

Flavonoid-Rich Fraction of *Alstonia boonei* Leaves Attenuates Haematological and Biochemical Changes Induced by *Plasmodium berghei*-Infection

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Abstract: Malaria has become a global scourge, particularly in the developing nations of the world. However, efforts to combat the malaria scourge have been hampered by the ability of plasmodium species to develop resistance to conventional anti-malarial drugs such as chloroquine and artemisinin. This necessitates the search for newer anti-malarial agents from other sources such as medicinal plants. This study investigated the anti-malarial activity of flavonoid-rich fraction of *Alstonia boonei* leaves (FRFABL) on haematological and various biochemical changes induced by *plasmodium berghei*-infected mice following a 5-day suppressive test. Forty eight (48) adult albino Wistar mice of average body weight of 30 ± 38 g were used for this study; 18 for the toxicity study while 30 mice consisting of six groups of five mice each were used for the anti-malarial study. Groups 4-6 were infected malaria and treated with 200, 400 and 600 mg/kg body weight of FRFABL respectively; group 3 mice was infected malaria and treated with 140 mg/kg body weight of Coartem® (standard anti-malarial drug); group 2 was infected malaria and no treatment (positive control) while group 1 was not infected and served as normal control. Quantitative phytochemical screening of FRFABL revealed high amounts of phenols, alkaloids and flavonoids. Tannins and terpenoids were detected in moderate amounts, whereas steroids and saponins were found in smaller amounts. After oral administration of 5000 mg/kg body weight, the toxicity test revealed that FRFABL was not toxic. It was observed that the percentage parasitemia of mice in Group 2 was significantly ($p < 0.05$) higher when compared to mice in parasitized and treated groups. When compared to the positive control (group 2), the treated groups showed significant ($p < 0.05$) increases in packed cell volume (PCV), haemoglobin (Hb) concentrations, and red blood cell (RBC) count, but the white blood cell (WBC) count decreased significantly ($p < 0.05$) in the treated groups when compared to the positive control (group 2). Similarly, FRFABL treatment of infected mice significantly ($p < 0.05$) restored some malaria-modified biochemical parameters of *plasmodium berghei*-infected mice. The results showed that FRFABL possesses good anti-malarial properties and will serve as an excellent anti-malarial agent.

Keywords: FRFABL, Malaria, Biochemical Parameters, FRFABL *Plasmodium berghei*

1. Introduction

Malaria is one of the most serious vector-borne diseases, resulting from the transmission of *Plasmodium* sporozoites from infected Anopheles mosquitoes to human beings [1]. Half of the world's population is currently at danger of malaria, according to the World Health Organization (WHO); in 2019, 229 million cases of malaria were reported, with 409,000 deaths [2]. Malaria continues to be a significant concern for healthcare providers; however, it may be controlled with early detection and immediate treatment [3, 4]. Among the available medications, chloroquine (CQ) was synthesized from quinoline and became the most widely used anti-malarial drug by the 1940s due to its low cost and wide range of efficacy [5, 6]. Resistance to this drug on the other hand, grew rapidly throughout Latin America, Asia and Africa [7, 8]. With a decrease in case incidence (i.e. cases per 1000 population) from 2000 to 2019, there was an upsurge in the number of cases during the COVID-19 pandemic [9]. Similarly, the number of deaths decreased from 2000 to 2019, but increased by 12% in 2020 of which Nigeria accounts for 27% of malaria cases globally [9]. Children under the age of 5 are most vulnerable to malaria, accounting for 67% of malaria deaths that occur in sub-Saharan Africa [10]. Malaria eradication in the world's most vulnerable populations (children and the poor) would have a beneficial impact on economy. However, resistance to anti-malarial drugs and insecticides has been identified as one of the threats facing its eradication. The ability of *Plasmodium* species to develop resistance to anti-malarial drugs, such as artemisinin, has become of great importance undermining the efficacy of these drugs, and these limitations highlight the need for innovative drug development [11]. Medicinal plants offer wide range bioactive compounds or secondary metabolites, various studies have shown that the crude extract of some medicinal plants showed higher antiplasmodia activity than some orthodox anti-malarial at equivalent dosage. [12-16]. Medicinal plants are known to contain a number of substances and are used in traditional medicine to treat a variety of diseases. Medicinal plants are the foundation of health care around the world and they are still used for numerous chemotherapeutic purposes in both developing and developed countries. Several traditional herbs have been studied have been studied and found to be effective in the prevention and treatment of malaria. *Alstonia boonei* De Wild is a huge deciduous evergreen tree that grows up to 45 meters tall and has a diameter of 1.2 meters. It is a member of *Apocynaceae* family, which has more than 40 species found on the continents of Asia, Africa and America [17, 18]. In Yoruba, *Alstonia boonei* is called *Ahun*, *Egbu-ora* in Igbo, *Ukhu* in Edo and *Ukpukunu* in Urhobo. In traditional medicine, different parts of the plant are used to treat a number of diseases, including malaria, fever,

