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Antimalarial effect of ethanol extract of *Parinari curatellifolia* stem-bark in *Plasmodium berghei*-NK 65 infected mice.

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Research Article

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Abstract

Background: The use of *Parinari curatellifolia* for the management of malaria by Africans without scientific validation, prompted this study, to investigate the antimalarial effect of ethanol extract of *Parinari curatellifolia* stem bark (EEPCSB) in *Plasmodium berghei (NK-65)*-infected mice.

Method: Thirty mice (16 -21 g) were grouped into six of five mice each. Group 1 (uninfected) served as normal control while groups 2-6 were infected. Group 2 (positive control) was untreated while group 3 (standard control) received 20/120 mg/kg b.w. of arthemeter/lumefantrine, groups 4-6 received 200, 400 and 600 mg/kg b.w. of EEPCSB respectively. The treatment lasted for four consecutive days. Parasitemia, heamatological and biochemical tests were carried out using established methods. The obtained data were analysed using one-way analysis of variance (ANOVA).

Result: The extract significantly ($p < 0.05$) suppressed the parasitemia, and produced significant ($p < 0.05$) increase in the PCV, RBC, and Hgb and a significant ($p < 0.05$) decrease in the WBC of groups 4-6 compared to the positive control. The liver function marker enzymes - AST, ALT, ALP, and total bilirubin were all significantly ($p < 0.05$) reduced in the treated animals, while there was a significant ($p < 0.05$) decrease in the total cholesterol, triacylglycerol and LDL, and a significant ($p < 0.05$) increase in the HDL of the infected animals compared to the positive control. While there was a significant ($p < 0.05$) reduction in lipid peroxidation marker MDA in the treated groups, the activities of the SOD and catalase, significantly ($p < 0.05$) increased compared to the positive control. The GSH concentration reduced significantly ($p < 0.05$) in the treated groups compared to the untreated group.

Conclusion: The extract showed excellent antimalarial effect by reducing parasitemia and reversing haematological and biochemical aberrations in *Plasmodium berghei*-infected mice as demonstrated in this study. Therefore, *Parinari curatellifolia* stem bark could be a source of lead compound for a novel and effective antimalarial drug.

Introduction

Malaria has continued to be a major public health problem despite efforts at controlling it. It is widespread throughout tropical and subtropical regions of the world, and Africa carries a disproportionately high share of the global malaria burden, both in terms of total malaria morbidity and mortality. World Health Organization (WHO) reported that, in 2021, 247 million cases of malaria and 619,000 deaths in 87 malaria endemic countries globally were recorded. Africa accounted for 95% of the total cases and deaths. Ninety-six percent (96%) of the cases and deaths took place in 29 of the 87 malaria endemic countries (WHO, 2022). According to the 2022 World Malaria Report, Nigeria had the highest number of global malaria cases (27 % of global malaria cases) in 2021 and accounted for the highest number of deaths (31 % of global malaria deaths).

Malaria is caused by *Plasmodium* parasites that are transmitted to humans through the bites of infected female *Anopheles* mosquitoes. *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale curtisi, Plasmodium ovale wallicker, Plasmodium vivax,* and *Plasmodium knowlesi*, are the five species of the parasite that infects humans, with *Plasmodium falciparum* being the deadliest (Milner, 2018; Prabhu *et al*., 2021). *Plasmodium* has a complex life cycle involving both sexual and asexual replications in both the insect and human hosts respectively (Garrido-Cardenas *et al*., 2019). During a blood meal, the female Anopheles mosquito transmits the sporozoites to humans. The sporozoites travels through the blood capillaries to the liver where it replicates asexually in the hepatocyte and mature into the liver schizont containing many merozoites, which are released into the blood stream. The merozoites invade the red blood cells and undergo intra-erythrocytic asexual replications and mature into the erythrocytic schizonts. The cycle continues, resulting in acute and chronic parasitemia. Some parasites commit to producing gametocytes, which are picked up by the mosquito during a blood meal. The sporozoites which are transmitted to another person upon blood meal, are produced through the sexual fusion of the gametocytes (Venugopal *et al*., 2020). The classic malaria paroxysms, which are marked by recurrent fevers and chills, are caused by the release of merozoites from infected red blood cells (iRBC) (Oliver *et al*., 2014).

Depending on the plasmodium species, the host's inherent and acquired immunity, and the selection of an appropriate and timely course of therapy, the clinical signs of malaria might differ significantly. Consequently, asymptomatic, uncomplicated and severe are three types of malaria seen in humans (Garrido-Cardenas *et al*., 2019). Malaria initially manifests as flu-like symptoms, including fever, chills, headaches, joint pains, nausea, vomiting, coughing, chest discomfort, myalgia, and weakness. Few patients go on to develop severe malaria which leads to coma, acute renal failure, severe anemia, acidosis, pulmonary edema and hypoglycemia (Su *et al*., 2019).

Quinine has been the foremost drug for treating severe malaria for more than 200 years. It was isolated from the bark of Cinchona species in 1820. Despite the significant limitations of quinine, it has nonetheless had long history of efficacy. The case-fatality rate in severe malaria, when treated promptly with quinine exceeds 20% (Li & Weina, 2010). Chloroquine, which is another important antimalarial drug, was synthesized in 1940. It remained the first line of treatment for uncomplicated malaria, until it was recently replaced with artemisinin-based therapy due to widespread resistance to chloroquine especially by *Plasmodium falciparum* (Saxena *et al*., 2003). Based on the recommendations of WHO, artemisinin combination therapy (ACT) was introduced in twenty African countries between 2001 and 2004, for treatment of malaria. Currently, ACT is the first-line treatment for uncomplicated *falciparum* malaria in Africa, Asia and South America (Maiga *et al*., 2021). Nevertheless, as was initially noted in Cambodia in 2006 and expanding to other regions of South-east Asia and Africa, resistance to artemisinin is

emerging (Maiga *et al*., 2021; Tajbakhsh *et al*., 2021), has necessitated the search for a novel antimalarial drug.

