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AQUEOUS STEM BARK EXTRACT OF *Mangifera indica* SUPPRESSES PARASITEMIA AND AMELIORATES ANAEMIA IN MICE MODEL OF MALARIAL INFECTION

Oche Okpe[1*], Emmanuel Eneojo Agada[1], Stanley Irobekhian Reuben Okoduwa[2*], Vincent Aondohemba Upev[1], Sunday Michael Abu[1] and Okopi Adakole[1]

[1]*Department of Biochemistry, Federal University of Agriculture, Makurdi, Benue State, Nigeria*
[2]*Department of Biochemistry, School of Basic Medical Sciences, Babcock University, Ilishan-Remo, Ogun State, Nigeria*

*Corresponding Author: okoduwas@babcock.edu.ng

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Abstract

Background: Malaria has continued to be a threat to human health over time despite the numerous efforts made in the development of novel drugs or effective vaccines. This study evaluates the anti-plasmodia activity of aqueous stem bark extract of *Mangifera indica* (AEMi) on *Plasmodium berghei*-infected mice. ***Methods***: *In vivo* anti-*Plasmodium berghei* activity of AEMi on *P. berghei* related anaemia was evaluated. Mice were infected with chloroquine-sensitive strain of *P. beghei* and were treated with AEMI or halofantrine for nine days. The levels of parasitemia, packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) were determined. ***Results***: The results obtained showed that AEMi significantly ($p<0.05$) suppressed the multiplication of *P. beghei*-induced anaemia among the AEMi (7.8×10^7) and halofantrine (8.91×10^7) treated groups as compared with the untreated infected group (43×10^7). The hematological analysis shows a significant ($p<0.5$) increased in RBC and decrease in WBC among the AEMI and halofantrine treated groups as compared with the untreated infected group. ***Conclusion***: Evidence from the present study suggests that AEMi probably has suppressive effects on *P. berghei* and can ameliorate some pathological damages. Therefore, it could be useful in malaria chemotherapy and the development of novel drugs for the treatment of malaria.

INTRODUCTION

Malaria, a parasitic infection caused by species of *Plasmodium,* poses serious health challenges worldwide. According to WHO [1], malaria account for over 200 million morbidities worldwide with over 400,000 deaths, in which Africa recorded 93% morbidity and 94% mortality respectively [1]. Various types of medication have been developed to combat malaria, including compounds like; quinine, chloroquine, sulphadoxine, pyrimethamine, artesunate and a combination of artemisinine and lumefanthrine (ACT) [2]. Although several drugs have been developed from these compounds, there is still an incidence of drug-resistant parasitic strain, which is partly responsible for the continuous spread of malaria. Hence, the search for a better remedy towards the development of new anti-malaria drugs and vaccine to combat the disease [3, 4]. More worrisome are the associated adverse side effects among the currently used drugs. The high resistance of the vector to the available insecticides also calls the reasons for the continuous search for better alternatives that could be proven to be more tolerable, cheaper and more accessible to people regardless of the social-economic gaps.

Historically, herbal concoctions have been used in the past by forefathers in various rural communities for the treatment of common ailments like fever, cold, typhoid, wound, and stomach pain before the advent of synthetic medicines [5]. Traditional knowledge and use of herbal medicines remain dominant in rural areas of Nigeria, and remain the first line treatment owing to the important

therapeutic roles they play in many disease conditions [5]. Hence the decision to find appropriate herbal that have demonstrated therapeutic efficacy against chloroquine sensitive strain of *Plasmodium falciparum* under *in vitro* condition.

Studies have shown that *Mangifera indica* possesses numerous therapeutic properties targeted amongst others for the treatment of rheumatism, diarrhea, inflammatory and fungal infection [6, 7, 8]. Consequently, the essence of investigation of the pharmacological properties of a plant is to pave way for the discovery of novel secondary metabolites [9, 10]. It is a known fact that certain bioactive components present in plants are yet to be explored for their medicinal property, and such compounds may be a very promising candidate for development of new drugs [11]. This study therefore focused on the effect(s) of *M. indica* in *Plasmodium berghei* infected mice (a model of malarial infection for pharmacological screening) and its possible therapeutic action, with the aim to scientifically justify its folkloric use in the treatment of malarial and anaemic conditions.

