



Efficacy of Mefloquine- Clindamycin in *Plasmodium Berghei* Infected-Mice

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Abstract

Mefloquine (MQ) is a quinoline methanol derivative that causes the malaria parasite to accumulate lethal heme by preventing hemozoin production. Clindamycin (CLD) is a lincosamide antibiotics which has powerful antiplasmodial action. This study evaluated the combined efficacy and potential of CLD and MQ for the treatment of malaria in mice infected with *Plasmodium berghei*. Both sexes of adult Swiss albino mice (27–42g) were used in a random grouping. The mice received oral treatments of MQ (15 mg/kg), CLD (10 mg/kg), and MQ-CLD after being injected with *Plasmodium berghei*. As the benchmark, 10 mg/kg of Chloroquine (CQ) was utilized. Blood samples were taken and evaluated for hematological indicators, inhibition, and parasitemia percentage at the end of treatment. Histological alterations were analyzed in liver samples. The mean survival time (MST) of the mice was also monitored. When compared to MFQ or CLD, MQ-CLD dramatically reduced the proportion of parasitemia in the curative, prophylactic, and suppressive tests, with a difference noted at $p < 0.05$. Curatively, compared to CQ 90.84% parasitemia inhibition, MQ, CLD, and MQ-CLD yielded 89.95%, 76.35%, and 94.43%, respectively. MST was significantly prolonged by MQ-CLD in the curative, prophylactic, and suppressive tests, with a difference of $p < 0.05$ when compared to MQ or CLD. MQ-CLD significantly ($p < 0.05$) restored *Plasmodium berghei* induced changes in packed cell volume, white blood cells, red blood cells, hemoglobin, monocytes, neutrophils, lymphocytes, high-density lipoprotein cholesterol, total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels. When compared to MQ or CLD, the results indicated that the levels of aspartate transferase, alanine transferase, alkaline phosphate, total protein, total bilirubin, urea, creatinine, and uric acid were slightly altered but were restored by MQ-CLD at $P < 0.05$. MQ-CLD diminishes the vascular congestion and inflammatory cells that are present in the liver of *Plasmodium berghei*-infected mice. The study showed that MQ and CLD can be exploited collectively for the treatment of malaria.

Keywords: Mefloquine, Clindamycin, Antiplasmodial, Infected Mice, Berghei

Introduction

Despite extensive intervention efforts, malaria infection has been a leading global public health menace, resulting to significant economic losses, morbidity, and fatality rate (WHO, 2020). As per the World Health Organization, an estimated 241 million and 627 million deaths from malaria were recorded globally in 2020. This corresponds to approximately 14 million additional cases and 69000 more deaths in contrast to 2019 (WHO, 2021). Children aged below five years old formed the most susceptible group to malaria in 2019, accounting up 67% (274 000) of all malaria fatalities worldwide (WHO, 2020).

In Sub-Saharan Africa, infections caused by malaria are prevalent, but there have been infrequently significant fatal outcomes from the parasite due to improved immune systems, compared to the spread of AIDS, HIV, or tuberculosis (World Health Organization, 2020). Nonetheless, there is a recent decrease in the worldwide occurrence of this illness

and consequently a lower rate of mortality, primarily in substantial part due to the increasingly widespread implementation of insecticide-treated nets and Artemisinin-based combination therapies. The World Health Organization and United Nations International Children's Emergency Fund predict that the total number of deaths resulting from malaria worldwide in 2015 decreased by 60% from a projected number of 985,000 in 2000 (Howitt et al., 2012; WHO, 2015).

Malaria negatively impacts the growth of the economy by 1.3 percent annually and is predicted to cost Africa \$12 billion in upfront expenses yearly. Impoverished rural households with limited opportunity to utilize modern preventive treatment and medical resources suffer a great deal of the expenses. Improved global awareness and financial support for the early detection and treatment of malaria continue to save lives regardless of the havoc the disease has left behind. Malaria-related death rates declined by 47% across the globe and 54% throughout Africa from 2000 to 2013, whereas cases of malaria decreased by 30% worldwide and 34% in the African continent (WHO, 2014). Families, healthcare systems, and revenue generation, along with development, have all been negatively impacted by the economic cost of malaria. Gathering information on the disease's monetary expense serves as an essential tool to ensure effective use of funds as well as the financial appraisal of malaria elimination initiatives. (Hailu et al., 2017). Malaria, alongside other tropical infections caused by worms, has previously been eradicated with artemisinin-based combination therapy (ACT). Although these are capable of eliminating parasites at every stage during their reproductive lifespan, and have greater advantages over alternative medications (WHO, 2015). Regarding every reported case of transmission of malaria, the World Health Organization (WHO) advocates using artemisinin with a few of its substitutes, recommended the initial therapy alongside an additional more prolonged partner prescription (WHO, 2015).