rheumatic pains, chronic diarrhea, sleeplessness, as anti-venom for snake bites and arrow poisoning [19-21]. Anti-malarial drug resistance to *P. falciparum* is an ongoing and growing problem, as has been demonstrated. In addition to rising morbidity and mortality, continuous efforts to improve the efficacy of antimalarial treatments have resulted in higher associated costs, indicating an urgent need for the discovery and development of novel anti-malarial agents. Medicinal plant approaches are certainly practical and have proven to be beneficial in the development of malaria drugs [22]. Therefore, the research study evaluated the anti-malarial activity of flavonoid-rich fraction of *Alstonia boonei* leaves (FRFABL) on hematological and various biochemical changes induced by *plasmodium berghei* in mice.

2. Materials and Methods

2.1. Collection of Plant Materials and Extraction Procedure

Fresh *Alstonia boonei* leaves were taken in January 2020 from Opanda-Nimbo in Uzo-Uwani Local Government Area of Enugu State, Nigeria. Mr. Alfred Ozioko, a taxonomist at the Bioresources Development and Conservation Programme (BDGP) Research Centre in Nsukka, Enugu State, was in charge of identifying and authenticating the plant materials. For reference, a voucher specimen with the voucher number, Intercedd/024 was placed in the herbarium. To avoid decomposition, the plant material was washed and air-dried for two weeks with regular turning. A mechanical grinder was used to crush the dry leaves into powder. Powdered sample (1.5 kg) was macerated in 3.5 L of absolute ethanol and left to stand for 72 hours, stirring occasionally. After that, the suspension was filtered through a mesh. To eliminate fine residues, further filtration was done with Whatman No. 1 filter paper. The filtrate was concentrated at 45°C using a rotary evaporator (IKA, Germany) to yield the crude extract which was stored at 4°C until needed.

2.2. Study Animals

This study used forty-eight (48) adult albino Wistar mice with an average body weight of 30 ± 38 g, 18 for the toxicity investigation and 30 for the anti-malarial study. They were purchased from the Animal Breeding Unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The mice were acclimatized in the Animal Farm of the Department of Biochemistry for two weeks prior to the start of the experiment under conventional laboratory conditions with a 24 hour light/dark cycle. They were fed a regular feed of commercial Rodent Chow from Chukun Feeds Nig. Ltd. and free access to water *ad libitum*. The animal received humane care all over the experimental period, in accordance with institutional, national, and international ethical recommendations for the care and use of laboratory animals (NIH publication #85-23, revised in

1985) and the International Guidelines for Handling of Laboratory Animals [23].

2.3. Chemicals, Reagents and Standard Drug

All of the chemicals used in this experiment were analytical grade. Sigma Aldrich (USA), British Drug House (BDH, England), Burgoyne (India), Harkin and Williams (England), Qualikems (India), Fluka (Germany), and May and Baker (England) produced these. Reagents utilized for the study were commercial products of Randox (USA) and Teco (USA) kits. The standard anti-malarial drug used in this study was Coartem® (Novartis, Basel Switzerland), an artemisinin-combination therapy (ACT) composed of arthemeter and lumefantrine in a ratio of 20:120. The drug was procured from a reputable retailer, Elofex Pharmaceutical and Drug Stores, Nsukka, Enugu state, Nigeria.

2.4. Preparation of Flavonoid-Rich Fraction

Extraction of FRFABL was carried out according to the method described by Chu *et al.* [24]. In a tiny flask, a measured amount of the crude extract was dissolved in 20 ml of 10% H₂SO₄ and was hydrolyzed by heating on a water bath for 30 minutes at 100°C. The mixture was placed on ice for 15 minutes to allow the flavonoids aglycones to precipitate. The cooled solution was filtered, and the filtrate (flavonoids aglycone mixture) was mixed in 50 ml warm 95 percent ethanol (50°C). The resulting solution was filtered once more into a 100 ml volumetric flask that had been filled with 95 percent ethanol. To make FRFABL, the filtrate was concentrated to dryness using a rotary evaporator.

