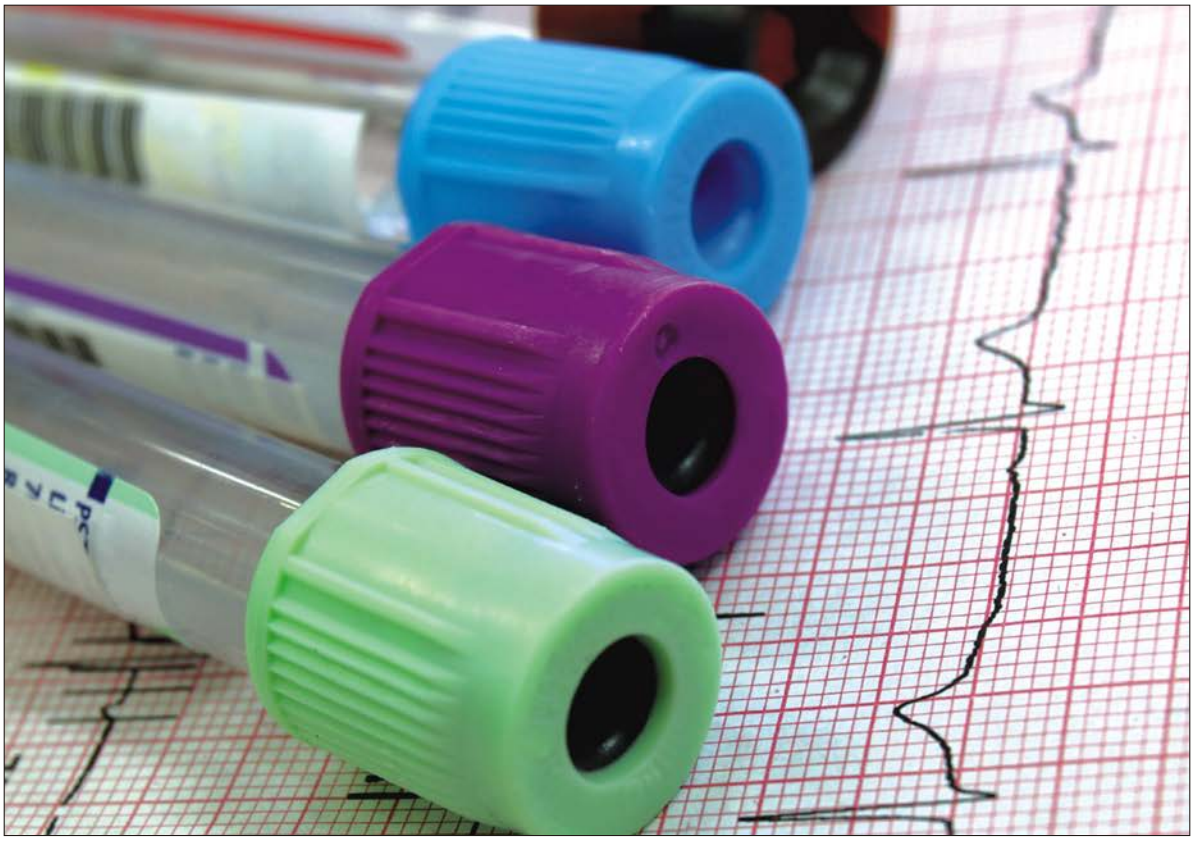

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Effect of the Aqueous Extracts of *Alstonia boonei* on the Haematological Profiles of Mice Experimentally Infected with the Chloroquine-Sensitive Strain of *Plasmodium berghei* NK-65

