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ACUTE TOXICITY, *IN VIVO* ANTIMALARIAL POTENTIAL AND HISTOLOGICAL EXAMINATION OF *Plasmodium berghei* INFECTED MICE TREATED WITH *Allanblackia floribunda* Oliv.

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ABSTRACT

Malaria is one of the oldest and most deadly diseases. In 2020, children below aged 5 years accounted for about 80% of malaria deaths and nearly 29% of global malaria mortality occurred in Nigeria. The present situation warrants accelerated action to combat the surge in malaria morbidity. This study therefore evaluated the acute toxicity, in vivo antimalarial potential and histological examination of Plasmodium berghei infected mice treated with Allanblackia floribunda extracts. Acute toxicity of the extracts was investigated using the Lorke's method. Antiplasmodial activity in established infection was assessed using the Rane's test while the Peter's 4-day suppressive test was used to investigate the effects of the extracts on early infection. There was no record of death in the acute toxicity study. Maximum parasite inhibition was recorded at 250mg/kg body weight for both the stem bark (72.57%) and leaves (52.75%) extracts in the Rane's test; similar trend was observed in the suppressive test. Maximum mean survival time was recorded at a dose of 250mg/kg body weight for the stem bark (21.4 days) and leaves (18.6 days) extracts respectively. Also, the stem bark and leaves extracts at low doses had better protection on the liver, brain and spleen histology of the P. berghei infected mice relative to the chloroquine treated group. This study has shown that methanol extracts of A. floribunda stem bark and leaves are relatively safe and they have very active antimalarial action.

KEYWORDS: *Plasmodium berghei*, *Allanblackia floribunda, Malaria, Leaves, Stem bark*

INTRODUCTION

Malaria is one of the oldest and most deadly diseases (WHO, 2021). In 2020, malaria cases increased to 228 million and death rate rose to about 602,000 compared to 2019 in which malaria episodes and mortalities were around 213 million and 534,000. In this same year, approximately 80% of malaria deaths were estimated in children aged under 5 years and nearly 29% of global malaria deaths occurred in Nigeria (WHO 2021). Factors like COVID-19 pandemic, Ebola virus infection, flooding and other humanitarian emergencies have contributed to the levelling off in the fight against malaria especially in sub-Saharan Africa (WHO, 2021). The present situation warrants accelerated action to combat the surge in malaria morbidity and one of WHO's strategies is to tailor existing approaches to prevention, diagnosis and treatment to local contexts.

Medicinal plants have been playing a pivotal role in the prevention and control of malaria infection by serving as sources of lead compounds for the development of excellent antimalaria drugs (WHO, 2021). Species of *Artimesia annua, Chromolaena Odorata, Azadirachta indica, Mangifera indica* etc have shown excellent antimalaria activity (Odugbemi *et al*., 2007). *Allanblackia floribunda* leaf and stem bark are equally used in ethnomedicinal practice for the treatment of malaria and other inflammatory related diseases in the southern regions of Nigeria. The analysis of the phytochemical composition using gas chromatography with flame ionization detection and the biological activity of these plant extracts have been reported (Irabor *et al*., 2021b). Scientific investigation that revealed its excellent *in vitro* antiplasmodial activity against chloroquine sensitive *Plasmodium* parasite has also been published (Irabor *et al*., 2021a). However, there is literature gap on the toxicity study and *in vivo* antimalarial potential that is corroborated with histological examination of tissues of animals exposed to this plant. Acute toxicity study, suppressive test, curative test and histological examination are some of the parameters that are used to ascertain the efficacy of plants used by herbalist to treat malaria parasite infection.

Acute toxicity examination of a potential antimalarial agent provides information on the lethal dose (LD_{50}, the dose that killed 50% of the test animals), the therapeutic index and the safety profile. Acute toxicity is defined as the unwanted effect(s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours (Chinedu *et al*., 2013). The LD_{50} data guide the appropriate dose used for Peter's 4-day suppressive and Rane's tests for an antimalarial candidate agent. The 4-day suppressive test does establish the antiplasmodial activity for plant of interest on early infections and Rane's test involves the curative capability of an antimalarial agent on established infections. Percentage inhibition of parasitemia is the most reliable parameter that is used to estimate the efficacy of the antimalarial agent for both methods. *In vivo* antimalarial activity of plants extract is rated using the dose in mg/kg body weight and the percentage growth

inhibition. At 1000 kg/mg body weight, a growth inhibition equal to 50% is moderate or inactive; at 500mg/kg body weight, a growth inhibition ≥ 50% is considered to have a moderate activity; at 250mg/kg body weight, a growth inhibition ≥ 50% is rated to have a good activity while at 100mg/kg body weight, a growth inhibition ≥ 50% is accepted as very active (Deharo *et al*., 2000). The data obtained from the percentage growth inhibition can be substantiated with histological examination of tissues and organs wreak havoc by malaria parasite because the integrity of the microscopic structure and architecture of the liver, brain and spleen speak volume of the extent of protection of antimalarial agents in plasmodiasis.

Therefore, to develop an alternative or complementary antimalarial therapy for local use and contribute to the scientific data available for the antimalaria property of *A. floribunda*, the acute toxicity, suppressive and curative *in vivo* antiplasmodial potential of the stem bark and leaves extracts were investigated. Furthermore, changes in the histology of the liver, brain and spleen of the mice infected with *Plasmodium berghei* and treated with the respective plant extracts were examined.

MATERIALS AND METHODS

Collection of Plant and Extraction

The *Allanblackia floribunda's* leaves and stem bark were collected at Ohogua community, Ovia North-east Local Government, Benin City, Edo State, Nigeria. The plant was authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria and the voucher specimen of the sample (UBHA361) was deposited at the herbarium of the same department. The plant scientific name was checked with http://www.theplantlist.org on 26th July, 2020. The stem bark and leaves of *A. floribunda* were extracted with methanol as described by Irabor *et al*. (2021a).

Animal for the Study

Mice of Swiss strain with weight range of 19 - 23g were bought from the Nigerian Institute of Medical Research (NIMR) located in Lagos State, Nigeria. The mice were kept at 26.5 ± 2ºC, they had access to commercial mash and water *ad-libitum*. The mice were acclimatized for two weeks before the commencement of the experiment. Ethical approval for this study was granted by the Institutional Ethics Review Committee, University of Benin (No: LS19114).

Acute Oral Toxicity Study

The acute toxicity of the extract was determined by establishing its median lethal dose (LD_{50}) using Lorke's method (Lorke, 1983). The appropriate amount of extract for each animal was obtained by calculation as shown below and reconstituted in carboxylmethylcellulose (CMC) before administering to the animals at their respective doses.

$$\text{Amount of Extract} = \frac{Dose}{1000} X \quad mean\ body\ weight(\text{g}) = \text{Xmg}$$

Phase I

Eighteen (18) mice were divided into six groups (A, B, C, D, E and F) of three mice each. The six groups were administered orally with graded concentrations (10, 100 and 1000 mg/kg body weight, respectively) of leaf and stem bark extracts. The animals were monitored for signs of toxicity such as tremors, weakness, loss of appetite and even death for a 24 hr period. Observation was continued for 7days.

Phase 2

Eighteen (18) mice were again divided into six (A, B, C, D, E and F) groups with each group consisting of three mice. These received graded concentration of 1600, 2900 and 5000 mg/kg body weight of the *A. floribunda* leaf and stem bark extracts, respectively. All the animals were monitored for signs of distress, behavioral alterations and most importantly, death for a period of 24 hr. Observation was continued for 7 days and LD_{50} was thereafter calculated according to the formula below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where: D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

Confidence Test

A confirmatory test was carried-out by administering 5000 mg/kg to two animals. Observation was done for 1 hr after administration and 10 minutes every 2 hours interval for 24 hours and result was documented.