MATERIALS AND METHODS

Plant Source

The stem bark of *M. indica* was collected from Pila village, Makurdi local government, Benue State, Nigeria. The botanical identification was done in the Botany Unit of the Department of Biological Science, Federal University of Agriculture, Makurdi, Nigeria.

Study Location

The entire study was conducted at the Department of Biochemistry, Federal University of Agriculture, Makurdi, Benue State, Nigeria. The plant extraction, acclimatization of the mice, inoculation of the parasite, determination of median lethal dose (LD_{50}) and other parameters including histological examination were carried out at the Veterinary Pharmacology/Physiology/Biochemistry Laboratory of the College of Veterinary Medicine.

Preparation of Plant Sample

Samples of the stem bark of *M. indica* collected were rinsed in clean water and dried under shade at room temperature. Using pestle and mortar, samples of the dried plants were pulverised into powder. The powder obtained was then used to prepare the extracts.

Preparation of Plant Extract

Exactly 100 g of the powdered sample was macerated in 1000 mL of distilled water. The cold maceration method described elsewhere was used with intermittent shaking for 3-hours interval at room temperature for period of 48 hours [12]. Watchman filter paper No.1 was used to filter the extract. The filtrate obtained was concentrated in temperature controlled water bath adjusted at fixed value of 45^{o}C. The weight of the concentrated extract was determined and then preserved in an air-tight sample bottle and kept in a refrigerator until it was needed for analysis.

Experimental Animals

The animals were bought from the animal house, Benue State University, Makurdi. The animals were kept in standard rodent cages and housed at College of Veterinary Medicine, Federal University of Agriculture, Makurdi for 2 weeks. The study was approved by the institutional animal care and ethic committee of Federal University of Agriculture Makurdi, Nigeria. The research conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [13]. The animals were acclimatized to ambient environmental condition. During the study period they were fed with standard pelleted diet and water *ad libitum.*

Malaria Parasite

The *Plasmodium* specie (*P. berghei*, chloroquine sensitive strain Nk65) was obtained from the Department of Parasitology, Ahmadu Bello University, Zaria were a stabilate is maintained by serial passage into healthy animals.

Determination of Median Lethal Dose of the Extract

The arithmetic-geometric-harmonic (AGH) methods of rough estimation of median lethal dose (LD_{50}), using up and down procedures modified by Saganuwan, [14] in combination with that of Lorke [15] were adopted. In brief, Mice were weighed (between 18-25 g each) and test doses of the extract, calculated in connection to the bodyweight of each overnight (12 hours) fasted mouse (only food withheld), were dissolved in distilled water and administered via oral gavage at dose ranges from 100 to 5000 mg/kg. The mice were then routinely and separately watched for behavioral changes and common toxicity signs after dosing for the primary 24 h with extraordinary attention being given amid the primary 4 hrs. The mice were further observed for up to 14 days following treatment for any signs of gross behavioural changes such as altering of feeding, lacrimation, hair erection, mortality, and other signs of toxicity appearance and the latency of death.

Inoculation of Parasite

The parasitized erythrocyte was obtained from the blood of donor mouse and diluted with 0.9% Normal Saline solution. Each Mouse was injected intraperitoneally with 0.5ml blood

suspension, which contains approximately 2.5×10^7 parasitized erythrocytes [16, 17].

Grouping of Experimental Animal and Treatment

Upon the establishment of parasitemia, the mice were divided at random into 4 sets each containing five (5) mice. They were treated for a period of 9 uninterrupted days with daily oral administration of the extract (350 mg/kg body weight) and standard malaria drug (Halofantrine 25mg/kg) [18]. From the preliminary study conducted to determine the minimum effective dose of the extract for use as treatment of the mice, it was observed that 350 mg/kg of the extract was the smallest dose that produced desired outcome hence was applied in this study.