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ABSTRACT

The anti-malaria effect of the aqueous extracts of *Alstonia boonei* was assessed and its influence on hematological profiles in treated and untreated mice. It is clear that *Alstonia boonei* showed marked antimalaria effects in a dose seeming fashion from the percentage chemosuppression and percentage parasitemia computed. The hematological indices of the mice assessed in this study includes the levels of White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), platelets, lymphocytes and neutrophils of the different groups of mice. The untreated group of mice infected with the chloroquine -sensitive strain of *Plasmodium berghei* recorded a significant ($p<0.05$) reduction in PCV, Hb, RBC, MCV and neutrophils observably from day 8-14 post infection while lymphocytes, WBC and platelets counts increased significantly ($p<0.05$) from day 8-14 postinfection in infected, untreated mice. Administration of the aqueous extracts of *A. boonei* at the different dosages 100, 200, 400 and 800 mg kg^{-1}, respectively to mice infected with *P. berghei* resulted in the normalization of hematological indices in the groups of mice infected and treated with the plant extracts when compared with the data obtained for the experimental control groups of mice. Although, it was observed that the MCV and MCHC values did not vary significantly in the treated groups when compared with experiment control groups. However, the plant extract indeed possess anti-malaria effect and our data on its influence on blood profiles during infection and treatment clearly illustrates its protective effect in malaria infection.

Key words: *Alstonia boonei*, aqueous extracts, anti malaria effect, *Plasmodium berghei*, haematological profiles

INTRODUCTION

The annual worldwide incidence of malaria exceeds 600 million clinical cases caused by the two most common malaria species, *Plasmodium falciparum* and *P. vivax* (Mendis *et al*., 2001; Snow *et al*., 2005; Tan *et al*., 2011), leading to 1.1-2.7 million deaths attributable primarily to *P. falciparum* (WHO, 2008). Malaria causes great suffering among people living in tropical and subtropical regions, with a disproportionate impact on infants, children and pregnant women (Abdullah *et al*., 2007; Dharmadasa *et al*., 2012).

Malaria is endemic in at least 87 countries, notably in a greater part of sub-Saharan Africa and large areas of Latin America, South Asia, Southeast Asia and the Western Pacific, currently inhabited by at least 40% of the world's population, placing 2.5 billion people at risk (Guerra *et al*., 2008; Hay and Snow, 2006).

The development of a malaria vaccine represents one of the most important scientific and public health enterprises at this time (Epstein *et al*., 2007; Kapoor and Banyal, 2011). Attempts began early in the twentieth century (Desowitz, 1991) but despite tremendous advances in the understanding of the parasite and the host immune response, the goal remains elusive.

Plant extracts have been very useful sources of medication for various disease conditions (Ataman *et al*., 2006; Gill *et al*., 2012). The use of medical plant extracts which are rich sources of natural inhibitors and pharmacologically active compounds can be very promising especially in the area of chemotherapeutic-based approaches against malaria (Gatsing *et al*., 2010).

A. boonei De Wild (Apocyanaceae) is a medicinal plant used extensively in West and Central Africa for the treatment of malaria, fever, intestinal helminths, rheumatism, hypertension (Terashima, 2003; Betti, 2004; Abel and Busia, 2005).

The antimalaria potential and insecticidal activity of *A. boonei* has been reported recently (Iyiola *et al*., 2011; Oigiangbe *et al*., 2007).

This study reports the anti-malaria effects of the aqueous extracts of *A. boonei* and the influence of treatment with this plant extracts on blood profiles of laboratory mice during infection and treatments.

MATERIALS AND METHODS

Laboratory mice: Albino mice weighing between 20-30 g were used for this study. The mice were obtained from Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria-Nigeria and were housed in the experimental animal house of Kogi State University, Anyigba-Nigeria and were fed with formulated feeds from vital feeds, Jos, Nigeria and water was administered *ad libitum*. The guide for care and use of laboratory Animals (1996) of the Institute of Laboratory Animal Research (ILAR) Commission on life Science, National Research Council was duly followed.

Plant material: The stem bark of *A. boonei* was collected from the wild in Anyigba, Kogi State, Nigeria and was identified at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria. The specimen was documented and assigned voucher number 9001.

Plant extracts preparation: Fresh samples of the stem bark of *A. boonei* were air dried in the laboratory at room temperature and pulverized to fine powder. Exactly 400 g of the fine powder obtained was percolated in 1600 mL for 72 h after which it was filtered. The filtrate collected evaporated to dryness using a temperature-regulated water bath pre-set at 40°C to yield the extract concentrate. The extract was stored at 4°C prior to use.

Parasite strain: The chloroquine-sensitive strain of *P. berghei* was obtained from the Institute of Malaria Research, Lagos, Nigeria and the strain was maintained in our laboratory for the period of this study by serial blood passage from mouse to mouse.

