0.17
HCO_3^-	20.50 ± 0.76	20.86 ± 0.51	19.83 ± 0.65	20.43 ± 0.48
Cl^-	95.57 ± 1.02	96.86 ± 0.70	95.50 ± 0.76	97.00 ± 0.95
Cr	0.65 ± 0.02	0.67 ± 0.03	0.67 ± 0.05	0.67 ± 0.03

Values were expressed as mean ± SEM,* n=10 rats. [a] $p < 0.001$ *vs* control. Na^+: Sodium ion; K^+: Potassium ion; HCO_3^- : Bicarbonate ion; Cl-: Chloride ion; Cr: Creatinine

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Table 5: Effect of aqueous extract of *Blighia sapida* on serum proteins in rats

Proteins	Control (mg/kg)	500 (mg/kg)	1000 (mg/kg)	2000 (mg/kg)
ALB (Units/l)	2.617 ± 0.07	3.000 ± 0.05[a]	2.800 ± 0.09	2.929 ± 0.78[b]
C.B (mg/dl)	0.150 ± 0.02	0.086 ± 0.03	0.100 ± 0.03	0.100 ± 0.00
T.P (mg/dl)	6.600 ± 0.12	6.900 ± 0.17	6.817 ± 0.09	6.900 ± 0.11
T.B (mg/dl)	0.317 ± 0.03	0.286 ± 0.03	0.283 ± 0.05	0.271 ± 0.03

Values were expressed as mean ± SEM. *n = 10 rats. [b] $p < 0.01$ *vs* control; [a] $p < 0.001$ *vs* control. ALB: Albumin; C.B: Conjugated bilirubin; T.P: Total protein; T.B: Total bilirubin

Table 5 shows no significant differences in TB, CB, and TP concentrations compared to the control. However, a significant increase in the levels of ALB at doses of 500 mg/kg/day and 2000 mg/kg/day compared to the control (Table 5) was seen in the study. Increases in serum ALB indicate hemoconcentration, possibly due to dehydration.[58] The serum ALB levels fall (2.9- 5.9 g/dl) within typical values in rats in this study.[45,59] The normal levels of the proteins studied may be indicative of some hepatoprotective function. This supports the results obtained from the liver enzymes (ALT and AST).

Histoarchitectural features in the parenchymal tissues of selected organs (heart, lungs, liver, kidney, spleen and brain) are shown in Figures 4-9, respectively after administering graded doses (500, 1000 and 2000 mg/kg/d) of aqueous extract of *Blighia sapida*. Some effects were observed in the organs, such as active interstitial congestion at 1000 and 2000 mg/kg doses in the heart of the treatment group compared to the control (Figure 4). The observed active interstitial congestion at doses of 1000 and 2000 mg/kg may be suggesting signs of toxicity. The histology of the lungs showed normal alveolar spaces, interstitial space, bronchial artery, and terminal bronchiole. Some beneficial effects were seen in the lungs such as the activation of cells of the mononuclear phagocyte system (local immune system) of the lungs (bronchioloalveolar lymphoid aggregates- a group of nucleated cells obtained from lung tissue which consists of alveolar macrophages, lymphocytes, plasma cells, neutrophilic and eosinophilic granulocytes) in the lungs[60] (Figure 5). These cells function by reacting to immune responses to antigen stimuli.[61] The presence of these activated cells in the mononuclear phagocyte system may result from the host defence function of the bronchoalveolar cells.

