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Effect of Methanol Extract of Costus Lucanusianus Root on Oxidative Stress, Liver Function and Haematological Markers in Malaria-infected Wistar Mice

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ABSTRACT

Background: Malaria is associated with oxidative stress, haematological alterations, and organ damage. *Costus lucanusianus* is traditionally used in the management of fever, but its antimalarial potential is not well established.

Materials and Methods: The methanol root extract of *C. lucanusianus* was evaluated in *Plasmodium berghei*-infected mice using the 4-day suppressive test. Acute toxicity (Lorke's method), parasitemia suppression, survival time, haematological indices, oxidative stress markers, liver function tests, and histopathology were assessed.

Results: The extract was safe up to 5000 mg/kg. It produced dose-dependent parasite suppression, with 500 mg/kg achieving 50% inhibition, though less effective than chloroquine (62.5%). Treated mice showed prolonged survival, improved antioxidant enzyme activity, and reduced lipid peroxidation. Haematological parameters indicated increased WBC and platelet counts with reduced neutrophils. Liver function markers remained largely stable, while histopathology showed milder hepatic distortion compared to untreated controls.

Conclusion: *Costus lucanusianus* root extract demonstrates significant antimalarial, antioxidant, and immunomodulatory activities, supporting its ethnomedicinal use and potential as a source for new antimalarial agents.

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INTRODUCTION

Malaria is a disease whose pathology is linked to inflammation and oxidative disease. During the blood stage of infection, the level of oxidative stress in plasma is frequently measured by determining the concentration of malondialdehyde (MDA), a lipid peroxide which is formed as a consequence of oxidation of unsaturated lipids and reflects the levels of free radicals in the circulation.[1] The increase in oxidative stress observed in malaria patients infected with either *P. falciparum* or *P. vivax* infections is often coupled with a decrease of anti-oxidant levels which leads to the loss of the homeostatic balance between free radicals and antioxidant capacity that is maintained in healthy tissues.[5]

Oxidative stress is caused by reactive oxygen or nitrogen atoms that have unpaired electrons in their outer shell. They are called reactive oxygen species (ROS) or reactive nitrogen species (RNS) and are commonly produced in cells. These radicals are oxidants that can damage cellular components, but are also involved in essential cellular processes, such as intracellular signaling and the oxidative burst in innate immune cells.[2] Oxidative stress can induce inflammation, since

ROS regulate the inflammatory response in immune cells through the activation of NF-kB, which results in the secretion of inflammatory cytokines.[3] ROS also serves as the first signal for the activation of the inflammasome,[4] further contributing to the inflammatory response.

Given that Plasmodium parasites are sensitive to ROS-mediated damage,[5] it is not surprising that various antimalarial treatments exploit this feature of the parasite to limit its growth within human hosts. For example, Quinolones, including chloroquine and amodiaquine, act by inhibiting the conversion of free heme to haemozoin within the infected erythrocyte, effectively increasing oxidative stress for Plasmodium parasites.[6]

Malaria is usually associated with various degrees of reduced blood counts, and mild to moderate thrombocytopenia is a common association of malaria but it is rarely associated with hemorrhagic manifestations or a component of disseminated intravascular coagulation.[7] Haematological parameters that are often affected include the relative numbers of circulating cell types such as erythrocytes, platelets, granulocytes and lymphocytes, as well as parameters like haemoglobin concentration.[8]

Malaria is known to cause liver and kidney damage especially is severe cases. In fact, it is the first parasitic infection to be clearly associated with glomerular diseases in the tropical region.[9] Hepatic dysfunction and jaundice are common features of severe malaria.[10]

Medicinal plants and natural products have been of scientific interest for their potential role in modulating oxidative stress and inflammatory-related disorders.[11] These resources are abundant in antioxidants, such as flavonoids, polyphenols, and vitamins which can neutralize harmful free radicals and reduce oxidative stress associated with various chronic diseases.[11] The plant of choice, *Costus lucanusianus* was selected as it was reported to possess anti-inflammatory activities.[12]

MATERIALS AND METHODS

Experimental animals

Thirty (30) wistar mice used for this study were 6-week-old wistar mice weighing 19-35 g obtained from the Animal house, Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt. They were housed in plastic cages with saw dust as beddings and were given food and water ad libitum. The animals were allowed to acclimatize for 7 days preceding the experiments. All experimental protocols followed internationally accepted principles for laboratory animal use and care.