The Inoculation of Mice with Malaria Parasites

Mice infected with *Plasmodium berghei* Nk65 chloroquine sensitive strain were obtained from Nigerian Institute of Medical Research (NIMR) Lagos, Nigeria and kept at the animal house in the Department of Biochemistry, University of Benin, Edo State, Nigeria. The animals were inoculated intraperitoneally with 0.2mL of the blood containing about $1x10^7$ infected erythrocytes.

Peter's 4- day Suppressive Test

The Peter's 4- day suppressive test against chloroquine sensitive *Plasmodium berghei* NK 65 infection in mice was employed (Peters, 1967). The mice were randomly divided into 7 groups (8 animals each). On the first day of the experiment (termed 'day 0'), all mice were injected intraperitoneally with *Plasmodium berghei* NK 65 infected erythrocytes, except the mice in the uninfected/ normal control group. Two hours after the infection of mice, the uninfected control (group 1) and the negative control (parasitized non-treated group 2) were given 0.2mL Carboxyl methyl cellulose (CMC-0.7%). The positive control (group 3) received 10mg/kg chloroquine. The four test groups were given 250mg/kg and 500mg/kg of methanol extracts of *A. floribunda* leaves and stem bark extracts respectively (Table 1). All administration was carried out orally by means of a cannula for four consecutive days (day 0 to day 3).

Percentage parasitaemia was monitored on a daily basis. On 'day 5' of the experiment, blood was collected from the tail of the mice and smeared onto a microscopic slide to make a thin film. The blood films were allowed to dry, fixed with in methanol (100%) and stained with giemsa (10%) for 20 minutes. The stained films were then

washed with water and allowed to dry. The slides were then examined microscopically using ×100 magnification in oil immersion. The parasitaemia was determined by counting minimum of eight fields per slide. The percentage parasitaemia was calculated using the modified Peter *et al*. (1993) formula:

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

The percentage parasitaemia was expressed as mean ± SEM. The average suppression of parasitaemia was calculated by comparing the average percentage parasitaemia in each group with that of negative control, using the formula

$$A = \frac{(B - C)}{B} \times 100$$

Where, A = Average percentage suppression of parasitaemia
B = Average percentage parasitaemia in the negative control group
C = Average percentage parasitaemia in the test group

Curative Test

Evaluation of the curative potential of the extracts was done using the method described by Ryley and Peters (1970). On the first day of the experiment (termed 'day 0'), all mice were injected intraperitoneally with standard inoculum of 0.2 mL of 1 x 10^7 *P. berghei* NK 65 infected erythrocytes, except the mice in the uninfected group. After which the animals were randomly assigned into 6 groups (groups 2 to 7; 8 animals per group). Seventy-two (72) hours post-infection, the uninfected control (group 1) and the negative control (parasitized non-treated, group 2) were given 0.2mL Carboxyl methyl cellulose (CMC- 0.7%); the positive control (group 3) received 10 mg/kg chloroquine and the four test groups were given 250 mg/kg and 500 mg/kg of methanol extracts of *A. floribunda* leaves and stem bark respectively (Table 1). The drug/extracts were administered once daily for 4 days (D_3 to D_7). Thin blood smears were prepared from the tail of each mouse every day for 4 days to monitor the parasitaemia level. The slides were then examined microscopically using × 100 magnification in oil immersion. The percentage parasitaemia was calculated using Peter *et al.* (1993) formula. The percentage of chemo inhibition was determined by the following formula:

$$\text{percentage inhibition} = \frac{A - B}{A} \times 100$$

Where: A= Average parasitaemia in negative control
B= Average parasitaemia in extract treated group

The Mean Survival Time (MST)

MST for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculum) in each group over a period of 28 days (D_0 to D_{27}) (Peter,1967).

$$\text{Mean Survival Time} = \frac{\text{Sum of days of survival X 100}}{\text{Total number of animals in the group}}$$

Table 1: Suppressive and Curative Test Grouping

S/N	Group	Substance Administered
1	nPnT (Normal control)	Vehicle (carboxylmethylcellulose) only
2	PnT (Negative control)	Parasitized + CMC
3	P +CqT (Positive control)	Parasitized + chloroquine (10mg/kg)
4	P+A.f $stem_{250}$	Parasitized+ *A. floribunda* stem (250mg/kg)
5	P + A.f $stem_{500}$	Parasitized + *A. floribunda* stem (500mg/kg)
6	P + A.f $leaf_{250}$	Parasitized + *A. floribunda* leaf (250mg/kg)
7	P + A.f$leaf_{500}$	Parasitized + *A. floribunda* (500mg/kg) leaf

Where nPnP = Non-parasitized, non-treated; PnT = Parasitized non-treated;CqT = Chloroquine treated and A.f = *Allanblackia floribunda*.

Histopathological Examination of Tissues

Animals were fasted overnight and sacrificed by cervical dislocation on 'day' 5 of the experiment for the suppressive test. After sacrificing the mice, parts of the liver, spleen and brain were collected for histological studies. Tissues harvested from animals and preserved using 10% neutral buffered formalin were placed in pre-labeled universal containers. Using all standard safety operating procedures, tissues were dissected and placed in labeled tissue cassettes. The thickness of tissues did not exceed 3-5mm thickness.

Tissues were subjected to automatic tissue processing using the Leica TP2010 automatic tissue processor for 18 h passing them through the four stages of tissue processing namely: fixation (using 10% Neutral buffered formalin), dehydration (using ascending grades of isopropyl alcohol), clearing or dealcoholisation (using xylene) and finally impregnation or infiltration (using molten paraffin wax). The tissues were then embedded in paraffin wax using the Leica automated tissue embedder and sectioned to get ultra-thin sections at five (5) microns, using the thermo scientific semi-automated rotary microtome. Tissues were floated out from the thermo scientific digital floating bath on frosted end pre-labeled slides and dried on the thermo scientific digital slim Line hot plate. Tissues were further dried in the hot air oven overnight and subjected to heamatoxylin and eosin (H & E) staining to demonstrate the general tissue structure. Stained slides were mounted in DPX (dibutylphtalate polystyrene xelene) and allowed to dry before viewing under the microscope using x10 and x40 magnifications. The microscopic features of the organs were compared with the control group.

Data Analysis

All data obtained from this study were expressed as mean ± SEM (standard error of mean). One way analysis of variance (ANOVA) followed by Tukey's HSD (honest significant difference) test were used to determine the significance differences

between the groups. Statistical significance was declared when *p* value was less than 0.05. The statistical analysis was performed using the statistical package for social science (SPSS) for windows, version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Acute Toxicity Study

Acute behavioural signs of toxicity as shown in Table 2 were not noticed in the mice administered various concentration range of 0 to 5,000mg/kg body weight of both plant extracts. There was no mortality recorded at all doses up to 72hrs. Physical and behavioural observations also showed no visible signs of extract toxicity such as hair erection, shivering and lacrimation. The oral median lethal dose (LD_{50}) was therefore estimated to be > 5,000mg/kg body weight for both the *A. floribunda* stem bark and leaves extracts.

In vivo antiplasmodial study

Percentage Parasite Inhibition and the Mean Survival Time of P. berghei Infected Mice Treated with A. floribunda Stem Bark and Leaves Extracts

Figure 1a compares the parasite inhibitory ability of the *P. berghei* infected mice treated with the standard antimalarial drug and the *A. floribunda* extracts after four days of treatment. Chloroquine recorded 95.93% chemosuppression while *A. floribunda* stem bark extract (250mg/kg body weight) demonstrated excellent activity similar to the standard antimalarial drug having 87.43% parasite suppression. The leaves extract at 250mg/kg body weight showed a very good activity chemosuppression (77.53%). According to the line graph in Fig. 1b, mice treated with chloroquine, 72 hours post infection had 95.96% inhibition on the *P. berghei* parasite. *A. floribunda* stem bark (250mg/kg body weight) demonstrated high potency as the standard antimalarial drug, having 72.57% parasite inhibition. *A. floribunda* leaves extract at 250 and 500 mg/kg body weight showed moderate *P. berghei* inhibitory activity of 52.75% and 50.40%, respectively.