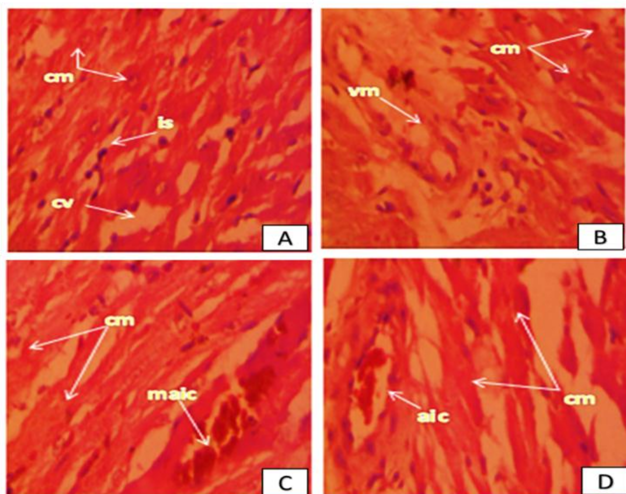

Figure 4: Representative photomicrographs of the heart of rats after AEBS administration for 21 days. (A) Control: showing normal cardiomyocytes (cm), interstitial space (is), and coronary vessel (cv). (B) AEBS 500 mg/kg/day: showing normal cardiomyocytes (cm) and normal vascular microvasculature (vm). (C) AEBS 1000 mg/kg/day: showing normal cardiomyocytes (cm) and mild active interstitial congestion. (D) 2000 mg/kg/day: showing normal cardiomyocytes (cm) and active interstitial congestion. (H and E staining, magnification x400).

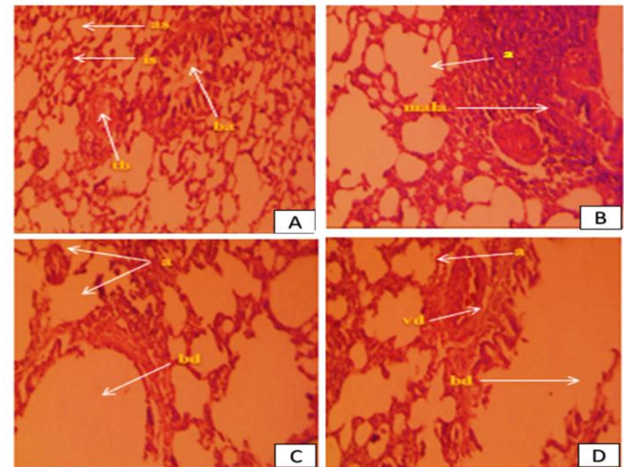

Figure 5: Representative photomicrographs of the lungs of rats after AEBS administration for 21 days. (A) Control: showing normal alveolar spaces (as), interstitial space (is), bronchial artery (ba), and terminal bronchiole (tb). (B) AEBS 500 mg/kg/day: showing normal alveoli (a) and mild active lymphoid aggregates (mala). (C) AEBS 1000 mg/kg/day: showing normal alveoli (a) and bronchiolar dilation (bd). (D) 2000 mg/kg/day: showing normal alveoli (a), vascular distortion (vd) and bronchiolar dilation (bd). (H and E staining, magnification x400).

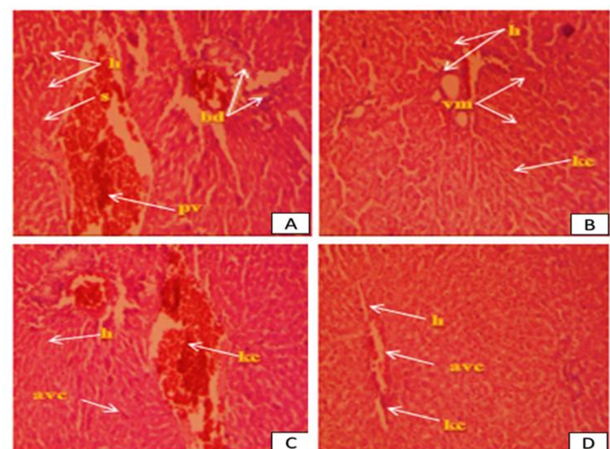

Figure 6: Representative photomicrographs of the liver of rats after AEBS administration for 21 days. (A) Control: showing normal hepatocytes (h), sinusoids (s), portal vein (pv), and bile ducts (bd). (B) AEBS 500 mg/kg/day: showing normal hepatocytes (h), normal vascular microstructure (vm), and mild activation of Kupffer cells (kc). (C) AEBS 1000 mg/kg/day: showing normal hepatocytes (h), active vascular congestion (avc) and Kupffer cell (kc) activation. (D) 2000 mg/kg/day: showing normal hepatocytes (h), active vascular congestion (avc) and Kupffer cell (kc) activation. (H and E staining, magnification x100).

