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Research article

# Sub-chronic toxicity study of ethanol stem-bark extract of *Blighia sapida* (Sapindaceae) in wistar rats

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ABSTRACT

*Blighia sapida* has been used in the treatment of different pathologies. The study aimed at evaluating the acute and sub-chronic toxicity of ethanol stem-bark extract of *B. sapida*. The acute toxicity was evaluated by gavage administration at single dose and the extract was also administered at doses of 250, 500 and 750 mg/kg body weight every other day for ninety day. No mortality or observable signs of toxicity were observed for acute and sub-chronic effects of the extract on the tested animals. No significant difference ($P > 0.05$) in haematological and biochemical parameters compared to the control group. However, histopathological observation revealed some derangements which could be due to continuous consumption of the extract by the animals. It implied that care must be exercised in the use of the plant for a long period of time to prevent its possible long-term toxic effects.

## 1. Introduction

*Blighia sapida* K. Kong (Family Sapindaceae) is popularly known as "Ackee'' (English), "Isin'' (Yoruba), "Gwanja kusa'' (Hausa), and "Okpu'' (Ibo). The seed is not edible whereas the fleshy aril is edible. The fruit is known to contain saponin, which have been reported to be hemolytic [1]. The pulp and the leaves are used to treat eye conjunctivitis. Similarly, the seeds and the jacket because of their oil and potash contents respectively, are burned and the ashes are used in making soap [1]. The stem-bark of *B. sapida* in combination with *Allium cepa* L. (Family Alliaceae) and the ripe fruits of *Capsicum frutescens* L. (Family Solanaceae), ground into powder, serve as cure for gonorrhea when taken with hot pap in northern Nigeria [2]. The edible aril fruits are fully opened naturally at maturity, the black seeds are discarded and the arils while still fresh and firm are best parboiled in salted water or milk and then lightly fried in butter for consumption.

*B. sapida* is referred to as multi-purpose herbal plant, belonging to Sapindaceae family and originated from Africa [3]. It is cultivated in countries like tropical America, India and West Indies [4]. It is an evergreen tree with a branchless straight bole for about 15 m. The tree can be planted purposely for its characteristics like ornamental and shade. At maturity, aril of the fruits serves as main food in countries like Jamaica probably because of the level of fat and protein contents [5]. Wood of the plant is resistant to termite and useful in furniture making, charcoal production and construction [6]. Extract obtained from the seed has been reported to be repellant of insects while combinations of different parts of the plant like bark, root, seeds, leaves and capsule treat different ailments like diabetes [7], yellow fever, dysentery and epilepsy [8]. The work aimed at studying the sub-chronic toxicity of ethanol extract of *B. sapida* on Wistar rat.

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## 2. Materials and methods

### 2.1. Plant materials

Fresh *Blighia sapida* stem-bark was collected during rainy season, from Sekona-Ede Road (Latitude: 7° 39 N, Longitude: 4° 27 E, and Elevation: 291 m), Osun State, Nigeria. The plant material was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The specimen copy was deposited at the Herbarium and specimen voucher number 17623 was collected.

#### 2.1.1. Preparation of plant extract

Fresh stem bark of *B. sapida* was cut into tiny pieces after removing the dead cells, dried under shade and ground into powder by electrical grinding machine at the Drug Research and Production Unit (DRPU), Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The powdered material (1 kg) was macerated in 70 % (v/v) of ethanol/water for 72 h at room temperature using the method of [9]. The resulting suspension was filtered and strained with muslin cloth, the process was carried out twice under the same conditions. The filtrates were pooled together, sieved by means of white cotton gauze, followed by using filter paper (Whatman No. 1) and concentrated with rotary evaporator (Edman High Vacuum Pump) at 40 °C to yield a residue termed ethanol extract (EE). The resulting extract was weighed, labelled and stored in the desiccator for further analysis.

### 2.2. Experimental animals

Wistar rats of both sexes, weighing between 150 and 220 g used were obtained from the Department of Anatomy, University of Ibadan, Ibadan, Nigeria. The animals were acclimatized to the laboratory conditions for two weeks in the Animal House, Department of Biochemistry, fed with standard pellet obtained from Feeds store in Ile-Ife and had free access to water. The principle of laboratory animal care (NIH publication No. 85–23) guidelines and procedures were followed in the study (NIH publication revised, 1985). Animal handling and care were in compliance with international laboratory animal use and care guidelines. The research was approved by Health Research and Ethics Committee, Institute of Public Health, Obafemi Awolowo University, PMB 045, Ile-Ife, Nigeria.

