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Antiplasmodial evaluation of aqueous extract of *Blighia sapida* K.D. Koenig leaves in *Plasmodium berghei* (NK65)-infected mice

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Abstract

Background: The study was designed to screen aqueous extract of *Bilghia sapida* leaves for its phytochemical constituents, in vivo antiplasmodial activity and biochemical changes in *Plasmodium berghei* (NK65)-infected female mice. Phytochemical screening was done using standard methods. In the acute toxicity test, three groups of mice received 1000, 2000 and 3000 mg/Kg/day of the extract respectively, and were observed for signs of toxicity, especially mortality for 24 h. Forty-eight mice were assigned into six groups of eight animals each. The uninfected group A (control) was administered distilled water, while groups B, C, D, E and F were inoculated intraperitoneally with about 10^7 parasitized erythrocytes and received distilled water, chloroquine (5 mg/Kg/day), 125, 250 and 500 mg/Kg/day of extract, respectively. The antiplasmodial activity was evaluated using Peter's 4 days suppressive test. Haematological indices, selected biochemical parameters and liver histology were evaluated.

Results: Screening revealed the presence of six phytochemicals in the aqueous extract of *B. sapida* leaves. Median lethal dose of the extract is > 5,000 mg/Kg/day. The aqueous extract of the leaves significantly ($P < 0.05$) reduced the level of parasitaemia dose-dependently with chemosuppression of 74.09% at 500 mg/Kg/day. The extract significantly ($P < 0.05$) prevented *P. berghei* infection-associated reduction in red blood cell indices. The significant ($P < 0.05$) *P. berghei*-induced alterations in liver function indices were improved in extract-treated mice. There were no visible lesions in the livers of animals that received 125 mg/Kg/day of extract.

Conclusion: The aqueous extract of *B. sapida* leaves has in vivo antiplasmodial activity and justifies its folkloric use in malarial treatment.

Keywords: *Blighia sapida*, *Plasmodium berghei*, Malaria, Chemosuppression, Antiplasmodial

1 Background

Malaria, an infectious disease of the blood caused by plasmodium parasites (*P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*), is associated with fever, anaemia, and other pathologies [37]. In severe cases, malaria leads to coma and death [6]. The deadliest of the five plasmodium species is *P. falciparum*, which is predominantly present in Africa [41]. With about 3.2 billion people at risk, malaria continues to be a major global public health challenge [12]. In 2020, about 228 million cases of malaria with about 602,000 deaths were reported in Africa. This represents about 95% of the 241 million global cases and 96% of the 627,000 deaths for the year, respectively [45]. Nigeria suffers approximately 30% of the malaria incidence in Africa, the greatest burden in the world [12]. With about 97% of Nigerians at risk of malaria, every part of the country is susceptible to the infection. Treatment of human malaria aims at interrupting the blood schizogony (intra-erythrocytic asexual multiplication) that causes the pathogenesis and clinical symptoms of the infection. Unfortunately, *P. falciparum,*

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P. vivax and *P. malariae* have developed resistance to many of the orthodox antimalarials [37]. Antimalarials in current use include quinolones, antifolates, artemisinin derivatives, and hydroxynaphthaquinones [34]. The problem of antimalarial drug resistance is compounded by cross-resistance, in which resistance to one drug confers resistance to other drugs that belong to the same chemical family or have similar modes of action. Apart from the issue of drug resistance, pharmaceuticals are expensive and inaccessible to most of the world's population [32]. Since the first antimalarial drug came from a plant and given the fact that there is a vast majority of plants with medicinal properties in the same region plagued with malaria, it is logical to explore more plants for prospective antimalarials to circumvent the challenges of drug resistance and high cost of orthodox treatment.

Blighia sapida is a woody, perennial, multipurpose fruit tree species that belongs to the Sapindaceae family. It is commonly known as "Ackee" (English). In Nigeria, it is called Gwanja Kusa (Hausa, Northern Nigeria), Isin (Yoruba, Western Nigeria) and Okpu (Igbo, Eastern Nigeria) [31]. *B. sapida* may reach 12–13 m high, with a spreading crown and ribbed branchlets. The stem is about 1.8 m in circumference with a grey and nearly smooth bark. The leaves are evergreen with an acute to rounded base that tapers to a point at the tip [15]. In folk medicine practice, the leaves and bark of *B. sapida* are used for treatment of many ailments as summarized in Table 1. Many researchers have also reported various biological activities for leaves and stem back of the plant (Table 1). However, there is a dearth of information regarding the in vivo antiplasmodial potential of *B. sapida* leaves. Hence, this study was designed to screen aqueous extract of the leaves of *B. sapida* for its phytochemical constituents, evaluate its in vivo antiplasmodial activity and biochemical changes associated with the infection.

