

Haematological Evaluations of the Antimalarial Activity of *Bridelia ferruginea* Benth Bark

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We investigated the antimalarial activity of the methanolic extract of *Bridelia ferruginea* benth bark at 400 mg/kg body weights in mice (*Mus musculus*) infected with chloroquine-sensitive *Plasmodium berghei* using the rane test. There was decreased in packed cell volume, RBC and Hb in infected groups from day zero to 14 with a corresponding increase in RBC of the uninfected -extract treated group ($p<0.05$). The infected – untreated showed continual decreased from day zero to 14 ($p<0.05$) compared to the infected – treated groups and the uninfected – untreated (control) group. However, decreased in MCHC was recorded by day 14 for the infected – untreated animals. WBC and lymphocytes indices revealed that there was no significant difference in all the groups by day zero, however by day 14, there was significant increased in the WBC and lymphocytes for infected – treated groups compared to all other groups ($p<0.05$). In the platelets count, by day 14 there was significant decreased in the infected – untreated group compared to others ($p<0.05$). Also, there was no significant difference in the neutrophils for the infected – extract treated, infected – chloroquine treated, control, and uninfected – extract treated groups ($p<0.05$). The haematological indices further substantiates the promising antimalarial activity of the bark extract.

Key Words: *Bridelia ferruginea*, Methanolic extract, Haematological indices, Antimalarial.

Malaria is one of the known vector-borne infectious disease caused by protozoan parasites. It's widespread is in tropical and subtropical regions including some parts of America, Asia and Africa. Every year, there are roughly 515 million cases of malaria, killing about one to three million people, with majority being young children in sub-Saharan Africa¹.

The challenges of producing an antimalarial vaccine and the global rise of chloroquine resistance in *Plasmodium falciparum* and *Plasmodium vivax*, have resulted in a pressing need for new antimalarials. The most promising targets for the development of a malaria vaccine is the *Plasmodium* liver stage. Previously, it has been repeatedly demonstrated in mice and humans that immunization with whole sporozoites attenuated by radiation or genetic modification can induce protective immune responses^{2,3}.

The inoculation of sporozoites into the dermis of a vertebrate host during the blood feeding of an infected Anopheline mosquito initiates the *Plasmodium* infection process. In essence, only about a third of these sporozoites passes the blood vessels to enter the blood stream and then travel

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to the liver⁴. The sporozoites transverse Kupffer cells, apparently being not recognized or attacked by them in order to gain access to the liver cells^{5,6,7}. However, a lot of stages of parasite development inside the liver cell are yet to be fully understood, particularly due to the poor inaccessibility and low features of these stages for detailed cellular and molecular investigations⁸.

Despite all that is known about malaria aetiology, biochemistry and immunology, very little progress has been achieved in its treatment at the community level. This burden is particularly critical in many parts of Africa, with increasing level of poverty. Several approaches have been proposed and implemented in contributing significantly to primary health care, among which the use of plant extracts has been often neglected⁹. In the past, medicinal plants have been the source of most successful antimalarial drugs such as the quinolones, the endoperoxides and the artemisinin derivatives¹⁰. However, some studies on Nigerian medicinal plants have yielded some highly active principles¹¹.

In the search for new antimalarial agents, a country like Nigeria endowed with wealth of unexplored natural resources could be the ideal place. One such plant is *Bridelia ferruginea* of the family Euphorbiaceae. It grows as a gnarled shrub and sometimes reaches the size of a tree in suitable environmental conditions^{12,13}. An aqueous extract of the leaves have been used to treat hypoglycaemia¹⁴ while the antimicrobial activity of the bark extract have also been reported¹⁵. Recently, the antimalarial activity of the methanolic bark extract has been reported in vivo at an LD₅₀ and optimum dose of 400 mg/kg body weight¹⁶.

To the best of our knowledge, pharmacological studies with respect to the haematological indices on the effect of the extract on the parasite at the blood stage have not been investigated. An highly specialized connecting tissue made up of cells and extracellular liquid medium which circulates through the heart and blood vessels supplying nutrients and carrying away waste products is the blood¹⁷. This study therefore intends to evaluate the activity of the bark extract using the haematological indices as an indicator of disease status of the animals.

