integrity Research Journals

Journal of Bioscience and Biotechnology Discovery
Volume 7(2), pages 10-23, July 2022
Article Number: D509E9AB1
ISSN: 2536-7064
https://doi.org/10.31248/JBBD2022.164
https://integrityresjournals.org/journal/JBBD

Full Length Research

Toxicity evaluation of the arils of the fruit of *Blighia sapida* KD Koenig (Sapindaceae) in Wistar rats

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Received 6th June 2022; Accepted 7th July 2022

ABSTRACT: The Methanol extract of the arils of fruits of *Blighia sapida* was studied for its toxic effects on certain hematological and biochemical indices as well as the histopathological examination of some organs of the Wistar rats. Preliminary phytochemical screening showed that the plant contains bioactive compounds such as alkaloids, flavonoids, polyphenols, cardiac glycosides, cyanogenic glycosides, steroid glycosides, cardenolides, terpenes and tannins. Three different concentrations of the extract were administered to different groups of the rats orally for 28 days in the sub-acute test and 90 days in the case of the Sub-chronic test. The extract showed no mortality even at a dose of 5000mg/kg. Other results obtained showed a significant increase in some hematological indices such as pack cell volume (PCV), hemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Others are mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), and lymphocytes (LYMP). However, white blood cells (WBC) and platelets (PLT) showed a significant decrease as the doses administered increased from low to high. There was also, a significant decrease in the levels of certain biochemical indices such as urea, creatinine, total bilirubin, conjugated bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) while the levels potassium (K^+), chloride (Cl^-), total protein and albumin were significantly increased. The methanol extract of the arils of fruits of *Blighia sapida* revealed mild distortion of hepatocytes, loose nuclei within the cells, and also complete loss of sinusoid with an increase in dose within the liver.

Keywords: Arils, *Blighia sapida*, Histopathological, methanol extract, toxicity, Wistar rats.

INTRODUCTION

The use of substances and chemicals in form that has pharmacological activity has increased over the years. Medicinal plants play vital roles in the treatment and management of different types of diseases (Olatunji and Atolani, 2009). Thus, many Countries have engaged tremendously in the investigation and evaluation of medicinal plants. The methodologies and safety profile of the constituents of the various plant materials have been discovered because of the continual search and interest developed in natural plant products according to Okoli and Iroegbu 2004.

Toxicity can result from long or short administration of a drug that can elicit toxic effects which could be mild or severe depending on the substance used. In Africa, Plants with known medicinal values have been used for a long time to treat various diseases and ailments. Some of the plant constituents can confer defense against a microbial attack on the plant while others exhibit other abilities on the plants, these plants exhibit various levels of toxicity (Duraipandiyan *et al.,* 2006).

Blighia sapida belongs to the family Sapindaceae. In Nigeria, it is known as “Ackee”, and it equally has several local names in Nigeria and other African Countries (Gardner *et al.,* 1998). The tree is usually densely branched and symmetrical with smooth gray bark. It possesses evergreen (rarely deciduous) alternate leaves.

Most parts of the plants have been used in traditional medicine in the treatment of different ailments. For example, the aqueous extract of the seeds is used as a parasite expellant (Kubmarawa *et al.,* 2007). The crushed new foliage applied on the forehead is used as headache relief and the juice from the leaf is applied as eye drops in cases of conjunctivitis (Kubmarawa *et al.,* 2007). Toxicity has been reported in the consumption of immature fruits of *Blighia sapida.* This includes but is not limited to serious vomiting which is usually referred to as Jamaica Vomiting syndrome (Brown, 1992). However, there is no known organ toxicity reported with mature arils of *Blighia sapida* (Brown 1992). This necessitates this evaluation to establish its safety profile

Various preparations and combinations have been used in the treatment of dysentery, epilepsy, yellow fever (Kubmarawa *et al.,* 2007), and diabetics (Kokwaro, 2000). The crushed seeds have been used to treat dental decay while the crushed bark is used for wound healing. Also, the decoction of the bark is used for constipation (Ekue *et al.,* 2010). The objective of this study is to evaluate the safety profile of the arils of the ripe fruits of *Blighia sapida* in rats.

MATERIALS AND METHODS

Chemicals

All reagents used for this experiment were from Sigma Company, St Louis, (USA) except for methanol was the analytical grade.

Plant collection and authentication

The Fruits arils of *Blighia sapida* were collected on the 25th ^{of} January 2022 from the Afana village of Zangon Kataf Local Government Area of Kaduna State, Nigeria. The authentication of the plants was done by Taxonomist Joseph Azila at the Federal College of Forestry, Jos, Nigeria with voucher number FHJ 26022. The plant voucher was kept at the herbarium of Federal College of Forestry, Jos, Nigeria.

Preparation and extraction of plant material

The Fruit arils of *B.sapida* was properly washed, chopped into pieces, and air-dried for two weeks. They were ground into powder and extracted using 70% Methanol. The extract was obtained by soaking 300 g of the dried powdery samples in 2500 ml of Methanol for 48 hours during which the mixture was intermittently shaken. It was later filtered through Whatman filter paper. The extracts were evaporated to dryness at 40^{o}C in a water bath. This temperature was maintained to avoid the chemical components from being destroyed. The residual methanol was removed by evaporating the methanol under a vacuum in a rotavapour. This was done just before evaporating the extract to dryness. Methanol is used because of its lower temperature to evaporate easily in the rotary vapour to avoid damage to the phytoconstituents of the extract. Also, its high polarity produces a high extraction yield.

Animals

The animals used for this study were male Wistar rats, weighing 60-200g. The animals were obtained from the National Veterinary Research Institute, Vom, Nigeria. They were maintained at the Experimental Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria to acclimatize to the experimental condition for one week before the commencement of the experiment. They were kept in rat cages and fed with standard pellet feeds and allowed free access to clean fresh water. All experimental protocols followed the Faculty of Pharmaceutical Sciences, University of Jos, Ethics on Research in Animals as well as internationally accepted principles for laboratory animal use and care. The ethical clearance with reference number REF/UJ/FPS/PCL/AEU/2 was approved and issued on the 16th of March 2020.

Phytochemical screening

Phytochemical screening was done to determine the chemical constituents of the plant extract. These include a test for alkaloids, cardiac glycosides, cyanogenic glycosides, flavonoids, resins, polyphenols (Trease and Evans, 2009), saponins, phlobatanins, anthraquinones, balsams (Sofowora, 2008), terpenes, and tannins.

