

# **IN VIVO ANTI-PLASMODIAL AND IN VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAF EXTRACT OF ALSTONIA BOONIE (EWE AHUN) AND ITS EFFECT ON SOME BIOCHEMICAL PARAMETERS IN SWISS ALBINO MICE INFECTED WITH PLASMODIUM BERGHEI NK 65**

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## **Abstract**

Malaria is an increasing worldwide threat, with more than three hundred million infections and one million deaths every year. The study was conducted to determine the effect of in vivo anti-plasmodial and in vitro antioxidant activity of ethanolic leaf extract of *Alstonia boonie* (Ewe ahun) and its effect on some biochemical parameters in Swiss albino mice infected with *Plasmodium berghei* NK65. Swiss albino mice were inoculated intraperitoneally with *Plasmodium berghei* NK65. The mice were grouped into six groups, five per group. Group I were not infected with *P.berghei*, Group II and III served both as negative and positive controls respectively, while Group IV, V, and VI were treated with 200, 400, and 800 mg/kg body weight of *A. boonie leaf* extract respectively. The phytochemical constituents of the extract showed the presence of secondary metabolites like tannin, flavonoids, steroids and saponin. The extract caused 37.9%, 34.7% and 45.8% suppression in parasitaemia at 200, 400 and 800mg/kg body weight respectively while Chloroquine exerted 100% suppression at 5mg/kg body weight. The curative test shows that the different concentration of the extract exert a growth inhibition of 41.8%, 43.3% and 37.5% at 200, 400, 800mg/kg body weight respectively while Chloroquine, cleared the parasites

by 93.1% at 5mg/kg body weight. The Hematological parameters showed that *A. boonie* had a significant increase ( $P<0.05$ ) in HGB, RBC and HCT values while their WBC count reduced significantly when compared to the negative control. Chloroquine and the extract significantly decreases ( $P<0.05$ ) plasma liver marker enzymes AST, ALT, ALP and GGT in the treated group compared to the untreated group. The total protein values significantly increased ( $P<0.05$ ) in the group treated with the extract and at a dose of 800mg/kg, the extract was nephrotoxic. In the in-vitro antioxidant assay, the extract significantly increase ( $P<0.05$ ) the level of CAT, SOD and GSH in the liver homogenate induced with oxidative stress using  $H_2O_2$  while the MDA values reduced significantly with the administration of the extract.

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**Keywords:** *Alstonia boonie* (Ewe ahun) , Anti-plasmodial, Biochemical parameters, *Plasmodium berghei* NK 65 infected Swiss mice

## Introduction

According to World Health Organization, malaria is an increasing worldwide threat, with more than three hundred million infections and one million deaths every year (WHO 2002). Malaria has been and is still the cause of major human morbidity and mortality. The classic symptoms of malaria are cyclical occurrence of sudden coldness followed by rigour, then fever and sweating lasting four to six hours, occurring every two days. Children with malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage. Malaria in Nigeria is holoendemic i.e. there is an intense all-year round transmission with greater intensity in the wet season than dry season. The most important problems associated with malaria is that Plasmodial parasites are resistant to most widely available and affordable drugs like Fansidar and Chloroquine (Kisame, 2005). Secondly, the control of mosquitoes which transmits malaria is made difficult by their resistance to a wide range of insecticides. Thirdly, is the production of fake antimalarial drugs. Lastly, most countries in Africa lack the necessary infrastructure and resources to manage and control malaria (WHO, 1994). *Plasmodium berghei* has been used in studying the activity of potential antimalarials in mice (Pedroni, *et al.* 2006 ) and in rats (English, *et al* 1996 ). It produces diseases similar to those of human plasmodium infection (Kumar *et al.* 2006 and Peter, 1998 ).

Medicinal plants are known to contain a variety of substances and are used in the treatment of many kinds of ailments in traditional medicine. Medicinal plants have the basis of health care throughout the world and remain relevant both in the developing and developed nations of the world for various chemotherapeutic purposes. A number of traditional herbs have been tested and used in the prevention and also treatment of malaria.