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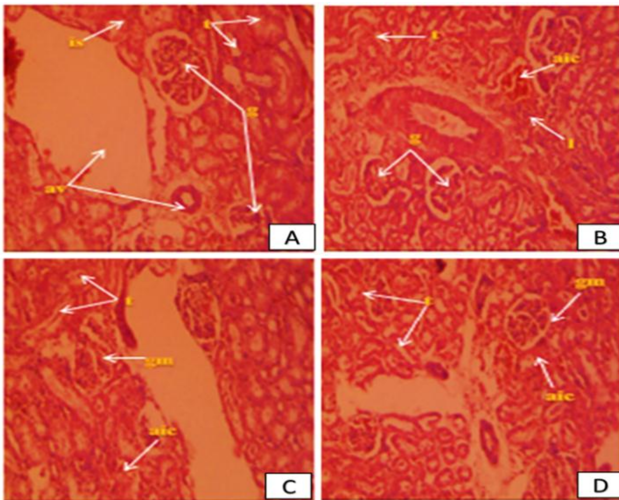

Figure 7: Representative photomicrographs of the kidney of rats after AEBS administration for 21 days. (A) Control: showing tubules (t), interstitial space (is), arcuate vessels (av) and glomeruli (g). (B) AEBS 500 mg/kg/day: showing normal tubules (t), normal glomeruli (g), active interstitial congestion (aic) and lymphocytes (l). (C) AEBS 1000 mg/kg/day: showing normal tubules (t), glomeruli microstructure (gm) and active interstitial congestion. (D) 2000 mg/kg/day: showing normal glomeruli (g), glomeruli microstructure (gm) and active interstitial congestion (aic). (H and E staining, magnification x100).

The histology of the liver showed normal hepatocytes and normal vascular microstructure in all the treatment groups, but active vascular congestion and activation of kupffer cells were observed in the liver at 1000 and 2000 mg/kg relative to the control (Figure 6). The activated kupffer cells observed in the liver at all the treatment doses may be hepatoprotective as activated Kupffer cells play a role in modulating immune and inflammatory responses, and these cells precipitate the synthesis of eicosanoids such as prostaglandin E2 (PGE2) and prostacyclin (PGI2).[62,63] These eicosanoids (PGI2 and PGE2) function to ensure protection against hepatocyte necrosis.[62] The histology of the kidney showed normal interstitial space, arcuate vessels, glomeruli microstructure, and tubules at 500 mg/kg, but the presence of active interstitial congestion and lymphocytes were observed in the kidneys at all the treatment doses relative to the control (Figure 7). These active interstitial congestion, and interstitial lymphocytes coupled with the increased amount of urea in the kidneys, indicates signs of harm to the kidneys. The spleen showed normal red and white pulp, arteriole, normal sinus histiocytes, and follicles which were comparable with the control (Figure 8). The normal histology of the spleen shows that the extract may not have conferred any form of damage to the spleen, irrespective of the increase in the organ weight. The histology of the brain showed a normal molecular layer, granular layer, and purkinje fibers at all the treatment doses except an active vascular congestion at a dose of 500 mg/kg in comparison with the control (Figure 9). Further investigation may be necessary to understand the reason for the active vascular congestion observed in the brain. In most of the organs, immune activation was optimal at the dose of 500 mg/kg. Beneficial effects were observed at this dose (500 mg/kg). Based on the histopathological findings, it is quite clear that at doses higher than 500 mg/kg, the leaf extract of *Blighia sapida* may be toxic.