Qualitative phytochemical analysis was carried out on the ripe aril (edible part) of the fruit of *Blighia sapida* to identify the phytoconstituents. The methods employed were as described by Trease and Evans (2009).

Test for alkaloids

For the test for alkaloids, 0.5g of the extract was stirred with 3ml of 1% hydrochloric acid over a water bath. The solution will be divided into 3 portions and tested with Dragendorffs, Mayers, and Wagner reagents for the presence of orange, creamy and reddish-brown precipitates respectively which indicates the presence of alkaloids (Trease and Evans, 2009).

Test for carbohydrates

The method for general test for carbohydrates was employed. 0.2 g of extract was heated with 1 ml of

concentrated sulphuric acid. Charring and effervescence indicate the presence of carbohydrates (Trease and Evans, 2009).

Test for glycosides

The Extract (0.2g) was placed in boiling water in a test tube. 5 ml of dilute sulphuric acid was added, and the content was boiled for 15 minutes over a water bath. Test tube contents were cooled and filtered. The filtrate was neutralized with 20%w/v/ potassium hydroxide solution (Trease and Evans, 2009).

Test for saponins

Two tests were carried out. Firstly, the frothing and foaming test was done by adding about 0.5g of extract of 5ml of distilled water in a test tube. The formation of froth or foam after vigorous shaking for a few minutes indicates the presence of saponins. If the extract showed positive foam and then it was subjected to an erythrocyte hemolysis test. Blood from the previously heparinize rat was added (A few drops) to a solution of the extract (made isotonic by shaking with 0.5g of extract in normal saline) and test tube contents were centrifuged for a minute. A red-colored supernatant after centrifugation confirms the presence of saponins (Trease and Evans, 2009).

Test for tannins

About 0.5g of the extract was stirred with 1ml of distilled water and filtered. A few drops of ferric chloride were added to the filtrate and a blue-black, green, or blue precipitate indicates the presence of tannins (Trease and Evans, 2009).

Test for flavonoids

The extract (0.5g) was dissolved in 5ml of distilled water in test tubes. 5ml of 20%w/v sodium hydroxide will then be added and mixed. A yellow-coloured solution indicates the presence of flavonoids (Trease and Evans, 2009).

Test for steroids

The extract (100mg) was dissolved in 2ml of chloroform. Sulphuric acid was added carefully to form a lowering layer. A reddish-brown colour at the interface is indicative of the presence of the steroidal ring (Sofowora, 1993).

Test for anthraquinones

Borntrager's test was used for the detection of anthraquinones. 0.5g of the extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 minutes. The extract was filtered, and the filtrate was shaken with an equal volume of dilute ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of free anthraquinones (Trease and Evans, 2009).

Toxicity studies

Acute toxicity test

Determination of Lethal dose (LD_{50}): The Lorke method was used to determine the concentration of the extracts of the Fruit arils of *Blighia sapida* that killed 50% of the test animal population (Lorke, 2003). Male Wistar rats were used for this experiment. The test was in two phases. Nine (9) Wistar rats were divided into 3 groups, with three animals per group. In the first phase, each group of the animals was administered 10, 100, and 1000 mg/kg of the extract respectively. The animals were monitored for 24 hours, and mortalities were noted. In the second phase, each group (of 1 animal each) was administered 1600, 2900, and 5000 mg/kg of the extract. They were monitored for 24 hours, and mortalities were noted. All extract administrations were done through the oral (gavage) route. The Lethal Dose (LD_{50}) was determined using the Highest dose that gave no mortality (Do) and the Lowest dose that produced mortality (D_{100}).

Sub-acute toxicity test

Twenty-four (24) male rats were randomly selected and weighed, and divided into four groups which were the Low Dose (LD), Middle Dose (MD), High Dose (HD), and Control groups. The low dose used was 250mg/kg and the middle dose used was 500 mg/kg while the high dose used was 750mg/kg. These were obtained by doubling and tripling the low dose respectively (American Association for Clinical Chemistry, 2014). The doses were administered orally via gavage daily for 28 days. The observation was done every 24 hours. After the test period, the animals were suffocated using chloroform and sacrificed. Sterile syringes with needles were used to collect the blood from the heart of the sacrificed animal by a process known as Cardiac Puncture. The blood samples were analyzed for the effect of the extracts on some hematological and biochemical indices using the Mindray 5-part Differential Hematological Auto-analyzer (BC-5300).

Hematological evaluation

Using the blood sample obtained, laboratory analysis was carried out to determine the effect of the extracts on certain

hematological parameters in the blood. They include Pack Cell Volume (PCV), Hemoglobin (HB), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Values (MCV), Mean Corpuscular Hemoglobin (MCH), Red Blood Cell (RBC), White Blood cell (WBC), Lymphocytes, Neutrophils, Monocytes, Eosinophil, and Platelets. The sample specimen bottle was properly labeled, imputed into the computer attached to the auto analyzer, and saved accordingly. 3 to 5 ml of blood in a specimen bottle was mixed thoroughly to avoid clusters or larger particles from blocking the tiny tubing in the auto analyzer as this will damage the machine. This is as described by Pincus *et al.* (2011). The Mindray 5-part Differential Hematological Auto-analyzer (BC-5300) was used to analyze these hematological parameters.

Biochemical evaluation

Laboratory analysis was also carried out to determine the following biochemical parameters; total protein, albumin, urea, globulin, Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), and Alanine Aminotransferase (ALT). This is as described by Pincus *et al.* (2011). The Mindray 5-part Differential Hematological Auto-analyzer (BC-5300) was used to analyze these biochemical parameters

Histopathological examination

Heart, liver, lungs, spleen, and kidney were collected from all animals at necropsy. The organs were dissected and retained in 10% neutral-buffered formalin. Sections of the tissues were embedded in paraffin, cut approximately 5 mm thick, stained with hematoxylin and eosin, and evaluated by a board-certified veterinary pathologist. Each lesion was listed and coded by the most specific topographic and morphologic diagnoses, severity, and distribution, using International Harmonization of Nomenclature and Diagnostic Criteria for Lesions as a guide. A 4-step grading system (minimal, mild, moderate, and marked) was used to define gradable lesions for comparison between treated and control groups.