2 Materials and methods

2.1 Materials

2.1.1 Plant material

Leaves of *B. sapida* (Fig. 1) were obtained, in July 2013 from Ayedaade area, Ogbomoso North Local Government of Oyo State, Nigeria (Latitude: 8° 8′ 31.7940″ N, Longitude: 4° 14′ 42.6696″ E, with average temperature and relative humidity of 21.4–35.1 °C and 43–85%, respectively), and authenticated at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen was deposited in the Departmental Herbarium under UIH001/1091.

2.1.2 Experimental animals

Female Swiss albino mice (*Mus musculus*) (21.04 ± 0.27 g) were obtained from the Animal Holding Unit of the

Fig. 1 Leaves and fruits of *B. sapida*

Table 1 Traditional uses and biological activities of *B. sapida*

S/no	Plant part(s)	Solvent(s)	Traditional medicinal use(s)	Reference(s)
A: Traditional medicinal uses				
1	Leaves, bark	Water	Malaria	Adebajo et al. [3], Abd El-Gani [2]
2	Leaves	Water	Diarrhoea, dysentery, conjunctivitis, fever, internal haemorrhage, cutaneous skin infections, and bacterial infections	Aloko and Bello [7]
B: Biological activities				
1	Leaves	Ethanol, water	Hypolipidemic and antiatherogenic in diabetic rats	Owolabi et al. [31]
2	Leaves		Inhibition of intestinal motility and enteropooling in castor oil-induced diarrhoea	Antwi et al. [8]
3	Leaves	Water Ethanol	Inhibition of amylase Inhibition of glucosidase in vivo	Kazeem et al. [19]
4	Leaves	Water	Larvicidal potential against some mosquito species	Ubulom et al. [42]
5	Leaves	Water	Antimicrobial activity	Ubulom et al. [43]
6	Stem bark	Ethanol	Prophylactic antimalarial activities	Otegbade et al. [30]

Institute of Advanced Malaria Research and Training, University College Hospital, Ibadan, Nigeria. The animals were housed in plastic cages under standard laboratory conditions (temperature: 28–31 °C; photoperiod: 12 h natural light and 12-h dark; humidity: 50–55%), fed with rat pellets (Grand Cereals Limited, Jos, Plateau State) and allowed free access to tap water. They were acclimatized for two weeks in the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

2.1.3 Malaria parasite

A mouse infected with *P. berghei* (NK65) was obtained from the Institute for the Advanced Malaria Research and Training, Ibadan. The parasite was subsequently maintained in the laboratory by serial blood passage from mouse to mouse.

2.1.4 Assay kits, chemicals and reagents

Chloroquine phosphate was manufactured by May and Baker Nigeria Plc, Otta, Nigeria, while Giemsa stain was a product of Sigma-Aldrich Chemical Company, St Louis, MO, USA. Lactate dehydrogenase (LDH) assay kit was a product of Biosystems S.A. Costa Brava, Barcelona, Spain, while those of albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were products of Randox Laboratories Ltd., United Kingdom.

2.2 Methods

2.2.1 Preparation of plant extract

The leaves of *B. sapida* were carefully separated from the stem, washed with tap water to remove debris and dust, and shade-dried. The dried leaves were then pulverized using an electrical Optimal Mixer Blender (MG 2053, Usha International, England). A known amount (300 g) of the powder was extracted in 3 L of distilled water for 72 h at 28^0C with intermittent shaking. The extract was filtered with Whatman No 1 (185 mm) filter paper. The filtrate was lyophilized to yield 14.46 g aqueous extract of *B. sapida* leaves which corresponded to 4.82%. The extract was then refrigerated at 4 °C and used within seven days.

2.2.2 Phytochemical screening

The extract was screened for the presence of alkaloids, steroids, saponins, flavonoids, phlobatannins, tannins, anthraquinones, cardiac glycosides, phenolics and, triterpenes [16, 36]. The phytochemicals were quantified as described for alkaloids, saponins, tannins phenolics, cardiac glycosides and phlobatannins [16].

2.2.3 Acute toxicity testing

Twelve female mice (13.62 ± 0.52 g) were assigned into three groups of four animals each. Animals in Groups 1, 2 and 3 received a single dose each of 1000, 2000 and 5000 mg/Kg/day of aqueous extract of *B. sapida* leaves, respectively. The mice were observed for signs of toxicity such as salivation, restlessness, vomiting, and mortality for 24 h and 14 days.