Set Grp1: Normal control (they were not infected and also not treated).

Set Grp2: Negative control (they were infected with *P. berghei* but were not treated).

Set Grp3: Infected with *P. berghei* and treated with 350 mg/kg extracts.

Set Grp4: Positive control (they were infected with *P. berghei* and treated with standard drug (25 mg/kg Halofantrine as standard antimalarial drug).

Determination of Haematological Parameters and Histological Analysis

The percentages of the parasitized erythrocytes, the white blood cell (WBC) and the red blood cell (RBC) counts were estimated using the Neubauer haemocytometer method described by Brown, [19]. Determination of the packed cell volume (PCV) was carried out using microhaematocrit method of Coles, [20]. Representative samples of excised liver were subjected to histological analysis and observed for pathological changes under a binocular microscope [21].

Statistical Analysis

Experimental data were presented as Mean ± Standard Error of Mean (SEM). Within groups comparisons were performed by the analysis of variance (ANOVA) using SPSS 20.0 for windows Computer Software Package). A significant difference in mean value was compared using the Duncan's new multiple range test. Significant difference was set at $p<0.05$ [22].

RESULTS

The Median Lethal Dose of the Stem Bark Extract of *M. indica* and Effect on *P. berghei*-Infected Mice

The median lethal dose (LD_{50}) was estimated to be greater than 5000 mg/kg (Table 1). Following the assumptions of Lorke [15] substances more toxic than 1 mg/kg are so highly toxic that it is not important to calculate the LD_{50} while substances whose LD50 value is greater than 5,000 mg/kg are regarded safe and not toxic.

As presented in the Figure 1, is the results for the percentage of parasitized erythrocyte in mice infected with *P. berghei* for the period of the study. The % parasitemia in sets 2, 3 and 4, were significantly ($p<0.05$) increased post infection. However, upon commencement of treatment, there was significant ($p<0.05$) reduction in parasite level in the treated groups as compared with the disease control, which present higher parasitemic level on Day 9.

Table 1: Acute Toxicity Test for Determination of Median Lethal Dose of Extract

S/N	Dose (mg/kg)	*Mortality	Toxicity Sign within 24 hrs and up to 14 days following treatment
1	50	0/3	No signs of toxicity appearance
2	100	0/3	No signs of toxicity appearance
3	200	0/3	No signs of toxicity appearance
4	500	0/3	No signs of toxicity appearance
5	1000	0/3	No signs of toxicity appearance
6	3000	0/3	No signs of toxicity appearance
7	5000	0/3	No signs of toxicity appearance