2.5. Phytochemical Analysis

FRFABL was subjected to quantitative phytochemical analysis to determine the presence of plant secondary metabolites in the plant extract, using the standard protocols as outlined by Trease and Evans [25] and Harborne [26].

2.6. Acute Toxicity Study

Acute toxicity study of FRFABL was done using method described by Lorke [27] to define the range of lethal dose and safe dose of FRFABL. A total of eighteen (18) mice were utilized in the acute toxicity study, which was conducted in two phases. They were divided into six (6) groups, each of which had three (3) mice. For the first phase, the FRFABL was given orally at dosages of 10, 100, 1000 mg/kg b.w and for the second phase, 1600, 2900 and 5000 mg/kg b.w. The mice were monitored for behavioral change (signs of toxicity) and mortality for 24 hours.

2.7. Induction Procedure

2.7.1. Parasite Inoculation

For inoculum preparation, donor mouse blood infected with the *Plasmodium berghei* NK-65 gotten from the animal farm faculty of Veterinary Medicine, University of Nigeria

Nsukka, Enugu State Nigeria, was utilized. Blood was taken from the donor mouse through heart puncture and serially diluted in Alsever's solution to generate a suspension containing approximately 1×10^7 infected RBCs per 0.2 ml suspension. This suspension (0.2 mL) was administered intraperitoneally into the experimental animals to initiate infection. Infection for malaria parasite was confirmed 72 h post infection (day 0).

2.7.2. Determination of the Initial Percentage Parasitemia Level

The determination of malaria parasitemia (Mp⁺) was according to Dacie and Lewis [28] method. Each mouse's tail was cut with a razor and gently squeezed to obtain a little drop of blood that was deposited in the centre of a microscope slide. A smooth edged slide spreader was used to distribute the thin film right away. A black lead pencil was used to name the slide, which was then air-dried in horizontal position. The thin blood films were fixed by placing the slide horizontally on a level staining rack. A drop swab was used to apply a little drop of 100% ethanol to the thin film. This was allowed to fix for 2 minutes. Giemsa Staining technique: 50 ml of buffered water (saline) pH 7.1-7.2 was added and mixed gently. Then, 1.5ml of Giemsa stain was added and mixed gently. In a staining rack, the slides were placed face down in a shallow tray supported by two rods. The diluted stain was then put into the shallow tray and stained for 30 minutes before being rinsed with clean water from the staining container. Finally, the slides back was wiped clean and placed in a draining rack to air dry. Dried stained film was observed microscopically with 100 x objectives using a counting chamber. By counting the percentage of infected red cells, the percentage parasitemia of the thin film was calculated. Counting the percentage of parasitized red cells: 100 x objectives were used to select an area of the thin film with a total number of red cells of about 250 per field. The number of parasitized red cells was counted in eight fields totaling about 2000 cells. The number of parasitized red cells was calculated by dividing the percentage of parasitized cells by 20.

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

2.8. Experimental Design

Thirty Wistar albino mice used for this study were divided into 6 groups of 5 mice each: Group 1, normal control, and received (0.5ml) normal saline. Group 2, positive control- was infected with malaria parasite and was untreated. Group 3, standard control- was infected with malaria parasite and treated with 140 mg/kg b.w of Coartem® drug. Groups 4-6 were infected with malaria parasite and treated with 200, 400 and 600 mg/kg b.w of the FRFABL for 5 days (from day 0-day 4). On the 6th day, after an overnight fast, blood samples were collected from all the mice in both EDTA and plain tubes. Samples in EDTA tubes were used for final percentage parasitemia count and hematological analyses while samples collected in plain tubes were allowed to clot for 10 min and

thereafter, spun for 15 min at 5000 rpm. The clear supernatant generated (serum) were used for analyses of some biochemical parameters.

2.9. Determination of Biochemical Parameters

Hematological indices were determined using the methods described by Ochei and Kolhatkar [29]; Briggs and Bain [30]. Aspartate and alanine aminotransferases (AST and ALT) activities were assayed using the method described by Reitman and Frankel [31] as contained in the Randox enzyme Kit. The activity of alkaline phosphatase (ALP) was assayed using the method described by Klein et al. [32] as contained in the Randox enzyme Kit. Total bilirubin concentration was determined by colorimetric method as described by Jendrassik and Grof [33]. Serum total cholesterol concentration was determined using the method of Allain et al. [34] as contained in Randox commercial kits. Serum high density lipoprotein (HDL) concentration was determined using the method of Albers et al. [35] as contained in Randox commercial kits. Serum triacylglycerols (TAG) concentration was determined using the method of Albers et al. [35] as contained in Randox commercial kit. Low density lipoprotein (LDL) was determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene glycol monomethyl ether. Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al. [36]. Superoxide dismutase (SOD) activity was assayed using method described by Xin et al. [37]. Catalase activity was assayed according to the method described by Aebi [38]. The reduced glutathione concentration was determined according to method described by Kings and Wootton [39].