Fig 1c shows the mean survival time (MST) of *P. berghei* infected mice treated with *A. floribunda* stem bark and leaves extracts. The mice infected without treatment (PnT) did not live beyond 16.8 days. The chloroquine treated group survived 28 days and beyond and did not show any clinical signs of malaria infection. Amongst the extract treated groups, the group treated with *A. floribunda* stem bark, at 250mg/kg, had the longest survival time (21.4 days) while the other extract treated groups could not live beyond approximately 20 days.

Variation in the Weights and Organ to Body Weight Ratio of P. berghei Infected Mice Treated with A. floribunda Extracts

Figure 2 shows a non-significant ($p > 0.05$) decrease in the mean body weight of the infected mice without treatment (PnT) between day 0 and 7. The mean body weights of the stem bark and leaf extracts treated groups, at varied concentrations between day 0 and 7 post infection, did not show any significant changes ($p > 0.05$). However, chloroquine treated and uninfected control recorded a significant increase ($p < 0.05$) in their mean body weights between day 0 and 7.

The organ to body weight ratio of *P. berghei* infected mice treated with *A. floribunda* stem bark and leaves extracts is presented in Fig. 3. The result shows hepatomegaly in the parasitized non-treated group (PnT). Also, the liver to body weight ratio of the mice in this group was significantly different ($p < 0.05$) from the chloroquine (CqT) treated group, uninfected (nPnT) group and 250mg/kg extract treated group. The spleen to body weight ratio showed similar trend with the liver to body weight ratio in that splenomegaly was prominent in the infected mice without treatment (PnT) and groups treated with higher dose of the plant. However, there were no significant differences ($p > 0.05$) in the brain to body weight ratios of the mice for all the groups evaluated.

Table 2: Acute lethal effect of methanol extracts of *A. floribunda* stem bark and leaves administered orally to mice

Experiment	Dose (mg/kg)	Stem bark	Leaf
Phase I	10	0/3	0/3
	100	0/3	0/3
	1000	0/3	0/3
Control	---	0/3	0/3
Phase II	1600	0/3	0/3
	2900	0/3	0/3
	5000	0/3	0/3
Confidence test	5000	0/3	0/3

Key: (0/3); 0 = number of deaths, 3 = number of rats used for the test

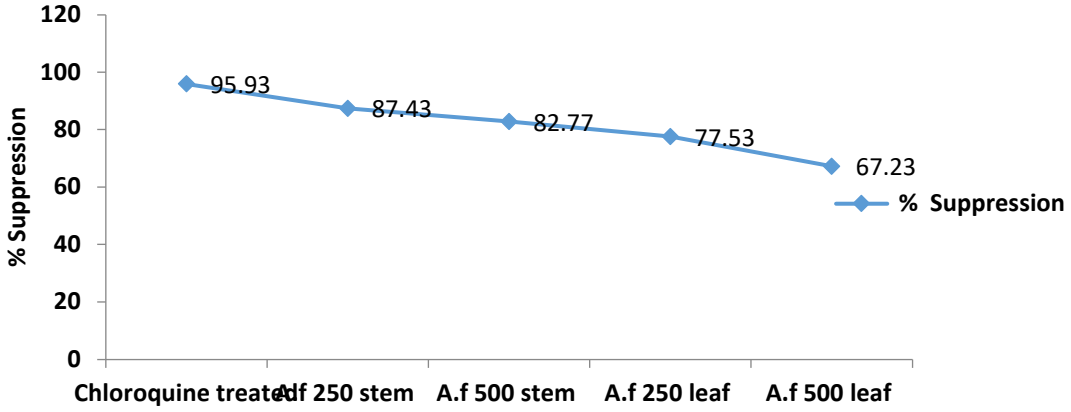

Fig. 1a: Percentage suppression of *P. berghei* in mice treated with *A. floribunda* stem bark, leaves and standard antimalarial drug, chloroquine

Values are mean ± SEM (n=6). Where: PnT= parasitized, non-treated; A.f leaf 250mg = *A. floribunda* leaves extract (250mg/kg); A.f leaf 500mg = *A. floribunda* leaves extract (500mg/kg); A.f stem 250mg = *A. floribunda* stem bark extract (250mg/kg); A.f stem 500mg = *A. floribunda* stem bark extract (500mg/kg)

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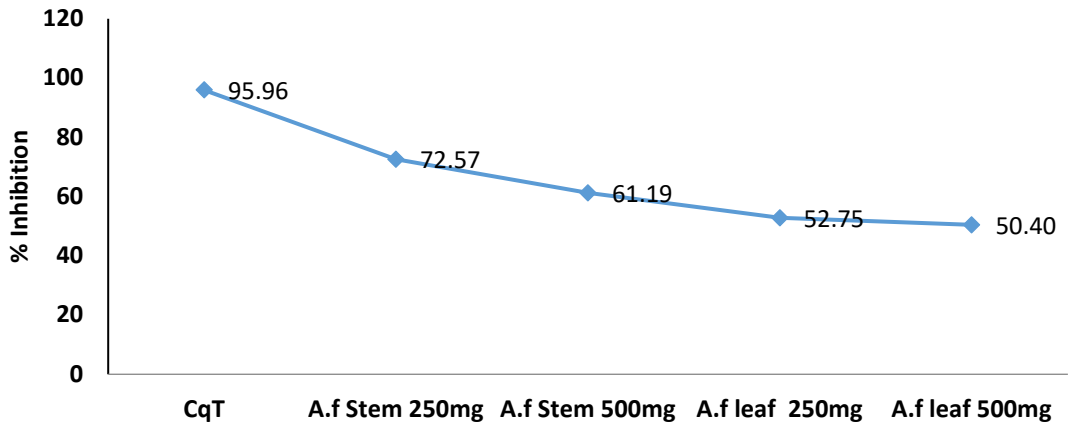


Fig. 1b: Percentage parasite inhibition at varied doses of *A. floribunda* extracts in P. berghei infected mice (curative test)

Values are mean ± SEM (n = 6). Where: CqT =chloroquine treated; A.f leaf 250mg = *A. floribunda* leaves extract (250mg/kg); A.f leaf 500mg = *A. floribunda* leaves extract (500mg/kg); A.f stem 250mg = *A. floribunda* stem bark extract (250mg/kg); A.f stem 500mg = *A. floribunda* stem bark extract (500mg/kg)

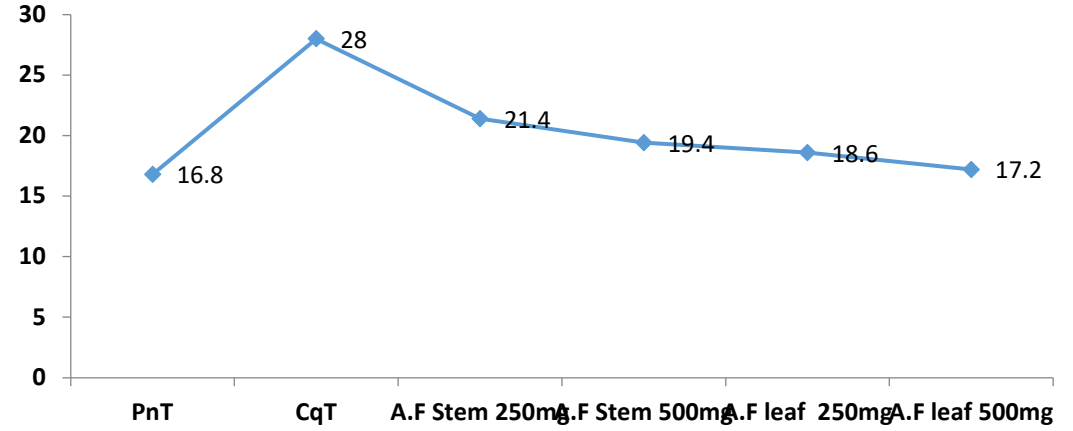


Fig. 1c: Mean survival time of *P. berghei* infected mice treated with *A. floribunda* extracts

Values are mean ± SEM (n = 6). Where: PnT= parasitized, non-treated; CqT =chloroquine treated; A.f leaf 250mg = *A. floribunda* leaves extract (250mg/kg); A.f leaf 500mg = *A. floribunda* leaves extract (500mg/kg); A.f stem 250mg = *A. floribunda* stem bark extract (250mg/kg); A.f stem 500mg = *A. floribunda* stem bark extract (500mg/kg).