*Alstonia boonei* De Wild is large deciduous evergreen tree, usually up to 45m tall and 1.2m in diameter. It belong to the family *Apocynaceae* which consist of more than 40 species widely distributed in the continents of Africa, Asia and America (Iwu, 1993 and Ojewole,1984). *Alstonia boonei* is known as Ahun in Yoruba, Egbu-ora in Igbo, Ukhu in Edo and Ukpukunu in Urhobo. Different parts of the plant are employed for the treatment of a variety of ailments in traditional medicine, to treat malaria, fever, rhematic pains, chronic diarrhea, insomnia, as anti-venom for snake bites and in the treatment of arrow poisoning (Koumaglo *et al*,1992, Asuzu, 1991 and Obih, 1985).

## **Materials and methods**

### **Collection and Preparation of Plant extract**

The leaves of *Alstonia boonei* were obtained from Abeokuta in Ogun State, Nigeria with the help of a traditional herbalist. The plant was authenticated by Miss Shokefun a botanist from SLT Department, Environmental biology Unit, Lagos State Polytechnic, Ikorodu. The leaves were washed, air dried under shade in the Biochemistry Laboratory, pulverised to coarse power using blender. Extraction was carried out by dispersing 200g of the grounded *Alstonia boonei* plant material in 1L of 70% ethanol and shaking was done with GFL shaker for 72 hours. This was followed by vacuum filtration and concentrated by rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was dried to complete dryness in an aerated oven at 40°C for 48 hours. The *Alstonia boonei* ethanolic extract was latter stored in a refrigerator at 4°C.

### **Phytochemical analysis**

Phytochemical analysis for bioactive constituents were carried out on the ethanolic extract of *Alstonia boonei* using standard phytochemical procedures (Sofowora (1993), Harborne (1973), Trease and Evans (1993)).

### **Experimental Swiss albino mice**

Seven (7) weeks old Swiss albino mice weighing 22- 32g were obtained from Nigeria Institute of Medical Research (NIMR), Lagos, Nigeria. They were acclimatized for seven days, housed in plastic cages with saw dust as beddings; food and water were given *ad libitum*. The mice were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

### **Acute toxicity test**

The acute toxicity test of ethanolic leaf extract of *Alstonia boonei* was carried out using modified Lorkes method (1993). Eighteen Swiss albino mice weighing 22 to 32 grams were randomized into three groups of six mice each and were given 1600, 2900 and 5000mg/Kg body weight of the extract orally. They were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. The oral median dose LD<sub>50</sub> was calculated

### **Animal grouping for infection and treatment**

The parasite *Plasmodium berghei* NK 65 was obtained from Nigeria Institute of Medical Research (NIMR), Lagos, Nigeria from Dr Aina, O.O. The parasites were kept alive by continuous intraperitoneal inoculation of known amount of the parasite into Swiss mice. 1ml of blood was taken from donor mice and diluted with 5ml phosphate buffer; such that 0.1ml contained standard inoculum of  $1 \times 10^7$  infected red blood cells (Maegraith *et al*, 1952). Thirty acclimatized Swiss albino mice were randomly selected and twenty five Swiss mice were inoculated intraperitoneally from the same source to avoid variability in parasitemia. The mice were randomly distributed into six groups of five per group as shown below:

GROUP I (Normal control ) Healthy Swiss mice

GROUP II (Negative control) mice infected with *P.berghei* NK65 received no treatment

GROUP III. (Positive control) = *P.berghei* +5mg/kg b,wt of chloroquine (Standard drug)

GROUP IV = *P. berghei* + 200mg/kg b.wt of *A. boonei* extract.

GROUP V = *P. berghei* + 400mg/kg b.wt of *A. boonei* extract.

GROUP VI=*P. berghei* + 800mg/kg b.wt of *A. boonei* extract.