*Mortality = Number of animals which died/number of animals used

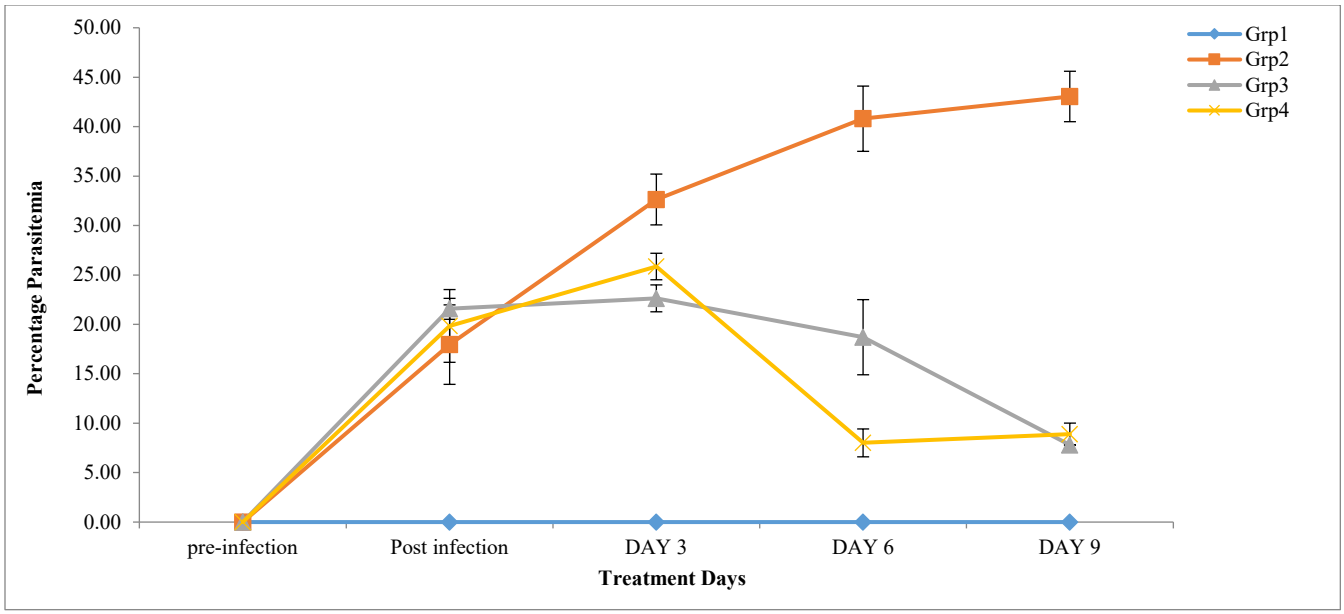


Figure 1: Percentage parasitemia level of mice infected with *Plasmodium berghei*

Effect of Stem Bark Extract of *M. indica* on the Mean Body Weight

The Figure 2 below is the presentation of the mean body weight of mice infected with *P. berghei* all through for the experimental duration. From the results the body weight of *P. berhgei* in sets Grp2, Grp3 and Grp4 were significantly ($P<0.05$) increased post infection. However, upon commencement of treatment, there was significant ($P<0.05$) decrease in the body weight of the treated groups on days 6 and 9.

Effects of Stem Bark Extract of *M. indica* on Packed Cell Volume

Figure 3 shows the levels of packed cell volume (PCV) among the infected mice. From the results, all the groups that were infected with *P. berghei* had significantly ($P<0.05$) decreased in PCV. However, the set Grp4 showed recovery in PCV as compared to the disease control set Grp1 and Grp3.