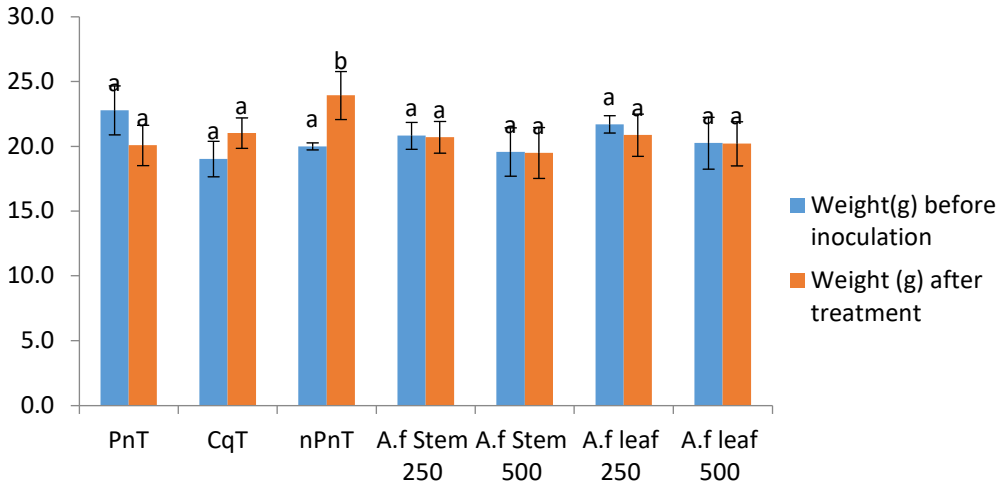


Fig. 2: Effect of *A. floribunda* extracts on the body weights of *P. berghei* infected mice between day 0 and 7

Values are mean ± SEM (n = 6). Bars with different alphabets are significantly different ($p < 0.05$). Where: PnT= parasitized, non-treated; CqT =chloroquine treated; nPnT= non- parasitized, non-treated; A.f leaf 250mg = *A. floribunda* leaves extract (250mg/kg); A.f leaf 500mg = *A. floribunda* leaves extract (500mg/kg); A.f stem 250mg = *A. floribunda* stem bark extract (250mg/kg); A.f stem 500mg = *A. floribunda* stem bark extract (500mg/kg).

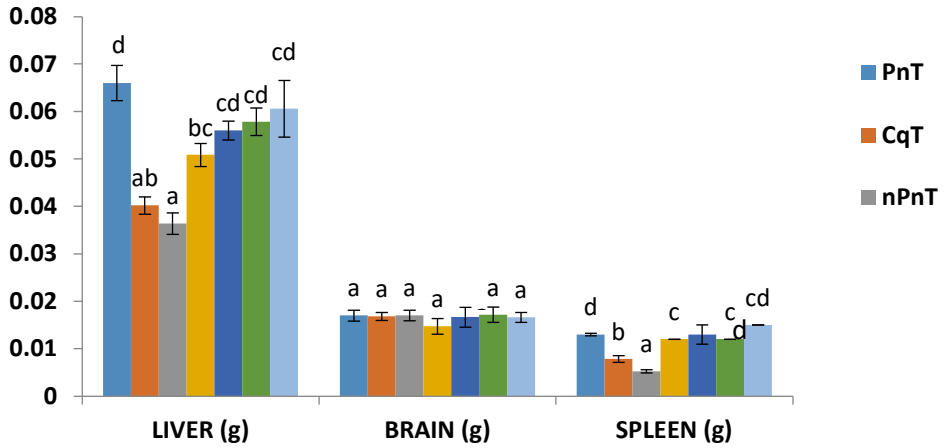


Fig. 3: Organ to body weight ratio of the liver, brain and spleen of *P. berghei* infected mice treated with *A. floribunda* extracts

Data are represented as mean ± SEM (n = 6). Bars with different alphabets are significantly different ($p < 0.05$). Where: nPnT= non- parasitized, non-treated; PnT= parasitized, non-treated; CqT = chloroquine treated; A flor leaf 250mg = *A. floribunda* leaves extract (250mg/kg); A flor leaf 500mg = *A. floribunda* leaves extract (500mg/kg); A flor stem 250mg = *A. floribunda* stem bark extract (250mg/kg); A flor stem 500mg = *A. floribunda* stem bark extract (500mg/k).

Histopathology of the Liver, Brain and Spleen of P. berghei Infected Mice Treated with A. floribunda Extracts

Liver Histology

Plate 1.1 represents the photomicrograph of the hepatocyte section of mice infected with *P. berghei* without treatment (PnT). Section of the hepatocytes shows diffused inflammation in the hepatic lobes (parenchyma) and portal spaces characterized by mononuclear cells exudation, lymphocytes and histiocytes in the portal and lobular compartments. There was evidence of mild fatty changes and focal microvesicular steatosis when compared with the normal uninfected control (Plate 1.3) which showed prominent hepatocytes with generally normal configuration. Plate 1.2 represents the photomicrograph section of the hepatocytes of the chloroquine treated mice (CqT). The section revealed hepatocytes with visible central vein and hepatocytes with mild kupffer cell activation and steatosis.

Plate 1.4 is the section of the hepatocytes of *P. berghei* infected mice treated with *A. floribunda* stem bark (250 mg/kg) revealed mild focal inflammation in the hepatic lobes (parenchyma) and/or portal spaces with prominent central vein. Hepatocytes of the *P. berghei* infected mice treated with *A. floribunda* stem bark (500 mg/kg) revealed visible atrophied central vein with visible fatty changes (Plate 1.5). Plate 1.6 represents the hepatocytes of mice infected with *P. berghei* and then treated with *A. floribunda* leaves (250 mg/kg). Section reveals prominent hepatocytes with visible pyknotic nucleus and centrioles. The liver section of mice infected with *P. berghei* and treated with *A. floribunda* leaves (500 mg/kg) indicated prominent hepatocytes with mild steatosis and centrioles (Plate 1.7).

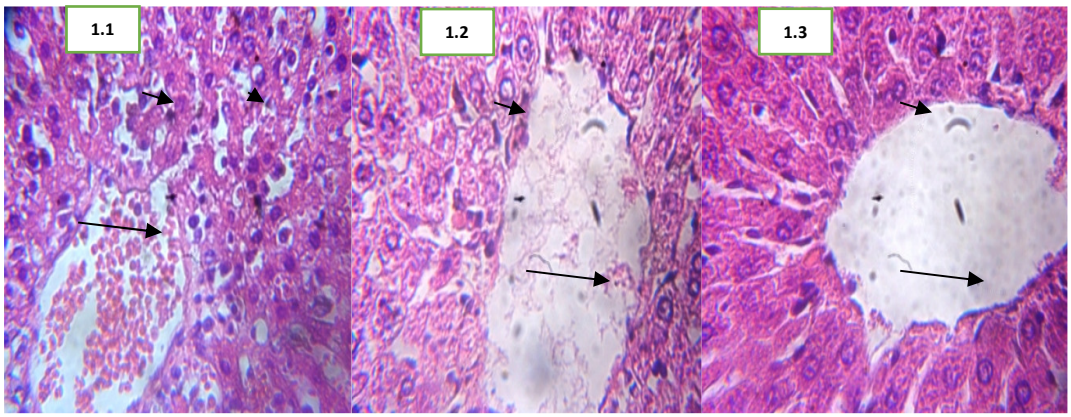
Brain Histology

Section of the brain tissue of mice infected with *P. berghei* without treatment (PnT) reveals granular and molecular cells layer with visible areas of haemorrhage and thickened white matter (Plate 2.1). Plate 2.2 shows the photomicrograph of the brain section of *P. berghei* infected mice treated with chloroquine. Histology features revealed molecular, granular and Purkinje cell layers Plate 2.3 depicts the photomicrograph of the brain section of uninfected mice showing normal molecular and granular layers with the white matter; also seen are Purkinje cell layers. Brain section of *P. berghei* infected mice treated with *A. floribunda* stem bark extract (250 and 500 mg/kg) and the leaves extract (250 and 500 mg/kg) showed prominent granular and molecular layers bounded by visible purkinje cells (Plates 2.4 to 2.7). The granular layer was split through by white matter. In addition, the stem bark treated mice at 500 mg/kg showed evidence of some haemorrhagic deposits (Plate 2.5).