Sub-chronic toxicity

This was determined after 90 days of oral (gavage) administration of the extracts of the Fruit arils of *Blighia sapida*. Twenty-eight (28) male rats were divided into four groups with seven animals in each group. Three different doses of the extract, HD, MD, and LD were administered to each of the test group and a control group. The dose of arils of the fruit of *Blighia sapida* extract administered was 750, 500, and 250 mg/kg respectively. After 90 days, the animals were suffocated using chloroform and sacrificed. A sterile syringe with a needle was used to collect the blood from the heart of the sacrificed animal (Pincus *et al.,* 2011). The blood sample was collected and stored in a heparinized specimen bottle with anti-coagulant and the specimen bottle with the blood sample was shaken briskly to prevent the blood from clotting. The blood samples were taken to the laboratory to be analyzed for hematological and biochemical parameters. Also, organs were harvested and treated the same way as described in the sub-acute toxicity examination method under histopathological examination.

Statistical analysis

The statistical analysis was done using the SPSS package (SPSS version 15.0). Data analysis was carried out using one-way ANOVA followed by Dunnett's post hoc test. The data were expressed as Mean ±SEM and valves of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The arils of fruits of *Blighia sapida* have shown some pharmacological activities which could be responsible for their medicinal use in traditional medicine (Tona, 2008; Sofowora, 1999; Olusegun *et al.,* 2013). The results show that the arils of the fruit of *Blighia sapida* contain Alkaloids, flavonoids, glycosides, polyphenols, tannins, terpenes, and cardenolides (Table 1). These results can be likened to that of Olusegun *et al.* (2013) and Ubulom *et al.* (2012), and like the results obtained by Ibraheem *et al.,* (2002). These constituents are responsible for their pharmacological actions and hence the basis for their use in medicine. The acute lethal dose toxicity showed that administration of the 70% methanol extract of arils of fruits of *Blighia sapida* produced no mortality up to a dose of 5000mg/kg body weight. This result can be likened to one reported by Owolabi *et al.* (2010) which states that aqueous and ethanol extract of the leaves of *Blighia sapida* has LD_{50} that is greater than 5000mg/kg as shown in Table 2, hence it can be deduced that it is safe or non-toxic. This is to the toxicity scale principle which states that any substance with LD_{50} greater than 5000mg/kg is practically nontoxic (Sandu *et al.,* 2012). Table 3 and 6 reveals an improvement in body weight of the animals which is expected for growing rats and could be an indication of well-being (Labie *et al.,* 2011).

The sub-acute and sub-chronic administration of the methanol extract of the arils of the fruit of *Blighia sapida* caused a significant increase in PCV (polycythemia) which could be an indication of dehydration. This is evident in the increase in RBC and Hb (Table 7).

The white blood cell count for sub-chronic toxicity (Table 7) decreased significantly while the lymphocytes increased

Table 1. Phytochemical Evaluation of the 70% Methanol extract of the aril of the fruit of *Blighia sapida.*

Constituents	Indications
Saponins	-
Polyphenols	+
Flavonoid	+
Steroid glycosides	+
Terpenes	+
Phlobatanins	-
Anthraquinone	-
Cyanogenic glycoside	+
Balsam	-
Resins	-
Alkaloids	+
Cardiac glycoside	+
Cardenolides	+
Tannins	+

(+) = Present and (-) = Absent.

Table 2. Acute toxicity test for the 70% Methanol extract of the arils of the fruit of *Blighia sapida*

Phases	Dose (mg/kg)	No. of Animals	Mortality	% Mortality
	10	**3**	0	0
1	100	**3**	0	0
	1000	**3**	0	0
	1600	**1**	0	0
2	2900	**1**	0	0
	5000	**1**	0	0

Table 3. Weight (g) of rats after the administration of the methanol extract of the arils of the fruits of *Blighia sapida.*

Parameters	Control	250 mg/kg	500 mg/kg	750 mg/kg
Onset	69.88±4.32	83.62±4.97	84.53±14.09	99.52±4.86
Week 1	83.55±3.05	94.53±5.13	101.30±18.82	114.00±6.10
Week 2	97.10±1.88	102.30±5.78	109.10±17.29	123.90±7.74
Week 3	111.00±4.47	127.60±8.97	133.60±19.99	149.20±11.19
Week 4	123.50±5.28	132.00±9.50	136.40±17.45	152.60±11.50

Values are presented as mean ± SEM.

significantly. This increase in lymphocytes could cause immunomodulation. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defense mechanism must be activated under the conditions of the impaired immune response or when selective immunosuppression is desired in situations like autoimmune disorders (Wang *et al.,* 2005; Wong *et al.,* 2004). Frequent consumption of some medicinal plants has been useful for enhancing immunological functions by potential enhancement of cell-mediated immunity in humans (Wang *et al.,* 2005; Wong *et al.,* 2004). It was observed that there was a significant decrease in Platelets after sub-chronic administration (Table 7). This suggests that there will be an impact on the clotting of the blood. These platelets are responsible for the formation of a blood clot, which is a meshwork of fibrin fibers, they adhere to damaged blood vessels and consequently prevent further blood loss (Wu *et al.,* 1996; Andrews *et al.,* 1997; Cox and Cox, 2000). This decrease could cause blood clot

Table 4. Sub-acute effect of the administration of methanol extract of arils of the fruit of *Blighia sapida* on Hematological parameters.

Parameters	Control	250 mg/kg	500 mg/kg	750 mg/kg
WBC ($x10^9$/L)	1.07±0.16	1.06±0.20	2.06±0.54	4.316±1.42*
LYM ($x10^9$/L)	0.6±0.15	0.505±0.14	0.60±0.22	2.460±0.72*
MON ($x10^9$/L)	0.29±0.12	0.27±0.11	0.07±0.01	0.3420±0.08
NEU ($x10^{-9}$/L)	0.18±0.07	0.24±0.07	1.26±0.52	1.094±0.51
EOS ($x10^{-9}$/L)	0.05±0.02	0.05±0.03	0.15±0.05	0.4086±.24
BAS ($x10^9$/L)	0.0030±0.00	0.004±0.00	0.01±0.01	0.0110±0.01
RBC ($x10^9$/L)	7.74±0.54	7.53±0.73	8.49±0.93	8.094±1.20
HGB (g/dl)	12.28±0.67	12.60±0.99	14.10±1.40	14.94±1.65
HCT (%)	43.48±3.12	47.10±2.59	51.12±5.58	52.22±7.84
MCV$x10^9$/L	56.18±0.88	64.22±3.43	60.55±2.12	64.76±2.19
MCH$x10^9$/L	15.88±0.33	16.88±0.37	16.63±0.22	18.80±1.02*
MCHC (G/dl)	28.40±0.99	26.57±0.86	27.72±1.05	29.10±1.00
PLT$x10^9$/L	175.80±58.95	272.30±65.49	258.8±96.12	243.0±43.41

Values presented as mean ± SEM, Significance relative to control *p<0.05.

