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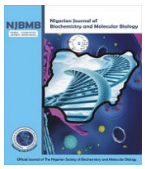

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Research Article

Synergistic Efficacy of *Moringa oleifera* and *Gossypium herbaceum* Co-Therapy against Malaria Infection in *Plasmodium berghei* Inoculated BALB/C Mice

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ABSTRACT

Despite significant progress in malaria treatment, it remains a major health challenge. The rising cost of conventional drugs in low-income countries has prompted interest in evaluating indigenous alternatives, specifically the leaves of *Moringa oleifera* and *Gossypium herbaceum*. The combined extracts of these plants, referred to as MOGH, possess various pharmacological activities, including antiplasmodial, antipyretic, and antianemic properties. This study aimed to assess the efficacy of the combined therapy (MOGH) against malaria. We induced malaria infection in mice by administering an intraperitoneal inoculum of *P. berghei* NK-65 infected red blood cells (RBCs) in a 1:1 ratio with normal saline. The infection was confirmed through daily microscopy of blood smears. Four days post-infection, the mice were grouped and treated as follows: 2% DMSO (vehicle control), 25 mg/kg/day chloroquine, 100, 200, and 300 mg/kg/day of MOGH extract. The uninfected group (Sham) received 2% DMSO. All treatments were administered for seven days. Standard procedures were used to analyze parasitemia, pyrexia, hematology, oxidative stress, cytokines, and other biochemical markers. Our results indicated that MOGH co-therapy significantly ($p < 0.05$) suppressed parasitemia and temperature, leading to improved survival rates. The treatment also increased levels of IL-6, IL-10, TNF-α, and malondialdehyde (MDA) while reducing the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Additionally, it exhibited hematopoietic effects. Although some biochemical irregularities were observed, the synergistic antiplasmodial effect of MOGH demonstrates potential as an alternative or adjunctive treatment for malaria infections.

Keywords: *Gossypium herbaceum, Moringa oleifera, malaria, hematological parameters, cytokines, oxidative stress indices*

INTRODUCTION

Malaria continues to pose a significant global health challenge, especially in tropical and subtropical regions (Antony and Parija, 2016; Dagen, 2020). The World Health Organization (WHO, 2023) estimated about 249 million cases of malaria worldwide, leading to over 600,000 deaths. These figures underscore the persistent threat of malaria despite notable progress in control efforts (WHO, 2023). The pathogenesis of malaria begins when infected mosquitoes inject sporozoites into the skin, from where they enter the bloodstream. They are then taken up by hepatocytes, where they develop into schizonts that release merozoites, which subsequently invade red blood cells (RBCs). After being

released into the bloodstream, these merozoites invade RBCs, leading to increased parasitemia levels (Meibalan and Marti, 2017). The rupture of infected RBCs (iRBCs) results in anemia; however, further loss of red blood cells occurs due to oxidative damage to the membranes of uninfected RBCs (Feldman *et al*., 2023). During malaria infection, by-products like hemozoin released from iRBCs activate immune cells, triggering secretion of cytokines and activation of oxidative stress (Pawłowska *et al.,* 2024). Cytokines such as interleukins (IL), tumor necrosis factor (TNF), chemokines, and other molecules are thought to contribute to the clinical symptoms of malaria (Percário *et al.,* 2012; Pawłowska *et al.,* 2024).

Although antimalarial drugs are effective, rising costs and increasing resistance limit their accessibility and efficacy in low-income countries. This situation calls for the exploration of alternative treatments. This study investigates alternative therapeutic approaches, with a focus on *Gossypium herbaceum* and *Moringa oleifera*, two plants widely recognized for their medicinal properties.

Gossypium herbaceum (GH), commonly known as Levant cotton, belongs to the *Malvaceae* family and has a rich history in traditional medicine across various cultures (Malhotra *et al.,* 2022). This plant contains a variety of bioactive compounds, including tannins, steroids, flavonoids, terpenoids, resins, saponins, and phenols (Malhotra *et al.,* 2022).

Moringa oleifera (MO), also known as the horseradish tree or miracle tree, is valued for its nutritional benefits and medicinal properties (Saini *et al.,* 2016). Key bioactive compounds in *M. oleifera* include glucomoringin, quercetin, kaempferol, phenolic acids, moringine, and terpenes (Leone *et al.,* 2021; Giacoppo *et al.,* 2017). In addition to their antiplasmodial effects, extracts of *M. oleifera* and *G. herbaceum* (MOGH) exhibit complementary pharmacological activities. These include antipyretic (Ahmad *et al.,* 2014; Bhattacharya *et al.,* 2014), anti-inflammatory (Martínez-González *et al.,* 2017), anti-anemic (Nurhayati *et al.,* 2023) properties. Other reported activities are hemozoin formation inhibition (Laksemi *et al.,* 2022), induction of reactive oxygen species (Guon *et al.,* 2017; Do *et al.,* 2021), antioxidative effects (Lawal *et al.,* 2017; Tao *et al.,* 2022), immunomodulation (Nassar *et al.,* 2024), and parasitic enzyme inhibition (Larayetan *et al.,* 2021; Bezerra *et al.,* 2023), among others. However, there is limited scientific information regarding their use as co-therapies for malaria in some indigenous communities in Nigeria.

