

Evaluation of *In Vivo* Anti-Malarial Activity of Methanolic Leaf Extract of *Persea Americana* against *Plasmodium Berghei*-in Mice

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ABSTRACT

Malaria is an increasing worldwide threat, with more than three hundred million infection and one million deaths every year. Phytomedicine and herbal medicine are culturally accepted and ubiquitously practiced. Traditional herbal medicines have been used for thousands of years to treat malaria worldwide, and about 1,277 medicinal plants from 160 families with antimalarial activities have been reported. It is therefore necessary to evaluate the antimalarial potentials of methanolic extract of *Persea americana* leaf on malaria infected mice in order to scientifically validate the folkloric use of the plant in the management of malaria. The results obtained from this present study show that the methanol extract of *Persea americana* leaf possesses significant ($p < 0.05$ and $p < 0.01$) antimalarial activity in *P. berghei* parasitized mice. The results also show that the extract exhibits excellent hematopoietic property by reversing and restoring the altered plasmodium-induced changes in hematological indices.

Keywords: Malaria, *Persea americana*, antimalarial and Mice.

INTRODUCTION

Malaria is an increasing worldwide threat, with more than three hundred million infection and one million deaths every year. According to the World Health Organization (WHO), malaria is a significant public health problem in more than 100 countries and causes an estimated 200 million infections each year, with more than 500 thousand deaths annually. Over 90% of these deaths occur in sub-Saharan Africa, where the disease is estimated to kill one child every 30 seconds [1]. The majority of those who die from malaria are infants, children and pregnant women living in sub-Saharan Africa and Asia [2]. This situation is worsened by the absence of malaria vaccine, lack of access and availability of effective anti-malarial drugs and presence of drug-resistant species. To add to this, the available antimalarial agents are expensive and have worrisome side effects. These situations demand for alternatives; one of which is search for plant materials with antimalarial

potentials. This situation has in recent times become aggravated by the progressive spread of *P. falciparum* resistant to the most commonly used and affordable antimalarial drugs such as chloroquine among others. Additionally, the emergence of the drug-resistant *Plasmodium* parasite has already compromised the efficacy of many anti malarias leaving artemisinin-based combination therapies as one of the few treatment regimens whose efficacy is assured. This necessitates a continuous effort to search for new drugs [3].

Worldwide, phytomedicine and herbal medicine are culturally accepted and ubiquitously practiced. Traditional herbal medicines have been used for thousands of years to treat malaria worldwide, and about 1,277 medicinal plants from 160 families with antimalarial activities have been reported [4]. Although modern medicine may be available in some communities, herbal medicines have often maintained popularity for historical

and cultural reasons, in addition to their cheaper costs [5]. Medicinal plants and formulations made from them have one or more parts of substances that can be useful for the therapeutic purpose [6]. Nigeria has rich flora diversity and many of the plant species are used by some indigenous people for medicinal purposes. A larger number of medicinal plants are used to treat malaria in the

MATERIALS AND METHODS

Plant material used in this study was fresh leaf of *Persea Americana*, which was collected within University of Nigeria, Nsukka community and was authenticated by Mr. Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka and deposited in the herbarium of the same institution with voucher number: PCG/UNN/0079.

Method of Extraction of Plant Materials

Fresh leaf of *P. americana* (1,000 g) was washed in running water to remove unwanted materials. The leaves were air-

Southern part of the country where rain forests exist and originate a humid tropical climate, with ideal conditions for persistent malaria transmission all year round [7]. It is therefore necessary to evaluate the antimalarial potentials of methanolic extract of *Persea americana* leaf on malaria infected mice in order to scientifically validate the folkloric use of the plant in the management of malaria.

dried for two weeks and ground into fine powder. Five hundred (500g) of ground sample was weighed and soaked in 1.5 litres of 95% methanol solution (BDH, England), stirred and allowed to stand for 24 hours. The suspension was filtered using Muslin bag followed by Whatman No. 42 filter paper. The filtrate was evaporated under reduced pressure and dried using a rotary evaporator at 55°C. The concentrated extract was stored in a labeled sterile screw capped bottle at 2-8°C. The percentage yield of the extract was as follows:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of Extract (g)}}{\text{Weight of Pulverized Sample (g)}} \times 100$$

Haematological assay

Determination of the haemoglobin (HB) concentration

Haemoglobin (HB) concentration was determined using cyanomethaemoglobin technique as outlined by [8].