#### 2.2.1. Determination of $LD_{50}$ of the extract

The lethal dose ($LD_{50}$) of the ethanol extract was designed in two phases according to [10]. Nine (9) rats were divided into three groups (3 rats per group) in phase one. Ethanol extract was administered by gavage at 10, 100 and 1000 mg/kg bwt to all the groups (1, 2 and 3) respectively once before feeding. The rats were checked regularly for signs of toxicity like withdrawal to corner of the cage, rough fur and salivation during the first 4 h. Then, they were observed for the next 24 h and every other day for 14 days, for effects of toxicity. In phase two, 3 rats were divided into 3 groups (1 rat per group) and 1,600, 2900 and 5000 mg/kg bwt of the extract were administered, this followed the procedure of the first phase.

#### 2.2.2. Sub-chronic toxicity study

The study was designed based on the earlier procedure of [11] as reported by [12]. Rats (20) with weight of (175.94 ± 3.72) were divided into four groups with each group consisted of five rats:

Group 1 served as control and was given 1.0 ml distilled water;
Group 2 was given 250 mg/kg body weight of ethanol extract.
Group 3 was given 500 mg/kg body weight of ethanol extract.
Group 4 was given 750 mg/kg body weight of ethanol extract.

The rats were weighed at the beginning of the experiment and every week throughout the duration (90 days) of the study. They were treated by gavage administration of the extract every other day. On day 91, all the surviving animals were fasted overnight, sacrificed and blood was obtained through cardiac puncture and kept in labelled heparinized bottles containing anticoagulant for estimation of biochemical parameters and hematological analysis. Also, liver and kidney were excised for biochemical analyses and histomorphological studies. The principle of laboratory animal care (NIH publication No. 85–23) guidelines and procedures were followed in the study (NIH publication revised, 1985). Animal handling and care were in compliance with international laboratory animal use and care guidelines [13]. The research was approved by Health Research and Ethics Committee, Institute of Public Health, Obafemi Awolowo University, PMB 045, Ile-Ife, Nigeria.

*2.2.2.1. Preparation of blood plasma.* Blood plasma was prepared according to the standard procedure of [14]. Typically, blood samples collected in heparinized bottles were centrifuged at 3000 rpm for 10 min in a Table Centrifuge (Model 90–2) at 25 °C. The plasma were collected in sterile bottles using sterile Pasteur pipettes, and kept for biochemical analyses.

*2.2.2.2. Investigation of haematological parameters.* The haematological parameters: RBC (Red blood cell count), WBC (white blood cell count), HGB (Haemoglobin concentration), PCV (Packed cell volume), MCHC (Mean Corpuscular Haemoglobin Concentration), MCH (Mean Corpuscular Haemoglobin) and MCV (Mean Corpuscular Volume) were analyzed using standard techniques reported by [15].

*2.2.2.3. Preparation of liver and kidney homogenates.* The liver and kidney homogenates were prepared as described by [16]. One gram (1 g) of tissue was homogenized with 100mM phosphate buffer, pH 7.2, to produce 10% (w/v) homogenates with pestle and mortar. The homogenates were carefully transferred into centrifuge tubes and volumes adjusted to 10 ml. This was centrifuged at 4000 rpm for 30 min in a Table Centrifuge (Model 90–2) at 25 °C. The supernatants were collected into clean bottles, labelled and kept in freezer for biomarker enzymes assay and other analyses.

*2.2.2.4. Determination of biochemical parameters.* The concentrations of the biochemical parameters were assayed according to the methods described by Lowry *et al.* [17] for total protein, albumin by Pinnell and Northam [18], total bilirubin, direct bilirubin by Jendrassik and Grof [19], urea by Fawcett and Scott [20], creatinine by Chawla. [14], the activities of ALT and AST as described by (Reitman and Frankel [21], while GGT was according to the method of Szasz [22] as reported by Adekola *et al.* [23].