MATERIALS AND METHODS

Sample collection and extraction preparation

G. herbaceum and *M. oleifera* leaves were purchased from Bekwarra, Cross River State, authenticated and assigned herbarium numbers (*G. herbaceum* -Bot/Herb/UCC/059 and *M. oleifera* - Bot/Herb/UCC/395). The leaves were shade-dried for three weeks, after which they were ground into a fine powder and cold macerated in 96% ethanol, separately for 72 h. The extracts were filtered using Whatman No. 1 filter paper and concentrated with a Soxhlet extractor, yielding percentage recoveries of 26.6% and 10.56% for *M. oleifera* and *G. herbaceum*, respectively.

Acute toxicity study

An acute toxicity study of *M. oleifera* and *G. herbaceum* leaf extracts, following a modified method of Chinedu *et al.* (2013) and Udoh *et al*. (2024), showed no deaths or behavioural changes in mice at the highest dose (5000 mg/kg; 1:1 MOGH), indicating high safety.

Experimental animals

A total of 30 male mice (weighing 23-30 g, aged 6-8 weeks) procured from the Pharmacology Department's animal house were allowed to acclimatize (temperature: 28 ± 5°C, humidity: 79 ± 5%, 12-hour light/dark cycle) and fed a standard rodent pellet diet and water *ad libitum*. Ethical approval (325PHA1924) was also obtained from the University of Calabar Ethical Committee on Faculty Animal Research Ethics (FAREC-FBMS).

Parasite inoculation

Male donor *P. berghei* NK65-infected BALB/c mice were obtained from the Nigerian Institute of Medical Research (NIMR), Lagos. When parasitemia reached 7% (Confirmed by microscope; Leica, Germany), donor mice were euthanized, and blood was collected by cardiac puncture and diluted with normal saline (1:1). 0.2 ml of the inoculum was injected intraperitoneally (i.p.) into the experimental BALB/c mice following the protocol described by Basir *et al.* (2012) and Pedroni *et al.* (2006) while the control was uninfected. Parasitemia levels were assessed via microscopy every 24 h post-infection.

Experimental design

The experimental animals were randomly divided into six groups (n = 5) and treated as follows:

Group 1 (Sham): Uninfected animals received 2% DMSO (Vehicle).

Group 2 (MI + Vehicle): Infected animals received 2% DMSO (Vehicle).

Group 3 (MI + 25 mg/kg CQ): Infected animals received 25 mg/kg chloroquine (CQ) in a 0.5% carboxymethylcellulose (CMC) solution.

Group 4 (MI + 100 mg/kg MOGH): Infected animals received 100 mg/kg MOGH (*Moringa oleifera* and *Gossypium herbaceum*, in a 1:1 ratio) in a 2% DMSO solution.

Group 5 (MI + 200 mg/kg MOGH): Infected animals received 200 mg/kg MOGH (*Moringa oleifera* and *Gossypium herbaceum*, in a 1:1 ratio) in a 2% DMSO solution.

Group 6 (MI + 300 mg/kg MOGH): Infected animals received 300 mg/kg MOGH (*Moringa oleifera* and *Gossypium herbaceum*, in a 1:1 ratio) in a 2% DMSO solution.

The selected doses of the MOGH extract were determined based on preliminary studies, while the dosage of chloroquine was established in accordance with World

Health Organization guidelines. Treatments commenced on day 5 post-infection, administered orally at 0.5 ml/day, and continued for 7 days.

Daily body weights, feed intake, and survival rate

Daily body weights were measured daily, while feed intake was determined by subtracting food remnants from a pre-weighed aliquot. The survival of the animals was monitored daily, along with observations for cognitive and behavioural abnormalities.

Parasitemia and pyrexia

Daily fever (pyrexia) was measured using a rectal thermometer, while malaria infection was confirmed by daily parasitemia estimation from blood smears. Blood collected from the lateral tail vein was stained with 10% Giemsa solution (4 ml stock + 36 ml buffer). The smears were examined under a microscope at 100x magnification to quantify parasitemia and observe morphological changes. Thick smears were used to concentrate red blood cells (RBCs) for improved sensitivity in detecting low parasitemia, while thin smears facilitated species identification (Vaughan *et al.,* 2015). Parasitemia was calculated using the formula (Cheesbrough, 2006):

$$\text{Parasitemia (\%)} = \frac{\text{Number of parasitized RBCs}}{\text{Total number of RBCs count}} \times 100$$

Samples collection

On day 12, the animals were pre-weighed and fasted for 12 hours. Thereafter, the animals were euthanized in accordance with approved protocols, after anesthesia with chloroform inhalation. Blood was obtained via cardiac puncture and used for further analyses, while the organs were harvested, weighed, and fixed in 10% buffered formalin solution.