Spleen Histology

Plate 3.1 represents the section of the spleen of *P. berghei* infected mice without treatment showing white and red pulp with visible central artery and prominent lymphoctes which appeared disintegrated. There were focal areas of heamorrhage. Plate 3.2 depicts section of the spleen of *P. berghei* infected mice treated with chloroquine (CqT). Prominent lymphoid follicles with

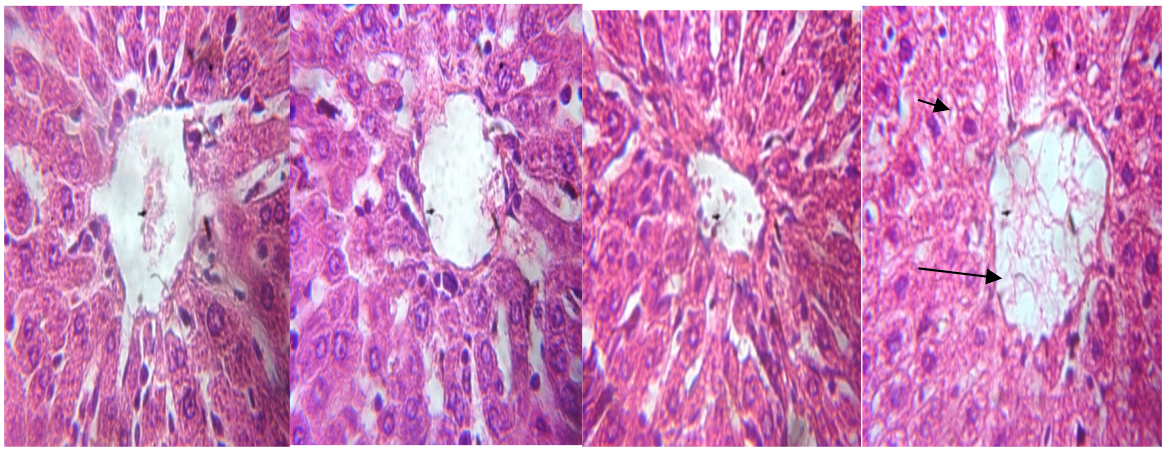
centrally to eccentrically located blood vessels which appeared large were seen. The follicles (white pulp) consisted of aggregates of lymphocytes and the red pulps were prominent with normal configuration. Plate 3.3 is the photomicrograph section of the spleen of uninfected normal control mice (nPnT), it shows white and red pulp, prominent central artery with mild dilation and prominent lymphoctes. There was observed increase in extramedullary haematopoiesis and a decrease in the lymphocytes in the white pulp. Section of the spleen of *P. berghei* infected mice treated with *A. floribunda* stem bark (250mg/kg) extract (Plate 3.4) and the leaf (250mg/kg) extract (Plate 3.6) also showed prominent lymphoid follicles with centrally to eccentrically located blood vessels. The follicles (white pulp) consisted of aggregates of lymphocytes, but the red pulps were prominent with increased configuration in the stem bark extract group and decreased configuration in the leaf extract group.



PnT CqT nPnT

Plate 1.1-1.3: Photomicrograph of the liver histology (X 400 Magnification)

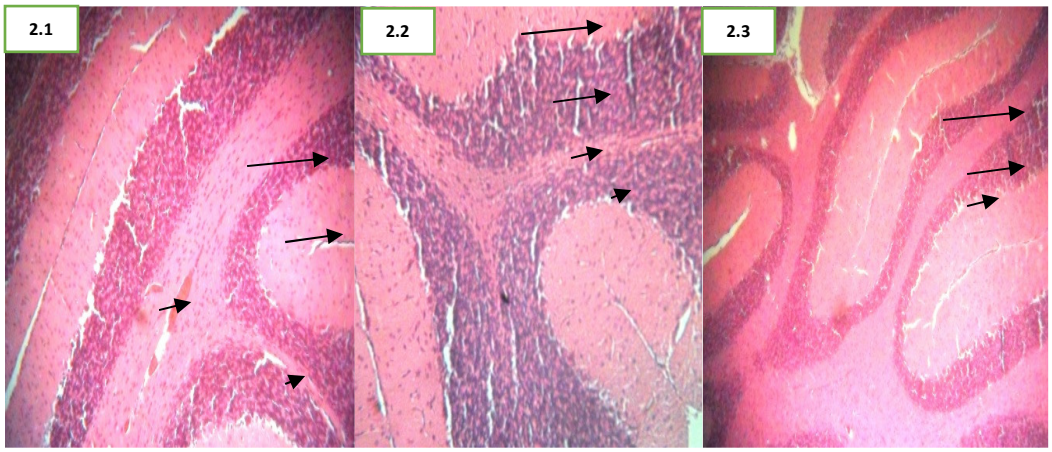
1.1-PnT (parasitized, non-treated) revealed diffuse inflammation in the hepatic lobes (parenchyma) and/or portal spaces (small arrow) characterized by mononuclear cells exudation lymphocytes and histiocytes in the portal and lobular compartments (long arrow). There were mild fatty changes and focal microvesicular steatosis (arrow head). 1.2-CqT (chloroquine treated) revealed visible central vein (long arrow) and hepatocytes (short arrow) there mild kupffer cell activation and steatosis. 1.3-nPnT (non- parasitized, non-treated) revealed prominent hepatocytes (short arrow) with visible pyknotic nucleus and centrioles (long arrow).



A.f stem 250 mg A.f stem 500 mg A.f leaf 250 mg A.f leaf 500 mg

Plate 1.4-1.7: Photomicrograph of the liver histology (X 400 Magnification)

1.4-Stem 250 mg reveals mild focal inflammation in the hepatic lobes (parenchyma) and/or portal spaces (small arrow) and prominent central vein (long arrow); 1.5-Stem 500 reveals visible atrophied central vein (long arrow) and hepatocytes (short arrow) there is visible fatty changes (short arrow) Leaf 1.6-leaves 250 mg reveals prominent hepatocytes (short arrow) with visible pyknotic nucleus and centrioles (long arrow); 1.7-Leaf 500 mg reveals prominent hepatocytes with mild steatosis (short arrow) with centrioles (long arrow). Where A.f = *Allanblackia floribunda*.



PnT CqT nPnT

Plate 2.1-2.3: Photomicrograph of the brain histology (X400 Magnification)

2.1-PnT (Parasitized, non-treated) features reveals granular cell layer (long arrow) and molecular layer (medium arrow), cell layer. There is visible area of haemorrhage (small arrow) with thickened white matter (arrow head); 2.2-CqT (Chloroquine treated) features reveals molecular layer (long arrow) and granule layer (medium arrow), Purkinje cell layer (short arrow). The white matter is not as prominent as nPnT; 2.3 nPnT (non-parasitized, non-treated) visible detailed features, molecular layer (long arrow) and granule layer (medium arrow) with white matter (short arrow). Also seen are Purkinje cell layers (arrow head).