Table 5. Sub-acute Effect of the administration of methanol extract of arils of the fruit of *Blighia sapida* on Biochemical parameters.

Parameters	Control	250 mg/kg	500 mg/kg	750 mg/kg
Na^+(mmol/L)	143.50±0.48	138.7±0.31*	140.50±0.28*	141.70±0.20*
K^+ (mmol/L)	3.98±0.05	4.796±0.06*	4.39±0.02*	3.85±0.03
Cl^-(mmol/L)	110.00±0.73	103.8±0.65*	101.90±0.46*	108.10±0.33
$HCO3^-$(mmol/L)	27.20±0.37	27.20±0.37*	24.80±0.20*	26.75±0.25
Urea (mmol/L)	1.46±0.05	3.796±0.05*	1.83±0.02*	1.01±0.01*
Cr (mmol/L)	38.25±0.55	57.65±0.86*	51.00±0.54*	42.96±1.00*
Total Bilirubin (µmol/L)	9.94±0.17	14.28±0.19*	12.60±0.04*	8.11±0.32*
Conjugated Bilirubin (µmol/L)	3.95±0.05	4.284±0.06*	3.30±0.06*	2.45±0.07*
ALP (IU/l)	245.20±2.33	513.4±18.96*	489.20±1.32*	270.00±1.84
ALT (U/l)	5.98±0.10	9.344±0.33*	7.71±0.12*	5.71±0.040
AST (U/l)	12.83±0.13	16.54±0.56*	14.11±0.12*	11.13±0.14*
Total Protein (g/L)	79.62±0.86	60.27±0.57*	66.23±0.34*	72.74±0.39*
Albumin (g/L)	39.81±0.43	30.13±0.28*	33.12±0.17*	36.36±0.19*
Globulin (g/L)	39.82±0.43	30.13±0.28*	33.12±0.17*	36.38±0.20*

Values presented as mean ± SEM, Significance relative to control *p<0.05.

Table 6. Weight (g) of rats after the administration of the 70% methanol extract of the arils of the fruits of *B. sapida.*

Parameters	Control	250 mg/kg	500 mg/kg	750 mg/kg
Onset	144.80±9.29	112.50±6.89	112.70±8.10	90.00±10.63
Week 1	160.20±7.69	136.60±7.47	142.50±10.38	104.70±12.22*
Week 2	173.10±7.75	142.60±8.77	143.90±9.54	110.10±12.34*
Week 3	187.40±8.93	164.30±9.84	178.00±12.28	128.40±12.95*
Week 4	201.80±10.03	171.80±9.94	178.50±10.36	141.90±12.58*
Week 5	216.20±10.16	185.70±11.04	185.60±10.28	149.90±12.90*
Week 6	225.60±10.92	197.70±11.21	192.60±9.90	154.10±13.63*
Week 7	232.00±11.02	207.50±10.78	201.30±11.25	152.00±11.83*
Week 8	239.70±12.33	212.00±9.43	204.30±14.02	152.30±12.06*
Week 9	249.00±12.90	214.60±8.59	205.10±16.81	147.40±13.07*
Week 10	253.50±12.18	218.00±5.95	205.10±17.13*	145.40±12.35*
Week 11	257.20±11.73	213.90±6.57	199.90±18.68*	138.20±12.77*
Week 12	263.50±10.96	210.00±7.56*	196.80±20.41*	133.80±12.95*

Values presented as mean ± SEM, Significance relative to control *p<0.05.

Table 7. Sub-chronic effect of the administration of 70% methanol extract of arils of the fruit of *B. sapida* on Hematological parameters.

Parameters	Control	250mg/kg	500mg/kg	750mg/kg
PCV (%)	48.20±0.20	31.60±0.68*	38.40±0.51*	42.80±0.37*
Hb (g/dl)	16.06±0.06	10.50±0.22*	12.76±0.18*	14.24±0.11*
Neut. ($x10^9$/L)	74.00±0.00	81.80±0.58*	78.00±0.00*	76.60±0.25*
Lymph ($x10^9$/L)	25.80±0.20	18.20±0.58*	22.00±0.00*	23.40±0.25*
WBC ($x10^9$/L)	3.46±0.15	5.59±0.33	5180±0.33	4950±0.22
PLT ($x10^9$/L)	179.60±0.51	261.40±0.51*	257.8±0.37*	248.80±0.58*
RBC ($x10^9$/L)	10.I3 ± 0.12	7.62± 0.50	8.91±0.54	10.32± 0.48
HCT (%)	45.34 ± 1.24	47.83± 2.81	49.23± 4.23	51.17± 3.56
MCV$x10^9$/L	53.23± 0.87	61.81± 2.46	63.11± 2.88	64.32±3.81
MCH (10^9/L)	17.99± 0.23	19.61±0.65	19.79±0.71	20.89±1.23*
MCHC (g/dl)	29.40±0.56	25.89±0.89	27.11±1.01	29.80± 1.01

Values presented as mean ± SEM, Significance relative to control *p<0.05.

Table 8. Sub-chronic effect of the administration of methanol extract of arils of the fruit of *B. sapida* on Biochemical parameters.

Parameters	Control	250mg/kg	500mg/kg	750mg/kg
Na^+(mmol/L)	145.40±0.29	133.2±0.47*	136.7±0.11*	141.10±0.27*
k^+ (mmol/L)	3.70±0.07	5.620±0.07	5.040±0.03	4.78±0.04
Cl^- (mmol/L)	110.30±0.30	97.74±0.38	101.10±0.46	105.70±0.27*
$HCO3^-$ (mmol/L)	25.98±0.12	19.34±0.22*	22.32±0.20*	23.62±0.10
Urea (mmol/L)	3.02±0.04	10.74±0.43*	7.68±0.09*	5.52±0.04*
Cr (mmol/L)	57.06±0.48	180.30±0.54*	150.30±0.46*	93.84±0.64*
ALP (IU/I)	290.00±1.14	499.8±1.07*	395.70±1.51*	322.90±0.35*
ALT(U/I)	20.44±0.16	50.02±0.61*	25.40±0.14*	23.60±0.16
AST (U/I)	29.98±0.50	58.56±0.49*	37.02±0.22*	33.08±0.19*
Total Protein (g/L)	81.78±0.44	68.28±0.24*	74.20±0.22*	77.10±0.24*
Albumin (g/L)	43.85±0.56	31.52±0.36*	37.14±0.14*	40.92±0.37
Globulin (g/L)	46.70±8.562	36.76±0.41*	37.06±0.21*	36.18±0.33*

Values presented as mean ± SEM, Significance relative to control *p<0.05.

impairment resulting in blood loss on injury in people that consume the fruit chronically.