In several regions of the world, particularly in malaria-endemic countries like Africa, numerous plants can be used to cure malaria. A good number of these plants have been documented in various ethnobotanical surveys of plants used in treating malaria (Iyamah and Idu, 2015; Ungogo *et al*., 2020; Enebeli-Ekwutoziam *et al*., 2021). As there has been an increasing resistance to the front-line antimalarial drugs, the quest to discover and develop new anti-malarial drugs has led to various *in vitro* and *in vivo* studies of plants species used ethno-medically in treating malaria in Africa. A considerable number of plant species have shown appreciable anti-plasmodial and antimalarial activity, however, not much success has been recorded in developing a new antimalarial drug, as only one clinical trial with *Cochlospermum planchonii* has been carried out till date (Tajbakhsh *et al*., 2021).

Parinari curatellifolia Planch ex Benth (Chrysobalanaceae), commonly known as Mbola or Mobola plum, is a large ever green, spreading tree up to 22 metres tall with a single bare stem and a dense, roundish to mushroom shaped crown. The plant's therapeutic qualities make it extremely valuable. In ethnomedicine, it is used to cure diabetes, fractures, wounds, cuts, and sores as well as fevers, pneumonia, and toothaches (Halilu *et al*., 2008; Ogbonnia *et al*., 2009). Various *in vitro* anti-malarial studies of root, root bark, and stem bark extracts of the plant using various solvents have been carried out with reports of good activity (Omoniwa *et al*., 2021; Tajbakhsh *et al*., 2021). However, there is no reported *in vivo* study on the anti-plasmodial/antimalarial activity of this plant till date.

Materials and methods

2.1 Materials

2.1.1 Plant Materials

Fresh stem-bark of *Parinari curatellifolia* was collected from Agu-orba in Udenu Local Government Area of Enugu State, Nigeria. The stem-bark was identified and authenticated by a taxonomist of the International Centre for Ethnomedicine and Drug Development, Nsukka Enugu State, Nigeria. It was assigned the voucher number InterCEDD/16291 and deposited in the Centre's herbarium.

2.1.2 Animals

Forty-eight (48) Albino mice of average body weight of 20 ± 5g mice were used for this study. The animals were purchased from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept under standard laboratory condition for one week prior to the commencement of the experiment, with a 12-hour light and dark cycle and maintained on a regular feed (commercial chicken grower's mash) and water *ad libitum*. They were given adequate care throughout the period of the experiment in accordance with the ethical rules and recommendations of the University of Nigeria Ethical committee on the care and use of laboratory animals.

2.1.3 Parasite

Chloroquine-sensitive *Plasmodium berghei* NK 65 strain which was used for the study was obtained from blood samples collected from infected donor mice from the faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. The blood sample was diluted with normal saline before inoculation into the animals.

2.1.4 Equipment and Instruments

The equipment used for this study was obtained from the Department of Biochemistry, the Department of Veterinary Medicine of the University of Nigeria Nsukka, and other scientific shops in Nsukka. They include: Test strips (Pyrex, England), PCV tubes (Pyrex, England), Haematocrit Centrifuge (Vickas Ltd, England), Colorimeter (El Scientific Co. India), Measuring Cylinder (Pyrex, England), Weighing Balance (Vickas Ltd, England), Centrifuge (Vickas Ltd, England), Spectrophotometer (E312 Model) (Jenway, UK), Timer test tubes (Pyrex, England), Haemoglobin graph (Jenway, UK), Whatman No 1 filter paper, Water bath (Gallenkamp, England), mechanical grinder (Vickas Ltd, England), Muslin cloth, Flat bottomed flask (Pyrex, England). Light microscope (Olympus), chemical balance (Gallenkamp, England), micro – pipettes (Perfect, USA), microscope slides, capillary tube, Refrigerator (Haier thermocool, England), microscope (XSZ – 107BN, India), counting chamber (MC Qiujing, China).

2.1.5 Chemicals and Reagents

All the chemicals that were used in this study were of analytical grade, and were products of Sigma Aldrich, USA, British Drug House (BDH) (England), Burgoyne, (India), Harkin and Williams, (England), Qualikems (India), Fluka (Germany), May and Baker (England). The commercial kits used for the assays were products of Randox, USA and Teco (TC), USA.

2.2 Methods

2.2.1 Extraction Procedure

The fresh stem-bark of *P. curatellifolia was* cut into smaller sizes and air-dried to a constant weight for three months. The dried stem bark was then pulverized into powdered form with the aid of a mechanical grinder. Thereafter, the coarsely powdered (700 g) bark was subjected to cold maceration using absolute ethanol (2.5 L) for seventy-two hours in a 5 L transparent plastic container. It was intermittently stirred with the aid of a glass rod. Thereafter, it was filtered with a muslin cloth to separate the marc from the micelle. Further filtration was carried out using Whatman grade 1 qualitative filter papers. The filtrate was concentrated using vacuum evaporation and stored in a sterile screw-capped bottle and stored until needed for analysis.

2.2.2 Determination of the Percentage Yield of the Ethanol Extract of *P. curatellifolia* Stem-bark

The percentage yield of the ethanol extract was determined from the weight of the dried pulverized stem-bark before maceration and the weight of the crude extract after concentration following the formula below;

$$\text{Percentage yield} = \frac{weight\ of\ crude}{weight\ of\ dried\ pulverized\ sample}\ x\ 100$$

2.2.5 Acute Toxicity Studies

A total of 18 albino mice weighing 14-21 g were used to determine the acute toxicity and the median lethal dose of the extract using the method of Lorke (1983). The study was done in two phases, with both phases having three mice each in three groups. In phase 1, groups 1, 2, and 3 received 10 mg/kg b.w, 100 mg/kg b.w, and 1000 mg/kg b.w respectively. Groups 1, 2, and 3 in phase 2 received 1,600 mg/kg b.w, 2,900 mg/kg b.w and 5,000 mg/kg b.w of the extract respectively.

The mice were then observed for 24 hours for lethality or any behavioural changes that shows signs of toxicity.

2.2.6 Parasite Inoculation

For inoculum preparation, donor mouse blood infected with *P. berghei* was used. Blood was drawn from the donor mouse via heart puncture and serially diluted in normal saline to create a suspension containing approximately $1x10^6$ infected RBCs per 0.2 ml suspension. This suspension (0.2 ml) was injected intraperitoneally into the experimental animals to initiate infection. Malaria infection was confirmed after 72 hours of inoculation.