The US Army discovered mefloquine (MFQ), a quinolinylethylamine with an approximate half-life of two to three weeks (Ravina, 2011). In accordance with Askling et al. (2012), mefloquine is a medication that should be considered towards the management of mild malaria. Similar to quinine and chloroquine, mefloquine is a bloodstream schizonticidal medication that is effective in destroying the reproductive phases and intraerythrocytic forms of vivax, ovale, and malaria parasite adult trophozoites. Mefloquine tends to interrupt the parasite erythrocyte hemoglobin synthesis and absorption (Shibeshi et al., 2020). It suppresses the formation of proteins and has schizonticidal effects by focusing on the *P. falciparum* 80S ribosome (Wong et al., 2017).

Clindamycin is a medication that predominantly functions as a bacterial inhibitor. The drug is a prescription medicine that became initially available in the early 1960s and is a semi-synthetic derivative of lincomycin. It suppresses the production of protein molecules in cells by attaching itself to the bacterial ribosome's 50s subunit (Aminov, 2013). This antibiotic is a lincosamide with anti-malarial properties and is a first-generation cephalosporin (Willison, 2014). In addition to treating bacterial infections like middle ear infections, strep throat, acne, pneumonia, pelvic inflammatory disorder, and bone and joint inflammation, it also serves as a drug for the management of gram-positive bacterial and anaerobic infections, which include toxoplasmosis, babesiosis, and *Pneumocystis carinii* pneumonia (Obonyo & Juma, 2012).

Despite being a slow-acting medication with an average parasitic elimination period ranging from approximately four to six days and a typical fever clearance period of between three and five days, clindamycin produces favorable results toward *P. falciparum* despite being used as monotherapy (Obonyo & Juma, 2012). Clindamycin treatment of *Plasmodium falciparum* infection is extremely effective and well-tolerated. In regions where resistance to chloroquine is prevalent, combination therapy remains the suggested therapy for pregnant women infected with the infection and proves successful for children (Khaja et al., 2021; Lell & Kremsner, 2002). This study assessed if combination of CLD and MQ would be effective for the treatment of malaria in mice infected with *Plasmodium berghei*.

Materials and Methods

Drug, Animals, and care

Adult Swiss albino mice of both sexes, weighing between 27 and 42g, were bought for this investigation from the facility of the department of pharmacology at the University of Port Harcourt in Rivers State, Nigeria. The animals were housed in a standard cage with pellet feed and unlimited access to water. In accordance with the National Institutes of Health's (NIH) guidelines for the care and management of laboratory animals, the mice were housed in standard laboratory settings for two weeks before the commencement of the investigation (NIH, 2011). MFQ

(15mg/kg) (Les et al., 2017), CQ (10mg/kg) (Somsak et al., 2018), and CLD (10mg/kg) (Gaillard et al., 2015) were used.

Malaria parasite strain

Plasmodium berghei (*P. berghei*) (NK65) strains that are sensitive to chloroquine (CQ) were purchased from the Nigerian Institute of Medical Research in Yaba, Lagos State. A tail blood sample was collected to confirm the parasite. Mice were utilized to nourish the parasites by constant blood flow. Each test mouse received an intraperitoneal (ip) injection of a standard inoculum of parasitized erythrocytes (1×10^7 made by diluting the donor mice's blood with normal.

Evaluation of Antiplasmodial Test

Evaluation of curative antiplasmodial activity of mefloquine-clindamycin.

The modified procedure described by Adikwu et al. (2022) was used. 25 albino mice (n=5) were divided into ten (5) groups at random, infected with *P. berghei* (1×10^7) intraperitoneally, and left untreated for three days. The mice in the test groups received daily oral treatments for 4 days. The normal (non-parasitized) control group received daily oral treatments of normal saline CQ (10mg/kg) for positive control, other groups received MQ (10mg/kg), CLD (10 mg/kg), and MQ-CLD 10mg/kg, respectively. Tail blood samples were collected from the mice and put on slides on day five after the treatments. Giemsa dye was applied to the slides, which were then examined under a light microscope.