The fruit of *Blighia sapida* resulted in a significantly decreased in AST and ALT concentration in both sub-chronic and sub-acute administration (Table 5 and 8). Both AST and ALT are found in the liver, muscles, and heart. They are usually used as biomarkers in assessing damages in any of the organs mentioned though not necessarily the extent of such damage (Rej, 1989). They are only increased when there is damage to the liver, muscle, and heart which eventually leaks into the blood. Therefore, the decrease in the level suggests that there is slight or no damage to any of the organs mentioned. Table 5 and 8 indicates a significant decrease in the level of ALP. Very less attention has been focused on clinical conditions associated with low or decrease ALP activity in humans. Various causes may attribute to low ALP activity such as hypophosphatasia, cardiac surgery, and cardiopulmonary bypass, blood collected with EDTA or oxalate anticoagulant, hypothyroidism, vitamin C and B_{12} deficiency, Milk alkali syndrome, protein/ calorie malnutrition, zinc, and magnesium deficiency (Simko, 1991; Lum, 1995).

The administration of the fruits of *Blighia sapida* for sub-chronic and sub-acute toxicity tests indicates a significant decrease in Urea concentration. The low blood urea concentration could suggest overhydration or cold weather when the body does not excrete much fluid from the system (Arise, 2012). More so, Jos, Nigeria where the studies was conducted is known to be very cold. Total protein and albumin after both chronic and sub-chronic administration revealed a significant increase in their levels. Tables 5 and 8 show a significant increase in total protein and albumin in both sub-chronic and sub-acute administration of the extract. The only clinical situation that causes an elevation in serum albumin is acute dehydration.

There is no significant injury to any of the organs studied during acute, sub-acute, and sub-chronic toxicity administration of the methanol extract (Figures 1 to 10).

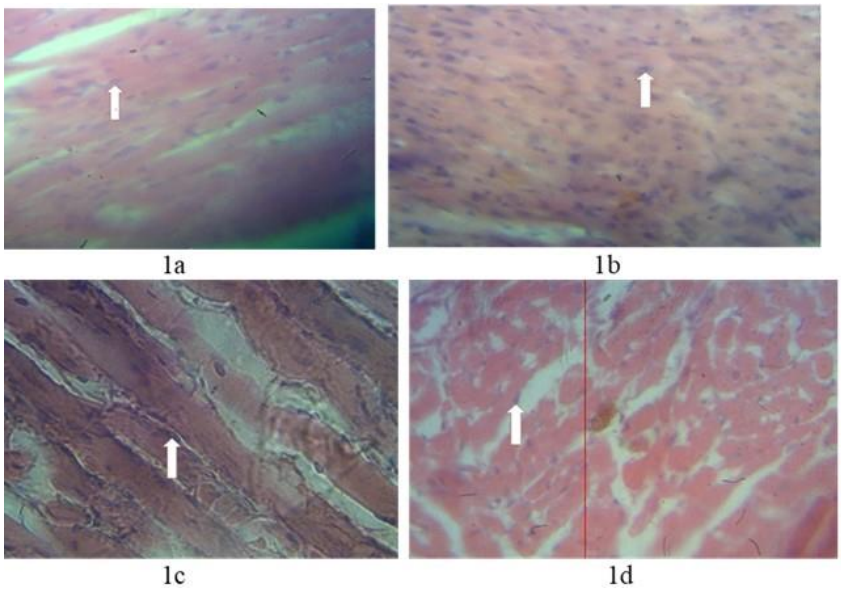

Figure 1. Photomicrographs of Heart sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida*. **1a:** Control group showing a normal nucleus within the muscle cell of the heart (Magnification x 40); **1b:** Group administered 250mg/kg showing a normal nucleus within the muscle cell of the heart. (Magnification x 40); **1c:** Group administered 500mg/kg of extract showing a normal nucleus within the muscle cell of the heart (Magnification x 40); **1d:** Group administered 750mg/kg of extract showing a normal nucleus within the muscle cell of the heart (Magnification x 40).

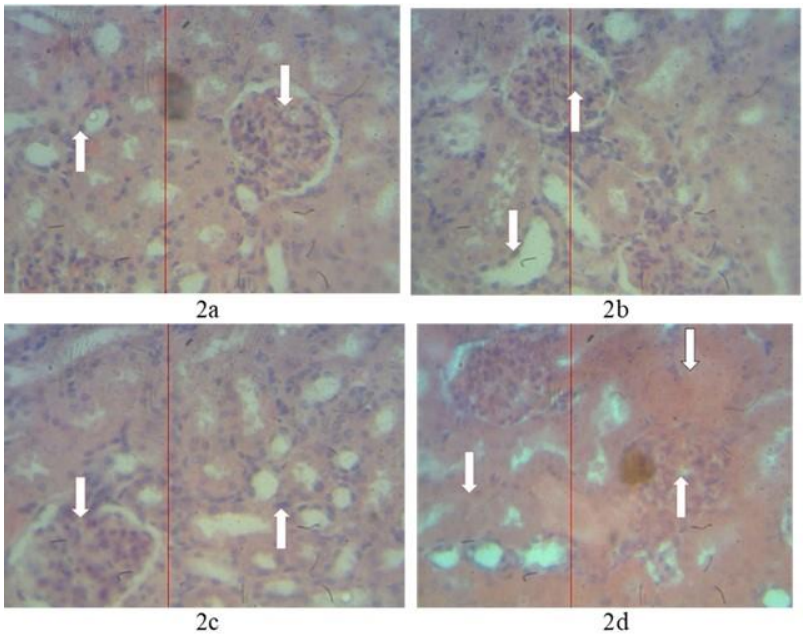

Figure 2. Photomicrographs of Kidney sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida*. **2a:** Control group showing a normal glomerulus and a normal nucleus within the collecting duct of the kidney (Magnification x 40); **2b:** Group administered 250mg/kg of extract showing a normal glomerulus and a normal nucleus within the collecting duct of the kidney (Magnification x 40); **2c:** Group administered 500mg/kg of extract showing normal glomeruli and a nucleus within the collecting duct of the kidney (Magnification x 40); **2d:** Group administered 750mg/kg of extract showing distortion of collecting duct forming hyperplasia and degenerated cell within the glomeruli of the kidney (Magnification x 40).