2.2.7 Experimental Design

The inoculated animals (30 in number) were then randomly divided into six (6) groups of five (5) mice each and treated for four consecutive days (Peters *et al.*, 1975). On the fifth day, the tail tip of each mice was cut with razor and blood collected for determination of parasitemia. Thereafter, blood samples for hematological and biochemical studies were collected via ocular puncture into EDTA bottles and plain bottles respectively. The animals were subsequently sacrificed and the liver homogenate was used for further biochemical studies.

Table 1: Experimental design

Groups	Description	Treatment
Group 1	Normal control	Administered with distilled water.
Group 2	Positive control	Administered with 0.2 ml *Plasmodium berghei*- infected blood and no treatment
Group 3	Standard control	Administered with 0.2 ml *Plasmodium berghei*- infected blood and treated with 20/120 mg/kg b.w. of arthemeter/lumefantrine (standard drug)
Group 4	Treatment group 1	Administered with 0.2 ml *Plasmodium berghei*- infected blood and treated with 200 mg/kg b.w. of *P. curatellifolia*
Group 5	Treatment group 2	Administered with 0.2 ml *Plasmodium berghei*- infected blood and treated with 400 mg/kg b.w. of *P. curatellifolia*
Group 6	Treatment group 3	Administered with 0.2 ml *Plasmodium berghei*- infected blood and treated with 600 mg/kg b.w. of *P. curatellifolia*

2.2.8 Determination of Malaria parasitemia

The method described by Dacie and Lewis (2011) was used to determine the malaria parasitemia (Mp+). The tip of the tail of each mouse was cut with a razor and gently squeezed to get a drop of blood unto the Centre of a microscopic slide. The blood was immediately spread on the slide with the aid of smooth-edged slide spreader to make a film of blood. The microscopic slide was then labelled at one end with black lead pencil and air dried in a horizontal position. The air-dried blood films were Placed on a staining

rack, flooded with Leishman stain and left for 30 s to 1 min to fix. Thereafter, twice as much buffered distilled water (pH 7.2) from a plastic wash bottle was poured on the films, and then left to stain. After 10 minutes the stain was washed off with water. Finally, the back of the slides was wiped and placed in draining rack and allowed to air dry. After drying, the stained films were placed on counting chamber and viewed under the microscope at 100x magnification. The percentage parasitemia was determined from this.

$$\text{Percentage parasitemia} = \frac{Number\ of\ infected\ RBC}{Total\ number\ of\ RBC}\ x\ 100$$

2.2.9 Statistical Analysis

The results were expressed as Mean ± standard deviation and test of statistical significance was carried out using one-way analysis of variance (ANOVA). The statistical package used was the Statistical Product and Service Solutions (SPSS) version 21, the result being considered significant at $p < 0.05$.

Results

3.1 Percentage Yield of Ethanol Extract of *Parinari curatellifolia* Stem-Bark

Seven hundred grams (700 g) of the coarsely powdered *Parinari curatellifolia* stem bark yielded 57.57 g of extract which is 8.23% extract after extraction with absolute ethanol.

3.2 Qualitative Phytochemical Screening of Ethanol Extract of *Parinari curatellifolia* Stem Bark

The phytochemical screening carried out on the ethanol extract of *Parinari curatellifolia* stem bark showed it contains the secondary metabolites as shown in Table 3.

Table 2: Secondary metabolites found in ethanol extract of *Parinari curatellifolia* stem bark

Phytochemical constituents	Presence
Tannin	+++
Phenolics	+++
Saponin	++
Flavonoids	++
Steroids	+
Glycosides	++

Terpenoids			++		
Alkaloid			++		
Key: Highly present	+++	Moderately present	++	Slightly present	+

3.3 Quantitative Phytochemical Composition of Ethanol Extract of *Parinari curatellifolia* Stem Bark

The table below shows the quantitative phytochemical composition of ethanol extract of *Parinari curatellifolia* stem bark.

Table 3. Quantitative phytochemical constituents of ethanol extract of *Parinari curatellifolia* stem bark

Phytochemical constituent	Concentration (mg/100g)
Tannin	1771.69
Phenols	1281.99
Saponin	123.10
Flavonoids	493.83
Steroid	51.58
Glycosides	645.68
Terpenoids	246.23
Alkaloids	709.59

3.4 Acute Toxicity of Ethanol Extract of *Parinari curatellifolia* Stem Bark

The result of the acute toxicity test of ethanol extract of *Parinari curatellifolia* stem bark showed there was no mortality recorded as a result of the extract at the doses tested, ranging from 10 mg/kg b.w to 5000 mg/kg b.w as can be seen in Table 4.

Table 4. Phases I & II of acute toxicity test of ethanol extract of *Parinari curatellifolia* stem bark extract

Phase/Groups	Dosage of extract (mg/kg b.w)	Mortality
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

n = 3

3.5 Effect of Ethanol Extract of *Parinari curatellifolia* Stem Bark on the Percentage parasitemia of *Plasmodium berghei*-infected Mice

Table 5 shows the percentage parasitemia of the mice treated with the ethanol extract of *Parinari curatellifolia* stem bark. Before the treatment, the percentage parasitemia of the mice that were infected with malaria (groups 2 – 6) were significantly higher ($p < 0.05$) than non-infected ones (normal control). There was non-significant ($p > 0.05$) difference among all the infected groups. After the treatment however, the percentage parasitemia in groups 3 to 6, were significantly lower ($p < 0.05$) compared with group 2 (untreated group). The treatment produced non-significant ($p > 0.05$) difference in the percentage parasitemia of the treated groups.

Table 5: Percentage parasitemia of *Plasmodium berghei*-infected mice treated with ethanol extract of *Parinari curatellifolia* stem bark

Group	Parasitemia (%)		
	Before treatment	After treatment	Reduction
1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00
2	70.20 ± 10.62^{b}	103.00 ± 3.32^{c}	$- 32.80 \pm 10.47$
3	69.80 ± 11.30^{b}	6.40 ± 1.95^{b}	63.40 ± 11.33
4	76.20 ± 6.87^{b}	8.40 ± 2.07^{b}	67.80 ± 7.19
5	74.00 ± 3.67^{b}	6.40 ± 1.67^{b}	67.60 ± 3.21
6	78.00 ± 5.66^{b}	5.40 ± 2.51^{b}	72.60 ± 6.87

