Ethanol Extract of *Blighia sapida* Stem Bark Show Remarkable Prophylactic Activity in Experimental *Plasmodium berghei*–Infected Mice

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ABSTRACT: This work explores the antiplasmodial potential of ethanol extract of *Blighia sapida* (Lin. Sapindaceae) in chloroquine (CQ)-resistant *Plasmodium berghei* (ANKA strain)–infected mice. Chloroquine-resistant (ANKA) strain of *P berghei* was inoculated intraperitoneally into Swiss albino mice. Mice were treated orally for 4 consecutive days, before and after inoculation (prophylactic, suppressive, and curative models) with graded doses of the plant extracts with Artemether-Lumefantrine (Coartem) as control. Prophylactically, the extract showed a remarkable activity in the chemosuppression of *P berghei* parasites ($P < .01$) ranging from 57% to 36.5% at doses of 200 to 800 mg/kg, respectively, whereas Coartem (10 mg/kg) produced 62.1% chemosuppression. No significant chemosuppression was observed in the curative and suppressive models. The plant extract appeared to be safe at the highest dose tested (5000 mg/kg) for acute toxicity, with no adverse effect on the different organs. The plant extract possesses prophylactic antimalarial activity, which supports its use in the prevention of malaria.

KEYWORDS: *Plasmodium berghei*, *Blighia sapida*, chemosuppression, curative, prophylaxis, efficacy and safety

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Introduction

Based on the latest World Health Organization (WHO) estimates of December 2016, there were 212 million cases of malaria in 2015 and 429 000 deaths, with sub-Saharan Africa accounting for 90% of all malaria deaths in the world.[1] This staggering number of deaths resulting from malaria is associated with the continuous spread of *Plasmodium falciparum* resistance to available antimalarial drugs, posing severe threats to existing malaria control programs. The lack of a deployable malaria vaccine coupled with increasing cases of drug-resistant infections makes the search for new antimalarials imperative.[2,3] Owing to the high cost of malaria treatment in developing countries in general and Nigeria in particular, many have resulted to consulting traditional herbalists or the use of medicinal plants for treatment of infection.[4]

For thousands of years, in various parts of the world, traditional herbal medicines have been used to treat malaria.[5–9] Two of the most important antimalarial drugs in use, artemisinin and quinine, were either derived directly from plants or synthesized using chemical structures of plant-derived compounds as templates.[5,9,10] Most of the people in malaria endemic countries still depend on traditional herbal remedies, which could be linked to limited availability as well as unaffordability of existing pharmaceutical drugs.[11]

Blighia sapida, also referred to as ackee or ackee apple, is a medicinal plant of the family Sapindaceae that naturally exists in the forests of most West African countries, where the fruits are used for several purposes, but rarely eaten.[12] When mixed with water, the green fruits of this plant produce lather and are used for laundry, in addition to its seeds being used for making soap due to its high oil content.[13] Some parts of this plant also serve medicinal purposes, such as the juice from the leaf is traditionally used to treat conjunctivitis,[12] whereas its twiggy leaves, once beaten into a pulp, are applied to the forehead for treating migraine or headaches.[14] For over 20 years, the Centre for Scientific Research into Plant Medicine (CSRPM) in Ghana has used this plant for the treatment of diarrhea.[15] The antidiarrheal activity of the stem bark of *B sapida* has also been reported,[16] including reports on its traditional use for the treatment of dysentery, yellow fever, burns, wounds, or conjunctivitis, etc.[14] Murine experiments have also demonstrated its utility in treating pancreatic β-cell dysfunction,[17] elevated blood glucose, dyslipidemia, and oxidative stress in alloxan-induced diabetic rats.[18]

In view of the significant challenges imposed by drug resistance, traditional plant extracts continue to be promising sources of new antimalarial compounds.[9] The use of traditional medicines for the treatment of malaria and other diseases continues to be a growing practice among many African families, despite the availability of orthodox method of treatment.[19] In addition, the short supply of artemisinin compounds leading to alternative

production methods,[9] as well as the desire for low-cost drug delivery strategies,[7] has led to significant research efforts to decipher alternative plant products that could serve as effective antimalarials, especially in sub-Saharan Africa. In this report, the antiplasmodial effect of ethanolic extract of *B sapida* stem bark in vivo is reported, using the suppressive, curative, and prophylactic model.

Materials and Methods

Plant collection, authentication, and extract preparation

The stem bark of *B sapida* was collected in December 2014 from Ipetumodu in the South West of Nigeria. Plant material was identified in the herbarium of the Botany Department of Obafemi Awolowo University, Ile-Ife, Osun State Nigeria with voucher number IFE-17629 by Mr GA Ademoriyo. The stem bark was air-dried at room temperature and powdered. About 50 g of the powdered plant was macerated in 500 mL of 99% methanol (1:10 W/V) for 48 hours and then filtered. With the aid of a rotatory evaporator, filtrate was concentrated to dryness and the residue obtained was stored until future use.