*2.2.2.5. Histology studies.* The tissues were fixed in 10 % formosaline to prevent putrefaction and autolysis. After fixation, the tissues were transferred into increasing concentrations of alcohol for dehydration at room temperature as follows: 50 %, 70 %, 80 %, 90 % and Absolute alcohol for period of 1 h interval at room temperature. Dehydrated tissues were cleared at room temperature in xylene to replace the alcohol. The tissues were then infiltrated in paraffin wax. Infiltrated tissues were embedded in paraffin wax to provide rigid support for microtomy. Sections of 5 μm thickness were prepared from the tissues using a LEICA rotary microtome (Bright B5143 Huntington, England). These sections were floated in a water bath (45 °C) to allow spreading of the folded sections. These sections were mounted on new clean glass slides. The sections were later dried at 40 °C on a slide drier to enhance adherence of the sections to the slide.

Sections on the slide were dewaxed in xylene, treated with descending grades of alcohol as follows: Absolute alcohol, 90 %, 80 %, 70 %, 50 % alcohol. Sections were stained with alum haematoxylin (Harris's). Excess stains were removed by rinsing in water. Sections were differentiated in a mixture of 1 % hydrochloric acid in 70 % alcohol to remove excess dye from the tissues. Sections were washed until nuclei were blue, stained with eosin and rinsed in water. Following a brief wash in water, sections were dehydrated rapidly in ascending grades of alcohol

as follows: 50 %, 70 %, 80 %, 90 %, and absolute alcohol and later cleared in xylene and mounted in DPX (Distrene Plasticizer and Xylene) with a cover slip. The stained tissues were observed under the microscope (LEICA DM750) interfaced with LEICA ($ICC_{50}$) camera.

### 2.3. Statistical analysis

The data obtained were analyzed using one way analysis of variance (ANOVA) followed by Tukey – kramer multiple comparisons test using the software Graphpad Prism 3. The statistical significance set at $p < 0.05$. Values were expressed as mean ± standard error of mean (SEM).

## 3. Results

### 3.1. Acute toxicity

The result of both phases of acute toxicity test revealed no indication of toxicity in the groups of rats that were used for the experiment when EE was administered up to 5000 mg/kg, monitored and observed for toxicity. The rate of feeding was normal, the rats were active and no mortality was recorded during the observation period of 72 h and then 14 days after the administration of the extract. Therefore, the acute toxicity of EE was above 5000 mg/kg and the EE could be speculated to be acutely non-toxic.

### 3.2. Sub-chronic toxicity

During the period of administration of ethanol extract, food consumption and water intake were normal, the animals were active and the appearance of their fur did not change as a result of EE that was administered up to the maximum dose of 750 mg/kg body weight by gavage administration for the period of 90 days, every other day. The extract had no effect on the weight gain in rats during and after administration for the period as shown in Table 1.

#### 3.2.1. Effects on biochemical parameters and histopathology of liver

The results indicates that ethanol extract of *B. sapida* had no significant implication on parameters considered except in total and direct bilirubin. Significant difference was observed in total bilirubin of groups (250 and 500) compared to control group (Table 2).

Also, Fig. 1 shown the histopathology of liver of the animals used for sub-chronic study. The micrograph revealed normal histoarchitecture with distinct hepatocytes, clear sinuosoids and central vein in the control group that was administered with distilled water only. It was observed that there is venous congestion and mild necrotic features which was revealed by mild vacuolation of the hepatocytes in the group administered with 500 mg/kg body weight. In group administered with 750 mg/kg body weight, the micrographs revealed necrotic features with vacuolation of the hepatocytes, there is also venous congestion. The histoarchitecture of the group administered with 750 mg/kg was not as good as what was observed in control, 250, and 500 mg/kg body weight groups. There are hepatocytes, central vein and bile duct as observed in other groups.

**Table 1**
Body weight of rats treated with the ethanol extract (EE) of *B. sapida.*

| Weight (g)/Group | Control | 250 mg/kg | 500 mg/kg | 750 mg/kg |
|---|---|---|---|---|
| Initial body weight | 188.00 ± 3.77 | 175.50 ± 3.80 | 176.50 ± 1.76 | 163.75 ± 5.56 |
| Final body weight | 225.33 ± 3.95 | 194.67 ± 4.46 | 202.00 ± 6.53 | 178.67 ± 7.31 |
| Percentage Weight Gain | 19.86 ± 0.24 | 10.92 ± 0.38 | 14.45 ± 0.52 | 9.11 ± 0.24 |
| Weight of Liver | 7.23 ± 0.37 | 6.85 ± 0.43 | 6.63 ± 0.26 | 6.04 ± 0.48 |
| Weight of Kidney | 1.19 ± 0.03 | 1.08 ± 0.03 | 1.13 ± 0.07 | 1.02 ± 0.07 |
| Liver-body weight Ratio | 0.03 ± 0.11 | 0.04 ± 0.14 | 0.03 ± 0.16 | 0.03 ± 0.14 |

Each value represented the mean ± S.E.M, (n = 5).