Parasitama and percentage parasitama inhibitions were calculated as shown below.

$$\% \text{Parasitemia} = \frac{\text{Number of parasitized red blood cells}}{\text{Total number of RBC count}} \times 100\%$$

$$\% \text{ Inhibition} = \frac{(\% \text{ Parasitemia of negative control} - \% \text{ Parasitemia of treated group})}{\% \text{ Parasitemia of negative control}}$$

Evaluation of suppressive antiplasmodial activity of mefloquine-clindamycin

A suppressive test was assessed using the modified procedure outlined by Nworgu et al. (2022). *P. berghei* (1×10^7) intraperitoneally was administered to twenty-five Swiss albino mice (n=5) and allowed for two hours before treatment. The unparasitized controls were treated with normal saline. MQ (15mg/kg), CQ (10mg/kg), CLD (10 mg/kg) and MQ-CLD (10mg/kg) were administered to the test group for four days. Day 5 after the course of treatment, tail blood samples from the mice were taken for parasitama and percentage parasitama inhibition as shown above.

Evaluation of prophylactic antiplasmodial activity of mefloquine-clindamycin

The modified method of Gboeloh and Nworgu (2023) was employed. The 25 Swiss albino mice (n=5) were randomly assigned to five groups at random. The negative and positive controls were normal saline for 4 days. The experimental mice received daily oral doses of CQ (10mg/kg), MQ (10mg/kg), CLD (10 mg/kg), and MFQ-CLD (10mg/kg), respectively, for 4 days. All the test group received a *P. berghei* (1×10^7) intraperitoneal inoculation on the fifth day and allowed for 72 hr. Tail blood samples were taken from the mice, and Parasitama and percentage parasitama inhibitions were determined as shown above.

Determination of mean survival time (MST)

Both the control and treatment groups of mice had their mortality rates monitored over a number of days from the moment of infection to death, and the results were reported. The following is the calculation of mortality expressed as MST (Haldar, 2018).

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

Assessment of hematological indices

Anesthesia was administered to the curative mice group, and blood samples were taken from the heart in tubes containing anticoagulant. Using a Cell-Dyn Model 331 430 autoanalyzer, the blood samples were analyzed for red

blood cells (RBCs), packed cell volume (PVC), hemoglobin (HB), and white blood cells (WBCs) using an automated hematology analyzer.

Evaluation of biochemical markers

Aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), creatinine (CR), urea (UR), total bilirubin (TB), uric acid and total protein (TP), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were assessed from blood samples taken from the mice in the curative group. employing laboratory chemicals in compliance with the guidelines provided by the manufacturer

Statistical analysis

The mean \pm standard error of the mean (SEM) is used to display the data. The differences in the means of the measured parameters were compared using a one-way ANOVA with SPSS (Statistical Package for Social Sciences) Version 20.0, followed by a Tukey's post hoc analysis. P values having a 95% confidence interval that were less than 0.05 were regarded as statistically significant.

Results

Curative effect of mefloquine-clindamycin on *Plasmodium berghei*-infected mice

MFQ-CLD showed significant $p < 0.05$ parasitemia reduction compared to single dose of CLD and MQ at $p < 0.05$. CLD, MQ, and MQ-CLD yielded parasitemia inhibition of 76.35%, 89.95%, and 94.43%, respectively; meanwhile, CQ generated 90.85% parasite clearance (Table 1). MFQ-CLD extended mean survival time MST compared to separate dosages of MQ and CLD with significance of $p < 0.05$ (Table 1).

Suppressive effect of mefloquine-clindamycin on *Plasmodium berghei*-infected mice

The suppressive test revealed the average percentage parasitemia inhibition observed on the fifth day and the inhibiting ability of each mouse group. The result showed a decreased percentage of parasite inhibition in the treated mice group compared to the untreated group. CQ had an inhibition of 90.30%, 76.13% for CLD, 90.20% for MFQ, 95.79% for MFQ-CLD, respectively. MFQ-CLD prolonged MST assessed against single doses of MQ and CLD with a significance level of $p < 0.05$ (Table 2).

Prophylactic effect of mefloquine-clindamycin on *Plasmodium berghei*-infected mice

The result of the prophylactic test indicated a significant difference $p < 0.05$ in the reduction in the treated mice group when compared to the untreated mice group at $p < 0.05$. The CQ group showed inhibition of 75.25%, 49.04% for CLD, 75.23% for MFQ, 82.59% for MFQ-CLD, respectively. MST was significantly prolonged by MQ-CLD when compared to each dose of MQ and CLD, with a significant difference observed at $p < 0.05$ (Table 3)

Effect of mefloquine-clindamycin on hematological indices of *Plasmodium berghei*-infected mice.

Hematological indices displayed a reduction in PCV, HB, and RBCs, whereas WBCs, NEU, LYM, and Mon were significantly ($p < 0.05$) increased in *Plasmodium berghei*-infected mice when compared to the control (Table 4). However, treatment with MFQ-CLD significantly ($p < 0.05$) increased PVC, HB, RBC, and significantly decreased WBCs, NEU, LYM, and Mon when compared to individual doses of CLD and MQ at $P < 0.05$ (Table 4).