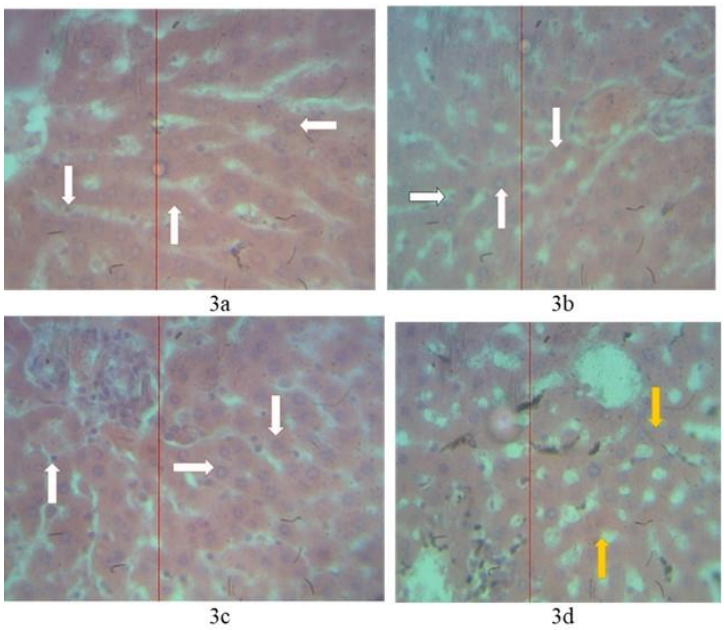

Figure 3. Photomicrographs of liver sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida.* **3a:** Control group showing a normal radial arrangement of hepatocytes, a normal nucleus within the hepatocytes, and presence of Kupffer cell within the sinusoid (Magnification x 40); **3b:** Group administered 250mg/kg of extract showing a normal nucleus within the hepatocytes, Kupffer cell and a redial arrangement of hepatocytes (Magnification x 40); **3c:** Group administered 500mg/kg of extract showing mild distortion of hepatocytes, a normal nucleus within hepatocytes and Kupffer cell within the sinusoid (Magnification x 40); **3d:** Group administered 750mg/kg of extract showing distortion of hepatocytes, loose nuclei within the cell, Kupffer cell and complete loss of sinusoid (Magnification x 40).

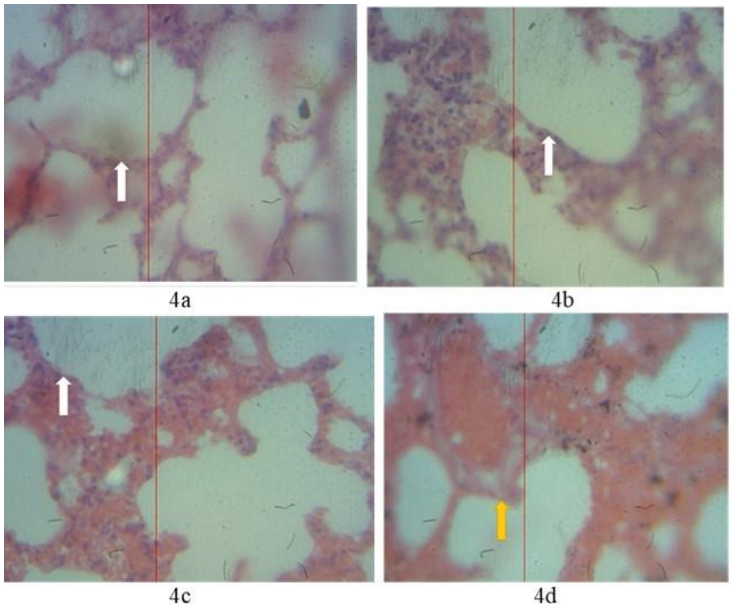

Figure 4. Photomicrographs of Lungs sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida.* **4a:** Control group showing a normal alveolar in the lungs (Magnification x 40); **4b:** Group administered 250mg/kg of extract showing a normal alveolar cell within the lungs (Magnification x 40); **4c:** Group administered 500mg/kg of extract. The arrow shows a normal alveolar within the lungs (Magnification x 40); **4d:** Group administered 750mg/kg extract showing a normal alveolar cell in the lungs (Magnification x 40).

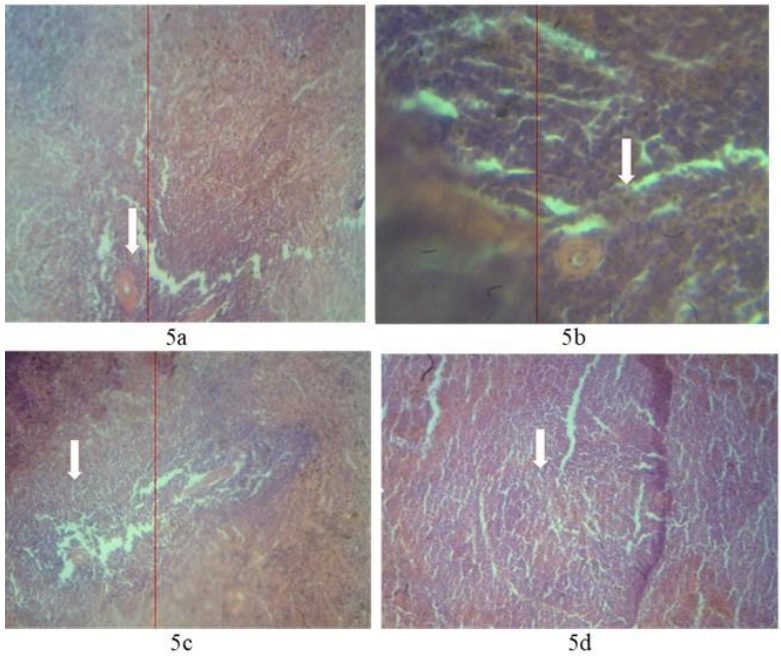

Figure 5. Photomicrographs of Spleen sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida.* **5a:** Control showing the normal white pulp within the spleen (Magnification x 40); **5b:** Group administered 250mg/kg of extract showing a normal white pulp within a spleen (Magnification x 40); **5c:** Group administered 500mg/kg of extract showing a normal white pulp within the spleen (Magnification x 40); **5d:** Group administered 750mg/mg of extract showing a normal white pulp within the spleen (Magnification x 40).