Determination of hematological parameters

Full blood count analysis was conducted using an automated hematology analyzer (Sysmex KX-21N, USA). Blood samples collected via cardiac puncture were analyzed for red blood cell (RBC) indices (RBC count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width), white blood cell (WBC) indices (WBC count, neutrophils, lymphocytes, and mixed cell percentage), and platelet indices (platelet count, mean platelet volume, and plateletcrit).

Estimation of some serum biochemical parameters, liver, kidney and lipid test

Liver function tests (ALT, AST, and ALP) were conducted following Lala *et al*. (2024), with modifications from Gowda et al. (2009). Kidney function tests (urea, creatinine, potassium, sodium, chloride, and bicarbonate) were analysed using the ci8200 Integrated System (Abbott, USA). Lipid profile parameters, including total cholesterol (TC), triglycerides (TG), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were determined following Vassault *et al*. (1999).

Determination of oxidative stress indices and cytokines

Oxidative stress analyses were performed following standard procedures for the determination of malondialdehyde (MDA) (CSB-E08558r), nitric oxide (NO) (MBS2604161), superoxide dismutase (SOD) (CSB-ELO22397RA), glutathione peroxidase (GPX) (CSB-E08907r), catalase (CAT) (CSB-E13439r), and reduced glutathione (GSH) (E4625-100). Cytokines were determined by flow cytometry using serum samples treated with cytokine extraction solution. CUSABIO kits were used to quantify the levels of interleukin (IL) 6 (IL-6) (CSB-E0463r), IL-8 (CSB-E04641r), IL-10 (CSB-E04595r), tumor necrosis factor (TNF) alpha (TNF-α) (CSB-E11987r), and TNF-β (CSB-E04626r), following the manufacturer's instructions.

Statistical analysis

The data obtained were analyzed and presented as mean ± standard deviation (SD). Differences among groups were assessed using one-way ANOVA, followed by the Tukey post-hoc test. Statistical analyses were conducted using GraphPad Prism software (version 9.5, USA), and p-values of less than 0.05 were considered significant.

RESULTS

MOGH effect on body weight change, feed intake, and percentage survival rate

The body weight change, feed intake, and percentage survival rate for the untreated MI + Vehicle group were significantly different compared to the Sham group. The MI + Vehicle group recorded the lowest body weight change (20 g), whereas other groups showed increases ranging from 30 to 45 g. Additionally, the feed intake in the MI + Vehicle group was 38 g, but all treatment groups showed higher values (Figure 1). The MI + Vehicle group had the lowest survival rate (40% at Day 12), while the MOGH groups showed dose-dependent improvements (Table 1).

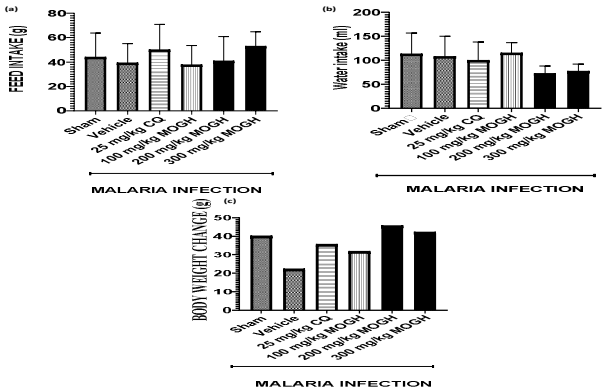

Figure 1. Effect of MOGH on (a) Feed intake, (b) Water intake, and (c) Body weight change of *P. berghei* infected BALB/c mice. Values are Mean ± Standard deviation (Analyzed using one-way ANOVA followed by Tukey's post-hoc test). Abbreviations: MOGH: *M. oleifera G. herbaceum* extract, CQ: chloroquine.

Table 1. Effect of MOGH on Percentage Survival Rate of *P. berghei* infected BALB/c mice.

Days	Sham	MI + Vehicle	MI + 25 mg/kg CQ	MI + 100 mg/kg MOGH	MI + 200 mg/kg MOGH	MI + 300 mg/kg MOGH
D0	100	100	100	100	100	100
D2	100	100	100	100	100	100
D4	100	100	100	100	100	100
D6	100	60	100	60	80	100
D8	100	60	100	60	60	100
D10	100	40	100	60	60	80
D12	100	40	100	60	60	80

Values are presented in percentages (n=5). Abbreviations: MI: malaria infection; CQ: chloroquine, MOGH: *M. oleifera G. herbaceum* extract

Pyrexia and parasitaemia suppression of MOGH

On Day 2, the average colonic temperature for all groups was 97.56 ºF. However, by Day 12 post-infection, the untreated MI + Vehicle group reached a peak temperature of 103 ºF, while treated groups remained within 96 to 97.6 ºF (Figure 2a). In terms of parasitemia estimation, the MI + Vehicle group peaked at a parasitemia level of 53.3% on Day 12. Furthermore, on Day 12, parasitemia levels were 1.1% in the 25 mg/kg CQ group, 4.0% in the 100 mg/kg MOGH group, 1.2% in the 200 mg/kg MOGH group, and 0.4% in the 300 mg/kg MOGH group (Figure 2b).