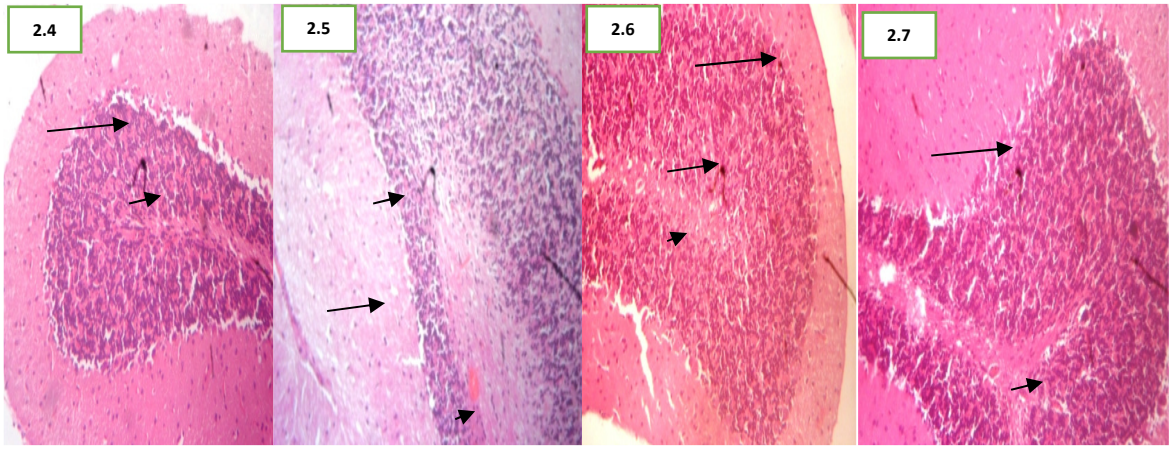


Plate 2.4-2.7: Photomicrograph of the brain histology (X400 Magnification)
2.4-Stem 250 mg, brain shows prominent granular layer (short arrow) and molecular layer (long arrow) bounded by visible purkinje cells. The granular layer is split through by white matter; 2.5-Stem 500 mg, brain presents prominent granular layer (short arrow) and molecular layer (long arrow) bounded by visible purkinje cells. There iare some haemorrhagic deposits seen (arrow head); 2.6-Leaf 250 mg, brain reveals prominent granular layer (short arrow) and molecular layer (long arrow) bounded by visible purkinje cells. The granular layer is split through by a widened white matter (arrow head); 2.7-Leaf 500 mg the brain reveals prominent granular layer (short arrow) and molecular layer (long arrow) bounded by visible purkinje cells. Where A.f = *Allanblackia floribunda*.

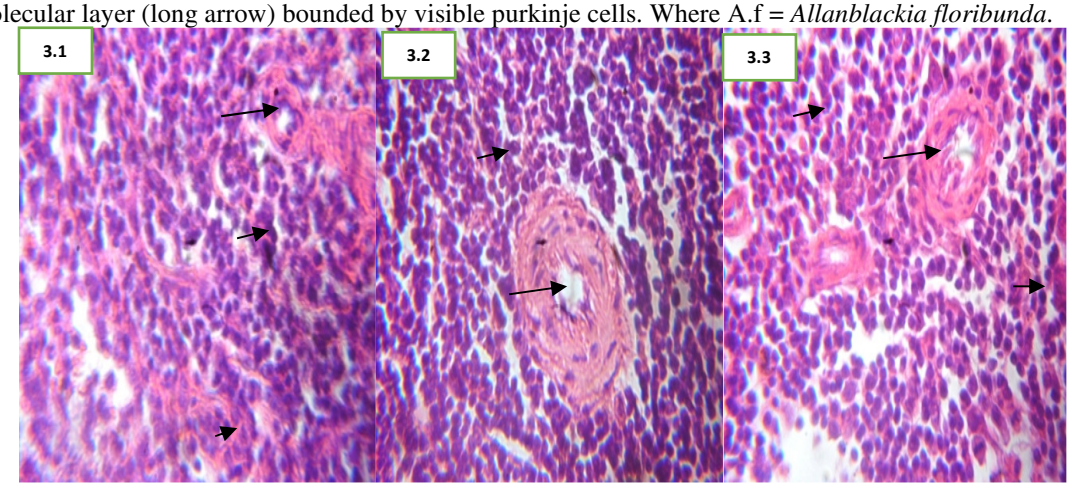
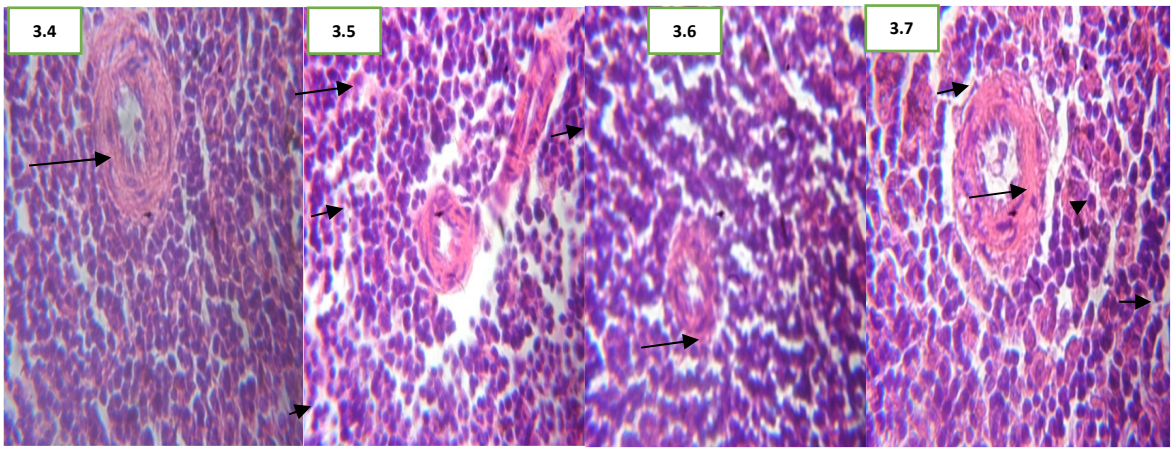


Plate 3.1-3.3: Photomicrograph of the spleen histology (X400 Magnification)
3.1-PnT (parasitized, non-treated) histology reveals white and red pulp with visible central artery (long arrow) and prominent lymphoctes which appear disintegrated (short arrow). There is focal areas of heamorrhage (arrow head);3.2- CqT (Chloroquine treated) shows prominent lymphoid follicles with centrally to eccentrically located blood vessels (long arrow) which appear large. The follicles (white pulp) consist of aggregates of lymphocytes (short arrow). The red pulps are prominent and shows a normal configuration (arrow head); 3.3-nPnT (non-parasitized, non-treated) Spleen histology reveals white (arrow head) and red pulp (short arrow) with prominent central artery with mild diation(long arrow) and prominent lymphoctes there is increase in extramedullary haematopoiesis and a decrease in the lymphocytes in the white pulp.



A.f stem 250 mg A.f stem 500 mg A.f leaf 25 0mg A.f leaf 500 mg

Plate 3.4-3.7: Photomicrograph of the spleen histology (X400 Magnification)
3.4-Stem 250 mg, spleen shows prominent lymphoid follicles with centrally to eccentrically located blood vessels (long arrow). The follicles (white pulp) consist of aggregates of lymphocytes (short arrow). The red pulps are prominent and shows increased configuration (arrow head); 3.5-Stem 500 mg, the spleen shows prominent lymphoid follicles with centrally to eccentrically located blood vessels (long arrow). The follicles (white pulp) consist of aggregates of lymphocytes (short arrow). The red pulps are prominent and shows a decreased configuration (arrow head); 3.6-Leaf 250 mg, the spleen shows lymphoid follicles with centrally to eccentrically located blood vessels (long arrow). The follicles (white pulp) consist of aggregates of lymphocytes (short arrow). The red pulps are prominent and increased (arrow head);3.7-Leaf 500 mg, the spleen shows prominent lymphoid follicles with centrally to eccentrically located blood vessels (long arrow). The follicles (white pulp) consist of aggregates of lymphocytes (short arrow). The red pulps are prominent and shows a distinct configuration (arrow head). Where A.f = *Allanblackia floribunda.*

DISCUSSION

The acute toxicity effect of *A. floribunda* stem bark and leaves extracts, administered at different concentrations (from 0 to 5000 mg/kg body weight) to normal mice, showed no significant physical evidence of behavioural changes (such as shivering, salivating, loosing of hair and sitting in corner of cages); and no mortality was recorded within 24 h and the next 7 days of the study. These observations suggest low toxicity and relative safety of *A. floribunda* extracts. According to the toxicity classes of Hodge and Sterner (2005), any compound with oral LD_{50} of 5000 mg/kg or more should be considered as practically harmless, this point was further buttressed by Lorke (1983) on toxicity testing of medicinal plants (Ajuru, *et al*., 2019).