MATERIALS AND METHODS

Chemicals

Absolute methanol (Riedel-de Haën) was obtained from Sigma-Aldrich Laborchemikalien GmbH, Germany. Chloroquine diphosphate salt was obtained from Sigma Chemical Company, St. Louis, Mo, USA. Other reagents used were of analytical grade and were prepared in all glass-distilled water.

Experimental Animals

Twenty five (25) adult Swiss albino mice (*Mus musculus*) with an average weight of 20 ± 2 g were obtained from the animal breeding unit of the Department of Pharmacology, University of Ibadan, Oyo state. The mice were housed in metabolic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad-libitum*. The study was conducted with approval from the University of Ilorin, animal ethics review committee in line with the Principles of Laboratory Animal Care¹⁸.

Parasite strain

A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Oyo state. Weekly blood passages in mice was carried out in order to maintained the parasites.

Plant source and Identification

The bark of *Bridelia ferruginea* benth were obtained from Idofian town of Kwara state, Nigeria in October, 2008 and were authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state.

Plant extract preparation

Fresh bark of *Bridelia ferruginea* were dried in the shade at room temperature and pulverized to powder using an electric blender. The extraction was done using the solvent methanol at ambient temperature (cold extraction) according to the method described elsewhere^{13,19}.

Animal grouping and extract administration

The animals were divided into five groups A, B, C, D, and E of five mice each. The percentage parasitaemia of the donor mouse was determined using an haemocytometer and an appropriate dilution of the infected blood in isotonic saline

was prepared. Animals in all the infected groups were inoculated intraperitoneally with 0.2ml of infected blood containing about 1×10^7 *Plasmodium berghei* parasitized red blood cells. All the animals were infected from the same donor mouse. The experiment was conducted according to the procedure described by²⁰ and an optimum dose of 400 mg/kg body weight of extract was used¹⁶.

Group A infected- extract treated (400 mg/kg body weight of the extract for 5 days 72 hrs post infection).

Group B infected- untreated

Group C infected- chloroquine treated (4 mg/kg body weight of chloroquine for 5 days 72 hrs post infection).

Group D uninfected- untreated (Control).

Group E uninfected- extract treated (400 mg/kg body weight of the extract for 5 days). Administration of all drugs was done orally with canula.

Sample collection and analyses

Tail blood of mice at days 0, 4, 8 and 14 were collected into EDTA bottles to prevent it from clotting; the samples were then used for haematological evaluations. Haematological analyses was done using the automated haematological analyzer SYSMEX KX21 (SYSMEX Corporation, Japan)²¹. Thus, red blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular

Table 1. Red Blood Cell Indices in the Experimental Animals (Day 0 and 4)

Days	Group	PCV (%)	RBC ($\times 10^{12}/L$)	Hb(g/L)	MCHC (g/dl)
Day 0	A	41.50 \pm 0.50 ^a	5.180 \pm 0.020 ^a	13.5300 \pm 0.150 ^a	33.010 \pm 0.010 ^a
	B	39.50 \pm 0.50 ^a	5.205 \pm 0.025 ^{ab}	13.0500 \pm 0.050 ^a	33.075 \pm 0.025 ^{ab}
	C	40.00 \pm 1.00 ^a	5.305 \pm 0.005 ^c	13.1950 \pm 0.195 ^a	33.065 \pm 0.065 ^{ab}
	D	39.50 \pm 0.50 ^a	5.220 \pm 0.010 ^{ab}	13.1100 \pm 0.060 ^a	33.165 \pm 0.065 ^{ab}
	E	41.00 \pm 1.00 ^a	5.255 \pm 0.035 ^{bc}	13.4700 \pm 0.340 ^a	33.155 \pm 0.025 ^{ab}
Day 4	A	37.50 \pm 1.50 ^a	5.400 \pm 0.050 ^{ab}	12.605 \pm 0.345 ^a	33.525 \pm 0.125 ^a
	B	36.00 \pm 2.00 ^a	4.930 \pm 0.010 ^a	12.050 \pm 0.450 ^a	33.215 \pm 0.025 ^a
	C	36.50 \pm 2.50 ^a	5.035 \pm 0.015 ^{abc}	12.075 \pm 0.785 ^a	33.285 \pm 0.015 ^a
	D	37.00 \pm 2.00 ^a	5.220 \pm 0.020 ^{de}	12.350 \pm 0.710 ^a	33.525 \pm 0.065 ^a
	E	40.00 \pm 1.00 ^a	5.270 \pm 0.040 ^e	13.315 \pm 0.145 ^a	33.500 \pm 0.375 ^a