**Table 2**
Effect of Ethanol Extract of *B. sapida* on Biochemical parameters.

| Biochemical parameters | Control | 250 mg/kg | 500 mg/kg | 750 mg/kg |
|---|---|---|---|---|
| Urea (mg/dl) | 76.74 ± 1.66[a] | 74.78 ± 1.36[a] | 70.59 ± 2.32[a] | 90.63 ± 5.05[a] |
| Creatinine (mg/dl) | 7.45 ± 0.01[a] | 5.80 ± 0.00[a] | 6.16 ± 0.00[a] | 5.65 ± 0.00[a] |
| Albumin (g/l) | 0.56 ± 0.68[a] | 0.53 ± 0.49[a] | 0.52 ± 0.50[a] | 0.53 ± 1.08[a] |
| T.bil (mg/dl) | 15.86 ± 0.27[a] | 18.56 ± 0.82[b] | 19.76 ± 0.23[b] | 16.22 ± 0.18[a] |
| D.bil (mg/dl) | 1.73 ± 0.52[a] | 1.81 ± 0.50[a] | 1.93 ± 0.08[a] | 0.72 ± 0.04[b] |
| TP (g/l) | 1.41 ± 0.01[a] | 1.17 ± 0.03[a] | 1.31 ± 0.01[a] | 1.51 ± 0.02[a] |
| TP (mg/g)- Liver | 3.98 ± 0.01[a] | 4.29 ± 0.18[a] | 3.04 ± 0.01[a] | 3.43 ± 0.09[a] |
| AST (U/L) | 848.81 ± 11.38[a] | 832.66 ± 14.94[a] | 833.17 ± 5.69[a] | 847.70 ± 17.84[a] |
| ALT (U/L) | 303.39 ± 0.67[a] | 296.57 ± 2.07[a] | 303.35 ± 0.41[a] | 299.63 ± 1.31[a] |
| ALT (U/L)-Liver | 376.25 ± 3.17[a] | 367.61 ± 2.98[a] | 363.30 ± 3.12[a] | 357.16 ± 4.32[b] |
| GGT (U/L) | 0.22 ± 0.03[a] | 0.10 ± 0.01[a] | 0.17 ± 0.01[a] | 0.14 ± 0.01[a] |

Values are represented the mean ± S.E.M of five Rats in each group (n = 5). Values with the same superscript were statistically compared with no significant different $p > 0.05$ while value with different superscript was significant at $p < 0.05$. T.bil: Total bilirubin, D. bil: Direct bilirubin, TP: Total protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: Gamma-Glutamyl transferase.

#### 3.2.2. Effects of ethanol extract of B. sapida on some heamatological parameters

Haematological parameters of the rats used in this study were investigated after 90 days administration of ethanol extract of *B. Sapida* so as to compare the parameters with control group. It was observed that the administration has little or no effect on the haematological parameters of the animals as the difference in the values of the parameters were not significant at $p > 0.05$ when the treated groups were compared with control group as shown in Table 3.

### 3.3. Discussion

The acute lethal dose toxicity revealed that gavage administration of *B. sapida* extract produced no mortality in animals up to the dose of 5000 mg/kg bwt. It has also been reported [24] that $LD_{50}$ values of both aqueous and ethanol extract of the leaves of *B. sapida* is greater than 5000 mg/kg which did not deviate from the observation of [25]. The rate of feeding was normal, the animals were active and no mortality was recorded. This method suggests the classification of the crude extract based on the prediction of the dose at which the animals must survive [26]. This is in accordance with [27] toxicity scale principle, any substance with an $LD_{50}$ value greater than 5000 mg/kg is practically nontoxic.

An improved body weight of the experimental animals during the subchronic study is indication of well-being of the animals [28]; otherwise, decrease in body weight of experimental animals could be sign of toxicity. There was increase in the body weight of animals in all the groups during the 90 days administration of *B. sapida* ethanol extract with the highest dose of 750 mg/kg body weight, which is part of indications that the plant extract may be safe in its usage for the treatment of diseases.