### **Anti-plasmodium studies**

#### **Suppressive test**

The Peter's 4-day suppressive test against *P. berghei* NK65 infection in Swiss mice was used (Peters, 1965). Adult Swiss mice weighing between 22 to 32g were inoculated by intraperitoneal injection with standard inoculum of *Plasmodium berghei* NK65 with  $1 \times 10^7$  infected red blood cells. The mice were divided into six groups as shown above and treated for 4 consecutive days with 5mg/kg.b.wt of Chloroquine, 200, 400, and 800mg/kg body weight of *Alstonia boonei* extract orally daily. On day 5 of

the experiment, blood was collected from the tail of each mouse and smeared onto microscope slide to make a film. The blood films were fixed with methanol, stained with Geimsa at pH 7.2 for 10 minutes and examined under the microscope for the presence of parasites. The parasite density was calculated for each group by comparing the parasitaemia in infected group (Group II) with those of control and those of treated mice.

### **Curative test**

The Curative test of ethanolic leaf extract of *Alstonia boonei* on another fresh infected Swiss albino mice were carried out according to the method described by Ryley and Peters, 1970. The mice were injected intraperitoneally with standard inoculums of  $1 \times 10^7$  *Plasmodium berghei* NK 65 infected erythrocytes on the first day (day 0). Seventy two hours later, thirty mice were divided into six groups of five mice per group as shown above. The groups were orally treated with 5mg/kg b.wt of chloroquine and *Alstonia boonei* leaf extract (200,400 and 800mg/kg b.wt respectively). The treatment was carried out once daily for 5 days, on each day of the treatment, blood was collected from the mice tail and smeared onto microscope slide to make thin and thick films. The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 minutes and examined microscopically to monitor the parasitaemia level. The parasite density was calculated for each group over a period of six days.

### **Hematological analysis**

The Swiss albino mice in the suppressive assay groups were sacrificed and their bloods were collected in EDTA bottles by ocular puncturing. The bloods in the EDTA bottles were assayed using BC -3200 Auto Hematology Analyzer in Lagos University Teaching Hospital in Lagos- Nigeria.

### **Determination of liver and kidney function test**

Plasma enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were determined by Randox diagnostic kits. The total protein, urea and creatinine were also determined using Randox diagnostic kits.

### ***In-vitro* antioxidant assay**

Three new healthy albino rats were scarified and their liver were removed and used for the assay. One gram of albino rat liver slice was taken in 4ml sterile PBS in flat bottom flask. H<sub>2</sub>O<sub>2</sub> at 0.5M concentration was used as an oxidant for the induction of oxidative stress in the liver slices. 100µl of

ethanolic leaf extract of *A. boonei* were added and later incubated for 1 hour at 37°C.

- Group A: 1g of untreated liver slice (negative control)
- B: 1g of liver slice + H<sub>2</sub>O<sub>2</sub> (positive control)
- C: 1g liver slice + 200mg of *A. boonei* extract
- D: 1g liver slice + H<sub>2</sub>O<sub>2</sub> + 200mg of *A. boonei* extract
- E: 1g liver slice + H<sub>2</sub>O<sub>2</sub> + 400mg of *A. boonei* extract
- F: 1g liver slice + H<sub>2</sub>O<sub>2</sub> + 800mg of *A. boonei* extract

After incubation for 1 hour, an homogenate was prepared from the slices using 10% (W/V) PBS buffer. The homogenate was centrifuged at 1500rpm for 10 minutes to clarify the debris and the supernatant was used for the antioxidant assay.

### **Estimation of Lipid peroxidative (LPO) indices**

Lipid peroxidation as evidenced by the formation of thiobarbituric acid (TBARS), malondialdehyde (MDA) and hydroperoxide (HP). These parameters were measured in the liver homogenate by the method of Niechaus and Sameulsson and Jiang *et al*, 1992.

### **Estimation of superoxide dismutase (SOD)**

The homogenate was assayed for the presence of SOD by utilizing the technique of magwere *et al* 1997 with slight modification.

### **Estimation of catalase (CAT)**

The liver homogenate was assayed for catalase colorimetrically at 620nm and expressed as μmoles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein as described by sinha.

### **Estimation of Reduced glutathione (GSH)**

Reduced glutathione (GSH) was determined in the liver homogenate using the method of Ellman.

### **Data analysis**

Data analysis was done using the Graph Pad prism computer software. Student's *t*-test and one-way analysis of variance (ANOVA) were used for comparison. A *P*-value < 0.05 was considered significant.