Infection and parasitaemia determination: Ten healthy albino mice were intraperitoneally infected by injection of 0.2 mL of infected blood diluted with sodium citrate solution to contain about 1×10^6 parasitized red blood cells (pRBC) of virulent *P. berghei* parasites.

Blood samples were collected by bleeding via the tail vein of *P. berghei*-infected mice and thin blood smears were made on microscope slides, fixed in methanol and stained with 10% Giemsa solution (Merck, Tokyo, Japan). The percentage parasitemia was determined by counting the percentage pRBC for at least ten different fields. At peak parasitaemia, blood was collected in heparinized syringe by cardiac plexus puncture, diluted as mentioned earlier and used for the infection of mice used in this study.

Animal groupings for infection and treatment: The mice were randomly divided into 8 groups (A-H) of ten (10) mice each. Animals in groups A and B were not inoculated with parasite while those in groups C-H were infected from the same donor mice.

The aqueous extract of *A. boonei* was formulated in distilled water corresponding to the dosages 100, 200, 400 and 800 mg kg^{-1} b.wt. of mice and the standard antimalarial Chloroquine (5 mg kg^{-1} b.wt. of mice) were also prepared.

Administration of the test extract and standard drugs were done by oral intubations and lasted for 4 days at a single dose per day according to the following groupings:

- Group A (uninfected mice): Received a proportionate volume of distilled water
- Group B (infected mice): Received a proportionate volume of distilled water
- Group C (infected mice): Received chloroquine diphosphate salt (5 mg kg^{-1} b.wt.)
- Group D (infected mice): Received *A. boonei* extract at a dose of 100 mg kg^{-1} b.wt.
- Group E (infected mice): Received *A. boonei* extract at a dose of 200 mg kg^{-1} b.wt.
- Group F (infected mice): Received *A. boonei* extract at a dose of 400 mg kg^{-1} b.wt.
- Group G (infected mice): Received *A. boonei* extract at a dose of 800 mg kg^{-1} b.wt.

Sample collection and analysis: Blood films were made from blood collected from the tail vein of all infected mice and the percentage parasitemia was obtained by microscopic examinations of Giemsa-stained thin blood smears.

The percentage chemosuppression was also done by subtracting the average percent parasitemia in the treated group from the average percent parasitemia in the control group (infected untreated), the value obtained was expressed as a percentage of the average percentage parasitemia in control group (infected untreated).

Haematological analysis: From the respective groups above, four mice were sacrificed from each of the groups on days 3, 8 and 14 post-infection and blood samples collected in heparinized sample bottles were submitted for haematological examination.

The haematological analysis was carried out using an automated hematological analyzer (SYSMEX KX21, SYSMEX Corporation, Japan). Red Blood Cell count (RBC), Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Mean Corpuscular Volume (MCV), White Blood Cell count (WBC), percentage neutrophils, percentage lymphocytes and platelet count were determined.

Statistical analysis: The data obtained were expressed as Mean+standard deviation were analyzed statistically using one-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test. Data from the test groups were compared with their respective controls and differences at $p<0.05$ were regarded as significant.

RESULTS

The anti-malaria effect of the aqueous extracts of *A. boonei* was assessed and its influence on hematological profiles in treated and untreated mice. It is clear from Table 1 and 2 that the test extract, *A. boonei* showed marked antimalaria effects in a dose seeming fashion as shown from the percentage chemosuppression and percentage parasitemia computed.

Chloroquine which was administered at 5 mg kg^{-1} suppressed parasitemia to undetectable levels (% chemosuppression 100) (Table 1).

The hematological indices assessed in this study includes the levels of White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets, lymphocytes and neutrophils of the different groups of mice are shown in Table 3 and 4.

The untreated group of mice infected with the chloroquine sensitive strain, *P. berghei* recorded a significant ($p<0.05$) reduction in PCV, Hb, RBC, MCV and neutrophils observably from day 8-14 post infection (Table 3, 4). While lymphocytes, WBC and platelets counts increased significantly ($p<0.05$) from day 8-14 postinfection in infected but untreated mice.

When the aqueous extracts of *A. boonei* was administered at the different dosages 100, 200, 400 and 800 mg kg^{-1} to mice infected with *P. berghei*, it was observed that the net effect of this extracts was such that it appears to normalize hematological indices in the groups of mice infected and treated with the plant extract when compared with the data obtained for the experimental control groups of mice (Table 3, 4).