Liver is usually exposed to injury (caused by toxicant) because of the roles it plays in the clearance and transformation of foreign chemicals, condition called hepatotoxicity [29, 30]. Generally it can be said that ethanol extract of *B. sapida* stem bark at the doses considered in this study

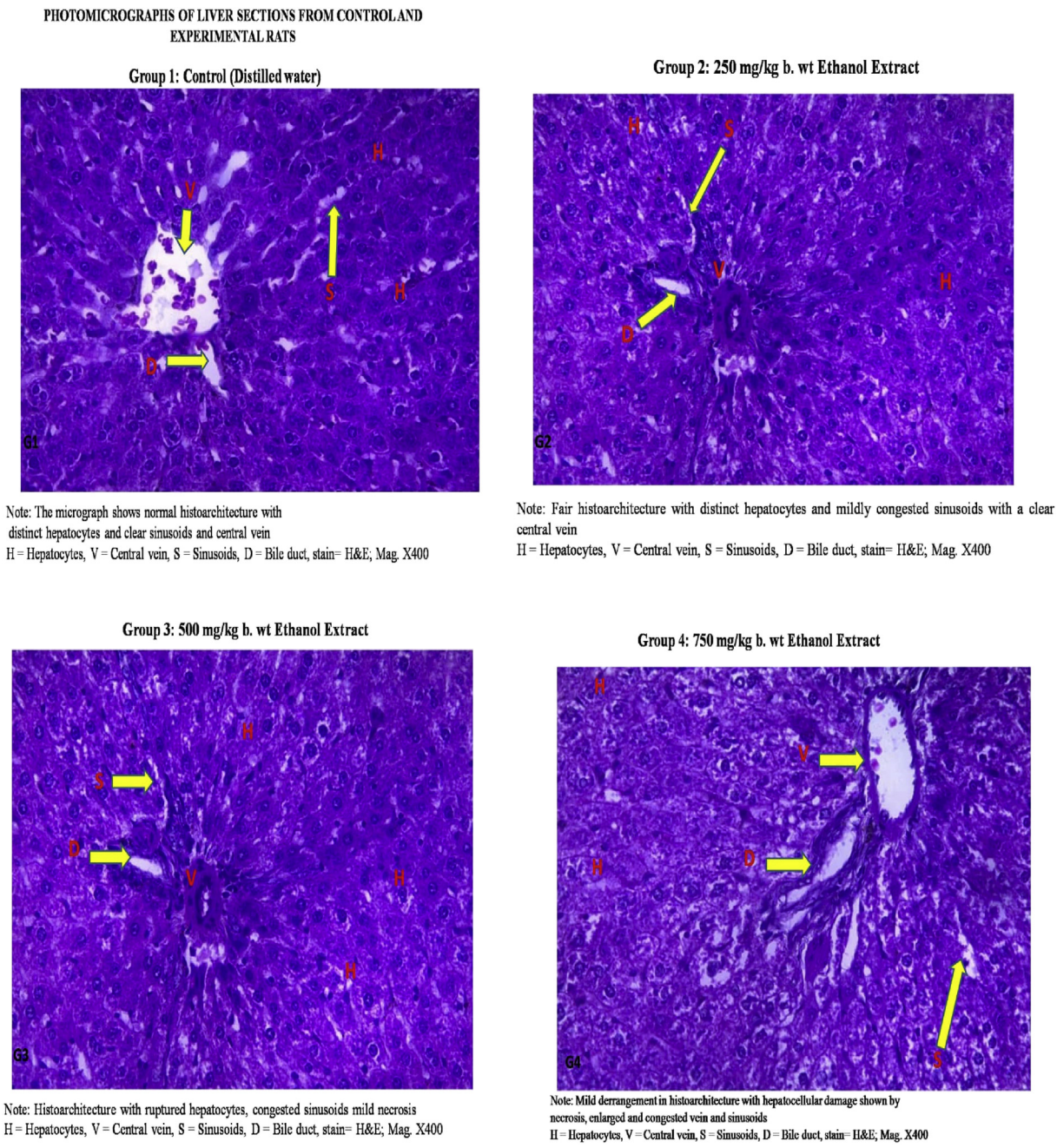


**Fig. 1.** The *in vivo* histomorphorlogy of liver of control group and the groups treated with different concentrations of ethanol extract of *B. sapida.* Note: Fig. 1 above should be printed colored.