Ethical consideration

Experimental procedures and protocols used in this study were in conformity with National Institute of Health's recommendations in guide for the care and use of laboratory animals and in accordance with the principles of Helsinki Declaration.

Parasite/infection

Chloroquine-resistant *P berghei* ANKA, obtained from Institute for Medical Research and Training (IMRAT), University College Hospital, Ibadan, Nigeria, was used to evaluate the antimalarial activity of *B sapida* in this study. Groups of Swiss albino mice (5 per cage) were infected intraperitoneally with 1×10^7 parasitized erythrocytes from an infected donor mouse. The day of infection was defined as day 0 (D0) and subsequent days D1, D2, etc.

Antimalarial medication

Standard Coartem (Artemether and Lumefantrine), used for treating malaria (Mekophar Chemical Pharmaceutical Joint-Stock Co, Ho Chi Minh City, Vietnam) and obtained from a pharmacy, was used as reference for the antimalarial screening in this study.

Acute toxicity test (assessment of minimum lethal dose)

The assessment of minimum lethal dose was done in 2 phases, following published methods.[20] In phase 1, groups of 3 mice in each group were given oral doses of 10, 100, and 1000 mg/kg body weight of *B sapida* ethanolic extract, respectively, and observed for 24 hours for mortality. In phase 2, 3 mice in each group groups were orally administered with 1600, 2900, and 5000 mg/kg body weight of the extract, respectively, and monitored for 24 hours, observing for mortality. The phase 2 study was conducted because we observed no deaths among animals in phase 1 study. The lethal dose and the penultimate dose to the lethal dose would indicate the value of the LD_{50}.[21,22]

In vivo antiplasmodial determination

Swiss albino mice (weight: 18-25 g), obtained from the Animal House, Department of Pharmacology, University of Ibadan, kept according to Institutional Animal Care and Use Committee (IACUC) standards, were allowed to acclimatize to the new environment for a week before study initiation. Each mouse was inoculated with 0.2 mL of infected blood containing about 1×10^7 dose of *P berghei berghei* (about 16.6%) from a donor mouse. Each mouse was inoculated on day 0 (D0) (intraperitoneally) for the suppressive and curative model and on the fifth day (D4) for the prophylactic model.

Suppressive test (4-day test)

The 4-day suppressive test was conducted according to the method of Peters (1975). In this, 25 mice distributed into 5 groups of 5 mice each were infected with 1×10^7 of parasitized red blood cells on day 0. Animals in group 1 received normal saline, those in groups 2 to 4 received 200, 400, and 800 mg/kg of ethanol extract of *B sapida* test extract, whereas those in group 5 (positive control) received 10 mg/kg of the standard drug Coartem. Treatment was administered orally and continued daily for the next 3 days for the group that received Coartem or 4 consecutive days for extract-treated group. On day 4, blood samples were collected from the caudal vein and stained with 10% Giemsa stain. Thereafter, the number of the parasitized cells was estimated under the microscope using ×100 objective.[23] The numbers of infected erythrocytes were counted until 1000 erythrocytes were achieved. The average percentage suppression of parasitemia was calculated in comparison with controls as follows:

$$\% \text{ Suppression} = \frac{\begin{array}{l}\% \text{ parasitemia in negative control} - \\ \% \text{ parasitemia in test group}\end{array}}{\% \text{ parasitemia in negative control}} \times 100$$

Prophylactic test

The prophylactic activity of the extract was performed as described previously.[24] In this, Swiss albino mice were randomized into 5 groups of 5 mice each: group 1 was treated with normal saline only, groups 2 to 4 were treated with 200, 400, and 800 mg/kg/d of the ethanol extract of *B sapida*, and group

5 served as positive control (treated with 10 mg/kg/d of the standard drug Coartem). Treatment started on day 0 and continued for 4 consecutive days, by which mice were inoculated intraperitoneally with 1×10^7 of the parasite. On day 7 (72 hours after inoculation), blood smears made from each mouse were stained with Giemsa, number of the parasitized cells was estimated, and the percentage suppression was evaluated.

Curative test

This was also performed with standard protocol,[24] as previously described. Twenty-five mice distributed into 5 groups of 5 were infected intraperitoneally on the first day (day 0) with 0.2 mL of parasite inoculum. After 72 hours of infection, all animals were treated with different regimens: group 1 received normal saline only; groups 2 to 4 received 200, 400, and 800 mg/kg body weight of plant extract, respectively; whereas group 5 received 10 mg/kg body weight of the standard antimalarial drug (Coartem). Treatment was continued for 5 consecutive days, blood smears were made and microscopically examined to monitor the degree of parasitemia as well as estimating the number of parasitized cells.

Histologic procedures

These were performed with hematoxylin and eosin procedures.[25] Briefly, organs such as liver, spleen, kidney, and testes from each animal were fixed in 10% formal saline, grossed and cut longitudinally into pieces of 4 mm in thickness, and processed for histologic study using standard procedure. Microtome sections were dried on a hot plate and later stained with hematoxylin and eosin stains for examination with a light microscope.