Conclusion

This study has provided insight into the toxicity and safety of aqueous extract of *Blighia sapida*. Based on our findings, the use of the leaf by traditional users in managing diabetes mellitus, hypertension and other forms of ailments may be relatively safe only at very low doses. The study has shown that repeated exposure of aqueous extract of *Blighia sapida* to the rats may be toxic to the organs at doses above 500 mg/kg, particularly the kidneys and the brain. Sub-chronic and chronic toxicological studies on the leaf extract using lower doses (<500 mg/kg) may be considered to elucidate the long-term toxic effect of the crude drug on the organs.

Conflict of Interest

The authors declare no conflict of interest.

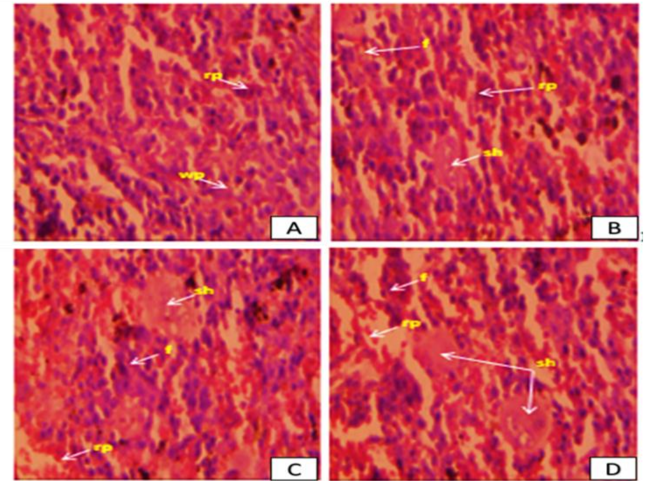

Figure 8: Representative photomicrographs of the spleen of rats after AEBS administration for 21 days. (A) Control: showing red pulp (rp), white pulp (wp), and arteriole (a). (B) AEBS 500 mg/kg/day: showing normal follicles (f), red pulp (rp), sinus histiocytes (sh). (C) AEBS 1000 mg/kg/day: showing normal follicles (f), normal red pulp (rp) and sinus histiocytes (sh). (D) 2000 mg/kg/day: showing normal follicles (f), normal red pulp (rp) and normal sinus histiocytes (sh). (H and E staining, magnification x100).

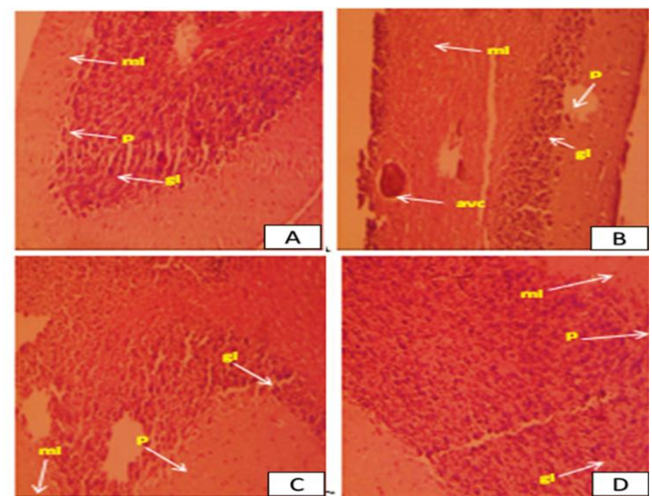

Figure 9: Representative photomicrographs of the brain of rats after AEBS administration for 21 days. (A) Control: showing molecular layer (ml), Purkinje (p), and granular layer (gl). (B) AEBS 500 mg/kg/day: showing normal molecular layer (ml), Purkinje (p), granular layers (gl) and active vascular congestion (avc). (C) AEBS 1000 mg/kg/day: showing normal molecular layer (ml), Purkinje (p) and granular layer (gl). (D) 2000 mg/kg/day: showing normal molecular layer (ml), normal purkinje (p) and granular layer (gl). (H and E staining, magnification x100).