may be relatively safe since it does not lead to an increase in the plasma enzymes. Because ALT and AST are normally localized within the cells of liver, muscles and heart, and are biomarkers for assessing damage to the liver, an increase in plasma levels of such enzymes serve as sign for significant hepatocellular injury [31], (GGT) also indicate clinical diagnosis of liver disorder [32]. The study by [24] revealed hypolipidemic and anti-atherogenic effects of ethanol and aqueous extract of *B. sapida* leaves extract in rat. Plasma/Liver Metabolites, like total protein, bilirubin and albumin can provide information on the synthetic capacity of liver [33]. The non-significant change observed in the treated groups compared to control indicates functionality of the hepatocytes. It was also observed that effect of the extract on the histology of liver was dose-dependent as the group with highest dose of 750 mg/kg revealed mild derangement.

The hematological parameters investigated in this study revealed that the administration of the extract had little or no effect on the haematological parameters of the animals at different doses. There was no destruction, no change in red blood cell production rate (erythropoiesis) and no issue about maturity. It was also observed that the extract does not improve process of erythropoiesis, humoral regulator of red blood cell production [34, 35].

Furthermore, in the present study, the results presented are a reflection of pulled set of data from rats of both sexes, which could serve as limitation to the study, as the results were not delineated for both sexes. Therefore, caution should be taken in the application of the report. Consequently, further studies are required to assess any sex-specific toxic effects of ethanol stem-bark extract of *Blighia sapida*.

**Table 3**
Effect of ethanol extract of *B. sapida* on Heamatological Parameters of Experimental Animals.

| Heamatological Parameters | Control | 250 mg/kg | 500 mg/kg | 750 mg/kg |
|---|---|---|---|---|
| RBC ($10^{12}$/L) | 5.22 ± 0.94[a] | 3.94 ± 0.74[a] | 4.29 ± 0.44[a] | 4.09 ± 0.30[a] |
| WBC ($10^{9}$/L) | 5.15 ± 1.38[a] | 4.22 ± 1.26[a] | 3.98 ± 0.93[a] | 5.02 ± 0.88[a] |
| Hb (g/dL) | 16.00 ± 0.71[a] | 14.5 ± 0.62[a] | 15.83 ± 0.95[a] | 13.92 ± 0.29[a] |
| PCV (%) | 48.75 ± 1.23[a] | 43.67 ± 1.66[a] | 45.50 ± 4.73[a] | 43.33 ± 4.25[a] |
| MCHC (g/dl) | 32.80 ± 0.64[a] | 33.20 ± 0.57[a] | 36.40 ± 5.62[a] | 33.07 ± 3.36[a] |
| MCV ($10^{-13}$/fl) | 3.70 ± 1.86[a] | 5.05 ± 2.13[a] | 3.67 ± 2.04[a] | 4.27 ± 1.98[a] |

Each values represented the mean ± S.E.M of five Rats in each group (n = 5).Values with the same superscript were statistically compared with no significant difference at $p > 0.05$. RBC: Red blood cell count, WBC: white blood cell count, Hb: Haemoglobin, PCV: Packed cell volume, MCHC: Mean Corpuscular Haemoglobin Concentration and MCV: Mean Corpuscular Volume.

[a] The [letter a] indicates that no significant difference was observed when the treated groups were compared with control group.

### 3.4. Conclusion

This study evaluated the acute and sub-chronic toxicity of ethanol stem-bark extract of *B. sapida.* The acute toxicity was evaluated by gavage administration of the extract at single doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg bodyweight. The extract was also administered at doses of 250, 500 and 750 mg/kg body weight every other day for ninety day. No mortality or observable signs of toxicity were observed for acute and sub-chronic effects of the extract on the tested animals. No significant difference ($P > 0.05$) in haematological and biochemical parameters in any animal of both sexes compared to the control group, however, histopathological observation revealed some derangements. The result supports its use in the treatment and management of different diseases as claimed ethno-medicinally. However, the use of the plant extract for long period of time against any form of ailment should be avoided in order to prevent possible toxic effects that could result from its continuous consumption.

## Declarations

### *Author contribution statement*

M. B. Adekola: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

J. O. Areola: Conceived and designed the experiments.

N. O. Omisore: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

F. T. Asaolu: Performed the experiments; Wrote the paper.

S. G. Ogunleye: Performed the experiments.

O. E. Apalowo: Performed the experiments; Analyzed and interpreted the data.

O. O. Babalola: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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### *Competing interest statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

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