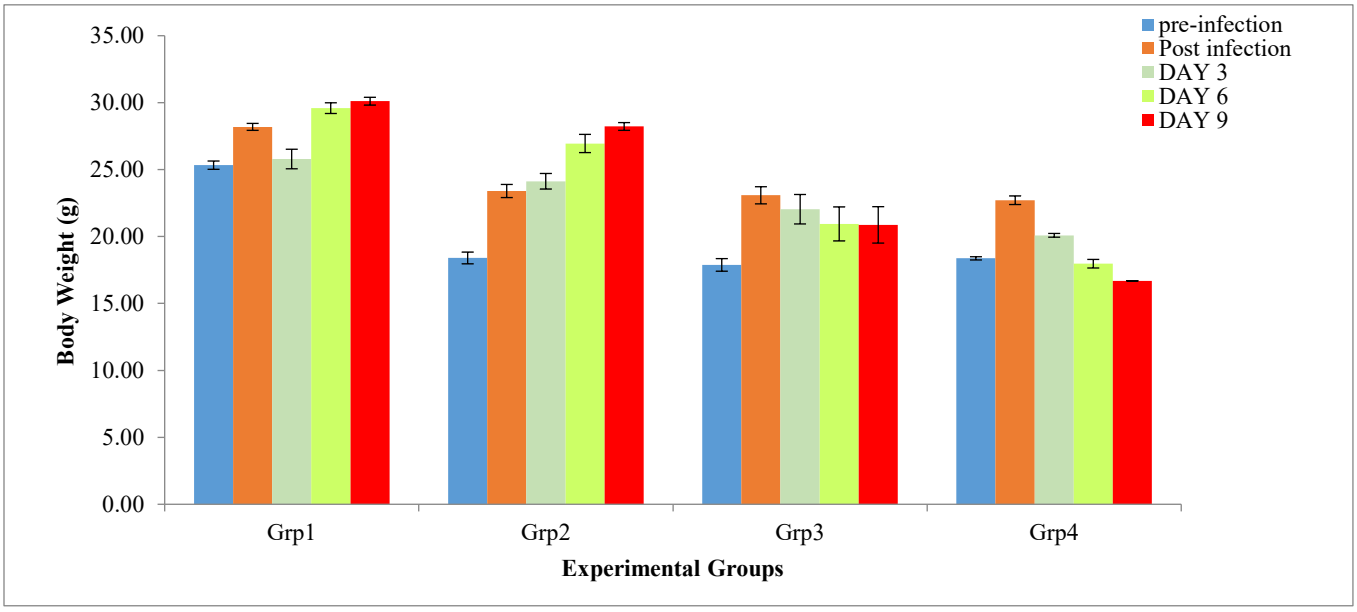


Figure 2: The Body weight of mice infected with *Plasmodium berghei*

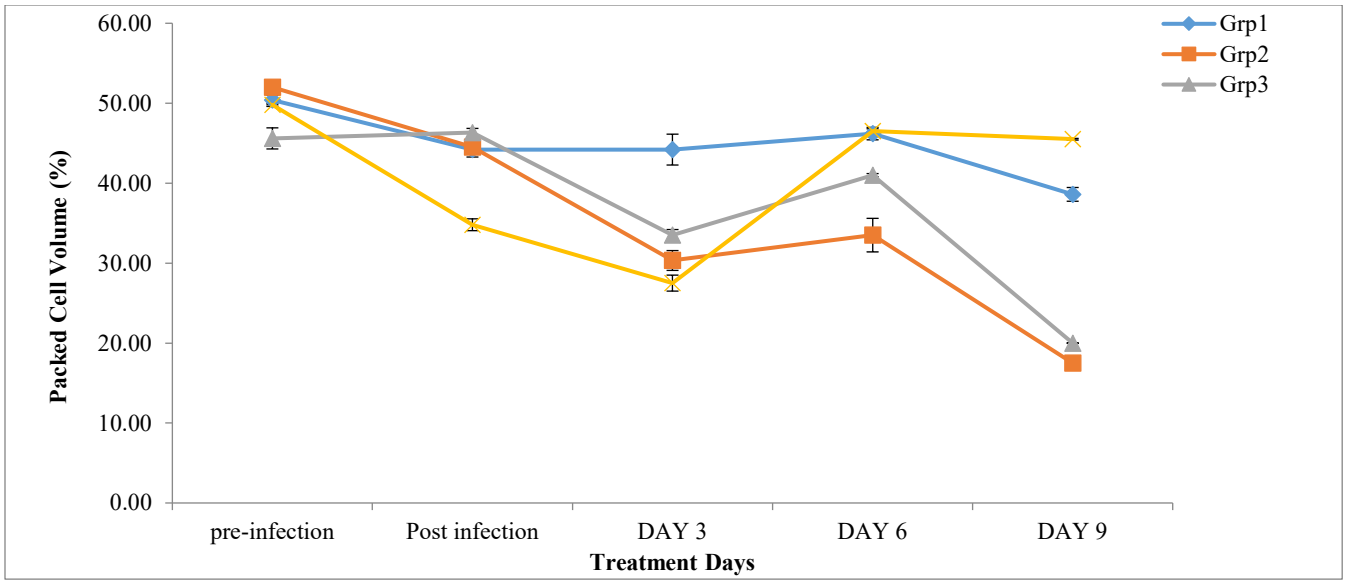


Figure 3: The Packed cell volume (PCV) of mice infected with *Plasmodium berghei*