2.2.4 Animal handling and evaluation of antiplasmodial activity of the extract

Forty-eight mice (21.04 ± 0.27 g) were completely randomized into six groups of eight animals each. Animals in Group A (normal control) were not infected with *P. berghei* but received 0.2 ml of distilled water while those in groups B (negative control), C, D, E and F were all inoculated with erythrocytes containing approximately 10^7 *P. berghei* and afterwards treated with 0.2 ml of distilled water, 0.2 ml corresponding to 5 mg/Kg/day of chloroquine phosphate, 125, 250 and 500 mg/Kg/day of aqueous extract of *B. sapida* leaves, respectively. A 4-day suppressive test was performed according to the procedure described by Peters and Robinson [33]. Each experimental mouse received the first dose of treatment 4-h post-infection (D_0) with *P. berghei* (NK65). Subsequent treatments were administered 24 (D_1), 48 (D_2), 72 (D_3) and 96 (D_4) hours after the first dose. Blood samples collected from the tails of the mice on D_4 were used for the preparation of thin films on microscope slides. The slides were thereafter fixed in methanol, stained with 5% Giemsa for 3 min, rinsed with water and left to dry. The blood smear was mounted on the microscope with immersion oil and viewed at a magnification of $\times 100$. Percentage parasitaemia was determined by counting the number of parasitized red blood cells and non-parasitized red blood cells in 4 fields of approximately 100 erythrocytes per field. Percentage parasitaemia and % inhibition of parasitaemia were computed using the following expressions:

$$\%\ \text{parasitaemia} = \frac{\text{Number of parasitized erythrocytes}}{\text{Number of parasitized erythrocytes} + \text{number of non - parasitized erythrocytes}} \times 100$$

$$\text{Mean \% chemo - suppression} = \frac{\text{Mean \%parasitaemia in control} - \text{mean \%parasitaemia in test}}{\text{Mean \%parasitaemia in control}} \times 100$$

2.2.5 Determination of haematological parameters

The animals were made unconscious and sacrificed under diethyl ether fumes. An aliquot of blood was collected by cardiac puncture into sample bottles containing EDTA. The haematological parameters analysed using the Sysmex Automated Haematology Diagnostic Machine (XP-300, Mundelein, USA) include red blood cell count (RBC), white blood cell count (WBC), haematocrit (% PCV), haemoglobin concentration (Hb) and platelet count (PLT).

2.2.6 Preparation of serum and liver supernatants

Blood was also collected into clean, dry centrifuge tubes and left for 10 min at 28^0C to clot. The tubes were then centrifuged at 3000 × g for 15 min. The serum was aspirated into sample bottles with the aid of Pasteur pipettes and used for biochemical assay. The liver was quickly excised from the animals, rinsed with ice-cold phosphate buffered saline (PBS) solution and blotted with blotting paper and weighed. A portion of the liver was homogenized in ice-cold PBS (1:10 w/v) [25]. The homogenates were centrifuged at 3000 × g for 15 min to obtain the supernatants which were used within 24 h for the analysis of the biochemical parameters.

2.2.7 Determination of biochemical parameters

The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) lactate dehydrogenase (LDH) and alkaline phosphatase (ALP), as well as total and direct bilirubin, were determined as described on the kits manufacturers' guidelines. The procedure described by Doumas et al. [13] was adopted for determining the level of serum albumin. Serum globulin level was obtained from the difference between serum albumin and the serum total protein content [40].

2.2.8 Histological examination of the liver

The liver of the animals was fixed in 10% (v/v) formal saline for 24 h, dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene and embedded in paraffin wax (melting point 56 °C). Tissue sections were prepared according to the method described by Drury and Wallington [14] and stained with haematoxylin/eosin (H&E). The processed histology slides were read with an Olympus light microscope (CX21FSI, Tokyo, Japan). Photomicrographs of the liver were captured at × 400 with Presto Image Folio package software.

2.2.9 Data analysis

Data were expressed as the means ± S.E.M. of eight determinations except for the phytochemicals. Means were analysed using Duncan's Multiple Range Tests. Statistical Package for Social Sciences, version 20.0 (SPSS Inc. Chicago, USA) was used for statistical analyses. Differences were considered statistically significant at $P < 0.05$.

3 Results

Screening revealed the presence of six phytochemicals in the aqueous extract of *B. sapida* leaves (Fig. 2). Alkaloids have the highest concentration while phlobatannins were the least abundant.

There was no mortality, stooling, respiratory distress, or salivation during the acute toxicity study when the graded doses (1000–5000 mg/Kg/day) of the extract were administered orally. Restlessness was observed in the animals that received 5000 mg/Kg/day at 10 min post-administration.

The aqueous extract of *B. Sapida* leaves significantly ($P < 0.05$) reduced the level of parasitaemia dose-dependently (Table 2). Chemosuppression of 54.76, 70.44 and 74.09% occurred at doses of 125, 250 and 500 mg/Kg/day, respectively.