The relative safety of *A. floribunda* extracts could be as a result of the route of administration of the extract because the channel through which substances are given to animals has a significant role to play with respect to bioavailability i.e., how much of the plant bioactive compounds are made available to the receiving subject. *A. floribunda* is rich in phytochemicals such as phenolics, flavonoids, alkaloids, saponins, tannins, quinones and other phytochemicals (Irabor *et al*., 2021b). These phytochemicals are probably present at concentrations that are not toxic to laboratory animals. Though phytochemicals such as saponins has been reported to cause haemolysis by

increasing the permeability of the plasma membrane and cardiac glycosides have been found to be toxic at high concentration (Dewick, 2002; Jones and Kinghorn, 2006). However, saponins can enhance nutrient absorption and aid in animal digestion while cardiac glycosides can improve circulation and normalize heartbeat in congestive heart failure (El-olemy *et al*., 1994). *A. floribunda* could have these phytochemicals in tolerable levels with respect to the highest dose (5000 mg/kg body weight) administered.

In the curative test and suppressive test, the extracts did not only inhibit the growth of the parasites but caused a decrease in the overall pathologic effect of the parasite in the mice. Nonetheless, the extracts at all doses could not give complete clearance of the parasite because the infected mice did not live beyond 28 days compared to the chloroquine treated group that recovered fully from the disease. This could be due to the reoccurrence of the infection after apparent cure in extract treated groups. Previous studies on *Croton macrostachyus, Dodonaea viscosa* and *Strychnos mitis* have also shown similar results in their mean survival time (Mengiste *et al*., 2012; Bantie *et al.,* 2014; Alelign *et al*., 2017).

The significant increase in survival time of the mice as a result of the administration of the graded doses of *A. floribunda* stem bark and leaves extracts could be associated to the presence of some bioactive secondary metabolites in the plant (Irabor *et al*., 2021b). As previously reported, plant's bioactive principles possess antiplasmodial activity (Hilou *et al*., 2006; Dharani *et al.,* 2008). The curative and suppressive potential of these pharmacologically active substances could be through single or synergistic action (Ayoola *et al*., 2008). Flavonoids act by interacting with the enzymes involved in the biosynthesis fatty acids in the parasite (Perozzo *et al*., 2002; Freundlich *et al*., 2005). Some flavonoids have been reported to inhibit the movement of L-glutamine and myo-inositol into infected red blood cell (Elford, 1986). The flavonoids present in the plant extracts could also counter redox imbalance associated with malaria infection hence increasing the mean survival time of the infected treated mice (Omoregie and Okugbo, 2014).

The reduction in the weights of the mice infected with *P. berghei* without treatment may be due to loss of appetite that might have resulted from parasite proliferation as well as rapid elimination of both infected and uninfected erythrocytes in the spleen. Complete clearance of parasites in chloroquine treated group may have been the reason for the non-significant increase in body weights of the mice in this group. There was neither an increase nor a decrease in the body weight of the extracts treated groups irrespective of the concentrations administered. The reason for this outcome could be that the plant extracts bioactive components challenged parasite growth, making the mice to maintain relatively stable weight post infection.

The liver to body weight ratio indicated hepatomegaly in the parasitized, non-treated (PnT) mice and the leaves (500mg/kg) extract treated mice in contrast to the non-parasitized non-treated (nPnT) normal control.

However, administration of chloroquine (CqT), the stem bark (250 and 500mg/kg) and leaves (250mg/kg) extracts resulted in normal or close to normal liver to body weight ratios when compared to the normal control. In severe *Plasmodium* parasite infection, liver enlargement is caused by many factors, namely fatty changes, portal tract inflammation, bile duct proliferation, sinusoidal congestion, hyperplastic kupffer cells and hemozoin deposition (Viriyavejakul *et al*., 2014).

Spleen enlargement is one of the markers used to estimate the severity of malaria infection in endemic areas (Chaves *et al*., 2011). The spleen is an organ that selectively filters the blood and eliminates infected and senescent erythrocytes. One of the common causes of splenomegaly as reported by Del Portillo1 *et al*. (2012) is malaria infection because the spleen is the primary organ that helps to mount immune response and it is also involved in the destruction of *Plasmodium* parasite infected red blood cells (Engwerda *et al*., 2006). Intense erythrophagocytosis and local infaction (Lubitz, 1949; Del Portillo1 *et al*., 2012) are the hallmark of splenic enlargement because during the parasite growth, erythrocyte infected cells forcefully passing through the slits of sinusal spleens leave behind deposit of cytoplasmic particulate matter like nuclear remnants, oxidized hemozoin and malarial parasite in the red pulp (Schnitzer *et al*., 1972). The spleen to body weight ratio showed similar trend with the liver to body weight ratio in which splenomegaly was prominent in the PnT group and in the infected mice treated with higher doses (500mg/kg) of the *A. floribunda* stem bark and leaves extracts in contrast to the normal control. Whereas the chloroquine as well as the *A. floribunda* stem bark and leaves extracts (250mg/kg) treated mice showed relatively normal spleen to body weight ratio which were comparable to that of basal control. The splenomegaly observed at higher doses of the extracts may be due to a reversal effect of the extracts as a result of higher levels of some inhibitory metabolites (flavonoids, alkaloids, saponins) that are capable of causing a decrease in their chemo-suppressive efficacy. Several studies have shown splenomegaly and hepatomegaly during malaria infection (Adachi *et al*., 2001; del Portillo *et al*., 2012). Inflammatory stimuli, including *Plasmodium* parasite and its hemozoin pigment can lead to enlargement in the liver and spleen (Vanderberg and Frevert, 2004).

In this study, the liver histology of *P. berghei* infected mice (negative control) revealed sinusoidal infiltration by kupffer cells, periportal infiltration by lymphocytes and polymorphonuclear cells, portal vein congestion, and central vein congestion, as well as vascular congestion. This could be as a result of the overwhelming immune response during parasite proliferation as well as cytotoxic substances accumulation and white blood cells proliferation (Rungruang *et al*., 2013). Though high doses of the stem and leaves extracts resulted in mild atrophied central vein and steatosis however, the extract at low concentration were able to conserve the architecture of the liver, spleen and brain tissues to a reasonable extent.

CONCLUSION

A. floribunda stem bark and leaves extracts were not toxic at the highest dose administered. Though the stem bark and leaves extracts were found to be very active at low doses against the chloroquine sensitive *P. berghei* however, it was observed that the stem bark extract has higher potency. Also, the stem bark and leaves extracts at low doses had better protection on the liver, brain and spleen tissue of the *P. berghei* infected mice relative to the chloroquine treated group.

REFERENCES

Adachi, K., Tsutsui, H. and Kashiwamura, S.I. (2001). *Plasmodium berghei* infection in mice induces liver injury by an IL-12- and Toll-like receptor/myeloid differentiation factor 88-dependent mechanism. *The Journal of Immunology*, 167(10): 5928–5934.

Ajuru, M. G., Ajuru, G., Nmom, F. W., Worlu, C.W. and Igoma, P. G. (2019). Acute toxicity study and determination of median lethal dose of *Catharanthus roseus* in Wistar rat albino rats. *Journal of Applied Sciences*, 19: 217-222.

Alelign, N., Giday, M., Teklehaymanot, T., Animut, A. (2018). Ethnobotanical survey of medicinal plants in Awash-Fentale district of Afar region of Ethiopia and *in vivo* evaluation of selected ones against *Plasmodium berghei. Asian Pacific Journal of Tropical Biomedicine*, 8: 73-78.