Data analysis

Percentage parasitemia and mean chemosuppression were determined using 1-way analysis of variance; Student-Newman-Keuls test was used for analysis and comparing of results at 95% confidence interval; $P < .05$ was set for statistical significance.

Results

About 11.10 g of brown crude extract was obtained from the extraction procedure, a percentage yield of 22%. The acute toxicity test revealed that the extract appeared to be nontoxic at the highest dose (5000 mg/kg) tested. No toxic symptoms or mortality was observed in any of the 3 animals in 3 groups both in phase 1 and phase 2 acute toxicity test study. All animals were still alive at the end of the acute toxicity test, with LD_{50} value greater than 5000 mg/kg body weight, showing the extract to be nontoxic. Histology sections of the liver, spleen, kidney, and testes reveal normal tissue morphology at all doses, except at 5000 mg/kg (black ring), where we observed a mild perivascular infiltration of liver inflammatory cells (Figure 1); otherwise, hepatocytes appeared normal.

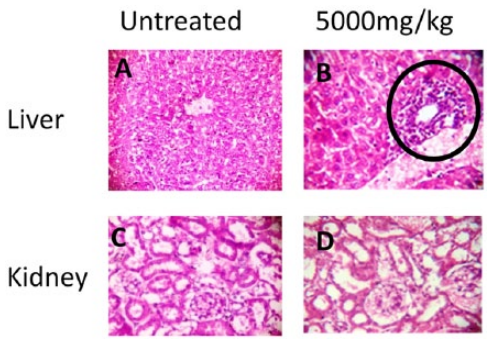

Figure 1. Photomicrographs of liver and kidney at the end of acute toxicity assessment. (A) Normal liver morphology. (B) (Black ring) mild perivascular infiltration of the liver inflammatory cells after treatment with 5000 mg/kg of the extract. (C) Normal kidney morphology. (D) Kidney remains normal after treatment with 5000 mg/kg of the extract. Haematoxylin and Eosin, 400x original magnification.

Suppressive effect of B sapida ethanolic extract on P berghei

In the suppressive test, mean parasitemia on day 4 after infection ranged from 14.20 ± 1.17 to 18.96 ± 0.60 for 200 and 800 mg/kg, respectively (Figure 2). The value for control was 19.02 ± 1.20, whereas for Coartem was 8.38 ± 0.39. The percentage chemosuppression of the extract ranged from 6.8% to 0.3% for 200 and 800 mg/kg, respectively, whereas that for standard antimalarial Coartem was 55.9%. Percentage chemosuppression showed increases with decrease in dose levels of the extract, indicating that maximal chemosuppression could be reached at the lowest dose, although intangible. Percentage chemosuppression in Coartem was very much higher than in control and all the doses of the extract, revealing that extract had little or no suppressive effect at all doses. Percentage parasitemia in control, compared with 400 and 800 mg/kg, was not statistically significant ($P > .05$), whereas that of 200 mg/kg compared with control, 400 and 800 mg/kg, was statistically significant ($P < .01$) (Figure 2). Percentage parasitemia in Coartem was much lower than in extract at the highest dose, showing statistical significance ($P < .001$), but percentage parasitemia of the lowest dose of the extract was slightly higher in comparison with Coartem, with the difference not significant (Figure 2).

Prophylactic effect of B sapida ethanolic extract on P berghei

The percentage parasitemia in mice infected and treated with 200, 400, and 800 mg/kg was 8.22 ± 0.32, 9.7 ± 0.31, and 12.12 ± 0.54, respectively (Figure 3). Untreated saline group had percentage parasitemia of 19.10 ± 0.33, whereas that of Coartem-treated positive control was 7.24 ± 0.91. Percentage chemosuppression of parasites in mice treated with 200, 400,