Principle

Drabkin's solution which contains potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate was mixed with the haemoglobin. The ferricyanide forms

methaemoglobin which is converted to cyanmethaemoglobin by the cyanide. The cyanmethaemoglobin produces a colour which is measured colorimetrically.

Procedure

Whole blood (20 µl) was added to 4 ml of Drabkin's solution in a test tube in a 1:250 dilution. This was well mixed, allowed to stand for 10 minutes at room temperature and the absorbance was read with colorimetrically at 540 nm with Drabkin's solution as a blank.

$$\text{Haemoglobin (HB)} = \frac{\text{Reading of test}}{\text{Reading of standard}} \times \frac{\text{conc standard}}{4}$$

Management of Experimental Animals

Animals used for the study were male albino mice of weight 20-25 g. The animals were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Before the experiment,

the animals were acclimatized under standard laboratory condition in the animal farm of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka for 7 days with free access to water and fed with pelletized growers feed *ad*

libitum. They received human care throughout the experimental period in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85-23, revised 1985) at the Animal house, Department of Veterinary Medicine.

Parasite Inoculation

Donor mouse blood infected with the *P. berghei* was obtained from the animal farm of Faculty of Veterinary Medicine,

University of Nigeria, Nsukka and was used for inoculum preparation. Blood was drawn from the donor mouse by heart puncture and diluted serially in Alsever's solution to make a suspension that contain about 1×10^6 infected RBC's in every 0.2 ml suspension. This 0.2 ml suspension was injected into the experimental animals intraperitoneally to initiate infection. Infection for malaria was confirmed 72 hours post infection (day 4). The inoculated animals were then randomized into six (6) groups of five (5) mice and treated as stated in Table 1.

Table 1: Experimental design

Groups	Description	Treatment
Group 1	Uninfected and untreated	Administered with DMSO, no infection, no treatment
Group 2	Parasitized and not treated(control)	Administered with 0.2 ml <i>Plasmodium berghei</i> infected blood and no treatment
Group 3	Infected and treated with 100mg/kg <i>P.americana</i>	Administered with 0.2 ml <i>Plasmodium berghei</i> infected blood and treated with 100 mg/kg b.w. of MEPL
Group 4	Infected and treated with 200mg/kg <i>P.americana</i>	Administered with 0.2 ml <i>Plasmodium berghei</i> infected blood and treated with 200 mg/kg b.w. of MEPL
Group 5	Infected and treated with 400mg/kg <i>P.americana</i>	Administered with 0.2 ml <i>Plasmodium berghei</i> infected blood and treated with 400 mg/kg b.w. of MEPL
Group 6	Infected and treated with ACT	Parasitized with 0.2 ml <i>Plasmodium berghei</i> infected blood and treated with 7mg/kg artemeter/lumefantrine

MEPL= methanol extract of *P. americana* leaf extract

Determination of Parasitaemia Level in Experimental Animals

The level of parasitaemia in the experimental animals was determined haematologically, using microscopic technique [9] with modification. Thick blood smears were collected daily from tail blood, stained with Giemsa's stain and examined under low powered microscope (x 10 resolution) to determine the parasitaemia level. The level of parasitaemia was determined on days 1, 4 and 8. On day 8 (4 days post-treatment),

mice were sacrificed and blood samples collected for biochemical and haematological analysis.

Principle and Procedure for Staining Thick Blood Film

Staining usually take place at a neutral pH (7.4, i.e. pH of blood), when buffered at pH 6.8, it brings the pH to neutral pH i.e. pH 7.0. Unlike charges of stain and blood will attract, the basic part of stain methylene blue stained the acidic part of the cell i.e. the nucleus while the acidic part of stain eosin stained the basic part

of the stain i.e. cytoplasm. The procedure was described by [10] was used. A 3% of stock Giemsa stain was diluted in buffered water immediately before use. Thick blood film was made on clean grease free glass slide, allowed to air-dry and stained with prepared Giemsa stain for 30 min. Stained slide was rinsed in clean water and allow air-drying before examined under microscope using X100

objective lens. Chromatin of malaria parasite stained dark red and cytoplasm stained blue with Giemsa's stain. Malaria parasitaemia was confirmed by microscopic examination using X100 objective lens (oil immersion lens). The percentage parasitaemia was determined by counting the parasitized red blood cells out of RBCs (red blood cells) in random fields of the microscope:

$$\% \text{ Parasitaemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100$$

Statistical Analysis

Data obtained from the laboratory were analyzed using IBM Statistical Product and Service Solutions (SPSS), version 18. The results were expressed as mean \pm standard error of mean (SEM). One-way

analysis of variance (ANOVA) with dunnet test for multiple comparison was used to compare means across the groups. Mean values with $P < 0.05$ were considered statistically significant compared to the positive control group.