Results are expressed in Mean ± SD (n = 5)
Mean values with different letters as superscript down the column are considered significant at $P < 0.05$.
Group 1 : Normal control (Unparasitized and treated with distilled water)
Group 2: Positive control (Parasitized and untreated)
Group 3: Standard control (Parasitized and treated with 20/120 mg/kg b.w Artemether/lumefantrine)
Group 4: Test group (Parasitized and treated with 200 mg/kg b. wt of EEPCSB)
Group 5: Test group (Parasitized and treated with 400 mg/kg b.w of EEPCSB)
Group 6: Test group (parasitized and treated with 600 mg/kg b.w of EEPCSB)

In this study, the extract significantly ($p < 0.05$) reduced the percentage parasitemia of the treated animals compared to the untreated group. This corroborates the earlier report of the antiplasmodial activity of *Parinari curatellifolia* stem bark extract (Omoniwa *et al*., 2021). This antimalarial effect of this extract could be attributed to the activities of bioactive compounds found in it, most likely the alkaloids, terpenoids and/or saponins. These compounds, acting singly or in synergy could have exerted a cytotoxic effect on the parasite. This result shows that *Parinari curatellifolia* stem bark is effective in the managing of malarial and can serve as source of a lead compound for a novel antimalarial drug.

3.6 Effect of Ethanol Extract of *Parinari curatellifolia* Stem Bark on Some Hematological Parameters in *Plasmodium berghei*-infected Mice

Table 6 below shows the effect of the ethanol extract of *Parinari curatellifolia* stem bark on mice induced with malaria.

Table 6: Some hematological indices of *Plasmodium berghei*-infected mice treated with the ethanol extract of *Parinari curatellifolia* stem bark.

Treatment Groups	Haematological indices			
	PCV(%)	RBC(10^6/mm^3)	WBC (x 10 mm^3)	HB (g/dl)
1	42.00 ± 1.58^{c}	10.79 ± 0.56^{d}	10.40 ± 0.37^{a}	10.64 ± 0.17^{de}
2	27.40 ± 2.41^{a}	5.72 ± 0.86^{a}	18.44 ± 1.11^{c}	6.50 ± 0.24^{a}
3	39.20 ± 0.84^{b}	$9.45 \pm 0.21b^{c}$	11.78 ± 0.71^{b}	9.40 ± 0.24^{b}
4	39.40 ± 1.15^{b}	$9.89 \pm 0.28b^{c}$	11.62 ± 0.84^{b}	10.08 ± 0.30^{c}
5	41.60 ± 1.52^{c}	10.09 ± 0.25^{bcd}	10.54 ± 0.34^{a}	10.44 ± 0.17^{d}
6	40.40 ± 1.67^{bc}	10.27 ± 0.66^{cd}	10.30 ± 0.65^{a}	10.74 ± 0.13^{e}

Results are expressed in Means ± Standard Deviation (n = 5)
Mean values with different letters as superscript down the column are considered significant at $p < 0.05$.
Group 1: Normal control (Unparasitized and treated with distilled water)
Group 2: Positive control (Parasitized and untreated)
Group 3: Standard control (Parasitized and treated with 20/120 mg/kg b.w Artemether/lumefantrine)
Group 4: Test group (Parasitized and treated with 200 mg/kg b. wt of EEPCSB)
Group 5: Test group (Parasitized and treated with 400 mg/kg b.w of EEPCSB)
Group 6: Test group (parasitized and treated with 600 mg/kg b.w of EEPCSB)

3.7 Effect of Ethanol Extract of *Parinari curatellifolia* Stem Bark on Some Liver Function Parameters of *Plasmodium berghei*-infected Mice

In the Table 7 below, the result of the liver function parameters of malaria infected mice treated with ethanol extract of *Parinari curatellifolia* stem bark (EEPCSB) is shown.

Table 7: Liver function parameters of *Plasmodium berghei* infected mice treated with ethanol extract of *Parinari curatellifolia*.

Treatment Group	Liver Function Parameters			
	AST activity (IU/L)	ALT activity (IU/L)	ALP activity (IU/L)	Bilirubin Conc. (mg/dl)
Group 1	19.00 ± 1.22^{a}	29.80 ± 1.48^{a}	43.20 ± 2.59^{a}	4.74 ± 0.70^{a}
Group 2	42.20 ± 1.79^{c}	66.68 ± 2.41^{c}	86.00 ± 4.74^{d}	8.02 ± 0.70^{b}
Group 3	23.20 ± 2.28^{b}	34.80 ± 3.03^{b}	49.60 ± 1.52^{bc}	4.66 ± 0.60^{a}
Group 4	23.60 ± 2.61^{b}	32.20 ± 1.78^{ab}	46.20 ± 1.48^{ab}	4.49 ± 0.19^{a}
Group 5	21.60 ± 1.52^{b}	32.20 ± 2.74^{ab}	47.40 ± 4.78^{abc}	4.60 ± 0.61^{a}
Group 6	23.20 ± 1.92^{b}	30.40 ± 2.30^{a}	50.80 ± 1.64^{c}	4.69 ± 0.61^{a}

Results are expressed in Means ± Standard Deviation (n = 5)
Mean values with different letters as superscript down the column are considered significant at $p < 0.05$.
Group 1: Normal control (Unparasitized and treated with distilled water)
Group 2: Positive control (Parasitized and untreated)
Group 3: Standard control (Parasitized and treated with 20/120 mg/kg b.w Artemether/lumefantrine)
Group 4: Test group (Parasitized and treated with 200 mg/kg b. wt of EEPCSB)
Group 5: Test group (Parasitized and treated with 400 mg/kg b.w of EEPCSB)
Group 6: Test group (parasitized and treated with 600 mg/kg b.w of EEPCSB)

3.8 Effect of Ethanol Extract of *Parinari curatellifolia* Stem Bark on the Lipid Profile of *Plasmodium berghei*-infected Mice

The lipid profile of the malaria infected mice treated with ethanol extract of *Parinari curatellifolia* is shown in the Table 8 below.