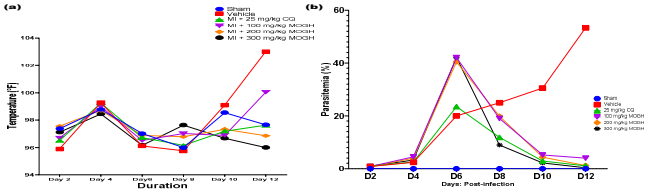

Figure 2. Effect of MOGH on (a) Pyrexia and (b) Parasitemia levels of *P. berghei* infected BALB/c mice. Values are expressed as Mean. Abbreviations: MOGH: *M. oleifera G. herbaceum* extract, CQ: chloroquine, D: day.

Hematopoietic effects of MOGH

The untreated malaria-infected group given vehicle showed significant ($p<0.05$) decreases in most red blood cell (RBC) indices compared to other groups. Notably, there were no significant ($p>0.05$) differences in RBC count and red cell distribution width-standard deviation (RDW-SD) between the Sham group and the 300 mg/kg MOGH group. Additionally, the hematocrit (HCT), hemoglobin (HGB), and mean corpuscular hemoglobin concentration (MCHC) levels in the 300 mg/kg MOGH group were not significantly ($p>0.05$) different from those in the 25 mg/kg chloroquine (CQ) group (Table 2).

On the other hand, the white blood cell (WBC) count in the untreated malaria-infected group given vehicle was significantly lower (2.63 x 10^-3/µL, $p<0.05$) compared to the other groups. Differential WBC counts were also significantly ($p<0.05$) reduced in this group compared with the others. In contrast, the MOGH-treated groups demonstrated dose-dependent improvements in WBC counts (Table 2).

Among the groups, the Sham group had the highest platelet (PLT) count at 1401 x 10^-3/µL, while the untreated malaria-infected group given vehicle recorded the lowest level at 31.7 x 10^-3/µL ($p<0.001$). The reduced platelet count in the untreated malaria-infected group was elevated in the 25 mg/kg CQ group (266 x 10^-3/µL) and further increased in the MOGH groups, ranging from 308 to 767 x 10^-3/µL (Table 2).

Table 2. Effect of MOGH on hematological parameters of malaria-infected BALB/c mice

Parameters	Sham	MI + Vehicle	MI + 25 mg/kg CQ	MI + 100 mg/kg MOGH	MI + 200 mg/kg MOGH	MI + 300 mg/kg MOGH
RBC indices						
RBC (10^{-6}/uL)	7.07 ± 0.45	0.76 ± 0.005 ^{a*}	7.35 ± 0.24 ^{b}	3.87 ± 1.37 ^{a,b,c}	5.18 ± 0.07 ^{b,c}	6.15 ± 0.77 ^{b}
HCT (%)	46.68 ± 2.71	3.87 ± 0.15 ^{a*}	39.58 ± 1.22 ^{a,b}	37.87 ± 1.95 ^{a,b}	40.8 ± 0.72 ^{a,b}	41.68 ± 0.91 ^{a,b}
HGB (g/dL)	13.1 ± 1.15	1.2 ± 0.08 ^{a*}	12.1 ± 0.11 ^{b}	8.1 ± 0.09 ^{a,b,c}	10.9 ± 0.48 ^{a,b}	12.6 ± 0.74 ^{b}
MCV (fL)	67.5 ± 1.23	50.5 ± 0.55 ^{a*}	54.4 ± 2.49 ^{a}	97.5 ± 1.23 ^{a,b,c}	80.1 ± 0.33 ^{a,b,c}	67.5 ± 2.37 ^{b,c}
MCH (pg)	18.4 ± 0.28	16.1 ± 0.13 ^{a}	16.4 ± 0.26 ^{a}	20.8 ± 1.03	21.1 ± 0.58 ^{a,b,c}	20.6 ± 0.84 ^{c}
MCHC (g/dL)	27.2 ± 0.91	31.7 ± 0.2 ^{a}	30.2 ± 0.72 ^{a}	21.4 ± 4.1 ^{b,c}	26.4 ± 0.54	30.5 ± 0.58 ^{a}
RDW-CV (%)	19.24 ± 0.17	20.0 ± 1.0	19.2 ± 0.59	18.5 ± 0.22 ^{a}	18.4 ± 0.21	16.03 ± 0.08 ^{a,c}
WBC indices						
WBC (10^{-3}/µL)	22.88 ± 0.61	2.63 ± 1.11 ^{a}	5.78 ± 0.29 ^{a,b}	5.33 ± 1.25 ^{a,b}	16.50 ± 0.51 ^{a,b,c}	42.75 ± 0.68 ^{a,b,c}
LYMP (10^{-3}/µL)	15.85 ± 0.69	1.84 ± 0.13 ^{a}	4.79 ± 0.42 ^{a,b}	1.85 ± 0.12 ^{a,c}	11.25 ± 0.75 ^{b,c}	38.70 ± 1.58 ^{a,b,c}
NEU (10^{-3}/µL)	4.77 ± 0.15	0.24 ± 0.01 ^{a}	0.58 ± 0.05 ^{a}	0.06 ± 0.01 ^{a}	2.27 ± 0.04 ^{a,b,c}	1.56 ± 0.05 ^{a,b,c}
MXD (10^{-3}/µL)	3.12 ± 0.0	0.25 ± 0.0 ^{a}	0.60 ± 0.03 ^{a,b}	0.02 ± 0.01 ^{a,c}	2.99 ± 0.01 ^{b,c}	2.54 ± 0.09 ^{a,b,c}
PLT indices						
PLT (10^{-3}/µL)	1401 ± 10.19	31.7 ± 1.2 ^{a}	266 ± 8.19 ^{a,b}	308 ± 4.55 ^{a,b}	545 ± 5.91 ^{a,b,c}	767 ± 21.61 ^{a,b,c}
MPV (fL)	10.6 ± 0.35	8.53 ± 0.18	10.82 ± 0.24	9.3 ± 0.27 ^{a,c}	9.5 ± 0.5	8.3 ± 0.67 ^{a,c}
PCT (%)	1.48 ± 0.65	0.03 ± 0.25 ^{a,b}	0.29 ± 0.11 ^{a,b}	0.29 ± 0.01 ^{a,b}	0.52 ± 0.11 ^{a,b,c}	0.64 ± 0.03 ^{a,b,c}