Table 1: Effect of treatment with the aqueous extract of *Alstonia boonei* (De wild) on parasitemia profiles in mice infected with the Chloroquine- sensitive strain of *Plasmodium berghei* NK-65

	Average parasitemia (%)						
	(Day post infection)						
Groups	4	5	6	7	8	14	21
A	-	-	-	-	-	-	-
B	7.68	8.42	12.64	16.20	21.50	33.30	54.20
C	3.60	1.90	1.35	1.26	1.10	0.35	0.00
D	7.00	8.20	10.12	10.90	11.33	10.30	10.10
E	7.22	7.85	9.06	10.20	9.11	7.02	3.43
F	7.01	7.03	8.45	9.71	7.05	2.30	0.00
G	6.80	7.05	8.80	10.10	7.80	3.31	4.02

Table 2: Percentage chemosuppression of the aqueous stem bark extracts of *Alstonia boonei* (De wild) in mice infected with the Chloroquine- seusitive strain of plasmodiun berghei (NK-65)

	Chemosuppression (%)						
	(Days post infection)						
Groups	4	5	6	7	8	14	21
A	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-
C	53.13	77.43	89.32	92.22	94.88	98.94	100.00
D	8.85	2.61	19.94	32.72	47.30	69.08	81.37
E	5.99	6.77	28.32	37.04	57.63	78.93	93.67
F	8.72	16.51	33.15	40.06	67.21	93.10	100.00
G	11.46	16.02	30.38	37.65	63.72	90.06	92.58

Table 3: Hematological profiles of mice infected with chloroquine sensitive *Plasmodium berghei* NK-65 treated groups (Group B-G), compared with uninfected experimental control group A

Days/groups	WBC ($\times 10^9$/L)	Platelets ($\times 10^9$/L)	Lymphocytes (%)	Neutrophils (%)
Day 3				
A	07.83±0.42	680.07±0.99	35.77±0.44	63.01±0.22
B	10.15±0.80	689.38±0.28	37.82±0.23	58.26±0.14
C	09.57±0.55	678.10±0.58	38.31±0.81	61.92±0.36
D	10.30±0.78	685.11±0.17	35.80±0.45	59.55±0.77
E	09.90±0.18	680.33±0.95	35.88±0.18	60.18±0.72
F	09.85±0.44	673.82±0.51	36.51±0.31	61.15±0.56
G	09.92±0.91	670.90±0.25	36.08±0.28	61.00±0.19
Day 4				
A	7.90±0.88	4.05±0.52	6.29±0.23	6.10±0.17
B	4.05±0.52	528.22±0.32	63.20±0.31	37.41±0.66
C	6.29±0.23	598.41±0.49	45.84±0.21	57.81±0.92
D	6.10±0.17	544.12±0.38	55.28±0.91	40.53±0.20
E	5.86±0.31	549.38±0.22	53.11±1.20	43.33±0.17
F	5.11±0.38[a]	565.71±0.18	48.80±1.01	46.62±0.86
G	5.70±0.22	558.11±0.25	51.14±0.33	42.11±0.23
Day 14				
A	7.94±0.91	676.24±0.96	37.82±0.50	61.81±0.82
B	2.62±0.11	228.32±0.18	65.10±0.28	60.02±0.61
C	6.38±1.01	654.24±0.28	42.05±0.11	60.02±0.61
D	4.08±0.24	485.50±0.71	52.11±0.81	40.80±0.12
E	5.01±0.48	490.13±0.81	50.80±0.55	44.45±0.18
F	5.52±0.76	579.52±0.13	45.41±0.61	59.43±0.22
G	5.81±0.81	570.05±0.62	48.13±0.12	56.14±0.41

It was also observed that the MCV and MCHC values did not differ significantly in the treated groups when compared with experiment control groups (Table 4).

DISCUSSION

Hematological and biochemical indices have been reported to be a reliable parameter for the assessment of the health status of animals (Saxena *et al.*, 2011; Obianime and Aprioku, 2011; Ohaeri and Eluwa, 2011). Also, the severity of hematopoietic changes depends on the specie and physiological state of the host and acuteness or chronicity of the infection (Jenkins and Facer, 1985; Stephen, 1986). Our data shows that the most significant changes in the hematological profiles were evident as parasitemia bouts increases.