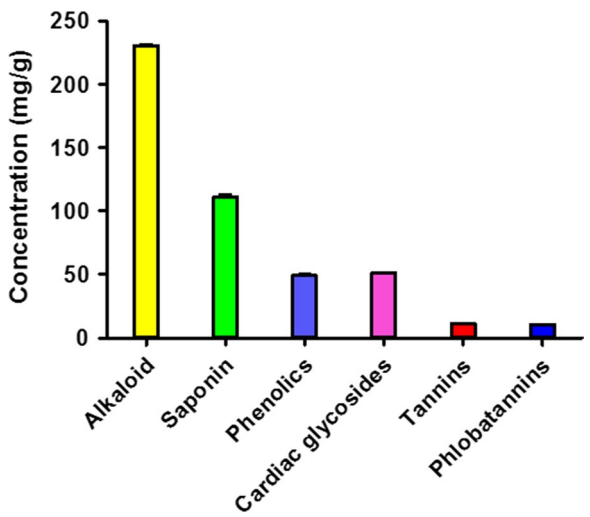

Fig. 2 Phytochemical contents of aqueous extract of *B. sapida* leaves

RBC, Hb and PCV were significantly ($P<0.05$) reduced by *P. berghei* infection. However, the extract reversed this trend significantly ($P<0.05$) at all the doses studied. Extract-induced increase in PCV was in a dose-dependent manner. RBC, Hb and PCV in the extract-treated animals were not significantly different from those of normal control animals. There was no significant difference ($P<0.05$) in WBC and PLT across all experimental groups. There was a significant ($P<0.05$) increase in the LYM (%) of extract-treated animals at 125 and 250 mg/Kg/day of extract relative to those of other groups (Table 3).

Serum ALT activity was significantly raised ($P<0.05$) by *P. berghei* infection. This rise was significantly suppressed in the 125 and 250 mg/Kg/day of extract-treated animals. The 500 mg/Kg/day dose elevated serum ALT activity when compared with the normal control animals. The activity of serum AST was significantly elevated in *P. berghei*-infected mice and this was suppressed in extract-treated mice compared with the

Table 2 Effect of 4-day post-infection administration of aqueous extract of *B. sapida* leaves on the % parasitaemia and % chemosuppression in *P. berghei*-infected female albino mice

Groups	Parasitaemia (%) on D_4	Chemosuppression (%) on D_4
Infected + distilled water	28.10 ± 0.15^{b}	–
Infected + 5 mg/Kg/day CQ	0.08 ± 0.12^{c}	97.31 ± 2.93^{a}
Infected + 125 mg/Kg/day of extract	1.27 ± 0.13^{d}	54.76 ± 4.62^{b}
Infected + 250 mg/Kg/day of extract	0.83 ± 0.12^{e}	70.44 ± 3.83^{c}
Infected + 500 mg/Kg/day of extract	0.73 ± 0.07^{e}	74.09 ± 5.97^{c}

Values are mean ± S.E.M ($n=8$)

CQ chloroquine

Values with different alphabet superscripts in the same column are significantly different ($P<0.05$)

Table 3 Effect of aqueous extract of *B. sapida* leaves on selected haematological parameters of *P. berghei*-infected female albino mice

Groups	RBC ($\times 10^6$/µl)	Hb (g/dL)	PCV (%)	PLT ($\times 10^3$/µL)	WBC ($\times 10^3$/µL)	LYM (%)
Not infected + distilled water	8.27 ± 0.89^{a}	12.87 ± 0.84^{a}	43.93 ± 4.42^{a}	672.33 ± 1.47^{a}	8.20 ± 0.87^{a}	82.90 ± 2.42^{a}
Infected + distilled water	4.05 ± 0.47^{b}	8.27 ± 0.72^{b}	20.87 ± 2.49^{b}	666.67 ± 3.24^{a}	6.70 ± 2.32^{a}	80.70 ± 0.52^{a}
Infected + 5 mg/Kg/day CQ	7.51 ± 0.31^{a}	14.72 ± 0.20^{a}	46.67 ± 0.92^{a}	676.00 ± 1.15^{a}	7.94 ± 1.57^{a}	82.33 ± 10.69^{a}
Infected + 125 mg/Kg/day of extract	8.32 ± 0.67^{a}	12.27 ± 0.94^{a}	43.50 ± 4.10^{a}	607.67 ± 1.16^{a}	10.87 ± 1.20^{b}	89.17 ± 2.93^{b}
Infected + 250 mg/Kg/day of extract	8.39 ± 0.34^{a}	12.80 ± 0.26^{a}	$47.10 \pm 1.21^{a,c}$	612.67 ± 1.21^{a}	10.85 ± 0.49^{b}	87.73 ± 2.51^{b}
Infected + 500 mg/Kg/day of extract	8.35 ± 0.04^{a}	12.85 ± 0.66^{a}	51.75 ± 1.90^{c}	722.00 ± 7.02^{a}	10.28 ± 0.03^{b}	83.87 ± 2.77^{a}