Effect of mefloquine-clindamycin on biochemical indices of *Plasmodium berghei*-infected mice.

AST, AT, ALP, TB, ALB, TP, UR, CRE, and Uric significantly ($p < 0.05$) increased in parasitized untreated mice when compared to the untreated control (Table 5). Thus, treatment with MFQ-CLD significantly ($P < 0.05$) decreased when compared to individual doses of CLD and MQ at $p < 0.05$ (Table 5 and 6).

Effect of mefloquine-clindamycin on lipid function in *Plasmodium berghei*-infected mice

The study showed a statistically significant ($p < 0.05$) increase in TC, TG, LDL, and VLDL, whereas HDL decreased significantly ($P < 0.05$) in the parasitized untreated mice when compared to the control group (Table 6). However, MQ-CLD significantly ($P < 0.05$) decreased TC, TG, LDL, and VLDL and increased HDL levels significantly ($P < 0.05$) when compared to individual doses of MQ and CLD, respectively, at $P < 0.05$ (Table 7).

Effect of mefloquine-clindamycin on liver histology in *Plasmodium berghei*-infected mice.

The liver tissue of the control mice showed normal sinusoids (SIN), normal central vein (CV), and normal hepatocytes (HEP). (Figure A). However, the liver of the parasitized mice showed congested sinusoids (CSS), congested central vein (CD), and steatosis (ST) (Figure B). The liver of mice treated with CQ (Figure C) and MQ (Figure D) showed normal sinusoids (SIN), normal hepatocytes (HEP), and inflammatory cell infiltration (INF) and congested central vein (CD). The liver of CLD revealed normal sinusoids (SIN), central vein (CV), and normal hepatocytes (HEP) (Figure E). Notwithstanding liver of MQ-CLD showed normal sinusoids (SIN), normal hepatocytes (HEP), and normal inflammatory cells infiltration (INF) (Figure F), respectively.

Table 1: Curative effect of mefloquine-clindamycin on *Plasmodium berghei*-infected mice

Treatment	Day1	Day2	Day4	%inhibition	MST
NEG	41.07±4.27	55.08±8.85	66.31±9.10	0.00	8.92±0.94
CQ	24.45±0.58 ^a	15.31±0.69 ^a	6.07±0.58 ^a	90.84	25.14±0.05 ^a
CLD	38.50±1.16 ^b	25.84±0.63 ^c	15.68±0.54 ^c	76.35	15.59±0.42 ^c
MFQ	25.68±0.47 ^a	15.69±0.69 ^a	6.66±0.71 ^a	89.95	25.09±0.05 ^a
MFQ-CLD	21.14±0.52 ^c	8.76±0.60 ^e	3.69±0.67 ^e	94.43	30.41±0.27 ^c

NEG: Negative control, CQ:

Chloroquine, CLD: Clindamycin, MQ: Mefloquine. Data are expressed as Mean ± SEM (Standard Error of Mean), n=5. Values with superscript a showed a significant difference (p<0.05) when compared with the negative control, values with superscript b and c showed a significant difference when compared to the positive control, while values with superscript d and e showed a significant difference between control groups and the mefloquine-treated group.

Table 2: Suppressive effect of mefloquine-clindamycin on *Plasmodium berghei*-infected mice

Treatment	Day1	Day2	Day4	%inhibition	MST
NEG	25.68±0.47	38.50±1.16	42.07±3.90	0.00	9.66±1.23
CQ	10.75±0.43 ^a	6.09±0.57 ^a	4.08±0.37 ^a	90.30	30.31±0.37 ^a
CLD	17.67±1.05 ^b	15.37±0.49 ^c	10.04±0.43 ^c	76.13	20.32±0.45 ^c
MFQ	10.77±0.43 ^a	6.14±0.56 ^a	4.12±0.37 ^a	90.20	30.28±0.38 ^a
MFQ-CLD	5.94±0.53 ^d	2.91±0.25 ^e	1.77±0.07 ^e	95.79	36.60±0.46 ^e

NEG: Negative control, CQ: Chloroquine, CLD: Clindamycin, MQ: Mefloquine. Data are expressed as Mean ± SEM (Standard Error of Mean), n=5. values with superscript a showed a significant difference (p<0.05) when compared with the negative control, values with superscript b and c showed a significant difference when compared to the positive control, while values with superscript d and e showed a significant difference between the control groups and the mefloquine-treated group.