### **Results**

The results obtained from the phytochemicals analysis of *Alstonia boonei* extract showed the presence of some secondary metabolite like tannins, saponins, steroids, flavonoids, protein, reducing sugar and fats

and oil. (Table I). The presence of these secondary metabolites in this extract may be responsible for the anti-plasmodial activity of *Alstonia boonei*.

Table I. The phytochemical screening of *Alstonia boonei*. leaf extract.

Phytochemical analysis test	Qualitative abundance
Alkaloids	+
Tannins (Ferric chloride test)	++
Saponins (Emulsion test)	+ +
Steroids (Salkowski test)	++
Flavonoids ( Ammonium test)	++
Protein (Million test)	+
Reducing Sugar (Fehling solution)	++
Fat and Oil (Stain test)	+

(+) present at low levels and (++) present at moderate levels.

**Table 2:** Suppressive and curative test showing the effect of chloroquine and *Alstonia boonei* ethanolic leaf extract against *Plasmodium berghei* NK65 infected Swiss mice.

Treatment	Dose (mg/kg)	Parasite Density for suppressive	(%) growth Inhibition for suppressive test	Parasite Density for curative test	(%) growth Inhibition for curative Test
Group I	NHC	NIL	NIL	NIL	NIL
Group II	NC	169982.52	00.00	1701652.21	00.00
Group III	5mg/kg (PC)	NIL from 3 <sup>rd</sup> day**	100**	NIL from 3 <sup>rd</sup> day**	93.1**
Group IV	200mg/kg	105519.73*	37.9	989618.80*	41.8
Group V	400mg/kg	110947.14*	34.7	964840.43*	43.3
Group VI	800mg/kg	92181.30*	45.8	1062974.5*	37.5

NHC=Normal healthy control group, NC=Negative control, PC=Positive control.

\*Significantly different from the NC at P<0.05, \*\* highly significant difference from the NC at P<0.05.

The ethanolic leaf extract of *A. boonei* caused 37.9%, 34.7% and 45.8% suppression in parasitaemia of *P.berghei* NK65 infected mice at 200, 400 and 800mg/kg body weight respectively (Table 2), while Chloroquine, a standard anti-malarial drug used exerted 100% suppression at 5mg/kg body weight. The curative test shows that the different concentration of the extract of *A. boonei* exert a growth inhibition of 41.8%, 43.3% and 37.5% at 200, 400, 800mg/kg body weight of the extract respectively while Chloroquine, the standard drug, cleared the parasites by 93.1% at 5mg/kg body weight.

**Table 3:** Suppressive effect of ethanolic leaf extract of *Alstonia boonie* and chloroquine on hematological parameters of Swiss albino mice infected with *P. berghei* NK65.

Hematological Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
WBC (X 10 <sup>9</sup> /L)	*7.6 ±1.1*	14.10 ±2.10	9.10 ±0.6*	10.5 ±1.03*	11.4 ±0.20*	10.4 ±0.23*
PCT (%)	0.458 ± 0.059	0.322 ± 0.071	0.391 ± 0.068	0.371 ± 0.035	0.501 ± 0.133	0.317 ± 0.051
MPV (fL)	6.6 ± 0.2	6.9 ± 0.2	6.7 ± 0.10	7.0 ± 0.10	6.9 ± 0.2	7.0 ± 0.2
PDW	14.20±0.10	14.50±0.20	14.1±0.2	14.3±0.20	14.90±0.30	14.60±0.20
HGB (g/dl)	14.3±1.60*	8.7±1.2	10.6±1.20*	12.10±0.20*	11.60±0.3*	13.4±0.4*
RBC (X 10 <sup>12</sup> /L)	9.10±0.5*	6.80 ± 0.4	8.20±0.43*	8.30±0.23*	8.68±0.22*	8.7±0.40*
HCT (%)	48.3±0.8*	32.2±6.5	44.1±0.4*	41.2±0.9*	40.11±2.1*	42.7±5.4*
MCV (fL)	54.22±3.4	53.30±3.2	54.10±2.4	52.11±0.50	52.00±0.30	52.20±0.70
MCH (Pg)	15.7±0.7	14.8±0.4	15.0±0.4	14.70±0.60	13.90±0.90	14.90±0.50
MCHC (g/dL)	31.10±0.20	29.10±1.20	31.40±0.30	29.00±0.20	27.23±0.37	28.80±0.23
RDW-CV (%)	17.70±0.50	18.10±0.10	17.90±0.20	17.70±0.41	17.60±0.40	17.20±0.22
RDW-SD (fL)	30.40±0.20	31.40±0.50	31.10±0.30	32.20±0.40	30.80±0.30	30.60±1.10