RESULTS

Table 2: Percentage of malaria parasitaemia in normal and malaria-induced mice treated with extract and Artemeter/Lumefantrine drug.

Groups	Treatment	Day 1 (%)	Day 4 (%)	Day 8 (%)
A	100 mg/kg	ND	70.80 \pm 3.71	46.80 \pm 3.44***
B	200mg/kg	ND	69.80 \pm 3.54	41.80 \pm 4.85***
C	400mg/kg	ND	69.40 \pm 1.44	33.20 \pm 4.91***
D	Infected and treated with ACT	ND	78.80 \pm 3.58	24.40 \pm 5.03***
E	Uninfected and untreated	ND	ND	ND
F	Infected and untreated (control)	ND	71.20 \pm 2.48	79.60 \pm 5.78

Data are mean \pm standard error of mean (SEM) (n = 5). p < 0.05 as compared with control group (one way ANOVA followed by Dunnet t-test, 2-sided).

MEPL= Methanolic extract of *P. americana* leaf; ND= Not detected

Pictorial representation of the malaria parasitemia in Days 1, 4 and 8 studies as shown in table 2

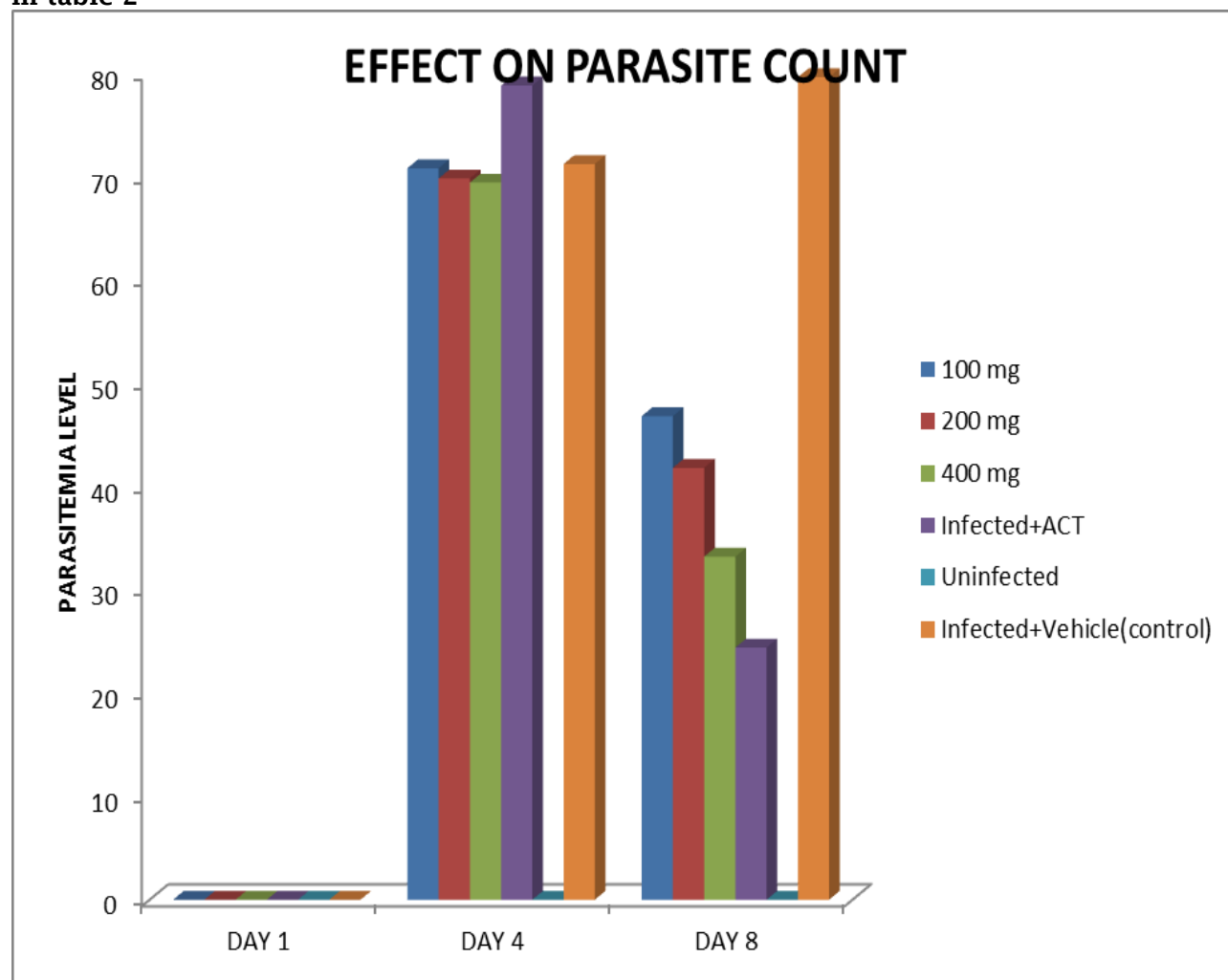


Fig. 1: Effect of *P. americana* leaf extract on the malaria parasitemia of parasitized mice

Table 3: Percentage haemoglobin concentration in normal and malaria-induced mice treated with extract and Artemeter/Lumefantrine drug.

Groups	Treatment	Day 1 (%)	Day 4 (%)	Day 8 (%)
A	100 mg/kg	11.00±0.25	7.06±0.28**	6.90±0.31***
B	200mg/kg	11.08±0.07	11.84±0.29*	11.68±0.14***
C	400mg/kg	11.00±0.25	9.24±0.11*	9.78±0.15***
D	Infected and treated with ACT	11.26±0.23	8.42±0.61*	10.28±0.21***
E	Uninfected and untreated	11.40±0.27	7.72±0.30***	10.34±0.18***
F	Infected and untreated (control)	11.28±0.17	7.84±0.29	11.22±0.14

Data are mean ± standard error of mean (SEM) (n = 5). p < 0.05 as compared with control group (one way ANOVA followed by Dunnet t-test, 2-sided).

Pictorial representation of the haemoglobin concentration in Days 1, 4 and 8 studies as shown in table 3.