Plant material and Authentication

Fresh roots of *Costus lucanusianus* were collected from the forest reserve of University of Port Harcourt, Nigeria (4.91°N, 6.92°E). The plant was identified by Prof. I. Agbagwa of the department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, and a sample was deposited at the University of Port Harcourt Herbarium with reference number UPH/V/1212

Preparation of Methanol extract of Costus lucanusianus root (MECL)

After collection of the plant, the roots were washed, chopped into smaller pieces and shade-dried at room temperature (32 – 35°C) to constant weight over a period of twenty-one (21) days. Bulk of the plant was collected between June 2023 and February 2024. The cold maceration extraction method was used. 500 g of dried *Costus lucanusianus* root was weighed and ground to fine powder and dissolved in 2500mL of methanol inside a 2.5L conical flask. The flask was shaken

vigorously at 30-minute intervals and left to stand for 72 hours at room temperature for effective extraction. The resultant mixture then was filtered with Whatman's No. 1 filter paper and cotton wool. The clear solution obtained was concentrated with rotary evaporator at 45°C under low pressure and later transferred to evaporating dish over a steam bath. The solid dried powder obtained was stored in sterile pre-weighed screw capped bottles and labelled accordingly. The extract was now stored at room temperature.

Acute Toxicity test in Mice

The toxic effect of methanol extract of *Costus lucanusianus* root was determined on wistar mice. Lorke's method was used for the study.[13] It had two phases. Phases 1 required nine animals and divided into three groups. Each group was administered 10, 100 and 1000mg/kg of the extract. The animals were placed under observation for 24 hours to monitor if mortality will occur.

Phase 2: Three animals were used in this phase. The animals were administered higher doses, 1600, 2900 and 5000 mg/kg of the extract respectively. They were then observed for 24 hours for mortality. Then the LD_{50} was calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

In vivo Antimalarial test of Fraction

The anti-malarial activity of methanol extract of *Costus lucanusianus* root was assessed by the classic 4-day suppressive test.[14] Mice were inoculated intraperitoneally (i.p) with blood containing 1 x 10^7 parasitized (CQ-sensitive *P. berghei berghei* NK 65 strain) erythrocytes contained in 0.2 mL inoculum on Day zero (D_0). The treatments commenced 1hour after the infection. Plant extract was solubilized in dimethyl suphoxide (DMSO) and administrated at different concentrations ($1/50^{th}$, $1/25^{th}$ and $1/10^{th}$ mg kg^{-1}/day of the LD_{50})[15] in a dose volume of 0.2 mL. The animals were placed in six groups with five mice in each group. Group 1, 2 and 3 served as the normal, negative and positive control respectively. Group 4-6 served as the treated groups. For the period of treatment (D_1 – D_3), blood samples were collected from tail snips of the mice daily and were made into thin films. The blood films were developed for microscopic examination to monitor their parasite densities. The % suppression (inhibition) of parasitaemia was calculated by comparing the parasitaemia present in negative control with those of treated group.

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

$$\%\ Suppression = \frac{\text{Mean parasitemia of negative control} - \text{Mean parasitemia of treated group}}{\text{Mean parasitemia of negative control}} \times 100$$

$$\text{MST} = \frac{\text{Total number of days mice survived}}{\text{Total number of mice}} \times 100$$

Liver function parameters

Blood plasma for liver function parameters were obtained from the blood samples following centrifugation at 3000 rpm for 5 min. Aspartate transaminase (AST) and Alanine transaminase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP) and Albumin were estimated using standard laboratory kits (Randox Laboratories Limited, Crumlin, County Antrim, BT294QY, United Kingdom), as per manufacturer's instructions.