2.10. Statistical Analysis

Raw data obtained from the laboratory were analyzed using both one and two-way analysis of variance (ANOVA) in Statistical package for Service Solution (SPSS) version 20 and presented as mean \pm SD in the tables. Mean values were separated by Duncan and were considered significant at $p < 0.05$.

3. Results

3.1. Quantitative Phytochemical Screening of the FRFABL

Quantitative phytochemical screening of FRFABL revealed high amounts of phenols, alkaloids and flavonoids. Tannins and terpenoids were found in moderate amounts while steroids and saponins were quantified in smaller amounts (table 1).

Table 1. Quantitative Phytochemical Screening of the FRFABL.

Phytochemical Constituents	Concentration (mg/100g)
Tannins	881.10 \pm 20.69
Steroids	95.79 \pm 3.19
Phenols	1406.52 \pm 53.43
Alkaloids	2625.61 \pm 188.67
Flavonoids	4260.88 \pm 355.03
Saponins	0.40 \pm 0.01
Terpenoids	484.37 \pm 30.35

Results are expressed in Mean \pm SD (n=3).

3.2. Result of the Acute Toxicity Test of the FRFABL

Table 2 shows the result of acute toxicity test of FRFABL. The result shows that the plant fraction was not lethal even at the highest dosage (5000 mg/kg b.w) administered. The median lethal dose (LD₅₀) of the extract should therefore be over 5000 mg/kg b.w.

Table 2. Results of Phase I and phase II of the acute toxicity test of the FRFABL.

Groups	Dose of extract (mg/kg b.w.)	Mortality	Behavioral changes	Body weight changes
Phase I				
Group 1	10	0/3	Nil	Not significant
Group 2	100	0/3	Nil	Not significant
Group 3	1000	0/3	Nil	Not significant
Phase II				
Group 1	1600	0/3	Nil	Not significant
Group 2	2900	0/3	Nil	Not significant
Group 3	5000	0/3	Nil	Not significant

Table 3. Effect of the FRFABL on Percentage parasitemia of *Plasmodium berghei*-infected albino Mice.

Groups	Day 0 Malaria parasitemia (%)	Day 4 Malaria parasitemia (%)
Group 1	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a
Group 2	44.75 \pm 7.18 ^b	78.00 \pm 4.08 ^c
Group 3	51.50 \pm 6.19 ^b	4.75 \pm 0.50 ^{ab}
Group 4	45.50 \pm 11.15 ^b	0.80 \pm 6.06 ^b
Group 5	50.25 \pm 6.40 ^b	4.75 \pm 1.26 ^{ab}
Group 6	55.75 \pm 9.27 ^b	3.75 \pm 1.50 ^{ab}

Results are expressed in means \pm SD; (n=5).

Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$. Group 1 administered with normal saline only; Group 2 *P. berghei*-infected and not treated; Group 3 *P. berghei*-infected + 140 mg/kg b.w coartem®; Group 4 *P. berghei*-infected + 200mg/kg b.w FRFABL; Group 5 *P. berghei*-infected + 400 mg/kg b.w FRFABL; Group 6 *P. berghei*-infected + 400 mg/kg b.w FRFABL.

3.3. Effect of FRFABL on Percentage Parasitemia of *Plasmodium berghei*-Infected Albino Mice

Table 3 shows the percentage parasitemia of mice treated with *A. boonei*. Before the administration of the FRFABL, the percentage parasitemia load of the test groups were found to be significantly ($p < 0.05$) higher compared to group 1 (negative control). There were no significant ($p > 0.05$) difference amongst the test groups ($p > 0.05$). However, after the treatment (day 4) the percentage parasitemia load of the treated groups were found to have a significant ($p < 0.05$)

decrease when compared to group 2 (untreated group).

3.4. Effect of the FRFABL on Haematological Indices of *Plasmodium berghei*-Infected Mice

The investigations of pack cell volume (PCV), red blood cell counts (RBC) and haemoglobin (Hb) concentrations reveal significant ($P < 0.05$) increases when their treated groups were compared to group 2 (untreated). However, white blood cells (WBC) count showed significant ($P < 0.05$) decreases on the treated groups compared to untreated group 2.