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Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Setzer RW, Kimmel CA. Use of NOAEL, benchmark dose, and other models for human risk assessment of hormonally active substances. Pure Appl Chem. 2003;75(11–12):2151–8.
2. Wilson NH, Hardisty JF HJ. Short-term, subchronic, and chronic toxicology studies. Principles and Methods of Toxicology. fourth edi. Francis T and, editor. Philadelphia: Elsevier; 2001. 917-57. p.
3. Anadón A, Martínez MA, Castellano V, Martínez-Larrañaga MR. The role of in vitro methods as alternatives to animals in toxicity testing. Expert Opin Drug Metab Toxicol. 2014;10(1):67–79.
4. Onuekwusi EC, Akanya HO, Evans EC. Phytochemical Constituents of Seeds of Ripe and Unripe *Blighia sapida* (K. Koenig) and Physicochemical Properties of the Seed Oil [Internet]. Vol. 3, Int. J Pharmaceutical Sci. Invent. ISSN. Online; 2014 [cited 2020 Dec 1]. Available from: www.ijpsi.org
5. Abolaji AO, Adebayo HA, Odesanmi OS. Nutritional Qualities of Three Medicinal Plant Parts (*Xylopia aethiopica*, *Blighia sapida* and *Parinari polyandra*) commonly used by Pregnant Women in the Western Part of Nigeria. 2007;
6. Marles RJ FN. Antidiabetic plants and their active constituents. Phytomedicine. 1995;2:137–189.
7. Okogun J. I. The chemistry of Nigerian medicinal plants. 1996;10:31–5.
8. Etukudo I. Ethnobotany. Conventional and Traditional Uses of Plants. Lippincott Williams & Wilkins; 2003.
9. Olayinka JN, Ozolua RI, Akhigbemen AM. Phytochemical screening of aqueous leaf extract of *Blighia sapida* KD Koenig (Sapindaceae) and its analgesic property in mice. J Ethnopharmacol. 2021;273:113977.
10. Parkinson AA. Phytochemical analysis of ackee (Blighia sapida) pods. City University of New York; 2007.
11. Mazzola EP, Parkinson A, Kennelly EJ, Coxon B, Einbond LS, Freedberg DI. Utility of coupled-HSQC experiments in the intact structural elucidation of three complex saponins from *Blighia sapida.* Carbohydr Res. 2011 May 1;346(6):759–68.
12. John-Dewole OO, Agunbiade SO, Alao OO, Arojojoye OA. Phytochemical and antimicrobial studies of extract of the fruit of *Xylopia aethiopica* for medicinal importance. E3 J Biotechnol Pharm Res. 2012;3(6):118–22.
13. Oloyede OB, Ajiboye TO, Abdussalam AF, Adeleye AO. Blighia sapida leaves halt elevated blood glucose, dyslipidemia and oxidative stress in alloxan-induced diabetic rats. J Ethnopharmacol. 2014;157:309–19.
14. Von Holt C. Methylenecyclopropaneacetic acid, a metabolite of hypoglycin. Biochim Biophys Acta (BBA)-Lipids Lipid Metab. 1966;125(1):1–10.
15. Moya J. Ackee (Blighia sapida) poisoning in the Northern Province, Haiti, 2001. Epidemiol Bull. 2001;22(2):8–9.