Haematological assays
Haematological analysis of the blood samples was performed using an automated haematology analyzer (2800 Haematology AutoAnalyzer). Parameters which were evaluated included white blood cell (WBC), red blood cell (RBC), haemoglobin concentration (Hg), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Oxidative stress markers
Catalase activity, superoxide dismutase, glutathione, glutathione peroxidase (GPX) activity and malondialdehyde (MDA) were estimated.

Histopathological examination
The liver specimens from each mouse were immediately stored in 10%v/v formalin in normal saline after gross histological examination and dehydrated using increasing concentrations of isopropyl alcohol (80-100%).

Data Analysis
Data from the study was analysed using GraphPad prism v. 9.02 statistical software. Data was presented as mean±SEM. Significance was considered at $p< 0.05$ using one-way analysis of Variance (ANOVA) followed by tukey's post hoc test.

Ethical Approval
The study was approved by the Research Ethics Committee of the University of Port Harcourt with approval number **UPH/CEREMAD /REC/MM105/080**.

RESULTS
Suppressive effect of Methanol root extract of Costus lucanusianus in *Plasmodium berghei* infested mice

Table 1 showed the Suppressive effect of methanol root extract of *C.lucanusianus* on *Plasmodium berghei* infested mice for day one. The result showed no significant difference between the negative control and the treated groups. Also, there was no significant difference between the positive control (5 mg/kg Chloroquine) and the treated groups. Table 2 showed significant difference when the treated groups were compared with the normal control (NC), negative control and positive control. It was also observed that the significance was dose dependent. As the dose of the extract increased, there was reduction in the parasite load and an increase in % inhibition. Table 3 showed further decrease in the % parasite load of the treated group when compared with the negative control. 500 mg/kg showed greater inhibition (50%) and its % parasite load (10.00±0.30) was close to the positive control (5 mg/kg CQ). The mean survival time (MST) showed an increase in survival time as the doses of the extract increased.

Table 1: Suppressive effect of Methanol root extract of *Costus lucanusianus* on *Plasmodium berghei* infested mice (DAY 1).

GROUPS	Parasite load (%)	% Inhibition	MST
NC	00±00		30.0±0.00
P.berghei	13.50±0.35		6.67±0.88
5 mg/kg CQ	12.00±0.80	11.1	29.00±0.58
100 mg/kg MECL	13.00±0.20[a]	3.7	19.35±0.77
200 mg/kg MECL	12.60±0.10[a]	6.7	21.34±0.88
500 mg/kg MECL	12.50±0.15[a]	7.41	25.01±1.02

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; MST: Mean Survival Time; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 2: Suppressive effect of Methanol root extract of Costus lucanusianus in Plasmodium berghei infested mice (DAY 2).

GROUPS	Parasite load (%)	% Inhibition	MST
NC	0.0±0.0		30.0±0.00
P.berghei	21.50±1.06		6.67±0.88
5 mg/kg CQ	11.00±0.40	48.8	29.00±0.58
100 mg/kg MECL	17.00±0.10[abc]	20.9	19.35±0.77
200 mg/kg MECL	15.00±0.50[abc]	30.2	21.34±0.88
500 mg/kg MECL	15.00±0.30[abc]	30.2	25.01±1.02

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; MST: Mean Survival Time; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 3: Suppressive effect of Methanol root extract of Costus lucanusianus in *Plasmodium berghei* infested mice (DAY 3).

GROUPS	Parasite load (%)	% Inhibition	MST
NC	0.00±0.00		30.0±0.00
P.berghei	20.00±1.56		6.67±0.88
5 mg/kg CQ	7.50±0.25	62.5	29.00±0.58
100 mg/kg MECL	15.00±0.20^{abc}	25	19.35±0.77
200 mg/kg MECL	13.00±0.10^{abc}	35	21.34±0.88
500 mg/kg MECL	10.00±0.30^{ab}	50	25.01±1.02

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; MST: Mean Survival Time; Values represented as mean±SEM; ^{a}p<0.05 when compared with NC; ^{b}p<0.05 when compared with P.berghei; ^{c}p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Effect of Methanol root extract of Costus lucanusianus on liver function, haematological and oxidative stress parameters of wistar mice

Table 4 showed no significant change in the levels of the liver function parameters. In table 5, it was observed that white blood cells significantly ($p<0.05$) increased in the treated groups when compared with the negative control. It was also noticed that the extract did not bring the WBC back to that of normal control (4.55±0.24). Platelet was also observed to significantly ($p<0.05$) change in the treated groups when compared with the negative control. Table 6 showed that haematological parameters were not significantly ($p<0.05$) changed except for neutrophils which was significantly ($p<0.05$) decreased in the treated groups when compared with the negative control.