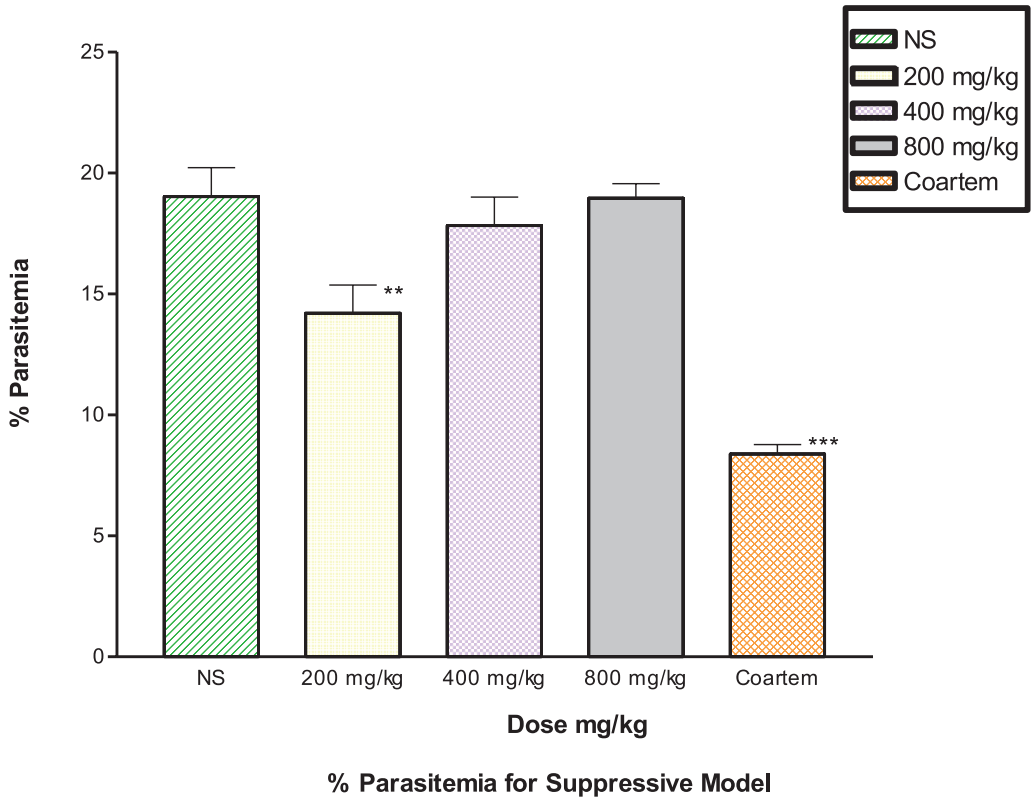

Figure 2. Suppressive effect of methanol extract of bark of *Blighia sapida* on *Plasmodium berghei.* Bars are expressed as mean ± SEM (n = 5); ***$P < .001$, Coartem versus normal saline (NS); **$P < .01$, 200 mg/kg versus normal saline.

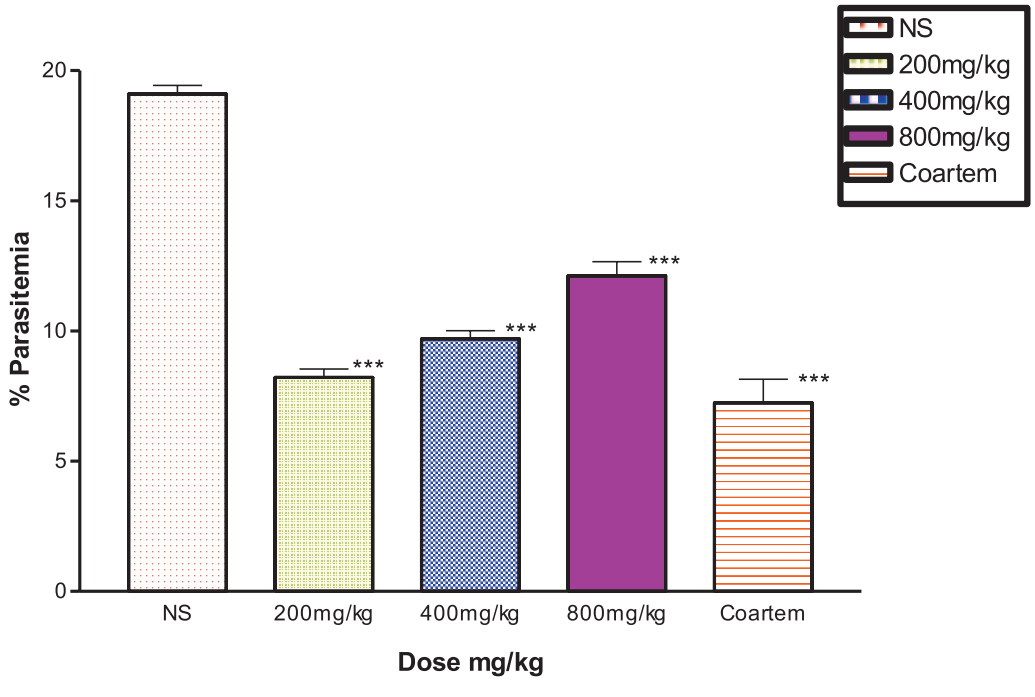

Figure 3. Prophylactic effect of ethanol extract of *Blighia sapida* on *Plasmodium berghei* in mice. Bars are expressed as mean ± SEM (n = 5); ***$P < .001$, test and control versus normal saline (NS).

and 800 mg/kg was 57%, 50.8%, and 36.5%, respectively, whereas that of Coartem was 62.1%. Unlike our observation in the suppressive model, percentage parasitemia decreased with a decrease in extract dose levels, showing that a tangible optimal