Values are mean ± S.E.M ($n=8$). Values with different alphabet superscripts in the same column are significantly different ($P<0.05$)

CQ chloroquine, *RBC* red blood cells count, *Hb* haemoglobin concentration, *PCV* packed cell volume, *PLT* platelet count, *WBC* white blood cells count, *LYM* (%) relative lymphocytes

Table 4 Effect of aqueous extract of *B. sapida* leaves on selected marker enzymes of *P. berghei*-infected female albino mice

Groups	ALT (U/L)	AST(U/L)	LDH ($\times 10^3$ U/L)	ALP(U/L)
Not infected + distilled water	58.81 ± 0.27^{b}	44.33 ± 1.69^{a}	0.59 ± 0.02^{a}	96.91 ± 5.75^{a}
Infected + distilled water	$66.32 \pm 1.14^{c,d}$	81.17 ± 2.52^{e}	$2.22 \pm 4.34^{b,c}$	200.10 ± 36.38^{b}
Infected + 5 mg/Kg/day CQ	66.88 ± 0.47^{d}	52.33 ± 1.17^{b}	$1.54 \pm 0.40^{a,b}$	102.58 ± 0.80^{a}
Infected + 125 mg/Kg/day of extract	42.75 ± 1.08^{a}	59.33 ± 1.64^{c}	3.00 ± 0.36^{c}	86.71 ± 13.50^{a}
Infected + 250 mg/Kg/day of extract	59.94 ± 1.57^{b}	67.00 ± 1.44^{d}	3.00 ± 0.78^{c}	82.19 ± 12.57^{a}
Infected + 500 mg/Kg/day of extract	$62.19 \pm 2.66^{b,c}$	72.33 ± 2.57^{d}	3.35 ± 0.02^{d}	71.61 ± 4.16^{a}

Values are mean ± S.E.M ($n=8$)

CQ chloroquine; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *LDH* lactate dehydrogenase; ALP alkaline phosphatase

Values with different alphabets superscripts in the same column are significantly different at $P<0.05$

Table 5 Effect of aqueous extract of *B. sapida* leaves on selected liver function indices of *P. berghei*-infected female albino mice

Groups	Liver: body weight (%)	Serum albumin (g/dL)	Serum globulin (g/dL)	Serum total bilirubin (mg/dL)	Serum direct bilirubin (mg/dL)
Not infected + distilled water	4.63 ± 0.19^{a}	4.30 ± 0.03^{a}	1.73 ± 0.20^{a}	22.57 ± 3.99^{a}	4.22 ± 1.62^{a}
Infected + distilled water	8.70 ± 0.32^{b}	0.97 ± 0.02^{b}	4.96 ± 0.24^{c}	23.65 ± 0.56^{a}	5.04 ± 1.75^{a}
Infected + 5 mg/Kg/day CQ	5.44 ± 0.04^{a}	$3.47 \pm 0.12^{a,c}$	2.80 ± 0.58^{b}	24.08 ± 5.74^{a}	$6.72 \pm 0.62^{a,b}$
Infected + 125 mg/Kg/day of extract	$7.20 \pm 1.00^{b,c}$	2.15 ± 0.60^{d}	2.57 ± 0.51^{a}	9.29 ± 3.63^{b}	4.37 ± 1.25^{a}
Infected + 250 mg/Kg/day of extract	6.63 ± 0.35^{c}	$3.88 \pm 0.68^{a,c}$	2.53 ± 0.82^{a}	$17.68 \pm 1.96^{a,b}$	9.79 ± 0.79^{b}
Infected + 500 mg/Kg/day of extract	6.67 ± 0.26^{c}	4.85 ± 0.16^{c}	2.50 ± 0.17^{a}	10.36 ± 1.70^{b}	$7.39 \pm 0.10^{a,b}$

Values are mean ± S.E.M ($n = 8$)

CQ chloroquine

Values with different alphabet superscripts in the same column are significantly different ($P < 0.05$)

untreated animals. However, within the treated groups, serum activity of AST increase significantly with dose. Serum ALP was significantly increased ($P < 0.05$) in the negative control animals but this was ameliorated following administration of the extract (Table 4).