Table 4. Effect of the FRFABL on haematological indices of *Plasmodium berghei*-infected mice.

Treatment Group	Haematological Indices			
	PCV (%)	RBC ($\times 10^6$)	WBC ($\times 10^3/\text{mm}^3$)	Hb (g/dl)
Group 1	39.50 \pm 2.38 ^b	10.66 \pm 0.14 ^b	10600.00 \pm 294.39 ^a	10.40 \pm 0.20 ^b
Group 2	32.25 \pm 2.63 ^a	9.46 \pm 0.47 ^a	13700.00 \pm 875.60 ^b	8.63 \pm 0.53 ^a
Group 3	43.50 \pm 2.38 ^c	10.40 \pm 0.28 ^b	10325.00 \pm 797.39 ^a	10.63 \pm 0.43 ^b
Group 4	43.00 \pm 1.83 ^{bc}	10.45 \pm 0.06 ^b	10900.00 \pm 454.61 ^a	10.43 \pm 0.34 ^b
Group 5	43.50 \pm 2.38 ^c	10.59 \pm 0.13 ^b	10825.00 \pm 287.23 ^a	10.70 \pm 0.28 ^b
Group 6	42.25 \pm 2.63 ^{bc}	10.42 \pm 0.02 ^b	10650.00 \pm 310.91 ^a	10.80 \pm 0.12 ^b

Results are expressed in means \pm SD; (n=5).

Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$. Group 1 administered with normal saline only; Group 2 *P. berghei*-infected and not treated; Group 3 *P. berghei*-infected + 140 mg/kg b.w coartem®; Group 4 *P. berghei*-infected + 200mg/kg b.w FRFABL; Group 5 *P. berghei*-infected + 400 mg/kg b.w FRFABL; Group 6 *P. berghei*-infected + 400 mg/kg b.w FRFABL.

3.5. Effect of the FRFABL on Liver Function Parameter of *Plasmodium berghei*-Infected Mice

Table 5 shows the effect of the plant fraction on liver function parameters. The ALT, ALP, AST activities and total

bilirubin concentration of the groups treated with FRFABL was found to have significant ($P < 0.05$) increases when they were compared to untreated groups 2.

Table 5. Effect of the FRFABL on the liver function parameters of *Plasmodium berghei*-infected Mice.

Treatment Group	Liver function tests			
	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	Total Bilirubin (mg/dl)
Group 1	20.00 \pm 1.86 ^a	47.25 \pm 3.40 ^a	30.73 \pm 2.50 ^a	6.43 \pm 0.26 ^d
Group 2	65.00 \pm 2.58 ^d	93.75 \pm 3.50 ^c	71.00 \pm 2.58 ^c	9.05 \pm 0.60 ^d
Group 3	24.25 \pm 1.71 ^b	56.50 \pm 5.00 ^b	35.75 \pm 1.71 ^b	5.28 \pm 0.54 ^{bc}
Group 4	28.00 \pm 1.63 ^b	50.00 \pm 1.83 ^b	36.50 \pm 2.65 ^b	4.80 \pm 0.60 ^{ab}
Group 5	24.00 \pm 0.82 ^b	50.50 \pm 2.08 ^b	37.00 \pm 1.83 ^b	5.93 \pm 0.55 ^{cd}
Group 6	24.00 \pm 3.56 ^b	50.50 \pm 5.00 ^b	34.75 \pm 0.96 ^b	4.45 \pm 0.19 ^a

Results are expressed in means \pm SD; (n=5).

Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$. Group 1 administered with normal saline only; Group 2 *P. berghei*-infected and not treated; Group 3 *P. berghei*-infected + 140 mg/kg b.w coartem®; Group 4 *P. berghei*-infected + 200mg/kg b.w FRFABL; Group 5 *P. berghei*-infected + 400 mg/kg b.w FRFABL; Group 6 *P. berghei*-infected + 400 mg/kg b.w FRFABL.

Table 6. Effect of the FRFABL on lipid profile of *Plasmodium berghei*-infected Mice.