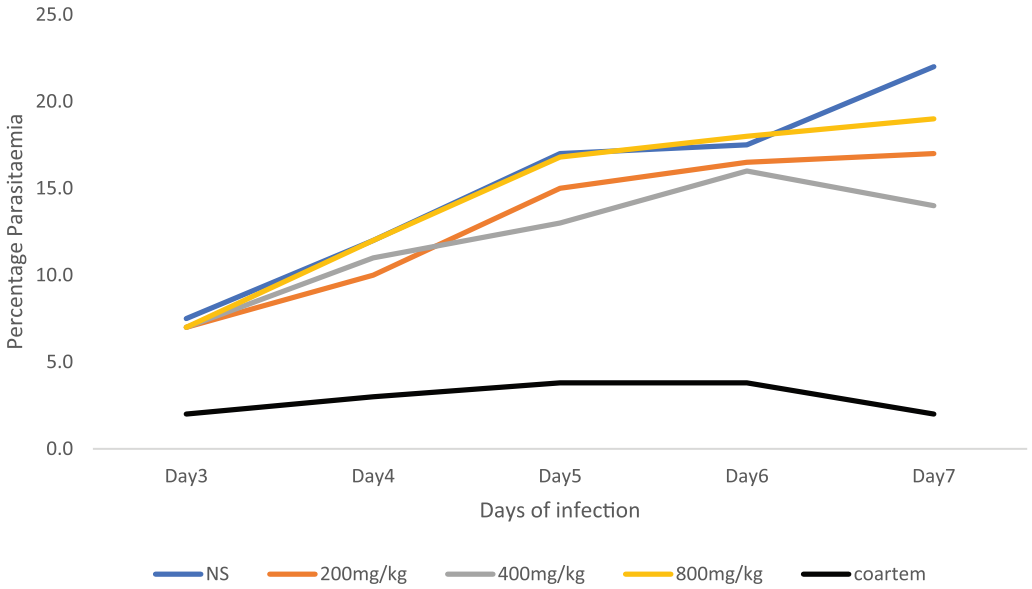

Figure 4. Curative effect of ethanol extract of *Blighia sapida* on *Plasmodium berghei* in mice. NS indicates normal saline.

Table 1. Weights of mice for prophylactic model.

DOSE, MG/KG	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
NS	21.40 ± 1.02	21.85 ± 0.99	22.08 ± 1.0	21.94 ± 0.96	22.28 ± 1.10	21.90 ± 0.93	19.45 ± 0.83	17.02 ± 0.75
200	20.78 ± 0.92	20.16 ± 0.94	21.02 ± 1.02	20.24 ± 1.10	20.96 ± 1.03	21.40 ± 1.20	20.16 ± 1.00	21.70 ± 1.08
400	17.28 ± 0.44	17.20 ± 0.39	17.46 ± 0.54	17.36 ± 0.51	17.62 ± 0.41	18.00 ± 0.47	17.32 ± 0.43	16.96 ± 0.40
800	22.28 ± 1.21	22.20 ± 1.40	22.50 ± 1.47	21.64 ± 1.33	22.12 ± 1.34	22.80 ± 1.52	21.90 ± 1.36	22.68 ± 1.44
Coartem	19.58 ± 1.00	19.88 ± 0.90	20.14 ± 0.78	19.78 ± 0.70	19.58 ± 0.70	20.12 ± 0.75	19.52 ± 0.63	20.42 ± 0.51

Abbreviation: NS, normal saline.
The baseline weights were taken before inoculation and subsequent weights taken before, during and after treatments. Data are expressed as mean ± SEM. Values do not indicate statistical significance to one another.

chemosuppression can be obtained at the lowest dose in this model and is statistically significant at all doses when compared with nontreated group ($P < .001$; Figure 3). The percentage of the extract at the lowest dose (200 mg/kg) was comparable with that of Coartem, although Coartem exhibited a highly significant ($P < .001$) decrease in percentage parasitemia compared with negative control and 800 mg/kg.

Curative effect of B sapida ethanolic extract on P berghei

Figure 4 shows curative effect of the extract at different graded doses. Unlike the suppressive and prophylactic models, none of the extract doses tested showed significant activity. The lowest dose (200 mg/kg) that had shown significant activity in other models showed no activity in our model. For day 3, the values for Coartem versus control versus 200 mg/kg of the extract was 1.74 ± 0.29 versus 7.58 ± 0.44 versus 7.18 ± 0.82, with similar findings on day 7 (21.48 ± 0.68, 17.00 ± 0.58, and 2.06 ± 0.39 for control), 200 mg/kg of extract and Coartem, respectively, revealing the extract had no significant activity against the parasite when compared with control.

Effect of malaria on weight of malaria-induced mice

The weights of all test animals decreased 24 hours after infection, with this decrease continuing among the suppressive, curative, and untreated groups 72 hours after treatment. However, by day 5 (21.40 ± 1.20), this progressive weight loss was turned around for the prophylactic group when compared with the weights obtained at the same dose on day 0 (20.78 ± 0.92) and day 7 (21.70 ± 1.08) (Table 1).

Effect of B sapida ethanolic extract on organs of P berghei–infected mice

The kidney, liver, and spleen of the mice revealed normal tissue architecture after treatment, demonstrating that this extract appears safe on the major organs of the mice (slides not shown).