Ayoola, A. G., Ipav, S. S., Sofidiya, O. M., Adepoju-Bello, A. A., Coker, A. B. H. and Odugbemi, O. T. (2008). Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda Oliv* (Guttiferae). *International Journal of Health Research* 1(2):87-93.

Bantie, L., Assefa, S., Teklehaimanot, T. and Engidawork, E. (2014). *In vivo* antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. *Alternative Med*icine, 14: 79.

Chaves, L. F., Taleo, G., Kalkoa, M. and Kaneko, A. (2011). Spleen rates in children: an old and new surveillance tool for malaria elimination initiatives in island settings. *Tropical Medicinal Hygiene,* 105: 226–231.

Chinedu, E., Arome, D. and Ameh, S. F. (2013). A new method for determining acute toxicity in animal models. *Toxicology International*, 20(3): 224-226.

Del Portillo1, H. A., Mireia, F., Thibaut, B., Lorena, M. J., Jean. L and Marcus, V. G. L. (2012). The role of the spleen in malaria. *Cellular Microbiology,* 14(3): 343–355.

Deharo, E., Mun˜oz, V., Sauvain, M., Bourdy, G., Callapa, J., Rojas, I., Vargas, L. and Tae, A. (2000). The search for natural bioactive compounds through a multidisciplinary approach in Bolivia. Part II. Antimalarial activity of some plants used by Mosetene Indians. *Journal of*

Ethnopharmacology, 69: 139–155.

Dewick, P. M. (2002). Medicinal natural products: *a biosynthetic approach*. 2nd ed., 220 p.

Dharani, N., Rukunga, G., Yenesew, A., Mbora, A., Mwaura, L. and Jamnadass, R. (2008). Common Antimalarial Trees and Shrubs of East Africa. *World Agroforestry Centre*, ISBN: 978-92-9059-238-9.

Elford, B. C. (1986): L-Glutamine influx in malaria-infected erythrocytes: a target for antimalarials? *Parasitology Today*, 2:309–312.

El-olemy, M. M., Al-muhtada, F. J., and Afifi, A. A. (1994). Experimental Phytochemistry. A Laboratory Manual. King Saud University Press Riyadh, Saudi Arabia, 6–65p.

Engwerda, C. R., Beattie, L., and Amante, F. H. (2005). The importance of the spleen in malaria. *Trends in Parasitology,* 21: 75–80.

Freundlich, J. S., Anderson, J. W., Sarantakis, D., Shieh, H. M., Yu, M., Valderramos, J. C., Lucumi, E., Kuo, M., Jacobs, W. R. Jr., Fidock, D. A., Schiehser, G. A., Jacobus, D. P. and Sacchettini, J. (2002). Synthesis, biological activity, and X-ray crystal structural analysis of diaryl ether inhibitors of malarial enoyl acyl carrier protein reductase: part 1: 4′-substituted triclosan derivatives. *Bioorganic and Medicinal Chemistry Letters***,** 15: 5247–5252.

Hilou, A., Nacoulma, O.G. and Guiguemde, T.R. (2006). *In vivo* antimalarial activities of extracts from *Amaranthus spinosus L.* and *Boerhaavia erecta L.* in mice. *Journal of Ethnopharmacology,* 103: 236–40.

Hodge, A. and Sterner, B. (2005). Toxicity classes. In Canadian centre for occupational Health and safety. Copy right @1997-2010. Retrieved from Shiavone, A., Gup, K. and Tassone, S. (2008). "Effects of a natural extract of chestnut wood on digestibility, performance traits and nitrogen balance of broiler chicks". *Poultry Science,* 87(3):521–7.

Irabor, F., Ebohon, O., Erhunse, N. Osariemen, T. O. and Omoregie, E. S. (2021a). *In vitro* antiplasmodial activity, cytotoxicity, antioxidant action and GC-FID analysis of *Allanblackia floribunda Oliv*. SN Applied Sciences 3, 820. https://doi.org/10.1007/s42452-021-04812-0

Irabor, F., Ebohon, O., Ibukun, O., Ayevbuomwan, E. M. and Omoregie, E. S. (2021b). Biological activity and subchronic toxicity study of *Allanblackia floribunda Oliv.* on experimental rats. *Scientific African,* 13: e00950 https://doi.org/10.1016/j.sciaf.2021.e00950.

Jones, P. and Kinghorn, D. (2006). Extraction of plant secondary metabolites. In: Sarker, D., Latif, Z. and Gray, A. (editors). *Methods in biotechnology natural*

products isolation. 2nd ed. Totowa: Human Press, 269–273 pp.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology,* 53: 275-287.

Lubitz, J. M. (1949). Pathology of the ruptured spleen in acute vivax malaria. *Blood,* 4: 1168–1176.

Mengiste, B., Makonnen, E. and Urga, K. (2012). *In vivo* antimalarial activity of Dodonaeaangustifolia seed extracts against *Plasmodium berghei* in mice model. *Momona Ethiopian Journal of Science*, 4: 47–63.

Odugbemi, T. O., Akinsulire, O. R., Aibinu, I. E. and Fabeku, O. P. (2007). Medicinal plants useful for malaria therapy in Okeigbo, Ondo state, southwest Nigeria. *African Journal of Traditional, Complementary and Alternative,* 4(2): 191- 198.

Omoregie, E. S. and Ehigbai, I. O. (2015). Comparative Studies on the Phytochemical Composition, Phenolic Content and Antioxidant Activities of Methanol Leaf Extracts of *Spondias mombin* and *Polyathia longifolia. Jordan Journal of Biological Sciences,* 8(2): 145-149.

Omoregie, E. S. and Okugbo, O. T. (2014). *In vitro* antioxidant activity and phytochemical screening of methanol extracts of *Ficus capensis* and *Dacryodes edulis* leaves *Journal of Pharmacy & Bioresources,* 11(2): 66-75.

Perozzo, R., Kuo, M., Sidhu, A. B. S., Valiyaveettil, J. T., Bittman, R., Jacobs, W. R. Jr., Fidock, D. A. and Sacchettini, J. C. (2002). Structural elucidation of the specificity of the antibacterial agent triclosan for malarial enoyl acyl carrier protein reductase. *Journal of Biological Chemistry,* 277: 13106–13114.

Peters, W., Robinson, B. L. and Milhous, W. K. (1993). The chemotherapy of rodent malaria. LI. Studies on a new 8-aminoquinoline, WR 238,605. *Pharmacokinetics,* 30(4): 263-299.

Peters, W. M. (1967). Rational methods in the search for antimalarial drugs. *Journal of the Royal Society of Tropical Medicine and Hygiene*, 61: 400-410.

Rungruang, T., Kaewkongkwan, Y., Sukakul, T., Kettawan, A., Chompoopong, S. and Boonmars, T. (2013). The effect of vitamin C on morphology and histology of liver and spleen of *Plasmodium* - infected mice. *International Food Research Journal*, 20(4): 1639-1643.

Ryley, J. F. and Peters, W. (1970). The anti-malaria activity of some quinolone esters. *Annals of Tropical Medicine and Parasitology*, 84: 209-222.

Schnitzer, B., Sodeman, T., Mead, M. L. and Contacos, P. G. (1972). Pitting function of the spleen in malaria: ultrastructural observations. *Science,* 177: 175–177.

Vanderberg, J. P. and Frevert, U. (2004). Intravital microscopy demonstrating antibody-mediated immobilisation of *Plasmodium*

berghei sporozoites injected into skin by mosquitoes," *International Journal for Parasitology*, 34(9): 991–996.

Viriyavejakul, P., Vasnt, K. and Chuchard, P. (2014). Liver changes in severe *Plasmodium falciparum* malaria: histopathology, apoptosis, and nuclear factor kappa B expression. *Malaria Journal* 106(11): 266-275.

World Health Organization (WHO). World malaria report 2021: Geneva: Licence: CC BY-NC-SA 3.0 IGO. 2021. 26 p.