Results are means of 4 determinations SEM. Means along the same column with different superscript are significantly different ($p < 0.05$). A= infected -extract treated; B= infected -untreated; C= infected -chloroquine treated; D= uninfected -untreated (control); E= uninfected -extract treated

Table 2. Red Blood Cell Indices in the Experimental Animals (Day 8 and 14)

Days	Group	PCV (%)	RBC ($\times 10^{12}/L$)	Hb(g/L)	MCHC (g/dl)
Day 8	A	29.0 \pm 01.00 ^b	3.440 \pm 0.040 ^b	9.800 \pm 0.200 ^b	33.760 \pm 0.230 ^b
	B	24.50 \pm 1.50 ^b	2.350 \pm 0.140 ^a	7.870 \pm 0.290 ^a	33.040 \pm 0.350 ^a
	C	32.50 \pm 0.50 ^b	4.160 \pm 0.040 ^d	10.790 \pm 0.190 ^c	33.500 \pm 0.070 ^b
	D	39.00 \pm 1.00 ^b	5.275 \pm 0.025 ^e	13.135 \pm 0.095 ^d	33.665 \pm 0.285 ^b
	E	38.50 \pm 0.50 ^b	6.040 \pm 0.010 ^f	13.020 \pm 0.020 ^d	33.775 \pm 0.135 ^b
Day 14	A	29.00 \pm 1.00 ^c	4.125 \pm 0.035 ^d	9.850 \pm 0.050 ^d	33.690 \pm 0.460 ^b
	B	11.75 \pm 0.25 ^a	1.080 \pm 0.010 ^a	3.755 \pm 0.075 ^d	29.960 \pm 0.040 ^a
	C	33.50 \pm 1.50 ^d	4.520 \pm 0.030 ^e	10.920 \pm 0.440 ^e	33.025 \pm 0.025 ^b
	D	40.00 \pm 1.50 ^e	5.215 \pm 0.035 ^f	13.280 \pm 0.280 ^f	33.500 \pm 0.130 ^b
	E	39.50 \pm 0.50 ^e	6.195 \pm 0.005 ^g	13.025 \pm 0.025 ^f	33.325 \pm 0.305 ^b

Results are means of 4 determinations SEM. Means along the same column with different superscript are significantly different ($p < 0.05$). A= infected -extract treated; B= infected -untreated; C= infected -chloroquine treated; D= uninfected -untreated (control); E= uninfected -extract treated

Table 3. White Blood Cell Indices and Platelets in the Experimental Animals (Day 0 and 4)

Days	Group	Platelets (X10 ⁹ /L)	Wbc (X10 ⁹ /L)	Neutrophil (%)	Lymphocytes (%)
Day 0	A	681.00 ± 0.50 ^a	6.300 ± 1.50 ^b	56.00 ± 2.00 ^a	44.00 ± 2.00 ^a
	B	678.00 ± 1.00 ^a	6.600 ± 2.40 ^b	54.00 ± 6.00 ^a	46.00 ± 6.00 ^a
	C	676.00 ± 2.00 ^a	6.050 ± 1.25 ^b	55.00 ± 1.00 ^a	45.00 ± 1.00 ^a
	D	681.00 ± 1.00 ^a	6.400 ± 0.90 ^b	56.50 ± 1.50 ^a	43.50 ± 1.50 ^a
	E	678.50 ± 0.50 ^a	6.600 ± 0.10 ^b	57.00 ± 1.00 ^a	43.00 ± 1.00 ^a
Day 4	A	679.00 ± 0.00 ^d	9.600 ± 0.20 ^b	41.00 ± 3.00 ^a	59.00 ± 3.00 ^b
	B	674.00 ± 2.00 ^c	7.700 ± 2.30 ^{ab}	38.00 ± 4.00 ^a	62.00 ± 4.00 ^b
	C	678.50 ± 0.50 ^d	6.000 ± 2.70 ^{ab}	46.00 ± 0.00 ^a	54.00 ± 0.00 ^b
	D	682.50 ± 2.50 ^{de}	4.750 ± 0.50 ^a	58.00 ± 2.00 ^b	42.00 ± 2.00 ^a
	E	684.50 ± 0.50 ^c	6.050 ± 2.55 ^{ab}	60.00 ± 7.00 ^b	42.00 ± 2.00 ^a