Table 3: Prophylactic effect of mefloquine-clindamycin on *Plasmodium berghei*-infected mice
NEG: Negative control, CQ: Chloroquine, CLD: Clindamycin, MQ: Mefloquine

Treatment	Day1	Day2	Day4	%inhibition	MST
NEG	29.98±0.27	38.50±1.16	50.44±0.08	0.00	9.82±2.31
CQ	5.76±0.47 ^a	8.42±0.04 ^a	12.48±0.25 ^a	75.25	32.55±0.37 ^a
CLD	10.97±0.38 ^c	20.87±0.32 ^c	25.70±0.47 ^c	49.04	21.55±0.58 ^c
MFQ	5.83±0.47 ^a	8.48±0.04 ^a	12.49±0.24 ^a	75.23	32.53±0.38 ^a
MFQ-CLD	1.20±0.27 ^e	4.12±0.36 ^e	8.78±0.36 ^e	82.59	37.43±0.38 ^e

Data are expressed as Mean ± SEM (Standard Error of Mean), n=5. values with superscript a showed a significant difference ($p<0.05$) when compared with the negative control, values with superscript b and c showed a significant difference when compared to the positive control, while values with superscript d and e showed a significant difference between control groups and the mefloquine-treated group.

Table 4: Effect of mefloquine-clindamycin on hematological indices on *Plasmodium berghei*-infected mice.
NC: Normal control, NEG: Negative control, CQ: Chloroquine, CLD: Clindamycin, MQ: Mefloquine

Treatment	PCV (%)	HB (g/dL)	RBCs (x10 ⁶)	WBCs (cells/L)	NEU(%)	LYMP(%)	MONO(%)
NC	59.51±0.26	19.20±0.43	6.39±0.07	5.23±0.08	74.8±0.3	20.50±0.29	0.35±0.00
NEG	20.33±0.30 ^a	5.21±0.24 ^a	1.06±0.02 ^a	25.17±0.38 ^a	220.5±0.6 ^a	82.75±0.25 ^a	5.47±0.09 ^a
CQ	40.80±0.03 ^b	14.35±0.05 ^b	3.68±0.07 ^b	10.44±0.19 ^b	86.8±0.3 ^b	27.00±0.41 ^b	1.51±0.08 ^b
CLD	27.34±0.12 ^d	7.53±0.17 ^d	1.71±0.21 ^d	18.32±0.17 ^d	176.0±1.1 ^d	41.75±0.63 ^d	2.84±0.03 ^d
MFQ	40.65±0.05 ^b	14.32±0.12 ^b	3.56±0.12 ^b	10.48±0.18 ^b	87.5±0.6 ^b	27.75±0.63 ^b	1.52±0.08 ^b
MFQ-CLD	47.89±0.24 ^f	15.18±0.02 ^f	5.01±0.01 ^f	7.71±0.11 ^f	81.8±0.5 ^f	27.50±0.29 ^f	1.18±0.00 ^f

Data are expressed as Mean ± SEM (Standard Error of Mean), n=5. values with superscript a showed significant difference ($p<0.05$) when compared with normal control, values with superscript b showed significant difference when compared to negative control, while values with superscript c and d showed significant difference when compared to negative control, values with superscript e and f showed significant difference when compared to positive control and mefloquine treated group.

Table 5: Effect of mefloquine-clindamycin on Liver function of mice infected with *Plasmodium berghei*.

Treatment	AST(u/l)	ALT(u/l)	ALP(u/l)	TB(g/dl)	ALB (g/dl)
NC	13.00±0.41	15.50±0.29	190.2±0.25	6.25±0.95	3.15±0.42
NEG	14.75±0.25 ^a	16.75±0.25 ^a	192.5±0.50 ^a	7.91±0.29 ^a	4.28±0.34 ^a
CQ	13.50±0.65 ^b	15.75±0.63 ^b	190.7±0.48 ^b	6.78±0.45 ^b	3.36±0.32 ^b
CLD	13.87±0.48 ^b	16.25±0.48 ^b	191.7±0.85 ^b	7.01±0.38 ^b	3.55±0.36 ^b
MFQ	13.50±0.50 ^b	15.75±0.63 ^b	191.0±0.41 ^b	6.80±0.46 ^b	3.31±0.30 ^{bb}
MFQ-CLD	13.50±0.29 ^b	15.50±0.65 ^b	190.7±0.25 ^b	6.84±0.44 ^b	3.32±0.35 ^b

NC: Normal control, NEG: Negative control, CQ: Chloroquine, CLD: Clindamycin, MQ: Mefloquine, AST: Aspartate Transferase, ALT: Alanine Transferase, ALP: Alkaline phosphate, TB: Total bilirubin, ALB: Albumin Data are expressed as Mean ± SEM (Standard Error of Mean), n=5. values with superscript a showed a significant difference (p<0.05) when compared with the normal control, while values with superscript b showed no significant difference when compared to the positive control.