Values are expressed as Mean ± Standard Deviation (Analyzed using One-way ANOVA; Tukey post hoc test). * $p<0.01$, a $p<0.05$ vs Sham, b $p<0.05$ vs MI + Vehicle; c $p<0.05$ vs MI + 25 mg/kg CQ. Abbreviations: MI: malaria infection; CQ: chloroquine, MOGH: *M. oleifera G. herbaceum* extract, RBC: red blood cell; RDW-SD: red cell distribution width- standard deviation; HCT: hematocrit; HGB: hemoglobin; MCV: mean cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell; LYMP: lymphocyte; NEU: neutrophils; MXD:

mixed cell percentage (monocytes-basophils-eosinophils), PLT: platelets; MPV: mean platelet volume; PDW: platelet distribution width; PCT: Plateletcrit.

MOGH effects on some biochemical parameters of malaria-infected BALB/c mice

Compared to the Sham group, the levels of ALT and ALP in the untreated MI + Vehicle group were significantly ($p < 0.05$) elevated, and further significant ($p < 0.05$) increases were observed across the MOGH treatment groups. The AST level in the MI + Vehicle group was not significantly ($p > 0.05$) different from that of the Sham group; however, it was significantly ($p < 0.05$) elevated in all MOGH-treated groups (Table 3).

The levels of urea, K^+, and Cl^- in the untreated MI + Vehicle group were significantly ($p < 0.05$) elevated, while the HCO^{3-} level was significantly ($p < 0.05$) reduced compared to the Sham group. In contrast, Na^+ and creatinine levels showed no significant ($p > 0.05$) difference compared to the Sham group. In the treatment groups, elevations were more pronounced, especially for urea, K^+, Cl^-, and creatinine in the 300 mg/kg MOGH group (Table 3).

Compared to the Sham group, the MI + Vehicle group showed no significant ($p > 0.05$) differences in TC, TG, and LDL levels. However, most MOGH-treated groups exhibited significant ($p < 0.05$), dose-dependent increases in lipid parameters (Table 3).

Table 3. Effect of MOGH on some biochemical parameters of malaria-infected BALB/c mice