Table 8: Lipid profile of *Plasmodium berghei* infected mice treated with ethanol extract of *Parinari curatellifolia* stem bark

Treatment Group	Lipid Profile			
	Total Cholesterol (mg/dl)	TAG Conc. (mg/dl)	HDL Conc. (mg/dl)	LDL Conc. (mg/dl)
Group 1	94.00 ± 3.16^{a}	104.80 ± 3.03^{a}	84.00 ± 3.16^{e}	20.20 ± 2.28^{a}
Group 2	116.20 ± 4.60^{d}	130.00 ± 6.20^{c}	33.20 ± 2.28^{a}	65.00 ± 3.16^{c}
Group 3	105.40 ± 2.41^{c}	113.60 ± 3.21^{b}	64.20 ± 1.79^{b}	25.00 ± 2.24^{b}
Group 4	$98.40 \pm 4.93a^{b}$	111.60 ± 3.29^{b}	69.20 ± 5.54^{bc}	26.40 ± 3.05^{b}
Group 5	103.00 ± 4.36^{bc}	109.00 ± 2.12^{ab}	73.20 ± 4.76^{cd}	24.60 ± 2.19^{b}
Group 6	100.00 ± 3.54^{b}	108.460 ± 2.19^{ab}	75.60 ± 4.83^{d}	26.00 ± 4.18^{b}

Results are expressed in Means ± Standard Deviation (n = 5)
Mean values with different letters as superscript down the column are considered significant at $p < 0.05$.
Group 1: Normal control (Unparasitized and treated with distilled water)
Group 2: Positive control (Parasitized and untreated)
Group 3: Standard control (Parasitized and treated with 20/120 mg/kg b.w Artemether/lumefantrine)
Group 4: Test group (Parasitized and treated with 200 mg/kg b. wt of EEPCSB)
Group 5: Test group (Parasitized and treated with 400 mg/kg b.w of EEPCSB)
Group 6: Test group (parasitized and treated with 600 mg/kg b.w of EEPCSB)

3.9 Effect of Ethanol Extract of *Parinari curatellifolia* Stem Bark on the Malondialdehyde Concentration and Antioxidant Markers in *Plasmodium berghei*-infected Mice

Table 9 shows the antioxidant markers and the malondialdehyde concentration of the *Plasmodium berghei*-infected mice treated with ethanol extract of *Parinari curatellifolia* stem bark.

Table 9: Malondialdehyde concentration and antioxidant markers of *Plasmodium berghei* infected mice treated with ethanol extract of *Parinari curatellifolia* stem bark

Treatment group	MDA Conc. (mg/dl)	SOD activity (IU/L)	Catalase (IU/L)	GSH Conc. (mg/dl)
Group 1	3.79 ± 0.90^{a}	8.64 ± 0.22^{c}	0.58 ± 0.32^{bc}	3.07 ± 0.60^{abc}
Group 2	8.85 ± 0.43^{c}	3.11 ± 0.73^{a}	0.23 ± 0.11^{a}	5.42 ± 0.57^{d}
Group 3	4.97 ± 0.72^{b}	7.32 ± 1.24^{b}	0.51 ± 0.07^{b}	3.66 ± 0.31^{c}
Group 4	4.63 ± 0.02^{ab}	8.26 ± 0.57^{b}	0.58 ± 0.03^{bc}	3.41 ± 0.73^{bc}
Group 5	4.80 ± 0.96^{b}	8.51 ± 0.85^{c}	0.60 ± 0.05^{c}	2.81 ± 0.67^{ab}
Group 6	4.79 ± 0.55^{b}	8.74 ± 0.44^{c}	0.56 ± 0.04^{bc}	2.35 ± 0.21^{a}

Results are expressed in Means ± Standard Deviation (n = 5)

Mean values with different letters as superscript down the column are considered significant at $p < 0.05$.

Group 1: Normal control (Unparasitized and treated with distilled water)

Group 2: Positive control (Parasitized and untreated)

Group 3: Standard control (Parasitized and treated with 20/120 mg/kg b.w Artemether/lumefantrine)

Group 4: Test group (Parasitized and treated with 200 mg/kg b. wt of EEPCSB)

Group 5: Test group (Parasitized and treated with 400 mg/kg b.w of EEPCSB)

Group 6: Test group (parasitized and treated with 600 mg/kg b.w of EEPCSB)

Discussion

The phytochemical screening of the ethanol extract of *Parinari curatellifolia* showed the presence of saponins, phenolics, flavonoids, alkaloids, glycosides, tannins, terpenoids and steroids. The quantity of steroids detected was negligible. Alkaloids, saponins, flavonoids, terpenoids, and glycosides were detected in moderate to high amounts. Our result was in agreement with that of (Halilu *et al*., 2008) which reported detection of tannins, saponins, flavonoids, steroids, glycosides in methanolic extract of *Parinari*

curatellifolia stem bark, but showed no detection of alkaloids. Furthermore, our findings is also comparable to those of (Sylvanus *et al*., 2014), differing only in the absence of flavonoids in ethanolic stem bark extract of Parinari curatellifolia, of their report. The report of Issakou *et al* (2022) also showed the detection tannins, saponins, alkaloids, flavnoids, terpenoids, and steroids which is in tandem with our result. In addition to phenolics, tannins, saponins, glycosides, alkaloids, terpenoids, flavonoids and steroids, (Omoniwa *et al*., 2021) reported the detection of resins in ethanolic extract of *P.curatellifolia* stem bark, which was not included in our study.

The plant extract's wide range of secondary metabolites may explain why it is used in ethnomedicine to treat a number of illnesses in Africa, such as malaria, fever, toothaches, fractures, pneumonia, cancer, inflammation, bacterial infections, and diabetes (Halilu *et al*., 2008; Kuntze *et al*., 2017; Mawire *et al*., 2021). Alkaloids are class of small nitrogen- containing organic molecules found in plants. Many alkaloids are great therapeutic agents which are used to treat various diseases including malaria, diabetes, cardiac dysfunction e.tc (Ain *et al*., 2016). Quinine, an alkaloid isolated from the bark of cinchona tree is an antimalarial drug. Isoquinoleine alkaloid extract from *Fumaria capreolata*, exerted antioxidant, analgesic, and intestinal anti-inflammatory activities in DNBS model of experimental colitis in mice. Antitumor efficacy is demonstrated by the alkaloids zephyrantine and narcyclasine-glucoside (Noureddine Bribi, 2018). Vincristine, an alkaloid from Vinca rosea, is an anticancer medicine, sanguinaria from Sanguinaria canadensis is antibacterial, and ementine from Carapichea ipecacuanha is antiprotozoal (Roy, 2017; Heinrich et al., 2021).