Results are means of 4 determinations SEM. Means along the same column with different superscript are significantly different ($p < 0.05$). A= infected –extract treated; B= infected –untreated; C= infected –chloroquine treated; D= uninfected –untreated (control); E= uninfected –extract treated

Table 4. White Blood Cell Indices and Platelets in the Experimental Animals (Day 8 and 14)

Days	Group	Platelets (X10 ⁹ /L)	Wbc (X10 ⁹ /L)	Neutrophil (%)	Lymphocytes (%)
Day 8	A	562.503.50 ^a	7.7501.25 ^{de}	42.001.00 ^b	59.001.00 ^c
	B	560.005.00 ^a	9.0500.15 ^c	33.500.50 ^a	66.500.50 ^d
	C	680.500.50 ^b	5.5000.10 ^{bc}	55.501.50 ^c	44.501.50 ^b
	D	683.501.50 ^b	4.8500.25 ^{ab}	66.004.00 ^d	34.004.00 ^a
	E	697.002.00 ^c	5.9000.50 ^{bc}	66.500.50 ^d	33.500.50 ^a
Day 14	A	577.502.50 ^c	4.9500.05 ^a	59.500.50 ^c	40.500.50 ^b
	B	233.501.50 ^a	12.0505.05 ^b	23.001.00 ^a	77.001.00 ^d
	C	642.500.50 ^d	4.4500.25 ^a	56.001.00 ^c	44.001.00 ^b
	D	678.000.00 ^c	3.5000.70 ^a	58.500.50 ^c	41.500.50 ^b
	E	687.002.00 ^e	4.0000.70 ^a	67.500.50 ^d	32.504.50 ^a

Results are means of 4 determinations SEM. Means along the same column with different superscript are significantly different ($p < 0.05$). A= infected –extract treated; B= infected –untreated; C= infected –chloroquine treated; D= uninfected –untreated (control); E= uninfected –extract treated

haemoglobin concentration (MCHC), white blood cell count (WBC), percentage neutrophils, percentage lymphocytes and platelet count were determined.

Statistical analysis

Data are expressed as mean ± SEM. The values were statistically analyzed using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test²². Data from the test groups were compared with their respective controls and differences at $p < 0.05$ were considered significant.

RESULTS

The findings revealed that there was decreased in packed cell volume, RBC and Hb in

infected groups from day zero to 14 ($p < 0.05$). The uninfected group experienced stability in this parameter, except the increase in RBC of the uninfected –extract treated group ($p < 0.05$). However, there was no statistical difference ($p < 0.05$) of these parameters in the infected –extract treated and infected –chloroquine treated. The infected –untreated showed significant continual decreased from day zero to 14 ($p < 0.05$) compared to the infected –treated groups and the uninfected –untreated (normal) group. There was also no significant difference ($p < 0.05$) in MCHC among all the groups from day zero to day 8. However, a marked significant decreased in MCHC was recorded by day 14 for the infected –untreated animals. This was statistically significant ($p < 0.05$)

compared to all other groups (Tables 1 to 2).