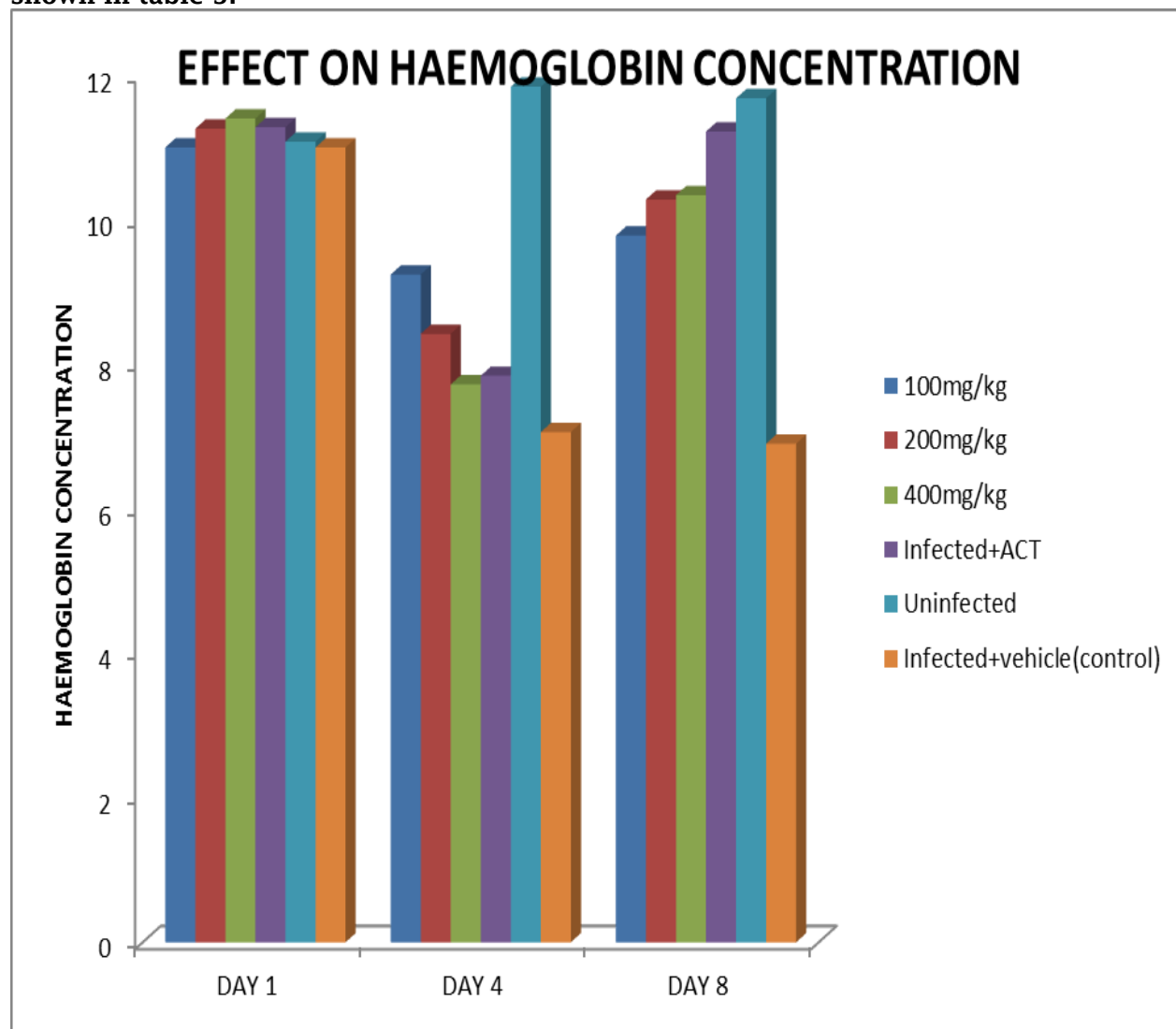


Fig. 2: Effect of *P. americana* leaf extract on the haemoglobin concentration of parasitized mice

DISCUSSION

The antimalarial activity of the methanolic extract of the leaf of *Persea americana* has not been established. The major aim of this study was to systematically evaluate the anti-malarial activity of *Persea americana* and to identify the constituents present which may possibly be responsible for the

antimalarial activity of the plant part extract.

The extraction procedure employed in this study was cold maceration. Although there are other extraction methods that can be employed, this method was adopted in the study because of its easy mode of operation and ability to saturate

the entire sample to be extracted. Methanol was chosen as an extraction solvent in this study due to its high elution strength and ability of partitioning both polar and non-polar constituents.

In this present study, malaria was induced using donor mouse blood infected with the *P. berghei* obtained from the animal farm of Faculty of Veterinary Medicine, University of Nigeria, Nsukka and was used for inoculum preparation. Blood was drawn from the donor mouse by heart puncture and diluted serially in Alsever's solution to make a suspension that contain about 1×10^6 infected RBC's in every 0.2 ml suspension. This 0.2 ml suspension was injected into the experimental animals intraperitoneally to initiate infection. The dose of the parasite used was able to significantly cause elevation in malaria parasitemia compared to the normal control mice that were not induced with malaria.

Methanol extract of *Persea Americana* was able to significantly ($p < 0.05$) reduce the parasitaemia at the doses used in this study indicating that the extract has potent antimalarial effect. The antimalarial activity exhibited by the plant extract was similar to that of the standard antimalarial drug (artemether/lumefantrine). The observed antimalarial activity is consistent with the traditional use of the plant as herbal medication against the disease and

The results obtained from this present study show that the methanol extract of *Persea americana* leaf possesses significant ($p < 0.05$ and $p < 0.01$) antimalarial activity in *P. berghei* parasitized mice.