Flavonoids have been reported to possess antioxidant effect, anti-bacterial effect, anti-cancer effect cardioprotective effect, anti-inflammatory effect, and immune system promoting effect (Tungmunnithum *et al*., 2018). Phenolics and flavonoids are the largest secondary metabolites from plants with antioxidant properties. Chrysosplenetin, a known polymethoxylated flavonoids in *Artemisia annua L*. and other Chinese herbal plants, was reported to boost the antimalarial activity of Artemisinin when co-administered (Patel, 2022). Many flavonoids including triglycoside of kaempferide, kaempferol-O-rutinoside, kaempferol-3-O-b-D-glucopyranosyl isoorientin, isovitexin, vitexin, 5,6-dimethoxyflavone, 2-hydroxyisoflavanone, naringenin, naringenin 7-O-b-D-glucoside, 5-hydroxy-7,8-dimethoxyflavanone 5-rhamnoside, kaempferol 3-rhamnoside-7-xylosyl-(1-2)-rhamnoside have been found to possess antimicrobial activities (Mierziak *et al*., 2014).

Plant tannins are polyphenols that are generically classed as hydrolyzed and condensed tannins. They have been reported to possess antioxidant, anti-bacterial, anti-inflammatory, antiparasitic and antiviral activities (Tong *et al*., 2022). Saponins are naturally occurring surface-active glycosides with a particular foaming property that are present in both wild and cultivated plants as steroidal and triterpenoid

saponins, respectively. They are reported to possess a variety of biological activities including membrane-permeabiliting, immunostimulant, hypocholesterolaemic and anticarcinogenic, antiparasitic, antiprotozoal, cytotoxic, antimicrobial and antioxidant (Guclu-Ustundag and Mazza, 2007; Desai *et al*., 2009).

The acute toxicity study of ethanol extract of *Parinari curatellifolia* stem bark indicated that the median lethal dose (LD_{50}) is above 5000 mg/kg b.w, as there was no mortality recorded due to the extract in mice up to 72 hours in all the doses tested. This indicates that the extract is safe for comsumption for both animals and humans. Ogbonnia et al 2008 had earlier reported the estimated LD_{50} of ethanol extract of *Parinari curatellifolia* seeds to be 7.2 g/kg b.w,which supports our result. However, (Atawodi *et al*., 2103) reported that the LD_{50} of methanolic root extract of *P. curatellifolia* was 1,265 mg/kg b.w in rats. It could therefore be inferred that stem bark is safer compared to the root extract.

The percentage parasitemia of the animals treated with the extract in this study was significantly ($p < 0.05$) reduced compared to the untreated group. This result is in tandem with that of (Omoniwa *et al*., 2021) which had earlier reported the *in vitro* antiplasmodial activity of ethanol extract of *Parinari curatellifolia* stem bark. The observed antimalarial effect of this extract is attributable to the activities of the constituent bioactive compounds including alkaloids, terpenoids, and/or saponins. These compounds could have acted singly or in synergy to exert cytotoxic effect on the malarial parasite. Further studies of the compounds in the extract could lead to isolation of lead compounds for novel antimalarial drug.

The result of this study indicated that the hematological parameters including packed cell volume (PCV), red blood cell (RBC) count, and haemoglobin (Hgb) concentration of the treated animals were significantly ($p < 0.05$) increased compared to the untreated animals, while the white blood cell (WBC) count of the treated animals was significantly ($p < 0.05$) lower compared to the untreated group. The observed decrease in the PCV, RBC count and Hgb concentration of the untreated infected animals was in accord with previous studies (Francis et al., 2014; Nefsu and Amsalu, 2019), which adduced that the phagocytosis of parasite-infected RBC, decreased RBC formation in the bone marrow, and mechanical destruction of the parasitized red blood cells accounted for the decline. In addition, the observed rise in the WBC count of the untreated infected mice was consistent with past studies (Francis *et al*., 2014; Kotepui *et al*., 2015; Sirak *et al*., 2016), which all suggested that such increase was attributable to enhanced leucocyte release in response to plasmodium infection. Hemolysis and anaemia are known features of malaria, due to the invasion of the red blood cells and digestion of heamoglobin by plasmodium parasite (Kavishe et al., 2017; Mawson, 2013). This accounts for the reduced PCV, RBC count and Hgb concentration in malarial infected subjects as observed in the untreated infected animals.

However, there was an increase in the PCV, RBC count, Hgb concentration and a decrease in WBC count of the animals when treated with the extract, indicating a reversal of the hematological aberrations caused by the Plasmodium parasites. This result suggests that the extract possesses antiplasmodial activity, leading to drastic reduction in parasitemia and hemolysis in the treated animals. The erythrocytic schizonts attack the red blood cells, damaging the membrane through the byproducts of its haemoglobin metabolism such as ferriprotoporphyrin 9, which exert oxidative stress and lipid peroxidation to the red blood cells, causing hemolytic anemia in malaria disease. Our result also agrees with the report of (Ajibola *et al*., 2012) which indicated that non-malaria infected persons has higher PCV and Hgb concentration than malaria patients. Sirak *et al*. (2016) had also reported that density of parasitemia determined the changes in hematological parameters in malaria patients. Patients with high malaria parasite density had lower PCV and Hgb concentration with higher WBC count while patients with low parasitemia had higher PCV and Hgb concentration with lower WBC count.

Anemia is determined by estimating the packed cell volume. A PCV value of less than 30% indicates anemia (Osonuga *et al*., 2006). The reduced PCV indicated anemia in the infected animals. Anemia is a common and frequently severe complication of malaria, particularly in young children and pregnant women, and accounts for more than 50% of malaria-associated mortality in endemic areas (Mohandas and An, 2013). Malaria-induced anemia is caused by multiple factors including lysis of parasitized erythrocytes, immune-mediated hemolysis, dyserythropoietic changes and shortened life span of erythrocytes (Osonuga *et al*., 2006) The ethanol extract of *Parinari curatellifolia* was able to restore the PCV of the treated animals in groups 5 and 6 to a level that is statistically non-significantly different compared to the normal control. This also demonstrates that the extract was able to stop hemolysis of red blood cells and may have the ability to increase erythrocyte synthesis in mice. This finding also indicates that the extract reversed anemia in mice to a comparably the same level as artemether/lumefantrine.