Table 6: Effect of mefloquine-clindamycin on the renal function of mice infected with *Plasmodium berghei*.

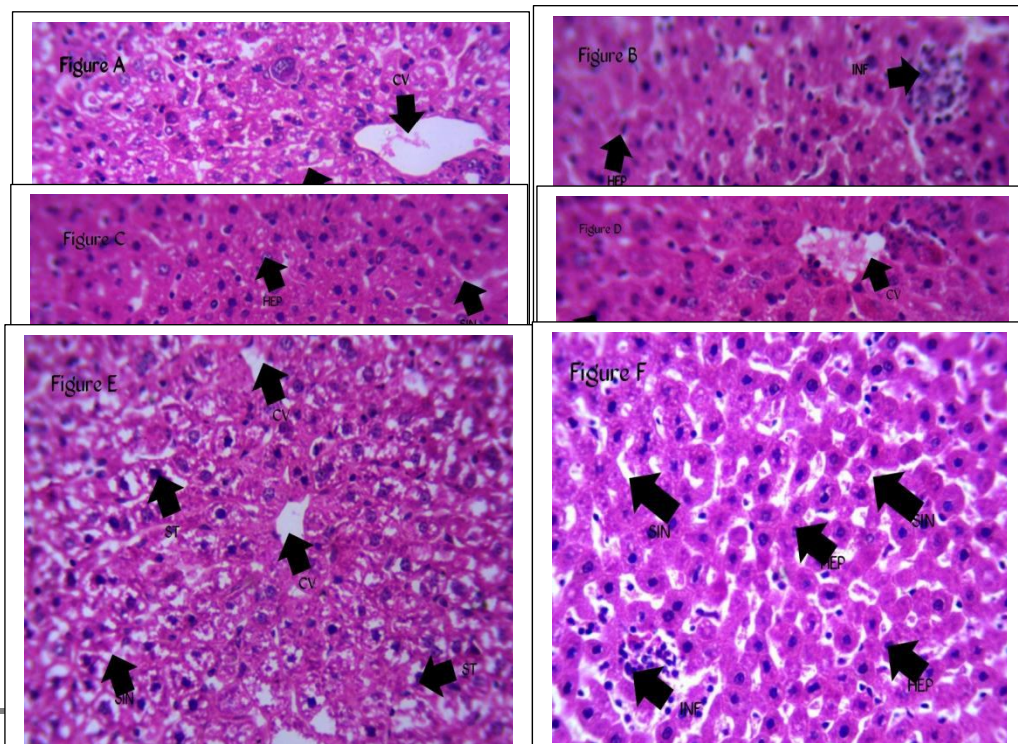
Treatment	CREA(mg/dl)	TP(g/dL)	UR(mg/dl)	UA(mg/dL)
NC	58.00±0.41	82.25±0.25	2.37±0.46	3.09±0.14
NEG	59.50±0.29 ^a	83.50±0.96 ^a	4.29±0.07 ^a	4.4±0.10 ^a
CQ	58.25±0.25 ^b	82.75±0.48 ^b	2.41±0.49 ^b	3.15±0.17 ^b
CLD	58.75±0.25 ^b	83.00±0.41 ^b	2.83±0.49 ^b	3.51±0.22 ^b
MFQ	58.25±0.25 ^b	82.75±0.48 ^b	2.42±0.49 ^b	3.16±0.17 ^b
MFQ-CLD	58.25±0.25 ^b	82.75±0.48 ^b	2.42±0.42 ^b	3.12±0.15 ^b

NC: Normal control, NEG: Negative control, CQ: Chloroquine, CLD: Clindamycin, MQ: Mefloquine, Creatinine (CRE), Total protein (TP), Urea (UR), Uric acid (UA). Data are expressed as Mean ± SEM (Standard Error of Mean), n=5. values with superscript a showed a significant difference (p<0.05) when compared with the normal control, while values with superscript b showed no significant difference when compared to the positive control.

Table 7: Effect of mefloquine-clindamycin on lipid function of mice infected with *Plasmodium berghei*.