Also, hematological profiles were normalized in the groups of mice infected and treated with effective dosages of *A. boonei* except for the MCV and MCHC values which did not vary significantly in the different groups of experimental mice, an indication of a typical feature of normocytic-normochromic anemia (Menezes *et al.*, 2004).

The extract prevented a drastic reduction in PCV, RBC and Hb values, features typical of the infection, malaria. This observation is supported by a report stating that anaemia is characterized by decreased values of RBC, Hb and hematocrit (Aleksandro *et al.*, 2009). In addition, we have also observed a decline in WBC counts in the different experimental groups of infected and treated mice when compared to experimental control mice. This observation is corroborated by a previous report

Table 4: Hematological profile of mice infected with chloroquine sensitive *Plasmodium berghei*, treated groups (Group B-G) compared with uninfected, experimental control group A

Days/Gps	PCV	Hb (g L^{-1})	RBC ($\times 10^{12}$/L)	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
Day 3						
A	41.98±0.33	13.82±0.42	7.85±0.92	55.00±0.80	18.44±0.65	33.84±0.22
B	38.20±0.54	13.32±0.48	7.02±0.61	53.30±0.21	18.30±1.10	34.00±0.80
C	41.85±0.68	13.89±0.33	7.61±0.40	56.75±0.90	18.98±0.84	33.60±0.70
D	40.22±0.40	13.41±0.46	7.45±0.90	55.10±0.19	18.42±0.32	33.15±0.33
E	40.51±0.34	13.55±0.41	7.50±0.21	55.45±0.51	18.50±0.58	33.21±0.55
F	41.11±0.75	13.75±0.52	7.55±0.81	56.12±0.30	18.65±0.62	33.40±0.61
G	41.32±0.41	13.48±0.55	7.52±0.88	56.41±0.17	18.60±0.91	33.10±0.64
Day 8						
A	41.88±0.43	13.79±0.46	7.80±0.33	55.60±0.58	18.50±0.29	34.22±0.80
B	20.12±0.64	08.02±0.31	4.60±0.54	53.00±0.75	16.91±0.80	33.80±0.32
C	34.08±0.58	12.26±0.81	6.62±0.82	54.81±0.80	18.02±0.99	33.90±0.27
D	23.88±0.46	08.10±0.41	4.98±0.21	53.87±0.28	17.10±0.61	33.10±0.48
E	25.40±0.60	11.06±0.62	5.20±0.54	54.50±0.51	17.28±0.30	33.05±0.31
F	30.18±0.63	12.10±0.28	6.10±0.81	56.50±0.40	17.94±0.71	33.18±0.87
G	30.62±0.55	12.01±0.90	5.80±0.44	56.01±0.83	17.80±0.40	33.09±0.45
Day 14						
A	41.09±0.67	18.25±0.82	13.70±0.31	7.77±0.94	55.81±0.12	18.40±0.75
B	32.50±0.92	36.04±0.27	04.58±0.22	3.51±0.80	38.21±0.26	09.85±0.42
C	27.08±1.02	25.15±0.30	12.92±0.50	6.96±0.51	59.00±0.91	20.02±0.83
D	32.85±0.80	29.50±0.33	08.36±0.89	3.85±0.90	49.28±0.70	17.41±0.33
E	33.02±0.32	32.40±0.41	10.06±0.25	4.40±0.52	52.03±0.90	17.80±0.60
F	33.10±0.68	30.04±0.35	12.03±0.62	6.03±0.33	56.48±0.84	18.50±0.38
G	33.20±0.32	11.56±0.81	5.24±0.55	55.52±0.32	18.00±0.20	33.15±0.13

Gps: Groups

on *Trypanosoma brucei rhodesiense* stating that the gradual decline and fluctuations of WBC counts are reflectors of persistent underlying infections (Ngotho *et al*., 2011).

We are aware of the possibility of the malaria parasite to sequester in the deep vascular beds of some vital organs even when plant extracts appear to clear parasites from the blood. Based on this, further study on tissue biopsy is ongoing which may be specific even when other parameters are inconclusive.

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