The clinical symptoms and pathologies associated with malaria are as a result of cellular and membrane changes in the human red blood cells (RBC) induced by the intra-erythrocytic parasite. These structural and biochemical changes, mediated by interactions between red cell membrane and parasite-expressed proteins, impart rigidity and adhesiveness to the infected red blood cells (iRBC) making them adhesive for a variety of cell types, including vascular endothelial cells (cytoadherence), platelets, and other infected or uninfected red cells. Infected red cells are eventually ruptured by the matured intra-erythrocytic parasite for another cycle of red cell invasion (Mohandas and An, 2013). Rigid RBCs contribute to disease pathology through sequestration of these cells and obstruction of the microvasculature, however its impact is primarily for the clearance of the parasitized cells in the spleen (Deng *et al*., 2015). The extract could have induced reduced cell deformability of the infected red cells thereby enhancing their host-

mediated clearance in the inter-endothelial cleft of the spleen. This idea is supported by the fact that reduced deformability of infected red blood cell was reported to be a common feature antimalarial drug. Artemether, the standard drug in this study, is thought to act by a mechanism which generate reactive oxygen species that contribute to reduced iRBC deformability through lipid peroxidation (Deng *et al*., 2015).

The malaria parasite digest haemoglobin, the iron containing protein of the red blood cells, during its 48-hour intra-erythrocytic cycle to obtain amino acids, leading to depletion of haemoglobin and accumulation of free heme (Joof *et al*., 2021). The result of this study showed that haemoglobin concentration in the untreated animals significantly ($p < 0.05$) reduced compared to the uninfected animals. The administration of the extract, on the other hand, significantly ($p < 0.05$) raised the haemoglobin concentration in the test groups. This result is consistent with prevous studies (Aragaw *et al*., 2020; Joshua *et al*., 2020; Enechi *et al*., 2021). The activity of some of the secondary metabolites in the extract could be responsible for the observed higher haemoglobin concentration in the treated animals. It could be adduced that some of the metabolites inhibited the digestion of haemoglobin by the parasite. This is as some quinoline drugs such as chloroquine and amodiaquine have been found to inhibit haemoglobin degradation (Famin and Ginsgurg, 2002). The increased white blood cell count found in the untreated animals indicates the immune response of the animals to the plasmodium infection. Reports have shown that severe malaria is characterized by leukocytosis. The extract however reduced the WBC count in the treated animals. This confirms that the extract was able to clear the infecting Plasmodium parasite thereby reducing the stimulation of leukocytosis.

The overall health of the liver is determined by a number of blood tests generally known as liver function tests. The tests evaluate the levels of several enzymes, proteins, and other chemicals produced by the liver. This test is employed in the assessment of liver damage, determine side effect of certain drugs, diagnosis of liver disease and monitoring of its treament. Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gammaglutamyl transferase, serum bilirubin, prothrombin time, and serum albumin are some of the commonly available liver function test. Several liver activities such as anion excretion (bilirubin), hepatocellular integrity (transaminases), bile production and subsequent free flow (bilirubin and ALP), and protein synthesis (albumin) are reflected by this test (Hoekstra *et al*., 2013; van Beek *et al*., 2014).

In this study, the activities of serum liver enzymes namely AST, ALT and ALP were assayed, while the concentration of the serum bilirubin was determined to assess hepatocellular damage and impairment of liver function due to malaria parasite, as well as the effect of the ethanol extract of *Parinari curatellifolia* stem bark on the liver. The result indicated that there was significant lowering of in the activities of AST,

ALT and ALP as well as the concentration of total bilirubin in all the test groups when compared to the untreated group. The untreated group demonstrated an increase in all of the assessed metrics when compared to the normal control group. The increase in the activities of aminotransferases (AST, ALT) and alkaline phosphatase may be as a result of possible hepatocellular damage (Gowda *et al*., 2009) while the increased hyperbilirubinemia could be due to parenchymal liver cell destruction and biliary blockage caused by malaria parasite, liver schizonts (Limdi and Hyde, 2003; Hoekstra *et al*., 2013). This finding is in agreement with earlier research (Enechi *et al*., 2021; Iyiola, 2020; Joshua *et al*., 2020) which discovered higher hepatic enzyme activity and hyperbilirubinemia in mice infected with *Plasmodium berghei*. The reduction in increased AST, ALT, and ALP levels demonstrated that the extract was efficient in correcting plasmodium parasite-induced hepatocellular damage. It is evident from this result that the has hepatoprotective ability which could be due to the synergistic action of the several secondary metabolites in the extract. The hepatoprotective effect could also be due to the action of a single phytochemical in the extract.

The concentration of bilirubin in plasma provides indirect information on the liver's uptake, conjugation, and excretion functions. Elevated plasma concentration of bilirubin are specific markers for serious liver injury and, consequently, liver function loss (Hoekstra *et al*., 2013). The reduction in plasma concentration of bilirubin of mice infected with *Plasmodium berghei* and treated with the extract shows that the extract reversed liver cell damage and restored liver cell function. This is congruent with the findings of Joshua *et al*. (2020) which demonstrated that in mice with *Plasmodium berghei* infection, *E. sengalensis* extract reduced the total bilirubin levels.

Visser *et al*., 2017 had reported changes in lipid profile associated with malaria infection. This study determined the total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides. The result of the showed that the ethanol extract of *Parinari curatellifolia* stem bark significantly ($p < 0.05$) reduced the total cholesterol, triglyceride and LDL cholesterol while significantly ($p < 0.05$) raising the HDL cholesterol in the treated animals compared to the untreated ones (positive control). Previous studies (Enechi *et al*., 2021; Joshua *et al*., 2020), supports this result. This study further revealed that malaria infection results in mixed hyperlipoproteinemia in mice, which is characterized by increased total cholesterol, triglyceride, LDL-cholesterol and decreased HDL-cholesterol (Natesan and Kim, 2021). However, the mechanism involved in the changes in plasma lipid profile related to malaria is yet to be completely understood. It has been suggested to be partly host-related (Acharya and Patil, 2009). This is in contrast with the reportedly observed dyslipidemia in human malaria which showed increased triacylglycerol and decreased HDL, LDL and total cholesterol plasma concentrations in infected patients (Visser *et al*., 2013; Dias *et al*., 2016; Sirak *et al*., 2016; Visser *et al*., 2017). This observed difference could be due to differing pattern of lipid metabolism in mice and humans. The accumulation of triglyceride and LDL-cholesterol causes damage to the arteries, contributing to cardiovascular disease, while on the other hand HDL-cholesterol is useful in

helping the body to get rid of bad cholesterol (Natesan and Kim, 2021). The finding of this study reveal that the extract possesses anti-lipidemia activity as it was able to reduce total cholesterol, triglyceride and LDL-cholesterol in the treated mice. The anti-lipidemia effect of this extract as found in this study also supports the previous report of Ogbonnia *et al*. (2008), which demonstrated that the hydro ethanolic extract of *P. curatellifolia* seed significantly reduced plasma lipid concentration in diabetic rats.