In table 7, there was no significant ($p<0.05$) difference in the values of Glutathione (GSH) when compared with negative control. However, the treated groups varied significantly ($p<0.05$) when compared with the normal control. There was also a significant ($p<0.05$) difference in the concentration of superoxide dismutase (SOD) between the treated groups and negative control. The positive control (5 mg/kg CQ) group showed a significant ($p<0.05$) difference in SOD when compared with the treated groups. Malondialdehyde (MDA) had no significant ($p<0.05$) change when the treated group was compared with the negative control. However, 500 mg/kg MECL reduced MDA significantly ($p<00.05$) when compared with the positive control (5 mg/kg CQ).

Table 4: Effect of Methanol root extract of Costus lucanusianus on liver function parameters of wistar mice.

GROUPS	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	TP (g/dl)	ALB (g/dl)
NC	28.00±2.00	23.00±2.00	33.50±2.12	64.00±1.41	42.00±1.41
P.berghei	25.50±1.50	22.00±1.00	32.00±1.41	53.00±2.83	34.50±2.12
5 mg/kg CQ	37.50±2.50	25.50±3.50	37.50±0.71	55.50±2.12	36.50±1.50
100 mg/kg MECL	25.50±2.29	18.00±1.22	32.00±3.59	51.50±4.21	34.02±1.87
200 mg/kg MECL	26.50±1.99	15.50±0.73	28.08±2.72	48.00±3.46	31.00±0.96
500 mg/kg MECL	28.50±3.18	19.00±1.85	29.00±3.11	63.50±1.21	42.00±3.12

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; AST: Aspartate transferase; ALT: Alanine transaminase; ALP: Alkaline Phosphatase; TP: Total Protein; ALB: Albumin; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 5: Effect of Methanol root extract of *Costus lucanusianus* on haematological parameters of wistar mice.

GROUPS	PCV (%)	Hb (g/dL)	RBC($\times 10^6/\mu l$)	WBC $\times 10^3/\mu l$)	PLT ($\times 10^3/\mu l$)	MCHC (g/dL)
NC	40.0±1.41	12.10±0.90	7.20±0.28	4.55±0.24[a]	510.0±87.0[a]	29.75±2.57
P.berghei	44.0±2.03	13.35±0.07	8.05±0.85	6.45±0.27[b]	254.0±52.0[b]	30.45±4.31
5 mg/kg CQ	37.0±1.41	11.65±1.45	6.65±0.07	7.95±0.40[c]	148.5±53.5[c]	31.15±4.45
100 mg/kg MECL	42.50±2.50	13.65±0.95	7.00±1.10	11.85±0.45[abc]	714.5±68.5[bc]	31.45±3.15
200 mg/kg MECL	38.50±0.71	11.80±1.40	6.50±0.20	7.15±0.13[a]	561.0±21.9[c]	30.00±4.24
500 mg/kg MECL	42.50±3.53	12.65±1.55	7.80±0.28	7.70±0.21[a]	495.0±63.51[c]	29.00±1.90

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; PCV: Packed Cell Volume; Hb: Hemoglobin; RBC: Red Blood Cells; WBC: White Blood Cells; PLT: Platelets; MCHC: Mean Corpuscular Hemoglobin Concentration; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 6: Effect of Methanol root extract of *Costus lucanusianus* on haematological parameters of wistar mice.