Parameters	Sham	MI + Vehicle	MI + 25 mg/kg CQ	MI + 100 mg/kg MOGH	MI + 200 mg/kg MOGH	MI + 300 mg/kg MOGH
Liver function tests						
AST (IU/l)	26.2 ± 2.39	31 ± 2.65	35.4 ± 1.67 [a]	39.67 ± 2.08 [a, b]	44.33 ± 2.08 [a, b, c]	48.5 ± 1.29 [a, b, c]
ALT (IU/l)	21.6 ± 1.34	26.8 ± 1.64 [a]	27.67 ± 0.88 [a]	33.33 ± 2.08 [a, c]	36.67 ± 2.08 [a, b, c]	41.5 ± 1.73 [a, b, c]
ALP (/l)	110.2 ± 2.17	124 ± 2.01 [a]	128.2 ± 2.59 [a]	135 ± 1.73 [a, b]	139.67 ± 2.08 [a, b]	152 ± 1.83 [a, b, c]
Kidney function tests						
Urea (mmol/l)	3.26 ± 0.18	3.77 ± 0.12 [a]	4.4 ± 0.20 [a]	4.93 ± 0.12 [a, b]	5.3 ± 0.17 [a, b, c]	5.83 ± 0.09 [a, b, c]
Na^+ (mmol/l)	135.4 ± 1.95	136 ± 2.65	136.67 ± 2.05	139.33 ± 1.53	140 ± 1.73 [c]	141.75 ± 2.06 [a, b]
K^+ (mmol/l)	3.84 ± 0.18	4.77 ± 0.23 [a]	5.32 ± 0.16 [a, b]	5.67 ± 0.12 [a]	6.27 ± 0.21 [a, b, c]	7.125 ± 0.15 [a, b, c]
Cl^- (mmol/l)	96.2 ± 2.17	104.33 ± 1.53 [a]	107.6 ± 1.95 [a]	112 ± 2.65 [a, b]	114.33 ± 0.58 [a, b]	116.25 ± 1.89 [a, b, c]
$HCO3^-$ (mmol/l)	20 ± 2.45	17.67 ± 2.08 [a]	16.2 ± 2.17	15.33 ± 2.31	14.33 ± 1.16	12.25 ± 2.22
Creatinine (mmol/l)	56.2 ± 1.79	59.07 ± 3.02	63.46 ± 2.23 [a]	67.33 ± 2.08	74.7 ± 4.51	80.53 ± 1.72 [a, b, c]
Lipid profile						
TC (mmol/l)	2.3 ± 0.24	2.67 ± 0.12	3.14 ± 0.25	3.53 ± 0.15 [b]	3.6 ± 0.27 [b]	3.8 ± 0.2 [b]
TG (mmol/l)	1.26 ± 0.11	1.5 ± 0.10	1.57 ± 0.16	1.83 ± 0.06 [a, b]	1.73 ± 0.06 [a]	1.87 ± 0.16 [a, b, c]
HDL (mmol/l)	0.61 ± 0.01	0.68 ± 0.02 [a]	0.81 ± 0.02 [a, b]	0.92 ± 0.01 [a, b]	0.95 ± 0.05 [a]	1.05 ± 0.17 [a]
VLDL (mmol/l)	0.59 ± 0.02	0.68 ± 0.03 [a]	0.72 ± 0.02 [a, b]	0.81 ± 0.03 [a, b, c]	0.77 ± 0.03 [a, b, c]	0.86 ± 0.02 [a, b, c]
LDL (mmol/l)	1.08 ± 0.13	1.23 ± 0.12	1.64 ± 0.23 [a]	1.83 ± 0.06 [a, b]	1.87 ± 0.12 [a, b]	2.0 ± 0.20 [a]

Values are expressed as Mean ± Standard Deviation (Analyzed using One-way ANOVA; Tukey post hoc test). * $p<0.01$, [a]$p<0.05$ vs Sham, [b]$p<0.05$ vs MI + Vehicle; [c]$p<0.05$ vs MI + 25 mg/kg CQ. Abbreviations: MI: malaria infection; CQ: chloroquine, MOGH: *M. oleifera G. herbaceum* extract, AST: aspartate transaminase; ALT: alanine transaminase, ALP: alkaline phosphatase, Na^+: sodium, K^+: potassium, Cl^-: chloride, HCO3-: bicarbonate, TC: total cholesterol, TG: triglyceride, HDL: high-density lipoprotein, VLDL: very low-density lipoprotein, LDL: low-density lipoprotein

MOGH effects on oxidative stress indices and cytokines of malaria-infected- BALB/c mice

In comparison to the Sham group, the levels of GPx, NO, CAT, and GSH in the untreated MI + Vehicle group were not significantly ($p > 0.05$) different. However, the level of SOD significantly ($p < 0.05$) decreased while MDA level significantly ($p < 0.05$) increased. In the treatment groups, the levels of SOD, CAT, and GPx significantly ($p < 0.05$) decreased, while MDA level was significantly ($p < 0.05$) elevated (Figure 3).

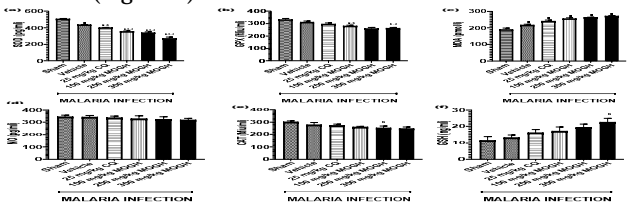

Figure 3. Effect of MOGH on Oxidative Stress Indices of *P. berghei*-infected BALB/c mice: a) SOD, b) GPX, c) MDA, d) NO, e) CAT, f) GSH. Values are expressed as Mean ± Standard deviation (Analyzed using One-way ANOVA; Tukey post hoc test). [a] $p<0.05$ vs Sham, [b] $p<0.05$ vs MI + Vehicle; [c] $p<0.05$ vs MI + 25 mg/kg CQ. Abbreviations: MI: malaria infection; CQ: chloroquine, MOGH: *M. oleifera G. herbaceum* extract, SOD: superoxide dismutase, GPX: Glutathione peroxidase, MDA: Malondialdehyde, NO: nitric oxide, CAT: Catalase, GSH: reduced Glutathione.