The results also show that the extract exhibits excellent hematopoietic property by reversing and restoring the altered plasmodium-induced changes in hematological indices.

There is need to however carryout further studies aimed at isolation, purification and characterization of the bioactive

indicative of its potential as an antimalarial agent. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. Malaria-infected patients tended to have lower white blood cells, lymphocytes, eosinophils, RBC counts and Hb concentration when compared to non-malaria-infected patients. This study found that haemoglobin concentration were significantly ($p < 0.05$) lower in the infected and untreated mice compared with the infected and treated and the uninfected mice (normal control). This is in line with the findings of Igbeneghu and Odaibo that showed that humans with acute malaria have lower haemoglobin concentration compared with apparently-healthy individuals. Malaria parasites in their erythrocytic stage invade RBCs and feed on haemoglobin, generating haemoglobin metabolites such as bilirubin in the plasma [11]. These result in lower haemoglobin concentration in malaria infection as observed in the present study. However, treatment of malaria-infected mice with the plant extract restored the altered haematological indices as seen in groups A, B, C and D.

The mechanism of action of the antimalarial effect of the extract was not investigated in this study and hence suggested for further investigation.

CONCLUSION

constituents of the plant under study. The results obtained from these further studies could be useful in the development of better and more tolerated antimalarial drug. Further studies could also be undertaken towards the possible structural modification of the pure phytoconstituents obtained from the methanolic leaf extract of *Persea americana*. This could serve as a preferred pharmacotherapeutic option in the treatment of malaria as well as contribute to a significant reduction in the worldwide incidence of malaria.

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