GROUPS	MCH (g/dL)	MCV (g/dL)	Neutrophil (%)	Lymphocytes (%)	Eosinophil (%)	Monocytes (%)
NC	16.85±1.65	56.55±2.15	22.00±1.41[a]	71.50±10.5	1.50±0.05	5.00±0.16
5 mg/kg CQ	17.35±2.05	55.70±1.27	21.00±1.81[b]	72.00±14.4	1.00±0.03	6.50±0.27
P.berghei	16.65±1.85	54.80±0.99	10.50±0.85[c]	83.50±12.50	1.50±0.01	4.50±0.15
100 mg/kg MECL	19.65±1.55	62.60±5.80	3.00±0.10[abc]	94.50±15.00	1.00±0.00	3.02±0.10
200 mg/kg MECL	17.90±2.12	59.85±0.95	3.50±0.50[abc]	92.50±21.21	1.00±0.00	3.01±0.11
500 mg/kg MECL	16.10±1.60	55.60±1.80	5.00±0.83[ab]	91.00±13.00	1.00±0.00	3.03±0.14

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; MCH: Mean Corpuscular Hemoglobin; MCV: Mean Corpuscular Volume; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Table 7: Effect of Methanol root extract of *Costus lucanusianus* on oxidative stress markers

GROUPS	GSH	GPx	CAT	SOD	MDA
NC	2.69±0.02[a]	0.081±0.0009[a]	1.18±0.11[a]	0.40±0.029[a]	0.35±0.038[a]
P.berghei	2.34±0.10[b]	0.073±0.0052[b]	0.95±0.16[b]	0.22±0.020[b]	0.55±0.021[b]
5 mg/kg CQ	2.09±0.08[c]	0.063±0.0009[c]	0.89±0.015[c]	0.19±0.012[c]	0.58±0.006[c]
100 mg/kg MECL	2.11±0.08[a]	0.062±0.0018[a]	1.43±0.124[bc]	0.41±0.023[bc]	0.41±0.035
200 mg/kg MECL	2.28±0.06[a]	0.072±0.0038	1.52±0.02[bc]	0.35±0.49[bc]	0.40±0.065
500 mg/kg MECL	2.31±0.06[a]	0.075±0.0044	1.70±0.04[abc]	0.40±0.019[bc]	0.36±0.048[c]

NC: Normal Control; P.berghei: Negative Control; CQ: Chloroquine; GSH: Glutathione; GPx: Glutathione peroxidase; CAT: Catalase; SOD: Superoxide Dismutase; MDA: Malondialdehyde; Values represented as mean±SEM; [a]p<0.05 when compared with NC; [b]p<0.05 when compared with P.berghei; [c]p<0.05 when compared with 5 mg/kg CQ; MECL: Methanol root extract of Costus lucanusianus.

Histology

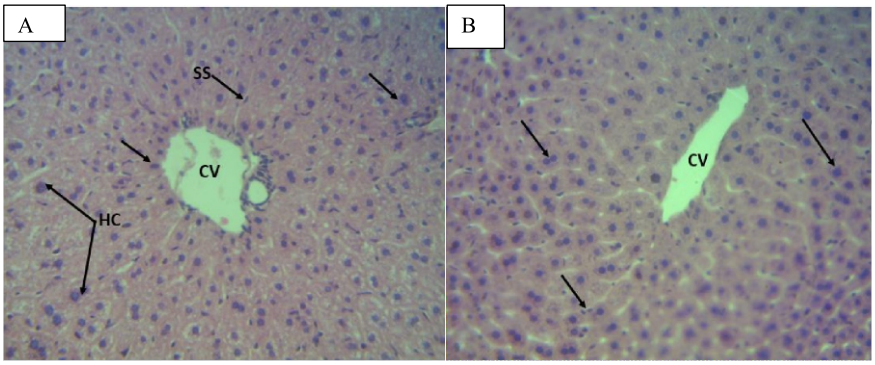

Figure 1: (A) NORMAL CONTROL: Photomicrograph (H&E X400) of the liver showing the centrilobar area of the central vein (CV): visible hepatocytes (HC) with kupfer cells within the sinusoids (SS) draining into the central vein. Livers tissues appear normal (arrows). Diagnosis: Normal liver tissue. (B) POSITIVE CONTROL: Photomicrograph (H&E X400) of the liver architecture showing minimal congestion of the central vein, sinusoids with kupfer cells and hepatocytes (arrows): tissue appears normal Diagnosis: Normal appearance of the liver parenchymal.