Treatment	TC(mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL(mg/dL)
NOR	62.70±0.63	35.90±0.04	15.15±0.42	40.50±0.25	20.33±0.49
NEG	173.81±0.37 ^a	115.69±0.85 ^a	3.63±0.25 ^a	147.11±0.71 ^a	80.53±0.29 ^a
CQ	86.77±0.30 ^b	50.38±0.25 ^b	9.73±0.05 ^b	66.91±0.41 ^b	35.83±0.01 ^b
CLD	129.51±0.06 ^d	70.91±0.04 ^d	5.28±0.04 ^d	110.14±0.01 ^d	60.31±0.05 ^d
MFQ	88.57±0.04 ^b	51.72±0.27 ^b	9.90±0.01 ^b	68.40±0.33 ^b	37.07±0.01 ^b
MFQ-CLD	76.59±0.21 ^f	45.71±0.06 ^f	11.02±0.01 ^f	56.46±0.25 ^f	27.93±0.40 ^f

NC: Normal control, NEG: Negative control, CQ: Chloroquine, CLD: Clindamycin, MQ: Mefloquine, TC: Total cholesterol, TG: Triglycerides, HDL: High density lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein
 Data are expressed as Mean ± SEM (Standard Error of Mean), n=5. values with superscript a showed a significant difference (p<0.05) when compared with the normal control, values with superscript b showed a significant difference when compared to the negative control, while values with superscript c and d showed a significant difference when compared to the negative control, values with superscript e and f showed a significant difference when compared to the positive control and mefloquine-treated group.



Livers of the control and experimental mice are shown in figures A-F. Control (Figure A), mice parasitized with *Plasmodium berghei* (Figures B), treatment with chloroquine (10mg/kg) (Figure C), treatment with mefloquine (10mg/kg) (Figure D), treatment with clindamycin (10mg/kg) (Figure E), treatment with mefloquine-clindamycin (10mg/kg) (Figure F). CV: Central vein congestion, INF: Inflammatory cells, HP: Normal Hepatocytes, SIN: Sinusoids, CS: Congested sinusoids. H and E X 400

Discussion

The prevalence of malaria poses significant health concerns in Southeast Asian and sub-Saharan African impoverished countries. According to Beeson et al. (2016) and Joseph et al. (2020), the struggle against malaria has grown exceedingly challenging due to the increasing susceptibility of different strains of *Plasmodium* against a wide range of antimalarial medications, the increase in resistance to pesticides in the mosquito population, especially with the inadequate supply of vaccinations. To tackle these problems, it will be imperative to discover new medications featuring distinctive biochemical interactions and combinations with present antimalarial medications that are currently available on the marketplace. However, this research intends to evaluate whether CLD may enhance MQ effectiveness against malaria in a *Plasmodium berghei*-infected mouse model. *P. berghei* has been utilized as an animal model for the scientific study of human parasitic infections owing to its evolutionary relationship to *Plasmodium* species, responsible for malaria in humans (Craig et al., 2012). Throughout the past few decades, the mouse model has been extensively employed to offer an understanding of the causes and mechanisms of root causes of illness, evaluate the beneficial effects of prospective drugs, and anticipate responses from patients (Seok et al., 2013). It possesses an identical developmental process to that of mice, alongside manifestations of symptoms associated with human malaria. The current research employed Rane's procedure, which evaluates the test compound of antiplasmodial effectiveness of a current infection, and a four-day suppressive protocol, which investigates actions of a bioactive agent on the initial phase of parasitemia (Mekonnen, 2015). In the present study, the curative test showed a reduction in percentage parasitemia levels which best reduction in parasite levels noticed in the MFQ-CLD treated groups against a single dosage of CLD and the MFQ treated group.

Furthermore, in both suppressive and prophylactic tests, a significant decrease in parasitemia inhibition was noticed in the MQ-CLD-treated mice group when compared to an individual dose of MQ and CLD. In the suppressive test, 76.13%, 90.20%, and 95.79% parasitemia inhibition were observed in CLD, MFQ, and MFQ-CLD in the treated mice group, respectively. This corroborates the study of Adikwu et al. (2022); Gboeloh & Nworgu (2023). Adikwu et al. (2022) stated that the antibacterial agent azithromycin has anti-plasmodial properties and exhibited the best results, whereas Gboeloh & Nworgu (2023) noted that cotrimoxazole is an effective and preventative antimalarial drug, particularly against schizonts at the liver stage. This could be attributed to their ability to target the parasite apicoplast and inhibit the parasite protein folate pathway (Co-Goldberg et al., 2012). MQ-CLD extended MST in mice across curative, suppressive, and prophylactic evaluation. When evaluating a possible antimalarial medication to prevent or lower mortality, MST is a crucial metric (Fidock et al., 2004). An effective test compound consists of anything that produces higher levels of MST than the control group without treatment (Duplessis et al., 2015).