The activity of serum LDH was significantly increased ($P < 0.05$) in a dose-dependent manner in the extract-treated animals relative to normal control animals (Table 4).

Infection with *P. berghei* produced a significant increase in the liver body weight ratio of the animals from 4.63% (in normal control) to 8.70% (negative control). This increase was remarkably averted by the extract at all doses (Table 5). *P. berghei* infection reduced the levels of serum albumin significantly in the negative control group ($P < 0.05$) while the extract increased the same in a dose-dependent manner with 500 mg/Kg/day having no significant ($P < 0.05$) difference compared to

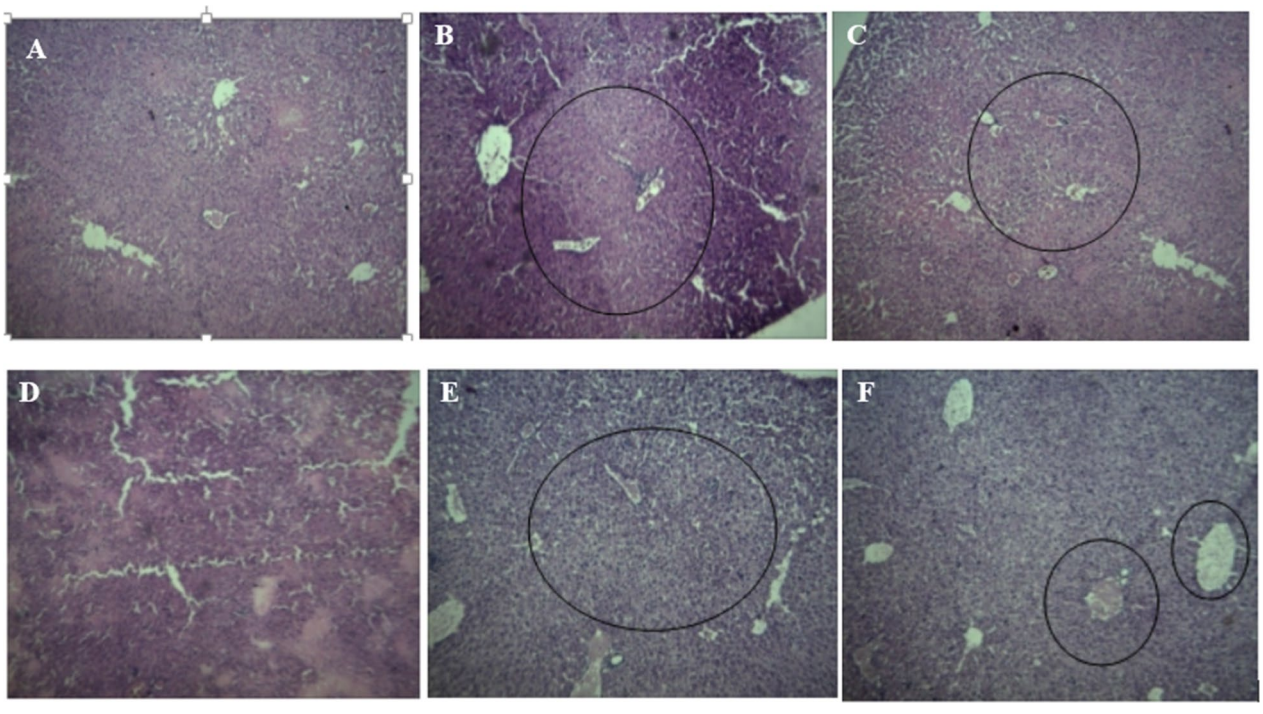

Fig. 3 Cross section of liver of female albino mice infected with *P. berghei* and administered **B** distilled water, **C** 5 mg/Kg/day of chloroquine, **D** 125 mg/Kg/day, **E** 250 mg/Kg/day, **F** 500 mg/Kg/day of aqueous extract of *Blighia sapida* leaves. **A** Normal control (×400; H&E)

normal control animals. There was a significant increase ($P < 0.05$) in serum globulin after infection with *P. berghei*. This was thereafter reduced significantly ($P < 0.05$) following the administration of the extract at all doses. Total bilirubin was significantly lowered by the administration of the extract ($P < 0.05$). Direct (conjugated) bilirubin was not altered in the group treated with the lowest dose of the extract (125 mg/Kg/day) but significantly raised ($P < 0.05$) in those that received higher doses of the extract (Table 5).

Histological examination revealed no visible lesions in the liver of animals in the normal control group (Fig. 3A); a very mild diffuse vacuolar degeneration of hepatocytes in *P. berghei*-infected animals (negative control) (Fig. 3B). There was also a mild-to-moderate periportal cellular infiltration by mononuclear cells. The animals that received 125 mg/Kg/day of extract had no visible lesions in the liver (Fig. 3D). The animals that received 250 and 500 mg/Kg/day of extract produced mild hydropic degeneration limited to the portal area and moderate diffuse vacuolar degeneration of hepatocytes (Fig. 3E and F).