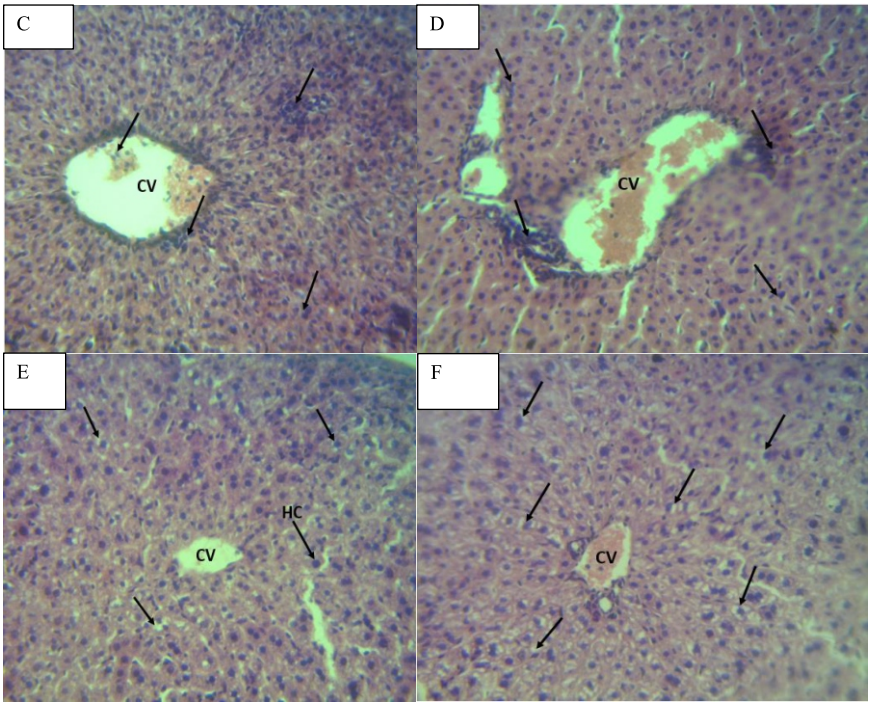

Figure 2: (C) NEGATIVE CONTROL: Photomicrograph (H&E x400) of the liver showing severely diffused mononuclear infiltration within the central vein and surrounding liver parenchymal (arrows) Diagnosis: Severe Inflammation of the liver tissue. (D) METHANOL LOW DOSE: Photomicrograph (H&E X400) of the liver showing mild sinusoidal dilation and mononuclear infiltration around zone 1 of the liver parenchymal (arrows). Diagnosis: mild inflammation of the liver tissue. (E) METHANOL MEDIUM DOSE: Photomicrograph (H&E X400) of the liver with congestion of the central vein with mild glycogen degeneration of the liver parenchymal (arrows). Diagnosis: mild fatty degeneration of the liver cytosol. (F) METHANOL HIGH DOSE: Photomicrograph (H&E X400) of the liver with congestion of the portal vessel with diffused glycogenesis of the liver parenchymal (arrows). Diagnosis: Glycogen degeneration of the liver parenchymal.

DISCUSSION

This study elucidated the effect of methanol extract of Costus lucanusianus root on liver function, haematological and oxidative stress markers. It adopted an in vivo model in order to factor in possible prodrug effect and involvement of the immune system in extermination of infection.[16] The acute toxicity result of the plant extract showed that it was safe to be taken at above 5000 mg/kg. Based on Erhirhie et al.,[17] the plant is practically non-toxic as the dose was within the range of 5000 – 15000 mg/kg.

The study found that different doses of the extract lowered parasite load in the animals and enhanced their survival time in a dose-dependent manner. It is hence concluded that the plant's antiplasmodial activity would be in the early infection stage where malaria's primary attack can be measured.[18] Although, 500 mg/kg of the extract reduced the parasite load significantly at day 3, chloroquine showed a better result and inhibited parasite load by 62.5%. This result is in contrast with Afshar et al[19] who stated that only dichloromethane (DCM) and n-hexane extracts of all aerial root of S. frigida indicated high to moderate anti-malarial potency in comparison with the reference control, while methanol extract of aerial root of S. frigida showed no significant anti-malarial activities. However, Uzor et al.[20] stated that the methanol fraction was the most active fraction of D. edulis.