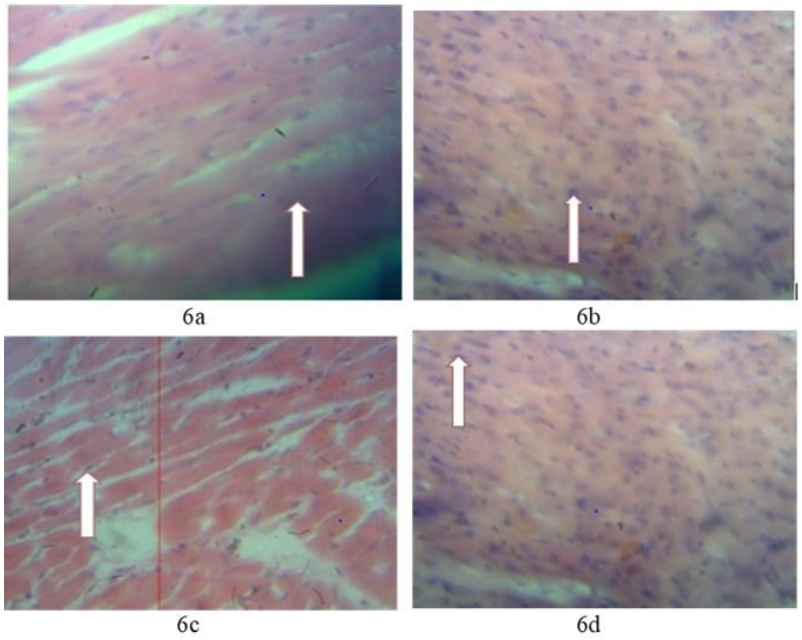

Figure 6. Photomicrographs of Heart sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida.* **6a:** Control group showing normal nucleus within the myocyte (Magnification x 40); **6b:** Group administered 250mg/kg of extract showing normal nucleus within the myocytes. (Magnification x 40); **6c:** Group administered 500mg/kg of extract showing a normal nucleus within the myocyte. (Magnification x 40); **6d:** Group administered 750mg/kg of extract showing a normal nucleus within the myocyte (Magnification x 40).

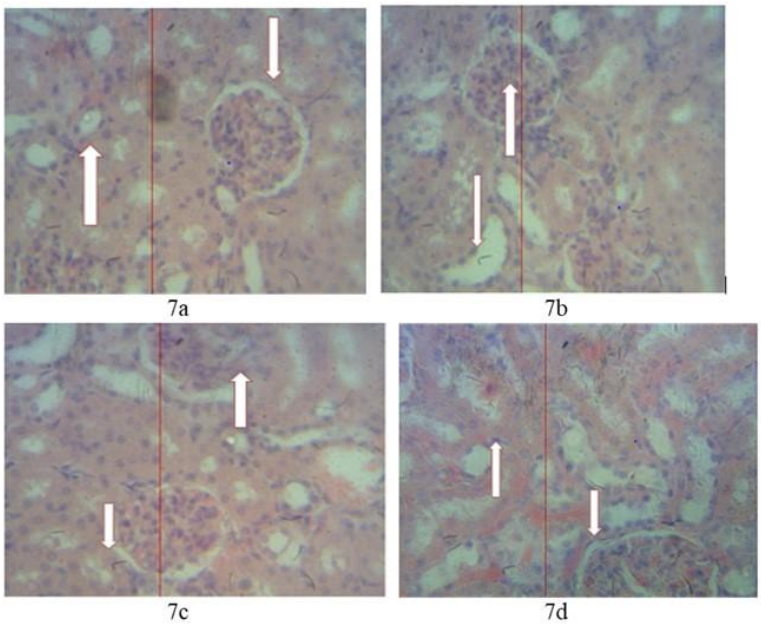

Figure 7. Photomicrographs of Kidney sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida*. **7a:** Control showing a normal glomerulus and a normal nucleus within a collecting duct in a kidney. (Magnification x 40); **7b:** Group administered 250mg/kg of extract showing a normal glomerulus and a normal nucleus within a collecting duct in a kidney (Magnification x40); **7c:** Group administered 500mg/kg of extract showing a normal glomerulus and a normal nucleus within a collecting duct in a kidney (Magnification x40); **7d:** Group administered 750mg/kg of extract showing a normal glomerulus and a normal nucleus within a collecting duct in a kidney (Magnification x40).

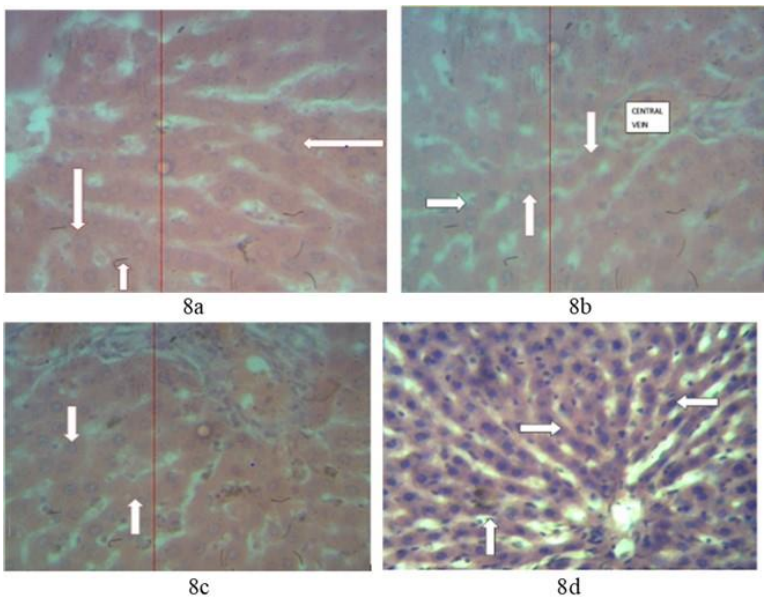

Figure 8. Photomicrographs of liver sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida.* **8a:** Control group showing a normal radial arrangement of hepatocytes, a normal nucleus (hepatic cell) within the hepatocyte, and Kupffer cell within the sinusoid (Magnification x 40); **8b:** Group administered 250mg/kg extract showing a normal hepatocyte, a normal nucleus and Kupffer cell within the sinusoid (Magnification x 40); **8c:** Group administered 500mg/kg body weight of methanol extract shows normal nuclei and radial arrangement of hepatocytes (Magnification x 40); **8d:** 750mg/kg body weight of methanol extract showing hepatocytes, a normal hepatocyte, and the presence of Kupffer cell within the sinusoid (Magnification x 40).