Mortality is a key complication in malaria, particularly in Children and pregnant women. The death of infected red blood cells and removal of uninfected red blood cells, and *Plasmodium* suppression of erythropoiesis, is the cause of anemia (White, 2018). The observation prompted the assessment of the antimalarial drug candidate's capacity to prevent anemia caused by malaria. In the present-day study, anemic signs marked decreased RBC, PCV, HB, and increased WBC, Neutrophils, Monocytes, and Lymphocytes were noticed in mice infected with *P. berghei*. This supports the earlier study of (Adikwu et al., 2022), but decreased anemia characterized by elevated RBC, PCV, HB, and decreased WBC was observed in mice treated with MQ-CLD. While an increase in PCV implies excessive synthesis of red blood cells, a reduced PCV indicates red blood cell destruction (Isacc et al., 2013). According to Isacc et al. (2013) hemoglobin's physiological duties include carrying carbon dioxide out of the body and delivering oxygen to human tissues so that food may be oxidized and energy can be released for other processes. Moisola et al. (2013). However, severe malaria is accompanied by increased WBCs, whereas high levels of NEU counts might be attributed to activated neutrophil secretion. An increase in monocyte count in malaria can activate mononuclear cells, which develop into inflammatory cytokines, like tumor necrosis factor, interleukin-1, and interleukin-6. These cytokines increase monocytes during *Plasmodium* infection, which enhances the hepatic synthesis of acute-phase inflammatory

proteins, including CRP (Wickramasinghe et al., 2000). The parasitized untreated mice showed high levels of AST, ALT, ALP, TB, ALB, TP, UR, CRE, and UA when compared to treated mice. Malaria-associated hepatocyte injury and organ damage may manifest significantly elevated serum levels, acute kidney injury, and loss of liver function with the involvement of parasitized erythrocyte adherence, pro-inflammatory response, and oxidative stress, which have been hypothesized (Al-salaby et al., 2016; Wichapoon et al., 2017). The consumption of hemoglobin by malaria parasites and the erythrocyte destruction give rise to toxic free heme that has the ability to induce oxidative stress during *P. Berghei* ANKA Infection (Kumar, 2017). High levels of these serum can lead to the breakdown of erythrocytes and other haem-containing proteins such as myoglobin and cytochromes, which can also be associated with hepatocellular damage, biliary tract obstruction, haemolysis, and neonatal jaundice (Mosab et al., 2018).

Elevated lipid indices of the mice, such as TC, TG, HDL, LDL, and VLDL, were noticed in the study. This is in consonance with the study of Georgewill et al. (2021), who reported similar results. Changes in serum lipid profile are associated with genetic infection and inflammatory diseases, as well as in chronic or acute conditions (Abdulkareem et al., 2017).

In cases of severe *Plasmodium* infection, liver involvement presents a substantial problem. An essential organ in the life cycle of the malaria parasite is the liver, where malaria sporozoites mature into merozoites. After being discharged into the bloodstream, the merozoites proceed to the erythrocytic stage (Viriyavejakul et al., 2014). The liver is hence vulnerable to potential malaria-related disease. Fatty alteration, hyperplastic Kupffer cells, portal tract inflammation, bile duct proliferation, sinusoidal congestion, and hemozoin deposition are some of the liver pathologies associated with malaria (Viriyavejakul et al., 2014). The evaluation of the dosing effect on histological analysis on parasitized untreated mice was marked with inflammatory cell infiltration, congested sinusoids, congested central vein, and disruption of normal hepatocytes and steatosis, which indicate cellular injury because of the attack of *Plasmodium*, which was not visible in treated mice with MQ-CLD. This also conforms with the study of Adikwu et al. (2022), which also showed similar disruption of the aforementioned. However, the antiplasmodial activity of MQ-CLD could be a result of the different mechanisms of action of the drugs used in the study. MQ is a quinolinylethylamine with an approximate half-life of two to three weeks (Ravina, 2011). which is similar to CQ, which is used for the treatment of malaria. It works by interrupting the parasite's erythrocyte hemoglobin synthesis and absorption (Shibeshi et al., 2020). CQ builds up in the food vacuoles of *Plasmodium* and forms a complex with haem, which causes the buildup of toxic haem product, leading to *Plasmodium* death. CLD is a first-generation cephalosporin (Willison, 2014) that works by suppressing the production of protein molecules in cells by attaching itself to the bacterial ribosome's 50s subunit (Aminov, 2013).

Conclusion

The study proved that the combined doses of the drug were more significantly effective than the individual doses for the management of malaria. However, malaria treatment should be accompanied by CLD as it potentiates the antiplasmodial activity of MQ on *Plasmodium berghei*-infected mice. This study recommends the use of CLD as a combined therapy with MQ for the treatment of malaria.

Conflict of interest: The authors declare no conflict of interest.

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