Hepatic dysfunction and jaundice are common features of severe malaria. In malaria infection, there is usually an increase in ALT, AST, ALP, Total bilirubin, and a decrease in albumin.[10] Table 4 showed that there was no significant change in the levels of the liver function parameters of animals treated with methanol root extract of Costus lucanusianus when compared with the negative control. The result gotten from this study is in contrast with Megabiaw et al[27] which stated that there was increased AST, ALT and ALP of malaria patients before drug treatment. Haematological abnormalities are considered a hallmark of malaria and are reported to be most pronounced in P. falciparum infections. The study revealed that white blood cells and platelet were significantly increased for 100 mg/kg methanol extract (Table 5). Also, neutrophils were significantly reduced for 100, 200 and 500 mg/kg methanol extract (Table 6). Other haematologic parameters were not significantly altered. The result was in contrast with a study that posited that leucopenia was frequently seen in the malaria-infected patients.[21] However, another study agreed with this research that leukocytosis was demonstrated in malaria infested patients.[22] This study aligned with a previous study that reported that malaria induced a reduction in neutrophil levels.[23] The underlying mechanisms include the marginalization of neutrophils to the sites of inflammation, splenic localization, serum lymphotoxic factors, and intercurrent bacterial infections.[23] Studies show that neutrophil numbers may be affected by antimalarials. [24,25] Thus, it explains the much reduction observed in neutrophil levels for the treated groups.

Since malaria is a highly inflammatory and oxidative disease, the incidence of oxidative stress is almost inevitable. Results show that high levels of oxidative stress were found in mice infected with P. berghei, P. yoelii or P. chabaudi,[26] indicating that oxidative stress is a major complication in Plasmodium infections. The result from this study indicated that oxidative stress markers for the treated groups were significantly different from that of the negative control except for GSH and GPx. The stress markers were seen to be restored in the animals administered with the extract. Malondialdehyde level was also seen to be restored to almost normal in the treated groups.
Histopathology of the liver indicated severe

inflammation of the liver cells while the costus treated groups expressed mild to moderate distortion of the liver.

CONCLUSION

The study revealed that methanol extract of C. lucanusianus root has promising antiplasmodial potential as it significantly reduced parasite load. The study also showed that liver function parameters did not significantly change but there was significant change in the levels of platelets, white blood cells and neutrophils. It was also shown that the extract restored oxidative stress markers to normal control.

REFERENCES

1. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438.
2. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757-72. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5927356/
3. Lingappan K. NF-κB in oxidative stress. Curr Opin Toxicol. 2018;7:81-6.
4. Ty MC, Zuniga M, Götz A, Kayal S, Sahu PK, Mohanty A, et al. Malaria inflammation by xanthine oxidase-produced reactive oxygen species. EMBO Mol Med. 2019;11(8):e10014.
5. Vasquez M, Zuniga M, Rodriguez A. Oxidative stress and pathogenesis in malaria. Front Cell Infect Microbiol. 2021;11:780827.
6. Kavishe RA, Koenderink JB, Alifrangis M. Oxidative stress in malaria and artemisinin combination therapy: pros and cons. FEBS J. 2017;284(16):2579-91.
7. Khattak AA, Venkatesan M, Nadeem MF, Satti HS, Yaqoob A, Strauss K, et al. Prevalence and distribution of human Plasmodium infection in Pakistan. Malar J. 2013;12:297.
8. Mutala AH, Badu K, Owusu C, Agordzo SK, Tweneboah A, Abbas DA, et al. Impact of malaria on haematological parameters of urban, peri-urban and rural residents in the Ashanti region of Ghana: a cross-sectional study. AAS Open Res. 2020;2:27.
9. Atanu FO, Rotimi D, Ilesanmi OB, Al Malki JS, Batiha GE, Idakwoji PA. Hydroethanolic extracts of Senna alata leaves possess antimalarial effects and reverse haematological and biochemical perturbation in Plasmodium berghei-infected mice. J Evid Based Integr Med. 2022;27:2515690X 2211164.
10. Kaeley N, Ahmad S, Shirazi N, Bhatia R, Bhat NK, Srivastava S, et al. Malarial hepatopathy: a 6-year retrospective observational study from Uttarakhand, North India. Trans R Soc Trop Med Hyg. 2017;111(5):220-5.
11. Agbor GA, Kuiaté JR, Sangiovanni E, Ojo OO. The role of medicinal plants and natural products in modulating oxidative stress and inflammatory related disorders, volume II. Front Pharmacol. 2023;14:PMC10641841. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10641841/
12. Owolabi OJ, Nworgu ZA. Anti-inflammatory and anti-nociceptive activities of Costus lucanusianus (Costaceae). Pharmacologyonline. 2009;1:1230-8. Available from: https://www. researchgate.net/ publication/290535384 _Anti-inflammatory_and_anti-nociceptive_activities_of_Costus_lucanusianus_Costaceae
13. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54(4): 275-87.
14. Peters W. Rational methods in the search for antimalarial drugs. Trans R Soc Trop Med Hyg. 1967;61:400-10.
15. Gad SC. Animal models in toxicology. CRC Press eBooks. Informa; 2015.