Values are expressed in mean ± SD of five mice in each group. \* indicate Significant difference when compared to negative control (P <0.05). WBC: White blood cell; PCT: Plateletcrit ; MPV: Mean platelet volume; PDW: Platelet Distribution Width; HGB: Hemoglobin Concentration; RBC: Red Blood Cell; HCT: Hematocrit ; MCV: Mean corpuscular volume; MCH: Mean Cell hemoglobin; MCHC: Mean Cell hemoglobin concentration; RDW–CV: RBC distribution width–coefficient of variation; RDW–SD: RBC distribution width–standard deviation.

The total WBC counts of the treated mice were significantly lowered following the administration of chloroquine and the extract of *Alstonia boonie*. The mean HGB, RBC and HCT values of the infected untreated mice (group II) were significantly ( P<0.05) lowered when compared to group I and all other groups ( Table 3). The other Hematological parameters show no significant differences between the untreated group and other groups.

**Table 4:** Determination of Plasma Total protein, Liver and Kidney function test of uninfected group and infected groups treated with chloroquine and the extract.

Hematological Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
AST (U/L)	5.20 ±1.50*	11.10 ±4.0	6.20 ± 2.10*	8.10 ±1.20*	8.00 ±1.0*	9.60 ±1.4*
ALT (U/L)	4.50±1.00*	17.10±5.10	6.80±1.10*	6.00±2.00*	8.80±1.20*	9.00±1.20*
ALP (U/L)	66.30±2.10*	109 ±4.30	68.40±1.40*	70.10±5.40*	68.20±4.2*	73.80±5.40*
GGT (U/L)	14.40 ± 3.2*	45.4 ±3.2	18.20±5.30*	32.0±2.30*	24.20±2.0*	28.10±2.1*
Creatinine (mg/dl)	0.81 ± 0.020*	1.71 ± 0.15	1.25 ± 0.20*	1.81 ± 0.240	1.90 ± 0.30	2.20 ± 0.21
Urea (mg/dl)	20.10±4.20*	29.50±1.50	24.5±2.30*	30.20±1.80	29.40±2.20	31.10±3.10
Total protein (g/l)	76.80±4.00*	58.50±5.60	67.10±3.20*	65.20±2.20*	64.20±3.4*	68.40±2.10*

\* indicate Significant difference (P <0.05) when compared to negative control animals.



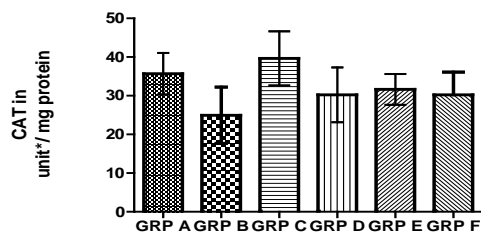


Figure 1: The effect of *A. boonei* on the activities of CAT in liver homogenate induced with oxidative stress using H<sub>2</sub>O<sub>2</sub>.

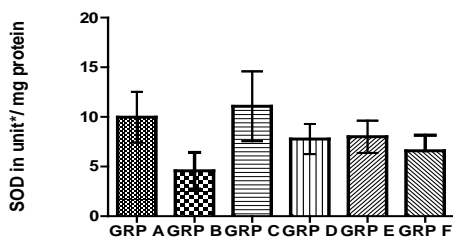


Figure 2: The effect of *A. boonei* on the activities of SOD in liver homogenate induced with oxidative stress using H<sub>2</sub>O<sub>2</sub>.