4 Discussion

Plant extracts contain different phytochemicals which are responsible for their biological, pharmacological and toxic effects. These phytochemicals and their corresponding activities confer medicinal advantages on plants. Results from this study showed the presence of alkaloids, saponins, tannins, phlobatannins, phenolics and cardiac glycosides in the aqueous extract of *B. sapida* leaves. Similar results were reported by Ologundudu et al. [27]. These phytochemicals are either individually, addictively or synergistically responsible for the effects observed afterwards. An acute toxicity study gives a clue on the range of doses that could be used in estimating the therapeutic index of drugs and xenobiotics as well as subsequent toxicity testing [35]. *B. sapida* leaf extract was found to be non-lethal up to an oral dose of 5000 mg/Kg/day. The same was reported in a similar work, where it was observed that the aqueous extract was not lethal up to 5000 mg/Kg/day in both rats and mice [26]. This creates a benchmark of doses that can be used for evaluating the biological activities of the plant extract and thus, does not implies that the extract will not produce systemic toxicity. One-tenth of this was used as the highest dose in the antiplasmodial experiment in other to minimize systemic toxicity.

In vivo models are usually employed in antimalarial studies because they take into account possible pro-drug effects and contributions of the innate immune system in the eradication of the pathogen [39]. The results of this study showed that the aqueous extract of *B. sapida* leaves suppressed the multiplication of malaria parasite at 250 and 500 mg/Kg/day up to 70% chemosuppression (Table 2). Compounds that reduce parasitaemia by 30% or more are considered active and are further evaluated in secondary screens [1], thus with a parasitaemia reduction of 54.76 ± 4.62, 70.44 ± 3.83 and $74.09 \pm 5.97\%$ by 125, 250 and 500 mg/Kg/day, respectively, aqueous extract of *B. sapida* qualifies for further evaluation for its antimalarial property. Although the %chemosuppression observed in extract-treated animals is lower than that of chloroquine, the extract is a cocktail of compounds constituting both the antiplasmodial and non-antiplasmodial principles, and isolation and purification of antiplasmodial principle(s) could improve the antiplasmodial activity. The %chemosuppression of *P. berghei* by the extract studied is higher than those achieved with 80% methanol extract of *Vernonia amygdalina,* a popular Nigerian plant traditionally used for malaria with only 37.67% suppression [11]. The observed antimalarial activity may be linked to the phytochemicals (Fig. 2) present in the extract. Alkaloids are one of the major classes of natural products that exhibit antimalarial activity. Indeed, quinine, the first antimalarial drug, belongs to this class. Previous studies have shown that plant-derived alkaloids have a great potential for antimalarial drug development [28]. A study reported that plants whose phytochemical compounds include alkaloids, anthraquinones and saponins may have antimalarial activities [10]. These reports are similar to those obtained in this study as aqueous extract of *B. sapida* leaves contains alkaloids, saponins, and tannins. Saponins are detrimental to several infectious protozoans, one of which is the *Plasmodium* species [4]. Some tannins have also shown antiplasmodial activity as reported by Keita et al. [20] with IC50 of 46.9 ± 1.12 and 67.8 ± 2.39 µg/mL for chloroquine-sensitive and -resistant strains of *P. falciparum*, respectively. Tannins, being phenolics, also act as antioxidants or free radical scavengers and may alleviate the oxidative stress associated with malaria, which plays an important role in malaria-associated anaemia [29]. The antiplasmodial activity may also be a result of a synergistic effect of two or more of the phytochemicals as phenolics have been reported to significantly act in synergy with some first-line malaria drugs [24]. Antiplasmodial activities have also been reported for *Paullinia pinnata* [5] which also belongs to the Sapindaceae family as *B. sapida*.