Effect of Stem Bark Extracts of *M. indica* on Red Blood Cell and White Blood Cell

The red blood cells (RBC) and white blood cells (WBC) counts of mice infected with *P. berghei* are presented in the Table 2 below. From the results a significant ($p<0.05$) reduction in RBC was observed among the experimental treated sets of animal post infection as compared with the normal control set. Interestingly, there was an observed significant ($p<0.5$) increase in the treated groups as compared with the disease control set upon treatment. Similarly, a significant ($p<0.05$) increased in WBC post-infection was observed among the same group as compared with the normal control set. However, after oral administration of plant extracts, it was observed that the plant exerted an ameliorative effect which culminated in significant ($p<0.05$) decreased in WBC when compared to the disease control set of animals.

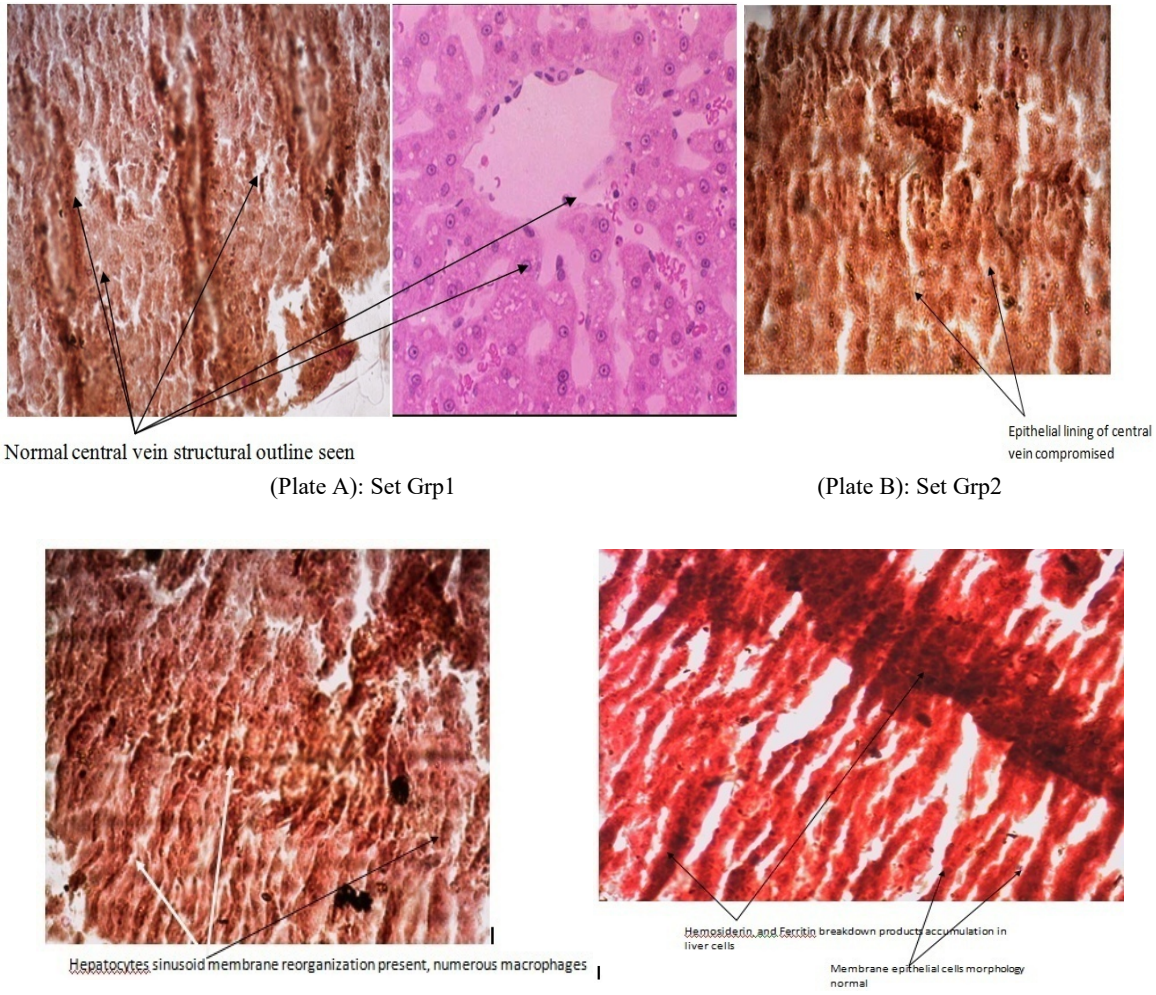
Table 2: The Red Blood Cell (RBC) and White Blood Cell (WBC) of mice infected with *Plasmodium berghei*