16. Ajayi AM, Ayodele EO, Ben-Azu B, Aderibigbe AO, Umukoro S. Evaluation of neurotoxicity and hepatotoxicity effects of acute and sub-acute oral administration of unripe ackee (Blighia sapida) fruit extract. Toxicol Reports. 2019;6:656–65.
17. Tanaka K, Ikeda Y. Hypoglycin and Jamaican vomiting sickness. Prog Clin Biol Res. 1990;321:167–84.
18. Barceloux DG. Akee fruit and Jamaican vomiting sickness (Blighia sapida Köenig). Dis DM. 2009;55(6):318–26.
19. Chang S-T, Wu J-H, Wang S-Y, Kang P-L, Yang N-S, Shyur L-F. Antioxidant activity of extracts from Acacia confusa bark and heartwood. J Agric Food Chem. 2001;49(7):3420–4.
20. Benzie IFF, Strain JJ. [2] Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In: Methods in enzymology. Elsevier; 1999. p. 15–27.
21. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm. 1990;58(3):237–40.
22. Stratil P, Klejdus B, Kubáň V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. J Agric Food Chem. 2006;54(3):607–16.
23. Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem. 1973;19(12):1350–6.
24. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20(4):470–5.
25. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem. 1977;23(5):882–4.
26. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982;28(10):2077–80.
27. Wieland H, Seidel D. A simple specific method for precipitation of low density lipoproteins. J Lipid Res. 1983;24(7):904–9.
28. Fawcett J, Scott J. A rapid and precise method for the determination of urea. J Clin Pathol. 1960;13(2):156–9.
29. Brod J, Sirota JH. The renal clearance of endogenous "creatinine" in man. J Clin Invest. 1948;27(5):645–54.
30. Onunogbo CC, Ohaeri OC, Eleazu CO. Effect of mistletoe (*Viscum album*) extract on the blood glucose, liver enzymes and electrolyte balance in alloxan induced diabetic rats. Am J Biochem Mol Biol. 2013;3:143–50.
31. Walter, K, Schutt C. Acid and alkaline phosphatase in serum (two point method). Bergmeyer HU, editor. Vol. 2, Methods of Enzymatic Analysis. Weinheim,: Verlag Chemie & Academic Press,; 1974.
32. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28(1):56–63.
33. Tietz NW, Finley PR, Pruden EL. Clinical guide to laboratory tests. Vol. 624. WB Saunders company Philadelphia; 1995.
34. Jendrassik L, Grof P. Colorimetric method of determination of bilirubin. Biochem z. 1938;297(81):e2.
35. Blanckaert N. Analysis of bilirubin and bilirubin mono-and di-conjugates. Determination of their relative amounts in biological samples. Biochem J. 1980;185(1):115–28.
36. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim acta. 1971;31(1):87–96.
37. Grant GH, Silverman LM, Chistenson RH. Amino acids and protein in: Tietz NW, ed. Fundamental of clinical chemistry. 3 rd. Philadelphia, WB Saunders Company; 1987.
38. Adefemi OA, Elujoba AA, Odesanmi WO. Evaluation of the toxicity potential of Cassia podocarpa with reference to