The extract also showed the ability to ameliorate anaemia due to malaria by maintaining the red blood cell counts (RBC), haemoglobin concentration (Hb) and packed cell volume (PCV) in *P. berghei*-infected and extract-treated mice. The observed prevention of anaemia by the extract was not significantly different from that of chloroquine. The clearance and/or destruction of

infected erythrocytes, the clearance of uninfected erythrocytes and erythropoietic suppression and dyserythropoiesis, all contribute to malaria anaemia [46]. Thus this anti-anaemic activity may be attributed to its ability to suppress parasite multiplication (as evidenced by a reduction in % parasitaemia) thereby reducing the number of parasitized erythrocytes billed for splenic destruction. It may also be that the extract has some stimulatory effect on the production of red blood cells (erythropoiesis). This aligns with the findings of Mboso et al. [22] who reported the erythropoietic effects of *Eremomastax polysperma* leaf extracts on female prepubertal and pubertal Wistar rats. Lymphocyte counts were increased by the extract. The observed increase in WBC in the extract-treated animals may result from stimulation of the immune system of the animals to fight the malaria parasites (Table 3). White blood cells function mainly to fight infection, defend the body by phagocytosis against invasion by foreign organisms, and produce, transport and distribute antibodies in the immune response. Many antimalarial herbal preparations may exert their antiinfective activity not only by affecting the parasite directly but may also stimulate the defensive system of the host by other mechanisms [39].

The liver plays a very crucial role in the pathology of malaria where sporozoites develop into merozoites [44]. It also plays a central role in the metabolism and excretion of xenobiotics and this makes it susceptible to their toxic effects. The extract lowered the enlargement of the liver caused by malaria infection in a dose-related manner (Table 5). An association has been established between the level of malaria parasitaemia and hepatomegaly. The extract at all doses was able to ameliorate the enlargement. This may be a direct effect of the ability of the extract to suppress the multiplication of the malaria parasite. Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are useful indicators of the well-being of the liver and are thus useful marker enzymes of liver cytolysis [23]. Although administration of the extract prevented the infection-associated increase in serum activities of the two aminotransferases at 125 mg/Kg/day body weight, a significant increase ($P < 0.05$) in the serum activities of ALT and AST at higher doses (250 and 500 mg/Kg/day body weight) when compared with those of the animals in the control group may imply that the extract at such doses may cause injury to the liver or other tissues with a significant amount of these cytosolic enzymes. The lack of significant change in the serum activity of ALP in the extract-treated group suggests that the extract may not cause hepatobiliary injury and cholestasis [38]. The activity of LDH significantly increased with extract administration in a dose-dependent manner, similar to what was obtained with *Cardiospermum helicacabum* [9]. LDH is present in all cells of the body with the highest concentration in the erythrocytes, heart, liver, kidney, lung and pancreas and can indicate injury in any of them [17]. Since the result of the chemosuppression test as well as the haematological parameters showed that the extract processes an antiplasmodial activity, the rise in LDH activity may not be linked to malaria haemolysis. Thus the dose-dependent, significant increase in serum LDH activity may need to be further investigated before the safety or otherwise of the extract would be determined.

The extract caused a significant increase and decrease in the level of serum albumin and globulin, respectively, which were altered by *P. berghei* infection. (Table 5). Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a useful indicator of the synthetic function of the liver. The reduction in albumin concentrations of *P. berghei*-infected animals may be attributed to decreased rate of hepatic synthesis of albumin which did not commensurate with the rate of its catabolism [21]. But the extract at all doses investigated maintained the levels of these proteins in levels not significantly different from the control meaning that the extract did not impair the synthetic function of the liver. Bilirubin, a marker for liver damage, is the major breakdown product of haemoglobin of red blood cells. The extract did not cause an increase in the total bilirubin level and it is an indication that the extract may not impair the excretory function of the liver [18]. Only the lowest dose of the extract (125 mg/Kg/day body weight) showed no visible lesion on the liver. Hepatic portal degeneration and congestion caused by higher doses (250 and 500 mg/Kg/day body weight) of the extract is an indication of damage to the liver. This further corroborates the elevated level of serum ALT and AST activities in the 500 mg/Kg/day body weight of extract-treated animals. These findings connote that aqueous extract of *B. sapida* leaves may not be save for consumption at doses beyond 125 mg/Kg/day bodyweight.

5 Conclusion

From the results of this study, the aqueous extract of *B. sapida* leaves has in vivo antiplasmodial activity as it suppresses the development of *Plasmodium berghe*i. This justifies the use of the plant in the treatment of malaria in folkloric medicine. The antiplasmodial activity could be due to any of the phytochemicals present in the extract or synergy between two or more of them. Further studies will be needed for isolation, characterization and identification of the active principle as well as safety evaluation.

Abbreviations

LD_{50}: Median lethal dose; LDH: Lactate dehydrogenase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase;

EDTA: Ethylenediamine tetraacetic acid; RBC: Red blood cell count; WBC: White blood cell count; % PCV: Haematocrit; Hb: Haemoglobin concentration; PLT: Platelet count; PBS: Phosphate buffered saline.

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Author contributions
TDO conceptualized the project, carried out the experiments, analysed the data and wrote the manuscript. The author read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not available. However, international guidelines for handling animals were strictly adhered to, according to The Basel Declaration issued on 30th November, 2010.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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