Parameters	Sets	Pre-infection	Post infection	DAY 3	DAY 6	DAY 9
RBC ($\times 10^{12}$ /L)	Grp1	13.60±0.19^a	13.08±0.21^a	12.06±0.25^a	10.58±0.11^a	11.46±0.25^a
	Grp2	12.20±0.33^a	4.93±0.21^b	1.23±0.08^b	1.40±0.16^b	2.15±0.01^b
	Grp3	12.28±0.06^a	5.63±0.13^b	3.05±0.35^b	7.50±0.22^c	4.90±0.00^c
	Grp4	11.56±0.08^a	2.70±0.09^a	1.38±0.03^b	5.45±0.37^c	6.55±0.07^c
WBC ($\times 10^9$ /L)	Grp1	4.68±0.24^a	4.32±0.23^a	4.56±0.22^a	7.44±0.05^a	6.04±0.25^a
	Grp2	4.20±0.24^a	6.75±0.48^b	17.93±1.63^b	13.00±0.08^b	13.20±0.12^b
	Grp3	4.56±0.09^a	7.67±0.04^b	5.20±0.36^a	7.50±0.22^a	9.40±0.00^c
	Grp4	4.68±0.57^a	15.36±0.57^c	12.10±0.73^c	4.60±0.16^c	7.80±0.04^c

Values are represented as the mean ± SEM of five replica determinations. Values with different superscripts along the row are considered significantly different at the value of $p<0.05$

Effect of Stem Bark Extracts of *M. indica* on Hepatic Region of Animals Infected with *P. berghei*

The histological section of the hepatocyte of *P. berghei* - infected mice are presented as plates A, B, C and D in the Figure 4 below. Plate A is the normal control set of animals with normal architecture of hepatic cells and a reference standard. The plate B is the malarial control set of animal, characterized with complete depletion of hepatic cells. The plate C, is the set of animal infected and treated with 350 mg/kg the aqueous extracts of *M. indica*. The plate present prominent sinusoids and sinusoidal spaces, along with areas of necrosis with slight restoration of diffused proliferated hepatic cells. Comparable slight restoration of diffused proliferated cells was observed in Plate D of the infected mice treated with standard drug (halofantrine).



(Plate A): Set Grp1 (Plate B): Set Grp2

(Plate C): Set Grp3 (Plate D): Set Grp4

Figure 4: The representative hepatic region of *P. berghei*-infected mice and treated with aqueous stem bark extract of *Mangifera indica* and Halofantrine.
Plate A: Normal architecture of hepatic cell with a reference standard.
Plate B: The epithelium lining of the central vein compromised (complete depletion of hepatic cells).
Plate C: Prominent sinusoids and sinusoidal spaces were seen, along with numerous macrophages.
Plate D: Hemosidenin and Ferritin breakdown products accumulation in hepatic cells, but there was normal membrane epithelial cell morphology.

DISCUSSION

Mangifera indica is one of the medicinal plants known for its numerous health benefits against many diseases. These health benefits are mainly accounted to the presence of many active phytochemicals in various part of this plant [23,24]. Screening of the various active phytochemicals present in the various part of a plant using a suitable animal model is the first step towards the isolation of the potent molecule for possible drug development [25]. In this study, mice model of malarial infection was used to validate the potency of *M. indica* against malarial infection. This model has been used previously for the screening of plants with anti-malarial activities [26].