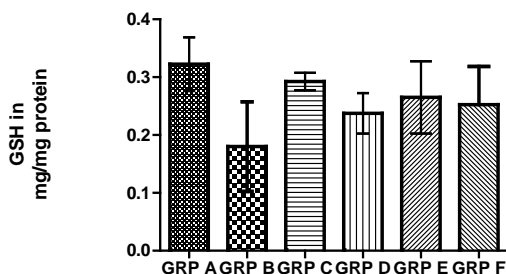


Figure 3: The effect of *A. boonei* on the level of GSH in liver homogenate induced with oxidative stress using H<sub>2</sub>O<sub>2</sub>.



Figure 4: The effect of *A. boonei* on the levels of lipid peroxidation indices in liver homogenate induced with oxidative stress using H<sub>2</sub>O<sub>2</sub>.

## Discussion

The presence of pharmacologically active phytochemicals like steroids, reducing sugar, saponins, tannins, protein, flavonoids , fats and oil have been shown to be present in the extract ( Table I). ). The presence of these secondary metabolites in *A. boonei*, may be responsible for their anti-*plasmodium* activity. Anti-plasmodial screening of plant substances have been shown to be caused by alkaloids, terpenes and flavonoids (Philipson, 1990, Milliken, 1997 and Christensen, 2001 ). These compounds could be acting singly or in synergy with one another to exert the anti-plasmodial activity observed in this study. It is evident by these findings that *A. boonei* possessed anti-plasmodium activity justifying its usage in the management of malaria in Nigeria. David, 2004 and Okokon, *et al.*2008 showed clearly that Saponin, flavonoids and tannins have been suggested to act as primary antioxidant or free radicals scavengers that can counteract the oxidative damage induced by malaria parasite.

Toxicity test shows that no death occurred at all the dose level used which shows that the extract does not have any toxic effect that can lead to the death of the mice. The median lethal dose LD<sub>50</sub> was estimated to be  $\geq$  5000mg/Kg body weight. Behavioural signs of toxicity like stretching, salivation, paw licking, severe reduced activities and respiratory distress were observed.

*Plasmodium berghei* parasite is used in predicting treatment outcomes of any suspected antimalaria agent due to its high sensitivity to chloroquine making it the appropriate parasite for this study (peter, 1998 and David, 2004). *Plasmodium berghei* has been used in studying the activity of potential antimalarials in mice (Pedronic,et al.2006 ) and in rats (English, et al 1996 ). It produces diseases similar to those of human plasmodium infection (Kumar at al,2006 and Peter,1998 ).

Suppressive test is a standard test commonly used for anti-malarial screening and the determination of percent inhibition of parasitaemia is the most reliable parameter. There were significant decrease ( $P < 0.05$ ) in parasite density in the treated group (group III to VI) compared to the untreated group (group II). Chloroquine drug used in this study exerted 100% suppression at 5mg/kg body weight (Table 2). Kiseko *et al*, 2000 showed that when a standard anti-malarial drug is used in mice infected with *P.berghei*, it suppresses the parasitaemia to a non-detectable level which is in line with our study. *A.boonie* ethanolic extract caused 37.9%, 34.7% and 45.8% suppression in parasitaemia of *P.berghei* infected mice at 200, 400 and 800mg/kg body weight respectively. The significant decrease in parasitaemia observed in this study was dose dependent.

Ethanolic leaf extract of *Alstonia boonie* showed marked anti-malarial effect in dose seeming fashion from the percentage parasitaemia

computed after carrying out curative test. The curative test showed that only Chloroquine (standard drug) cleared the parasites by 93.1% while the different concentration of the extracts exert a growth inhibition of 41.8%, 43.3% and 37.5% at 200, 400, 800mg/kg body weight of the extract respectively (Table 2).