Treatment Group	Lipid Profile			
	LDL (mg/dl)	HDL (mg/dl)	Total Cholesterol (mg/dl)	TAG (mg/dl)
Group 1	26.00 \pm 2.16 ^a	67.00 \pm 2.58 ^{bc}	78.75 \pm 6.70 ^b	97.25 \pm 3.59 ^a
Group 2	84.50 \pm 3.41 ^c	34.25 \pm 3.30 ^a	105.25 \pm 4.11 ^d	119.75 \pm 1.70 ^b
Group 3	32.75 \pm 2.21 ^b	72.25 \pm 3.09 ^c	87.25 \pm 7.27 ^c	99.25 \pm 2.75 ^a
Group 4	33.50 \pm 3.11 ^b	71.75 \pm 5.05 ^c	71.50 \pm 8.22 ^{ab}	95.00 \pm 5.71 ^a
Group 5	33.75 \pm 0.96 ^b	68.75 \pm 4.85 ^{bc}	73.00 \pm 4.76 ^{ab}	98.00 \pm 6.97 ^a
Group 6	33.75 \pm 3.86 ^b	63.25 \pm 3.40 ^b	68.75 \pm 4.85 ^a	98.25 \pm 6.02 ^a

Results are expressed in means \pm SD; (n=5).

Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$. Group 1 administered with normal saline only; Group 2 *P. berghei*-infected and not treated; Group 3 *P. berghei*-infected + 140 mg/kg b.w coartem®; Group 4 *P. berghei*-infected + 200mg/kg b.w FRFABL; Group 5 *P. berghei*-infected + 400 mg/kg b.w FRFABL; Group 6 *P. berghei*-infected + 400 mg/kg b.w FRFABL.

3.6. Effect of the FRFABL on Lipid Profile of *Plasmodium berghei* - Infected Mice

Table 6 shows the lipid profile of mice treated with FRFABL. The results of the low density lipoprotein (LDL), cholesterol and triacylglycerol (TAG) concentrations revealed a significance ($p < 0.05$) decreases when the treated groups were compared to untreated group 2. Meanwhile high density lipoprotein (HDL) concentration was found to be significantly ($p < 0.05$) higher when the treated groups were compared to untreated group 2.

3.7. Effect of FRFABL on MDA and Antioxidant Parameters of *Plasmodium berghei*-Infected Mice

Table 7 shows the pro-and anti-oxidant indices in Albino mice treated with extract of FRFABL. The concentration of Malondialdehyde (MDA) result was found to be significantly ($p > 0.05$) lower when the treated groups were compared to untreated group 2. However, the activities of superoxide dismutase (SOD), catalase and reduced glutathione (GSH) were observed to have a significant ($p < 0.05$) increases when the treated groups were compared to untreated group 2.

Table 7. Effect of the FRFABL on MDA and anti-oxidant parameters of *Plasmodium berghei*-infected Mice.

Treatment Group	Anti-oxidant Levels			
	MDA (mg/dl)	SOD (IU/L)	Catalase (IU/L)	GSH (mg/dl)
Group 1	1.59±0.11 ^a	8.75±0.67 ^b	0.51±0.17 ^c	0.17±0.67 ^a
Group 2	4.84±0.63 ^c	3.93±0.50 ^a	0.18±0.55 ^a	0.15±0.67 ^c
Group 3	1.88±0.56 ^{ab}	8.68±0.33 ^b	0.53±0.22 ^c	0.23±0.30 ^{ab}
Group 4	2.23±0.29 ^{ab}	7.82±1.05 ^b	0.50±0.36 ^c	0.23±0.40 ^{ab}
Group 5	2.30±0.59 ^{ab}	8.04±0.47 ^b	0.51±0.57 ^c	0.25±0.52 ^b
Group 6	2.45±0.22 ^b	8.01±0.47 ^b	0.44±0.13 ^b	0.23±0.53 ^{ab}

Results are expressed in means ± SD; (n=5).

Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$. Group 1 administered with normal saline only; Group 2 *P. berghei*-infected and not treated; Group 3 *P. berghei*-infected + 140 mg/kg b.w coartem®; Group 4 *P. berghei*-infected + 200mg/kg b.w FRFABL; Group 5 *P. berghei*-infected + 400 mg/kg b.w FRFABL; Group 6 *P. berghei*-infected + 400 mg/kg b.w FRFABL.

4. Discussion

Medicinal plants are known to contain a variety of substances and are used in traditional medicine to treat a variety of ailments. It has been the foundation of health care throughout the world and continues to be relevant for various chemotherapeutic purposes in both developing and developed countries. *Alstonia boonei* De Wild is very huge deciduous evergreen tree that belongs to the *Apocynaceae* family, which includes over 40 species found across Africa, Asia and America. In traditional medicine, different parts of the plant are used to treat a number of diseases, including malaria, fever, rhematic pains, chronic diarrhea, insomnia, anti-venom for snake bites and arrow poisoning treatment. The research study evaluated the anti-malarial activity of FRFABL and its effect on hematological and various biochemical changes induced by *plasmodium berghei* infection in mice.