The acute toxicity test in this study showed that the leaf extract of *M. indica* caused neither mortality within the first 24 hours nor obvious signs of acute toxicity such as hair erection, lacrimation, tremor, salivation, diarrhea and the misfortune of craving within the following 14 days at the dosages administered. In the present study, there were significant increases in the parasite load in disease control set of animals as the duration of experiment progresses, compared to the set of animals treated which had significant reduction in the parasite load in the course of treatment. The pathogenesis of malaria is characterized by haematological changes involving major cell types such as erythrocytes, leukocytes, and thrombocytes [27-29]. The results obtained from this study shows some levels of haematological alterations. These observations were confirmed through

statistical analysis that revealed decrease in PCV and RBC with a concomitant increase in WBC as the pathogenesis of the infection progresses without in the absence intervention. Nonetheless, the negative haematological alterations were partly reversed in the animal within the sets Grp3 and 4. This observation could be attributed to the treatment intervention. However, several studies have alluded to the fact that medicinal plants have restorative ability to PCV, RBC and WBC in disease state. Different parts of *M. indica* such as leaves, stem bark and root bark are being used in the treatment of ailments [8, 10, 23, 24, 30]. Tannins, saponins, flavonoids, terpenoids, phenols, and cardiac glycosides are present in the stem bark of *M. indica* [31,32], which probably confer on the plant its ameliorative ability [28]. This result was supported by the work of Okunola *et al*, [32], who reported recovery in PCV, RBC and WBC when infected animals are treated with medicinal plants.

The attenuation in parasitemia upon administration of the aqueous stem bark extract of *M. indica* correlates with a concomitant increase in PCV. This suggests a direct inhibition on the multiplication of the parasites, which is in concordance with report of Lydia *et al*, [33]. Van-Wolfswinkel *et al.* [34] however noted that the increase in peripheral total WBC count as well as in differential lymphocytes and monocytes takes place during the liver phase of infection after which a pronounced decrease occurs. Thus, this observation in the establishment of leukocyte alteration during episodes of malaria infection occurs as a result of different infection stages presented when WBC count was analyzed [35]. Modulation of immune system as indicated by alterations in WBC count occurs due to the presence of flavonoids [36], which are also contained in *M. indica* as reported by Udem *et al*. [24]. The elicitation of immune response by the *Plasmodium* parasite is characterized by increased leukocytosis post infection and upon the administration of the extract of *M. indica*, signaling the presence of foreign agents and thus the mobilization of the necessary defense machineries for in order to combat the infesting agent. Flavonoids accounts for the activities associated with numerous bioactive compounds known to possess antiplasmodial activity [37-40].

The histological studies indicated that animals treated with the extract of *M. indica* had a mild anatomical recovery of the hepatocytes, which shows its hepatoprotective potential, in agreement with the reports of Adeneye *et al*. [41]. Infected mice treated with 350 mg/kg of *M. indica* showed improved hepatocytes when compared with the infected mice that were not treated, thus, suggesting the hepatoprotective activity of the plant.

CONCLUSION

From the observations seen in this study, the extract of *Mangifera indica* demonstrates ability to reduce *P. berghei in-vivo* through mechanisms yet to be ascertained. The different possible mechanisms exploited by the plants confers hepatoprotection, as well as improvement in hematological parameters such as PCV, RBC and WBC. This therapeutic potency exhibited by the plant therefore give credence to the plant for its traditionally use by herbal medicine practitioners in the treatment of malaria. This investigation therefore has scientifically justified the use of *M. indica* in folkloric. Hence, as malaria continues to remain a threat to human health coupled with the incessant resistance of the present conventional antimalarial drugs, the search for better alternatives from among traditionally used medicinal plants as alternative remedy becomes imperative for scientific validation. This could lead to the identification of new potential candidates for development drugs with distinct mechanism of action.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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