Studies have showed that hematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals (Sexena ,*et al* 2011 and Ohaeri, 2011). WBC functions in the body defense against foreign bodies and this is often achieved through leucocytosis and antibody production (Marieb, 1995). There were significant increases

( $P < 0.05$ ) in the WBC count in group II compared to group III and all the groups treated with *A.boonie* extract (Table 3). Leukocytosis observed in group II may be due to bone marrow tumor, leukemia, tissue damage, and inflammatory disease of the mice infected with *P.berghei* NK65. The values of parasitaemia were significantly high ( $P<0.05$ ) in the treated group (group III to VI) compared to the healthy group because some of the parasites were still present in the mice. There were significant increase ( $P<0.05$ ) in HGB, RBC and HCT of the group treated with Chloroquine and *A.boonie* extract compared to the untreated infected mice. The extracts prevented a drastic reduction in HGB, RBC and HCT values, features signifying severe anemic conditions. The hemolysis of blood may be due to non-immune destruction of parasitized red cells in case of high parasitemia or immune mediated destruction of parasitized cell as well as non-parasitized red cells because the changes in the red cell antigen structure brought about by the parasitic invasion stimulate the production of antibodies against the red cell. This triggers immune immediate red cell lysis. This study is in accordance with the report of Aleksandro, *et al* 2009. They observed that anemia is characterized by decreased values of HGB, RBC and HCT Gavigan *et al.*, 2001 observed that the growing parasite consumes and degrades the intracellular proteins which are mainly hemoglobin, this may account for the decreased in Hemoglobin.

There were also significant increase ( $P<0.05$ ) in HGB, RBC and HCT of the healthy mice (group I) compared to the group treated with chloroquine and *A.boonie* extract (group III-VI ) respectively. This may be due to the destruction of RBC and HGB by the *P. berghei* during treatment. There were no significant change ( $P<0.05$ ) in the MPV, PCT, MCV, MCH, MCHC, PDW, RDW-CV.and RDW-SD values in the entire experimental groups

Table 4 shows that there is a significant increase ( $P>0.05$ ) in plasma AST, ALT, ALP and GGT in the untreated mice compared to other treated mice. This shows that the mice in group I have liver impairment or

hepatocellular damage compared to other mice in other groups. Increase in the serum levels of AST and ALT (especially ALT) are reported to be associated with liver damage (Mukherjee, 2003). Halim, *et al*, 1997, Momoh *et al*, 2014, Momoh and Manuwa, 2014 showed clearly that increase in plasma ALP and GGT levels is associated with liver damage. There were significant increase ( $P>0.05$ ) in the plasma creatinine and Urea values obtained in the infected untreated group compared to group I, III, IV and V. Mice treated with 800mg/kg body weight of the extract causes significant increase in creatinine and urea values compared to the untreated infected mice. This is an indication that *Alstonia boonei* leaf extract may be nephrotoxic at high dose and could cause kidney damage. There were significant decrease ( $P<0.05$ ) in the plasma total protein in group II compared to other groups. This may be due to the reduction in protein synthesis. Since malaria parasite causes the destruction of cells that are responsible for protein synthesis. This finding agrees with an earlier report that showed that chronic infections and autoimmune diseases may lead to reduced protein synthesis.

Liver is the major organ used for removing xenobiotic substances from the body and as such is subjected to many substances causing oxidative stress. In the in-vitro antioxidant assay, there were a significant reduction ( $P<0.05$ ) in activities of CAT and SOD in liver homogenate in group B compared to group A, C, D, E, F (Figure I and 2). The reduced levels of antioxidant enzymes (SOD and CAT) in group B liver homogenate clearly indicate hepatocytes necrosis due to production of reactive oxygen species. Group A, C, D, E and F have higher GSH values ( $P<0.05$ ) compared to group B (Figure 3). Low GSH value has been implicated with oxidative stress. MDA values significantly increase ( $P<0.05$ ) in the liver homogenate of group B compared to group A, C, D, E and F (Figure 4). This indicates hepatic injury as a result of oxidative stress caused by  $H_2O_2$ . Increase in MDA value indicates increased in lipid peroxidation which could have resulted from the depletion of GSH concentration.

## Conclusion

The result obtained from this study reveals that 200, 400 and 800mg/kg body weight of *Alstonia boonie* ethanolic leaf extract suppresses *Plasmodium berghei* NK 65 and could be used in the management of malaria. The extract has positive effect on some biochemical parameters and may cause kidney damage at high dose.

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