The cytokines levels in the untreated MI + Vehicle group were not significantly ($p > 0.05$) different compared to the Sham group. Additionally, the levels of IL-8 and TNF-β remained insignificant ($p > 0.05$) across all groups. In contrast, the MOGH groups showed dose-dependent increases in IL-6, IL-10, and TNF-α compared to both the Sham and MI + Vehicle groups (Figure 4).

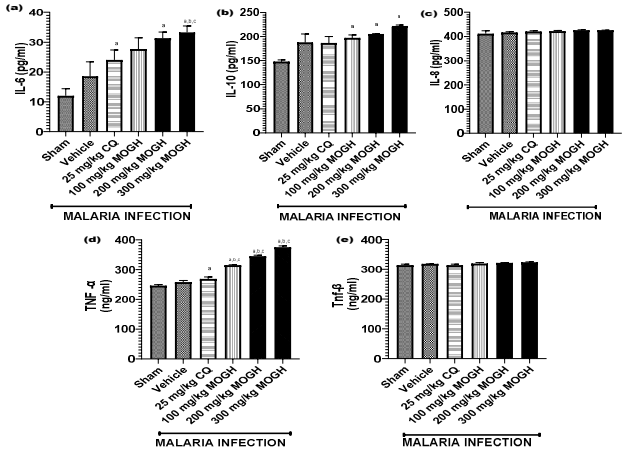

Figure 4. Effect of MOGH on Cytokine levels of *P. berghei* infected BALB/c mice: a) IL-6, b) IL-10, c) IL-8, d) TNF-α, e) TNF-β. Values are expressed as Mean ± Standard Deviation (Analysed using One-way ANOVA; Tukey post hoc test). [a] $p<0.05$ vs Sham, [b] $p<0.05$ vs MI + Vehicle; [c] $p<0.05$ vs MI + 25 mg/kg CQ. Abbreviations: MI: malaria infection; CQ: chloroquine, MOGH: *M. oleifera G. herbaceum* extract; IL: interleukin, TNF: tumour necrosis factor.

DISCUSSION Malaria infection presents a variety of symptoms, including fever, lethargy, weight loss, convulsions, and, if untreated, death (Shiff, 2002). In this study, the untreated group displayed these symptoms, along with notable behavioural and cognitive changes, high parasitemia, and pyrexia on day 12. Minimal changes in body weight were also observed, consistent with reduced feed intake. Conversely, groups treated with Chloroquine and MOGH showed significant improvement, with marked reductions in parasitemia and fever, leading to higher survival rates.

Previous studies have shown that malaria-infected red blood cells (iRBCs) have shorter lifespans due to the build-up of toxic metabolites produced during the malaria parasite's breakdown of haemoglobin (Cowman et al., 2016). This process impairs the deformability of red blood cells (RBCs), promoting their premature removal from circulation and affecting non-parasitized RBCs as well (Becker et al., 2004; Meireles et al., 2020). Additionally, dyserythropoiesis may occur as a consequence of parasite-derived metabolites and immune mediators, leading to anaemia and, in severe cases, pancytopenia, as observed in the untreated Vehicle group of this study.

Clinically, anaemia is characterised by reduced red blood cell (RBC) counts and haemoglobin (HGB) levels, accompanied by decreased mean corpuscular volume (MCV) and haematocrit (HCT) levels, indicating a microcytic hypochromic state (Cowman et al., 2016). Treatment with 300 mg/kg MOGH significantly alleviated malaria-induced anaemia, as shown by the restoration of RBC indices. Furthermore, the red cell distribution width–coefficient of variation (RDW-CV), which indicates the degree of variation in RBC sizes (anisocytosis) commonly seen in severe malaria infections, was elevated in the untreated malaria group but significantly reduced in all treatment groups, most notably in the 300 mg/kg MOGH group.

Leucopenia, observed in the untreated group, also involves reduced white blood cell (WBC) counts and may result from WBC redistribution rather than direct destruction (McKenzie et al., 2005; Lampah et al., 2015). WBC counts tend to vary during different stages of malaria, with reports of both leucopenia and leucocytosis depending on the infection severity and immune response (Hänscheid et al., 2008; Kotepui et al., 2014).

Thrombocytopenia is also a common haematological feature of Plasmodia infection, often associated with platelet aggregation, immune-mediated destruction, and splenic sequestration (Ladhani et al., 2002). The untreated Vehicle group showed a marked decline in platelet counts, whereas MOGH treatment significantly restored platelet levels, especially at the 300 mg/kg dose.