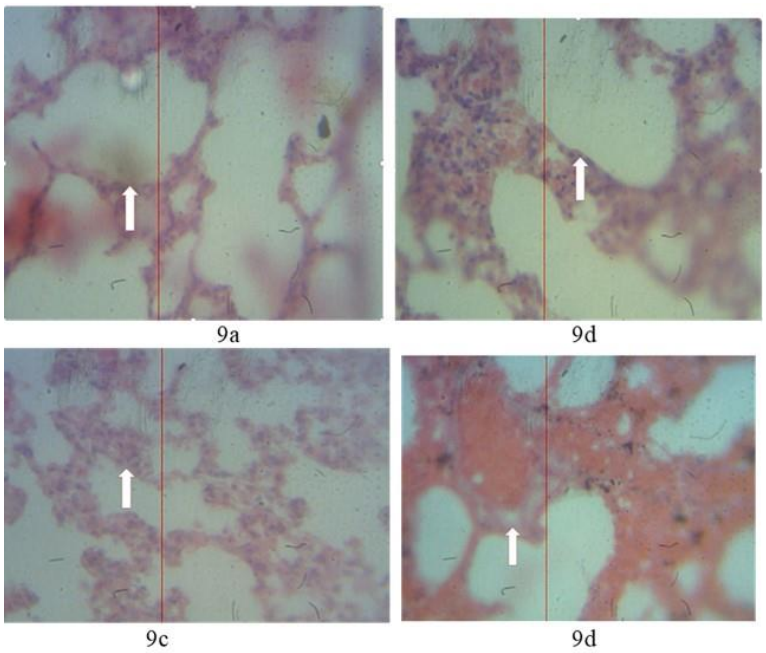

Figure 9. Photomicrographs of Lungs sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida*. **9a:** Control group showing a normal alveolar (Magnification x 40); **9b:** Group administered 250mg/kg of extract shows a normal alveolar cell (Magnification x 40); **9c:** Group administered 500mg/kg body extract showing normal alveolar cell (Magnification x 40); **9d:** Group administered 750mg/kg of extract shows normal alveolar cell (Magnification x 40).

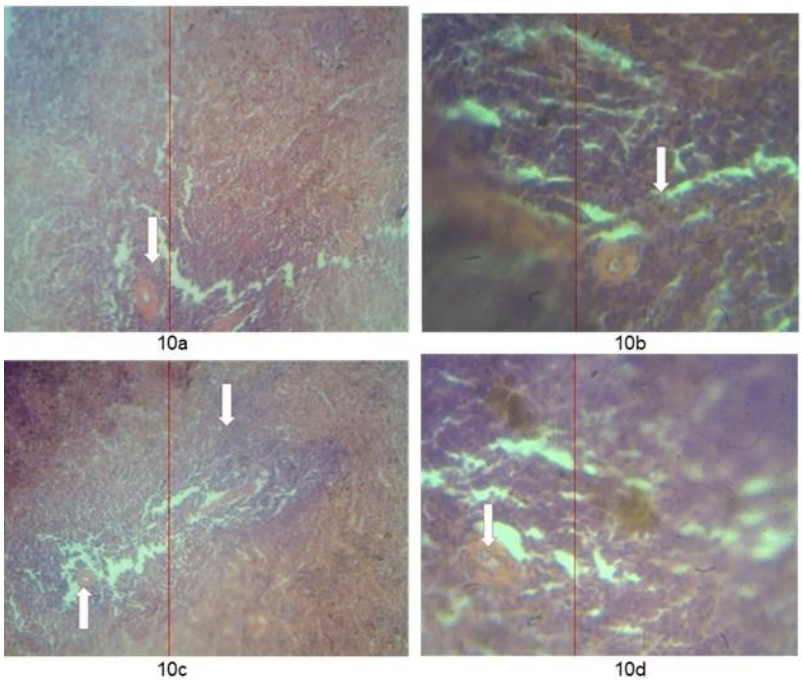

Figure 10. Photomicrographs of Spleen sections from control and experimental rats after the administration of methanol extract of the arils of fruits of *Blighia sapida*. **10a:** Control group showing normal white pulp within a spleen (Magnification x 40); **10b:** Group administered 250mg/kg of extract showing a normal white pulp within a spleen (Magnification x 40); **10c:** Group administered 500mg/kg of extract showing a normal white palp and splenic artery within a spleen (Magnification x 40); **10d:** Group administered 750mg/kg of extract showing splenic artery within the spleen (Magnification x 40).

However, there was a slight distortion of the collecting duct forming hyperplasia and degeneration of cells within the glomeruli (Figure 2d). From Figure 3, there was a mild distortion of hepatocytes, loose nuclei within the Kupffer cells, and loss of sinusoid (Figures 3c and 3d). This could be that the liver, which is usually exposed to injury (caused by toxicants) because of the role it plays in the clearance and transformation of foreign chemicals (Saukkonen *et al.,* 2006; Greenhough and Hay, 2012) is revealed to be distorted at increasing doses of 500mg/kg and 750mg/kg. This is an indication that the extract could be toxic at a high dose and for prolonged use.

Conclusion

This study evaluated the acute, sub-acute, and sub-chronic toxicity of methanol extract in the arils of the fruits of *Blighia sapida.* Finding from this study shows that the methanol extract of the arils of the fruit of *B.sapida* is safe in low concentration as toxicity begins to manifest with the increase in dose. It indicates its ability to cause polycythemia and thrombocytopenia.

Recommendation

Further studies should be conducted on the chronic toxicological effects on the organs studied. This will give a more extensive safety profile of the fruits.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest in this study.

ACKNOWLEDGMENT

The authors acknowledge Mr. Sunday Azi, Technician, Department of Pharmacology and Toxicology, and Mr. Yusuf, Department of Veterinary Medicine, University of Jos, Nigeria. Also, the Hard-working Mr. Gbenga O. Department of Pharmacology, Bingham University, Jos Nigeria for their technical support. I am solely responsible for the funding of this work with no source of funding from anyone else.

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