Results of the WBC and lymphocytes revealed that there was no significant difference in all the groups by day zero, however by day 4 there was a sharp increase in these parameters in the infected –extract treated group and the infected untreated group. This was statistically significant compared to the other groups. On day 8, there was recorded decrease in white blood cells and lymphocytes in infected –extract treated group with a corresponding increase in the infected –untreated group ($p<0.05$). On day 14, there was a significant increase in the WBC and lymphocytes for infected –treated groups compared to all other groups ($p<0.05$) (Tables 3 and 4). In the platelets count, there was no significant difference ($p<0.05$) on day 0 to 8 for all the groups. However, by day 14 there was a sharp significant decrease in the infected –untreated group compared to others ($p<0.05$) (Table 4). There was also no significant difference ($p<0.05$) in neutrophils among all the groups at day zero. By day 4 and 8, there was a recorded significant decrease in the neutrophils for all the infected treated groups. However, by day 14 there was no significant difference in the neutrophils for the infected –extract treated, infected –chloroquine treated, normal, and uninfected –extract treated ($p<0.05$). The infected –untreated recorded a significant decrease in neutrophils compared to all other groups ($p<0.05$) (Table 4).

DISCUSSION

In malaria infection, the most pronounced changes involve the blood and the blood forming system. Anemia is commonly found as a problem related to malaria. The decrease in packed cells volume (PCV), red blood cells (RBCs) and haemoglobin in all the infected mice groups was possibly an indication of anemia. This was obvious and persisting for the infected untreated mice from day 8 to 14 of infections. The observed decrease could also be due to repeated haemolysis of infected red blood cells with massive destruction of red blood cells accounting for rapid development of anemia in plasmodial infection^{23,24}.

The increase in RBC of the uninfected –extract treated possibly connotes that the extract may have been able to stimulate increase in the rate of production of red blood cells

(erythropoiesis). Yakubu and co-workers²⁵ have reported that humoral regulator of red blood cell production is due to erythropoietin released in the kidney. The non-significant difference of the indices relating to RBC in the infected –extract treated and infected –chloroquine treated suggests that the extract compared favourably with the reference drug. This also showed that the methanolic extract of *Bridelia ferruginea* does not possess any potential of inducing anemia at the concentrations used for the number of days administered.

The marked sharp decrease in MCHC recorded by day 14 for the infected –untreated mice could be possibly due to changes in the red cell antigen structures brought about by the parasitic invasion which stimulates the production of antibodies against the red cell. This triggers the immune mediated red cell lysis²⁶ (Tables 1 and 2). The sharp increase in WBC and lymphocytes by day 4 in the infected –extract treated group and the infected untreated group could be as a result of stimulation of the immune system of the mice to fight the invasion of the malaria parasites. White blood cells particularly helps to fight infection and defend the body by phagocytosis against invading foreign organisms. They also help to produce, transport and distribute antibodies in the immune mechanisms of reaction²⁴ (Table 3).

The decrease in WBC and lymphocytes on day 8 in the infected –extract treated and infected –chloroquine treated groups could be as a reduction in the ability of the animals to respond to the malaria parasite infection. This could be an indication of high parasitaemia (Tables 3 and 4)^{16, 27}. The significant increase in WBC and lymphocytes at day 14 in the infected –treated groups was a display of a boost in the immune status of the animals due to treatment with the extract and the reference drug.

The sharp significant decrease of the platelets count in the infected –untreated group by day 14 could be possibly due to thrombocytopenia which is common in malaria infection. The observed significant decrease in the neutrophils for all the infected treated groups could possibly be due to neutropenia which is a common feature of malaria infection. The non-significant difference observed in the neutrophils count for all groups except the infected –untreated

group demonstrated a plausible increased in the neutrophils at day 14 due to stimulatory effect of thrombopoietin. This finding is in agreement with the work reported elsewhere^{24,28}.

We have previously reported the antimalarial activities of the plant at 400 mg/kg body weights in mice giving about 100% reduction in parasitaemia¹⁶. The present study has been able to substantiate our earlier findings of the antimalarial activities of the plant at the same concentration with the established mode of treatment (Rane test) using the haematological indices as an indicator of disease status in the animals.

Conclusively, the plant indeed has exhibited promising antimalarial activity confirming its traditional use in the treatment of malaria. Investigation into its *in vitro* activity against *Plasmodium* parasite is an on going study in our group.

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