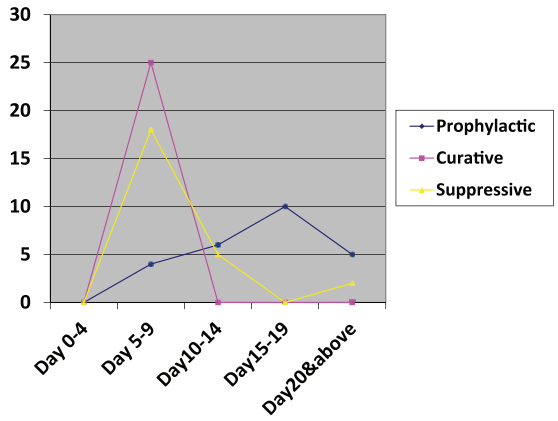

Figure 5. Graph showing survival time of mice in the 3 models.

Survival time of mice in the 3 models

The survival time for mice in the prophylactic group was longer than those in suppressive and curative groups (Figure 5). Mice in prophylactic group started dying on day 9, whereas most were still alive at day 20 and beyond. However, animals in curative group started dying on day 5 and were all dead by day 9, whereas those in the suppressive group started dying on day 5 with 98% dead by day 11. These results clearly demonstrate the prophylactic effect of this extract, at different doses, on malaria-infected animal.

Discussion

Despite the many and varying efforts at malaria control, this disease is still a global threat of enormous proportion and a significant contributor to health and economic inequities in endemic countries. Inhabitants of these countries, under the scourge of disease, face the conundrum of finding appropriate, effective, and cheap means to protect themselves against infection, whereas the parasite, however, has shown an increasing resistance to available antimalarial drugs. This resistance is also favored by population movement and human migration leading to the introduction of resistant parasite to areas previously free of drug resistance. The possibility of synthesizing new antimalarials from plants has become a major policy goal in the control arena, becoming more urgent, in light of the limited number of antimalarials in development, the potential of parasite resistance to the only available therapeutic drugs (artemisinin and its derivatives), and the poor solubility of artemisinin. These are some of the factors driving the design of alternative methods of artemisinin delivery that could potentially slow the process of resistance evolution.[10,26,27] We demonstrate that *B sapida* investigated in this study possesses antimalarial properties that deserve further analysis and could become a cheap, readily available antimalarial in developing countries.

This study shows that *B sapida* has remarkable activity (almost equivalent with Coartem), when used as a prophylactic agent in chloroquine-resistant *P berghei*–infected mice, with the 7-day assay leading to a significant parasitemia suppression. Maximum activity was recorded at the lowest dose (200 mg/kg), with a decrease with increasing dosage, implying that the extract had no pronounced activity at higher doses, and that the small dose suffices to produce significant antimalarial activity. This observation concurs with previous result, where the minimum inhibitory activity shown by the methanolic extract of *B sapida* against *Staphylococcus aureus* started at a dose of 200 mg/mL, but further increase in activity was seen as the dose was decreased to 100 mg/mL, suggesting that the lower the dose, the higher the activity displayed.[28] It is worth noting that *B sapida* extract exhibited the most active antiplasmodial properties at the lowest dosage tested. This dose-dependent activity potentially shows that response at low dose is more effective than response inhibition at high dose.[29] Our results show that *B sapida* extracts will serve as excellent antimalarial agent in the prophylactic model of treatment.

The absence of death after administering 5000 mg/kg body weight of *B sapida* extract in the acute toxicity test suggests that the extract appears to be nontoxic, an observation supported by the toxicity scale principle, which states that any chemical showing an LD_{50} greater than 5000 mg/kg is practically nontoxic.[30] In this report, the acute toxicity test conducted with the extracts demonstrate their safety because the highest dose caused no death. Interestingly, the highest dose used to treat parasite-infected mice from which antimalarial activity was elicited was much lower than the highest acute dose, thus making the extract quite selective and safe.

Loss of appetite, ultimately leading to weight loss, is one of the characteristics of malaria infection.[31] We observed weight loss in *P berghei*–infected animals after infection, with the initial weight loss recorded reversed after 5 days of treatment with the extracts in the prophylactic group, but not in the curative and suppressive groups, which showed progressive weight loss. This result in the suppressive and curative groups agrees with earlier report of continuous weight loss among treated animals compared with nontreated animals[32] but disagrees with our previous study using extracts from *Russelia equisetiformis*, which showed a reversal in body weight after treatment.[3] However, the prophylactic group in this study where we observed slight increase after treatment agrees with our previous study.

The survival time for mice in the prophylactic group extended beyond day 20, whereas those in the curative model did not survive beyond day 9. About 98% of the mice in the suppressive model were dead by day 15, with only 2% surviving till day 20 but not beyond, further confirming the prophylactic activity of *B sapida* ethanol extract.