Quantitative phytochemical screening of FRFABL shows it contains significant amounts of flavonoids, phenols and alkaloids. The finding gives credence to the report of Obiagwu *et al.* [40] on the phytochemical analysis of the crude precipitate, fractions, and compound from *A. boonei* methanol root-bark extract. Although the molecular mechanism of action of flavonoids in combating malaria has not been clearly elucidated, several authors have attributed the antimalarial activity of flavonoids to its ability in inhibiting fatty acid biosynthesis of the parasite [41, 42]. Scientific reports have shown that plant extracts containing flavonoids possess anti-malarial and antioxidant activities, thereby acting as free radical scavengers which help to

subdue oxidative damage induced by malaria parasite. Flavonoids have also been reported to show non-oxidative effect on haemoglobin which renders it an effective pharmacological agent for treating anemia, one of the conditions mostly observed during malaria infection [43].

The result on the acute toxicity test indicates that *Alstonia boonei* is safe even at the highest dose (5000 mg/kg b.wt) of the FRFABL given to the mice. The mice show normal physical activities, no behavioral change was observed and survived beyond 24 hours.

In comparison to the untreated group (Group 2), treatment of malaria parasite infected-mice with the FRFABL significantly ($p < 0.05$) reduced the percentage parasitemia level in a dose-dependent manner. This could be due to the presence of bioactive substances which include phenols, flavonoids and alkaloids in large concentrations found in the plant that possess anti-malarial activity. The findings backed up previous research by Onwusonye and Uwakwe [44] and Onyishi *et al.* [45] who found *A. boonei* to have some anti-plasmodial activity in different extracts in a dose-dependent manner. Flavonoids present in the FRFABL may help to prevent malaria induced oxidative stress by scavenging free radicals and serving as reducing agents, as well as hydrogen atom donating molecules or singlet oxygen quenchers; chelating metal ions, and sparing other antioxidants (e.g. vitamin C, vitamin E and carotene). In addition, several plant derived-flavonoids such as citflavanone, lonchocar- pol A, 8-prenylldaidzein, luteolin, β -hydroxydihydrochalcone, deguelin and obovatol, have also been shown to have anti-malarial properties [46, 47].

In this study, it was discovered that the parasitized-

treated groups had significantly ($p < 0.05$) increased red blood cell (RBC), packed cell volume (PCV) and haemoglobin (Hb) concentrations than the parasitized-untreated group mice. This is consistent with the findings of Igbenegbu and Odaibo [48] and Onyishi et al. [49] who found that humans with acute malaria have lower RBC, PCV and Hb concentrations than apparently healthy humans. The parasitized-treated mice had a significantly ($p < 0.05$) lower white blood cell (WBC) count than the parasitized-untreated group of mice. According to Francis et al. [50] lower packed cell volume concentrations are linked to parasitized red cell mechanical devastation. Eledo and Izah [51] discovered that *plasmodium* parasite causes a change in hematological status, thus causing a significant reduction in neutrophils, lymphocytes, monocytes, haemoglobin and packed cell volume, as well as an increase in erythrocyte sedimentation rate and white blood cell count. The observed increase in WBC in the parasitized-untreated group could be attributed to the stimulation of immune system of the animal to combat the malaria parasite. Therefore, the oral dosage of FRFABL ameliorated the investigational alteration found in the hematological indices.

The effect of FRFABL on the liver function parameters of *plasmodium berghei* infected mice, showed that the activities of AST, ALP, and ALT in the parasitized-treated groups reduced significantly ($P < 0.05$) when compared to the parasitized-untreated group mice. This may be as a result of inducing the activity of the enzyme by the plant fraction and the standard drug which was in consistent with the report of Momoh et al. [52] that the AST and ALT values of Control group were higher due to the normality of the enzyme activity by the extracts. The FRFABL when taken orally, tends to stabilize the activity of these enzymes (AST, ALP, and ALT), which were previously elevated due to the disruption of the liver membrane by the *plasmodium* parasite. However, the significant ($p < 0.05$) decrease observed in the level of total serum bilirubin of parasitized-treated groups compared to the parasitized-untreated group mice could be as a result of destruction of red blood cells by the malaria parasite and this compliments the works done by Amah et al. [17] and Enechi et al. [53] When the integrity of the membrane of the hepatocytes is compromised, certain enzymes located in the cytosol are released into the blood and their estimation in the serum is a useful quantitative biomarker for the evaluation of liver damage [54]. Treatment with FRFABL in this study has shown that the plant fraction protects hepatocyte integrity of parasitized-treated mice.