16. Waako PJ, Gumede B, Smith P, Folb PI. The in vitro and in vivo antimalarial activity of Cardiospermum halicacabum L. and Momordica foetida Schumch. et Thonn. J Ethnopharmacol. 2005;99(1):137-43.
17. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol. 2018;11(1):5-12. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6117820/
18. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria, XXII. Ann Trop Med Parasitol. 1975;69(2):155-71.
19. Afshar FH, Delazar A, Asnaashari S, Vaez H, Zolali E, Asgharian P. Screening of anti-malarial activity of different extracts obtained from three species of Scrophularia growing in Iran. Iran J Pharm Res. 2018;17(2):668. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5985184.
20. Uzor PF, Onyishi CK, Omaliko AP, Nworgu SA, Ugwu OH, Nwodo NJ. Study of the antimalarial activity of the leaf extracts and fractions of Persea americana and Dacryodes edulis and their HPLC analysis. Evid Based Complement Alternat Med. 2021;2021:e5218294. Available from: https://www.hindawi.com/journals/ecam/2021/5218294/
21. Kotepui M, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S. Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border. Malar J. 2014;13:123.
22. Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, et al. Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya. Malar J. 2010;9(Suppl 3):S3.
23. Kotepui M, Piwkham D, PhunPhuech B, Phiwklam N, Chupeerach C, Duangmano S. Effects of malaria parasite density on blood cell parameters. PLoS One. 2015;10(3):e0121057.
24. Bethell D, Se Y, Lon C, Socheat D, Saunders D, Teja-Isavadharm P, et al. Dose-dependent risk of neutropenia after 7-day courses of artesunate monotherapy in Cambodian patients with acute Plasmodium falciparum malaria. Clin Infect Dis. 2010;51(12):e105-14.
25. Zwang J, Ndiaye JL, Djimdé A, Dorsey G, Mårtensson A, Karema C, et al. Comparing changes in haematologic parameters occurring in patients included in randomized controlled trials of artesunate-amodiaquine vs single and combination treatments of uncomplicated falciparum in sub-Saharan Africa. Malar J. 2012;11:1.
26. Nneji CM, Adaramoye OA, Falade CO, Ademowo OG. Effect of chloroquine, methylene blue and artemether on red cell and hepatic antioxidant defence system in mice infected with Plasmodium yoelii nigeriensis. Parasitol Res. 2013;112(7):2619-25.
27. Megabiaw F, Eshetu T, Kassahun Z, Aemero M. Liver enzymes and lipid profile of malaria patients before and after antimalarial drug treatment at Dembia Primary Hospital and Teda Health Center, Northwest, Ethiopia. Res Rep Trop Med. 2022;13:11-23. Available from: https://www.dovepress.com/liver-enzymes-and-lipid-profile-of-malaria-patients-before-and-after-a-peer-reviewed-fulltext-article-RRTM

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