Although there have been some reports of *B sapida* poisoning in people who consumed the fruit (ackee) in particular,[33–37] such poisonous effect was not observed with the stem bark used in our study. Histologic examination of the liver, kidney, and spleen showed no toxicity to the organs after treatment with the at all doses used, agreeing with the results

of a different study using *Blighia unijugata*, which showed no toxicity to the liver and kidney when administered in a dose-dependent fashion to Wistar rats more than 4 weeks.[38] Histology of the testes reveals that extract has no toxic effect on male animals and as such not inhibitory to fertility. The difference in the reported toxicity may be due to the part of the plant examined. The seed is toxic, whereas the stem bark appears safe.

Conclusively, our results suggest that the ethanol bark extract of *B sapida* show some intrinsic antimalarial activities by its prophylactic ability against chloroquine-resistant *P berghei* parasites. This performance can surely be improved on in future studies if the crude extract is purified and the active substituents are identified and the doses further fractionated. The extracts have considerably low toxicities in experimental mice, this result supporting the traditional use of this plant for the prophylactic treatment of malaria only.

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Author Contributions

OO conceived and designed the experiments. OOO, JAO, and DIA performed the laboratory work. JAO and DIA analyzed data. OOO wrote first draft of manuscript. JAO, DIA, and BNT contributed to writing of manuscript. BNT, OOA, and OO made critical revisions and approved final version. All authors reviewed and approved the final manuscript.

Disclosures and Ethics

The authors also confirmed that this article is unique and not under consideration or published in any other journal. Experimental procedures and protocols are in conformity with National Institute of Health's recommendations in guide for the care and use of laboratory animals and the principles of the Declaration of Helsinki.