Oxidative stress is another key aspect of malaria pathogenesis, resulting from reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide generated during parasite-related metabolic activities (Pohanka, 2013). These ROS can induce endothelial cell apoptosis, contributing to microvascular damage and severe malaria complications (Ty et al., 2019). Unsaturated phospholipids in cellular membranes are highly vulnerable to oxidation, leading to lipid peroxidation products such as malondialdehyde (MDA), a recognized biomarker of oxidative stress (Percário et al., 2012; Ty et al., 2019). The antioxidant enzyme system—comprising superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—is essential in counteracting oxidative damage, with SOD transforming superoxide radicals into hydrogen peroxide, and CAT and GPx further detoxifying it. In this study, activities of these enzymes declined progressively with higher MOGH doses, which may be linked to increased levels of interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), indicating ongoing oxidative stress activities during parasite clearance. Correspondingly, MDA levels increased with higher MOGH concentrations, whilst nitric oxide (NO) levels remained unchanged. These findings align with previous research on the oxidative stress-modulating effects of *Moringa oleifera* and *Gossypium herbaceum* extracts (Giacoppo et al., 2017; Larayetan et al., 2021).

To better understand the potential mechanism of action of MOGH extract on oxidative stress activation, the activities of cytokines and chemokines were further examined. Cytokines are signalling molecules produced by various cells that have both local and systemic effects, coordinating immune responses through complex interactions, whilst chemokines such as interleukin-8 (IL-8) are essential for guiding immune cell migration and recruiting additional immune cells to infection sites (Percário et al., 2012). Pro-inflammatory cytokines, including interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and tumour necrosis factor-beta (TNF-β), are released during infection to aid parasite elimination; however, excessive production can cause harmful inflammation (Percário et al., 2012). While these cytokines assist in parasite clearance and maintaining immune homeostasis, prolonged or excessive inflammation

can result in tissue damage and complications. Conversely, anti-inflammatory cytokines like interleukin-10 (IL-10) help counteract pro-inflammatory responses and prevent immune-mediated harm (Carlini et al., 2023). In this study, higher doses of MOGH were associated with increased levels of pro-inflammatory cytokines IL-6 and TNF-α, along with elevated IL-10. Meanwhile, levels of IL-8 and TNF-β remained unchanged. This pattern indicates that MOGH may facilitate malaria parasite clearance by activating pro-inflammatory cytokines, while the rise in IL-10 suggests a host feedback mechanism to regulate inflammation and restore immune balance.

Malaria infection in this study also induced marked biochemical alterations. Elevated plasma levels of liver enzymes were observed, which may result from decreased hepatic clearance due to reduced blood volume (Frevert et al., 2005). Increased alkaline phosphatase (ALP) activity can be indicative of hepatic or bone disorders, whereas elevated alanine aminotransferase (ALT) levels suggest hepatocellular injury or biliary dysfunction (Frevert et al., 2005). The presence of hyperkalaemia and hyperchloremia likely reflects renal impairment and dehydration, particularly in animals administered higher doses of MOGH, as illustrated in Figure 1b and Table 3. Moreover, malaria-associated metabolic disturbances such as haemolysis and hypoxia-induced acidosis may precipitate metabolic acidosis, temporarily reducing bicarbonate levels. This reduction signifies the body's adaptive response to maintain acid–base equilibrium through ongoing metabolic adjustments and renal compensation (Plewes et al., 2018; Katsoulis et al., 2021).

Conversely, in a related study investigating the organ-specific effects of MOGH, histopathological evaluations of MOGH-treated groups revealed signs of organ stress and damage, consistent with underlying biochemical alterations (Udoh et al., 2025). The study demonstrated that MOGH co-therapy possessed notable antimalarial efficacy, effectively reducing parasite burden at the organ level. However, it also induced morphological changes such as degeneration of the brain choroid plexus and cerebral frontal cortex, hepatic lesions, splenic parenchymal fibrosis, and erosion of renal glomeruli—factors likely contributing to the observed biochemical disruptions. Furthermore, the inverse patterns of high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL) levels may indicate compensatory lipid regulation in response to treatment, suggesting possible adverse effects of MOGH, particularly at higher doses.

CONCLUSION

Inducing malaria in mice led to various haematological issues, including pancytopenia, fever, and increased mortality. This was accompanied by elevated levels of pro-inflammatory cytokines and markers of oxidative stress. Treatment with MOGH caused changes in liver enzymes, kidney function, and lipid profiles, suggesting possible organ toxicity. While MOGH increased pro-inflammatory cytokine levels and reduced essential antioxidants, it also demonstrated dose-dependent curative effects by lowering parasitaemia and improving both pancytopenia and survival rates. Therefore, MOGH may serve as a potential alternative or adjunctive treatment for malaria, particularly in regions where anaemia is a major concern. However, its safety profile needs comprehensive evaluation.

AUTHORS' CONTRIBUTIONS

Conceptualization and study design were by ESU, ARA, and BEI. ESU, AEI, and BFJ researched while BEI, FKO, and AGO analyzed the results. The original manuscript draft was written by ESU while its review and editing were done by FKO, BEI, and SCO. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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