There was a significantly ($p < 0.05$) higher HDL concentration in Groups treated with FRFABL compared to parasitized-treated mice. Also, mice in groups 3–6 parasitized-treated mice had significant ($p < 0.05$) lower LDL, TAG and TC concentrations in comparison with mice in Group 2 parasitized-untreated. This study supports the findings of Ogugua et al. [55] who observed that Commercial herbal preparations caused increase on the level of HDL on the treated mice compared to the untreated

and decrease on the level of LDL, TAG and TC treated mice compared to the untreated. Lipid and lipoprotein abnormalities are widespread in both physiological and nonphysiological conditions. In both phases of *plasmodium* life cycle in the human host, lipids play a critical role in its metabolism. These organisms use cholesterol and phospholipids from the host to their metabolic requirements, such as membrane or haemozoin formation [56]. Additionally, it is likely that the parasite modifies metabolic pathways of lipids in the hepatocytes. Finally, oxidative stress has been associated with oxidation of lipoproteins, contributing to the abnormalities of lipids levels [57].

Levels of lipid-peroxidation products, such as malondialdehyde (MDA) indicate the presence of oxidative stress, which leads to malarial pathogenesis and other associated problems [58], as well as a reduction in the antioxidant capacity of the infected individuals. According to previous research, high MDA levels can be seen as a possible indicator of the severity of malaria infection [59, 60]. The increased lipid peroxidation observed in the group 2 untreated mice may be due to the inefficient antioxidant system prevalent in malaria, the result showed a significant ($p < 0.05$) decrease in the groups treated with FRFABL compared to the untreated groups. However, the superoxide dismutase (SOD) and catalase activities and reduced glutathione (GSH) concentration significantly ($p < 0.05$) increased the anti-oxidant level in mice treated with FRFABL compared to the untreated mice. The findings were in agreement with the report of Onyishi et al. [46] who reported that the extracts of *A. boonei* significantly reduced the degree of tissue peroxidation, increased the level of reduced glutathione (GSH), as well as superoxide dismutase and catalase activities.

5. Conclusion

Results from the present study demonstrated that FRFABL possesses good antimalarial properties at the varied doses of the fraction administered and present evidence in reducing the parasite load in infected mice. The findings also showed that FRFABL has protective effects on alterations in hematological indices, liver function parameters, lipid profile as well as oxidative stress and lipid peroxidation associated with malaria infection in mice. This suggests that the plant is a likely reserve of novel antimalarial agents; hence, justifies the ethno-medicinal use of the plant in the management of malaria. This outcome might be attributed to the existence of pharmacologically active principles in the fraction which may have acted singly to exert the antimalarial activity observed in this study.

6. Suggestion for Further Study

- 1) To understand the long-term effect of the plant, further study on the toxicity should therefore focus on the acute and chronic test.

- 2) Further research on the histological conditions on the liver should be carried out to ascertain the level of oxidative stress put in the cause of *plasmodium berghei* infection in mice.
- 3) Further purification, isolation and characterization should be carried out to confirm the bioactive compounds present in the plant.

Credit Authorship Contribution Statement

OCE: Conceptualization, Supervision and Editing of the manuscript.

CCA: Methodology, Supervision, Writing of the original draft of the manuscript, Editing of the manuscript, Analyzed and Interpreted the Data, Handling the peer reviews.

JIO: Editing of the manuscript Analyzed and Interpreted the Data.

UCO: Editing of the manuscript, Analyzed and Interpreted the Data.

ECO: Funding acquisition, Methodology, Investigation, Writing of the original draft of the manuscript.

TUN: Funding acquisition, Methodology, Investigation, Analyzed and Interpreted the Data.

MCU: Funding acquisition, Methodology, Investigation, Analyzed and Interpreted the Data.

SCE: Funding acquisition, Methodology, Investigation, Analyzed and Interpreted the Data.

PCI: Funding acquisition, Methodology, Investigation, Analyzed and Interpreted the Data.

LNE: Funding acquisition, Methodology, Investigation, Analyzed and Interpreted the Data.

CFO: Funding acquisition, Methodology, Investigation, Analyzed and Interpreted the Data.

Data Availability

The statistical data used to support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest

The authors declared no competing interest that could have appeared to influence the work reported in this research paper.

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