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

official Senna. West Afr J Pharmacol Drug Res. 1988;8:41–8.
39. Nirogi R, Goyal VK, Jana S, Pandey SK, Gothi A. What suits best for organ weight analysis: review of relationship between organ weight and body/brain weight for rodent toxicity studies. Int J Pharm Sci Res. 2014;5(4):1525–32.
40. Kern WF. PDQ hematology. PMPH-USA; 2002.
41. Gotoh S, Hata J, Ninomiya T, Hirakawa Y, Nagata M, Mukai N, et al. Hematocrit and the risk of cardiovascular disease in a Japanese community: The Hisayama Study. Atherosclerosis. 2015;242(1):199–204.
42. Kishimoto S, Maruhashi T, Kajikawa M, Matsui S, Hashimoto H, Takaeko Y, et al. Hematocrit, hemoglobin and red blood cells are associated with vascular function and vascular structure in men. Sci Rep. 2020;10(1):1–9.
43. Radaelli F, Colombi M, Calori R, Zilioli VR, Bramanti S, Iurlo A, et al. Analysis of risk factors predicting thrombotic and/or haemorrhagic complications in 306 patients with essential thrombocythemia. Hematol Oncol. 2007;25(3):115–20.
44. Xu QQ, Xu YJ, Yang C, Tang Y, Li L, Cai H Bin, et al. Sodium Tanshinone IIA Sulfonate Attenuates Scopolamine-Induced Cognitive Dysfunctions via Improving Cholinergic System. Biomed Res Int. 2016;2016.
45. LaRegina MC, Sharp PE. The Laboratory Rat: A Volume in the Laboratory Animal Pocket Reference Series. CRC Press USA Hal. 1998;1(9):17–8.
46. Oakenfull DG, Fenwick DE. Adsorption of bile salts from aqueous solution by plant fibre and cholestyramine. Br J Nutr. 1978;40(2):299–309.
47. Wan C, Wong CN, Pin W, Wong MH, Kwok C, Chan RY, et al. Chlorogenic acid exhibits cholesterol lowering and fatty liver attenuating properties by up-regulating the gene expression of PPAR-α in hypercholesterolemic rats induced with a high-cholesterol diet. Phyther Res. 2013;27(4):545–51.
48. Huang K, Liang X, Zhong Y, He W, Wang Z. 5-Caffeoylquinic acid decreases diet-induced obesity in rats by modulating PPARα and LXRα transcription. J Sci Food Agric. 2015;95(9):1903–10.
49. Baeza G, Sarriá B, Mateos R, Bravo L. Dihydrocaffeic acid, a major microbial metabolite of chlorogenic acids, shows similar protective effect than a yerba mate phenolic extract against oxidative stress in HepG2 cells. Food Res Int. 2016;87:25–33.
50. Del Hierro JN, Casado-Hidalgo G, Reglero G, Martin D. The hydrolysis of saponin-rich extracts from fenugreek and quinoa improves their pancreatic lipase inhibitory activity and hypocholesterolemic effect. Food Chem. 2021;338:128113.
51. Hosseini A, Razavi BM, Banach M, Hosseinzadeh H. Quercetin and metabolic syndrome: A review. Phyther Res. 2021;35(10):5352–64.
52. Pagana KD. Mosby's Manual of Diagnostic and Laboratory Tests. In: St. Louis Mosby, Inc. 1998 and Rebecca J.F Gale, editor. Encyclopedia of Medicine. Springer; 2002.
53. Higgins C. Urea and the clinical value of measuring blood urea concentration. Acutecaretesting Org. 2016;1–6.
54. Singbartl K, Formeck CL, Kellum JA. Kidney-immune system crosstalk in AKI. In: Seminars in nephrology. Elsevier; 2019. p. 96–106.
55. El Hilaly J, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of Ajuga iva in experimental animals. J Ethnopharmacol. 2004;91(1):43–50.
56. Whitfield JB, Zhu G, Madden PAF, Montgomery GW, Heath AC, Martin NG. Biomarker and genomic risk factors for liver function test abnormality in hazardous drinkers. Alcohol Clin Exp Res. 2019;43(3):473–82.
57. Lala V, Goyal A, Bansal P M DA. Liver Function Tests [Internet]. Treasure Island: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482489/
58. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin Chem. 2000;46(12):2027–49.
59. Kumar V, Gill KD. Basic concepts in clinical biochemistry: a practical guide. Springer; 2018.
60. Reynolds HY. Analysis of protein and respiratory cells obtained from human lungs by bronchial lavage. J Lab Clin Med. 1974;84:559–73.
61. Rigano D, Formisano C, Basile A, Lavitola A, Senatore F, Rosselli S, et al. Antibacterial activity of flavonoids and phenylpropanoids from Marrubium globosum ssp. libanoticum. Phyther Res An Int J Devoted to Pharmacol Toxicol Eval Nat Prod Deriv. 2007;21(4):395–7.
62. Crafa F, Gugenheim J, Saint-Paul M-C, Cavanel C, Lapalus F, Ouzan D, et al. Protective Effects of Prostaglandin E1on Normothermic Liver Ischemia. Eur Surg Res. 1991;23(5–6):278–84.
63. Artru F, McPhail MJW, Triantafyllou E, Trovato FM. Lipids in liver failure syndromes: a focus on eicosanoids, specialized pro-resolvinglipid mediators and lysophospholipids. 2022;

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