Malondialdehyde (MDA) is used as an indirect measure of the cumulative lipid peroxidation as it is a unstable end product of lipid peroxidation (Mao *et al*., 2019). This aldehyde known for its high cytotoxicity and carcinogenicity is a product of free radicals' attack on phospholipids. Increased level of MDA is associated with decreased antioxidant parameters (Maurya *et al*., 2021), and this is as a result of increased production of oxidative species (Percário *et al*., 2012), associated with malaria infection. The result of this study indicated that MDA concentration significantly ($p < 0.05$) reduced in the treated mice compared to the positive control (the untreated infected mice). This shows that the extract has free radical scavenging activity which could be attributed to the phytochemical constituents in the extract including flavonoids, phenols, tannins, and terpenoids. This finding is consistent with previous studies which showed *Parinari curatellifolia* to be a potent scavenger of nitrite free radicals (Boora *et al*., 2014) and inhibitor of phospholipid peroxidation due to free radical activity (Ndhlala *et al*., 2006).

Superoxide dismutase (SOD) is utilized both the human host and *Plasmodium* parasite as first line of defense against superoxide radicals. They metalloproteins that dismutate superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2) using copper/zinc (Cu/Zn), iron (Fe), or manganese (Mn) as cofactors (Kavishe *et al*., 2017). The SOD activity of the mice treated with the extract of *Parinari curatellifolia* stem bark was found to significantly ($p < 0.05$) increase compared to the untreated animals. The SOD activity of the untreated group decreased significantly compared to the uninfected group. This could be due to increased concentration of oxidants generated by the host cell in response to malaria infection, leading to the depletion of the enzyme and reduced activity observed. The extract however restored the activity of the enzyme to a level that is non-significantly different from the normal control in all the treated groups. This effect could be attributed to the antioxidant activity of the extract. The extract contains antioxidative phytochemicals such as flavonoids, phenols and tannins. Previous studies in rats (Olaleye *et al*., 2014; Yakubu, 2012; Atawodi *et al*., 2013), has showed that *Parinari curatellifolia* possess antioxidant activity, is consistent with the result of our study.

The catalase activity of the treated animals in this study was also found to increase significantly ($p < 0.05$) in the group of mice treated with the extract compared to the untreated group. The catalase activity in the untreated group significantly decreased compared to the normal control, possibly due to excessive

generation of hydrogen peroxide (H_2O_2). Catalase limits the accumulation of H_2O_2 (Kavishe *et al*., 2017). Previous studies has shown that malaria infection is associated with decreased activity of catalase (Vasquez *et al*., 2021). The result of this study antioxidative activity of the ethanol extract of *Parinari curatellifolia* stem bark. However, the result showed that the reduced glutathione (GSH) concentration significantly increased in the untreated group compared to the normal control (unparasitized mice). This observation is consistent with that of Stocker *et al*., 1985, which reported increased GSH concentration in malaria patient with high number of infected red blood cells due to the enhancement of glutathione reductase. They suggested that significant portion of the increase was associated with the production of GSH by the parasite itself. The administration of the extract resulted in significant ($p < 0.05$) decrease in GSH concentration of the treated group compared to the untreated group. The effect might be due to generation of oxidative stress by a phytochemical component of the extract, as it is known that some anti-malarial drugs exert their antiplasmodial effect by increasing oxidative stress on the parasite. A study by (Akanbi *et al*., 2010) had reported lower levels of GSH in malarial patients treated with anti-malarial drug compared to patients who had not taken drug.

Conclusion

The findings from this study so far have shown that the ethanol extract of *Parinari curatellifolia* stem bark can serve as an effective and safe regimen for the treatment of malaria in the mouse model. The extract is composed of phytochemicals that can be beneficial to human health, and caused no mortality in the acute toxicity test in mice, showing its safety for human use. The extract also effectively reduced parasitemia in infected mice, while restoring hematological aberrations including PCV, RBC count, WBC count, haemoglobin concentration and biochemical aberrations including liver enzymes activities, bilirubin concentration and lipid profile, induced by the *Plasmodium* parasite. This study also showed that the extract countered lipid peroxidation by reducing the MDA level in treated animals as well as restored the antioxidant markers to the levels found in the uninfected animals. This study has so far demonstrated that ethanol extract of *Parinari curatellifolia* stem bark is effective and safe for the treatment of malaria, thereby justifying its use for managing malaria in various part of the African continent. This plant can as well serve as a source of lead compound for the development of a novel, safe and effective antimalarial drug if exploited.

Abbreviations

ACT	Artemisinin combination therapy
ALT	Alaninine aminotranferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
CAT	Catalase

DCM-MeOH	Dichloromethame-methanol
EEPCSB	Ethanol extract of *Parinari curatellifolia* stem bark
FFA	Free fatty acids
FP	Ferriprotoporphyrin
GGT	Gamma-glutamyl transferase
GGTP	Gamma glutamyl transpeptidase
G6PD	Glucose-6-phosphate dehydrogenase
GSH	Reduced glutathione
HDL	High density lipoprotein
Hgb	Haemoglobin
IC50	Median inhibitory concentration
LDL	Low density lipoprotein
MCV	Mean Cell Volume
MDA	Malondialdehyde
PCV	Packed cell volume
RBC	Red blood cell
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SPSS	Statistical Product and Service Solution
SOD	Superoxide dismutase
VLDL	Very low density lipoprotein
WBC	White blood cell
WHO	World Health Organization

Declarations

Ethics approval

This study was conducted in line with the ethical rules and recommendations of the University of Nigeria Ethical committee on the care and use of laboratory animals.

Consent for publication

Not Applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

LOM, PEJ, CCA; Conceptualization, Methodology, Software, LOM: data curation, writing – original manuscript preparation, PEJ: supervision, PEJ, CCA, LOM: investigation, validation PEJ, CCA, IO, JOF: writing – reviewing and editing. All authors read and approve the final manuscript.

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