REFERENCES

1. WHO. *WHO—World Malaria Report 2015*. WHO; http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/. Published 2015. Accessed February 5, 2017.
2. Ogungbamigbe TO, Ojurongbe O, Ogunro PS, et al. Chloroquine resistant *Plasmodium falciparum* malaria in Osogbo Nigeria: efficacy of amodiaquine + sulfadoxine-pyrimethamine and chloroquine + chlorpheniramine for treatment. *Mem Inst Oswaldo Cruz*. 2008;103:79–84.
3. Ojurongbe O, Ojo JA, Adefokun DI, Abiodun OO, Odewale G, Awe EO. In vivo antimalarial activities of *Russelia equisetiformis* in *Plasmodium berghei* infected mice. *Indian J Pharm Sci*. 2015;77:504–510.
4. Awe EO, Adeloye A, Idowu T, Olajide OA, Makinde J. Antinociceptive effect of *Russelia equisetiformis* leave extracts: identification of its active constituents. *Phytomedicine*. 2008;15:301–305.
5. Basco LK, Mitaku S, Skaltsounis AL, et al. In vitro activities of furoquinoline and acridone alkaloids against *Plasmodium falciparum*. *Antimicrob Agents Chemother*. 1994;38:1169–1171.
6. Ekué MRM, Sinsin B, Eyog-Matig O, Finkeldey R. Uses, traditional management, perception of variation and preferences in ackee (*Blighia sapida* K.D. Koenig) fruit traits in Benin: implications for domestication and conservation. *J Ethnobiol Ethnomedicine*. 2010;6:12.
7. Weathers PJ, Arsenault PR, Covello PS, McMickle A, Teoh KH, Reed DW. Artemisinin production in *Artemisia annua*: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases. *Phytochem Rev*. 2011;10:173–183.
8. Elfawal MA, Towler MJ, Reich NG, et al. Dried whole plant *Artemisia annua* as an antimalarial therapy. *PLoS ONE*. 2012;7:e52746.
9. Weathers PJ, Elfawal MA, Towler MJ, Golenbock D, Weathers PJ, Rich SM. Pharmacokinetics of artemisinin delivered by oral consumption of *Artemisia annua* dried leaves in healthy vs. *Plasmodium chabaudi*-infected mice. *J Ethnopharmacol*. 2014;153:732–736.
10. Desrosiers MR, Weathers PJ. Effect of leaf digestion and artemisinin solubility for use in oral consumption of dried *Artemisia annua* leaves to treat malaria. *J Ethnopharmacol*. 2016;190:313–318.
11. Zirihi GN, Mambu L, Guédé-Guina F, Bodo B, Grellier P. In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *J Ethnopharmacol*. 2005;98:281–285.
12. Rashford J. Those that do not smile will kill me: the ethnobotany of the ackee in Jamaica. *Econ Bot*. 2001;55:190–211.
13. Aderinola O, Farinu G, Akinlade J, et al. Nutritional potential of *Blighia sapida* K Konig (Ackee ackee) leaves as a dry season feed resource for West African dwarf goats in the derived savanna zone of Nigeria. *Livest Res Rural Dev*. 2007;19. http://www.lrrd.org/lrrd19/6/ader19078.htm.
14. Etukudo I. *Ethnobotany: Conventional and Traditional Uses of Plants*. Uyo, Nigeria: The Verdict Press; 2003.
15. Abbiw D. *Useful Plants of Ghana*. London: Intermediate Technology Publications & The Royal Botanic Gardens Kew; 1990.
16. Antwi S, Martey O, Donkor K, Nii-Ayitey Okine LK. Anti-diarrhoeal activity of *Blighia sapida* (Sapindaceae) in rats and mice. *J Pharmacol Toxicol*. 2009;4:117–125.
17. Ojo OA, Ajiboye BO, Ojo AB, Oyinloye BE, Imiere OD, Adeyonu O. Ameliorative potential of *Blighia sapida* K.D. Koenig bark against pancreatic β-cell dysfunction in alloxan-induced diabetic rats [published online ahead of print 17 March 2017]. *J Complement Integr Med*. Epub ahead of print doi:10.1515/jcim-2016-0145.
18. Oloyede OB, Ajiboye TO, Abdussalam AF, et al. *Blighia sapida* leaves halt elevated blood glucose, dyslipidemia and oxidative stress in alloxan-induced diabetic rats. *J Ethnopharmacol*. 2014;157:309–319.
19. Maroyi A. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. *J Ethnobiol Ethnomedicine*. 2013;9:31.
20. Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol*. 1983;54:275–287.
21. Matsumura F. *Toxicology of Insecticides*. Springer; 1975. http://www.springer.com/us/book/9781461344124. Accessed May 8, 2017.
22. Corbett J, Wright K, Baillie A. *The Biochemical Mode of Action of Pesticides*. 2nd ed. London and New York: Academic Press; 1984.
23. Ljungström I, Perlmann H, Schlichtherle M, et al. *Method in Malaria Research*. Manassas, VA: MR4/ATCC; 2004.
24. Peters W. The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of P. berghei in screening for blood schizontocidal activity. *Ann Trop Med Parasitol*. 1975;69:155–171.
25. Layton C, Bancroft JD. *Bancroft's Theory and Practice of Histological Techniques, Expert Consult: Online and Print*. 7th ed. London, England: Elsevier Health Sciences; 2013.
26. van der Kooy F, Verpoorte R. The content of artemisinin in the *Artemisia annua* tea infusion. *Planta Med*. 2011;77:1754–1756.
27. Suberu JO, Gorka AP, Jacobs L, et al. Anti-plasmodial polyvalent interactions in *Artemisia annua* L. aqueous extract–possible synergistic and resistance mechanisms. *PLoS ONE*. 2013;8: e80790.
28. Ubulom PM, Udobi CE, Akpabio E, et al. Antimicrobial activities of leaf and stem bark extracts of *Blighia sapida*. *J Plant Stud*. 2013;2:47.
29. Calabrese EJ, Baldwin LA. The hormetic dose-response model is more common than the threshold model in toxicology. *Toxicol Sci Off J Soc Toxicol*. 2003;71:246–250.
30. Sandu RB, Tarțău L, Miron A, Zagnat M, Ghiciuc CM, Lupușoru CE. Experimental researches on acute toxicity of a *Bidens tripartita* extract in mice—preliminary investigations. *Rev Med Chir Soc Med Nat Iasi*. 2012;116:1230–1234.
31. Dikasso D, Makonnen E, Debella A, et al. Anti-malarial activity of *Withania somnifera* L. Dunal extracts in mice. *Ethiop Med J*. 2006;44:279–285.
32. Chinchilla M, Guerrero OM, Abarca G, et al. An in vivo model to study the anti-malaric capacity of plant extracts. *Rev Biol Trop*. 1998;46:35–39.
33. Meda HA, Diallo B, Buchet JP, et al. Epidemic of fatal encephalopathy in preschool children in Burkina Faso and consumption of unripe ackee (*Blighia sapida*) fruit. *Lancet*. 1999;353:536–540.

34. Joskow R, Belson M, Vesper H, et al. Ackee fruit poisoning: an outbreak investigation in Haiti 2000-2001, and review of the literature. *Clin Toxicol (Phil)*. 2006;44:267–273.
35. Moya J. Ackee (*Blighia sapida*) poisoning in the Northern Province, *Haiti*, 2001. *Epidemiol Bull*. 2001(22):8–9.
36. Katibi OS, Olaosebikan R, Abdulkadir MB, Ogunkunle TO, Ibraheem RM, Murtala R. Ackee fruit poisoning in eight siblings: implications for public health awareness. *Am J Trop Med Hyg*. 2015;93:1122–1123.
37. Gaillard Y, Carlier J, Berscht M, et al. Fatal intoxication due to ackee (*Blighia sapida*) in Suriname and French Guyana. GC-MS detection and quantification of hypoglycin-A. *Forensic Sci Int*. 2011;206:e103–e107.
38. Aquaisua AN, Bassey RB, Ikpeme BM, et al. Effect of crude extracts of *Blighia unijugata* on histology of the liver and kidney of adult Wistar rats. *Int Res J Pharm Pharmacol*. 2011;1:17–22. http://www.interesjournals.org/irjpp/april-2011-vol-1-issue-2/effect-of-crude-extracts-of-blighia-unijugata-on-histology-of-the-liver-and-kidney-of-adult-wistar-rats. Accessed May 8, 2017.