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Repurpose of Mefloquine-Cotrimoxazole Combination as Anti-Plasmodial Agents in Mice Infected with *Pasmodium Berghei*

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ABSTRACT

The keystone for the treatment of malaria is artemisinin-based combination therapy (ACT). The vulnerability of parasites to ACT has, regrettably, declined. Determining new medications or drug combinations that would mitigate parasite resistance is therefore pertinent. This study assessed the antimalarial property of mefloquine-cotrimoxazole in parasitized mice. Thirty adult Swiss albino mice of (18-30g) were grouped into five of 6 mice each. All the mice were inoculated with *Plasmodium berghei* and orally treated. Group 1 was not treated while groups 2,3, 4 and 5 were treated with chloroquine (10mg/kg), mefloquine (10mg/kg), cotrimoxazole (10mg/kg) and a combination of mefloquine/cotrimoxazole (10mg/kg) respectively for five (5) days. The mean survival time was determined and blood samples were collected and evaluated for percentage parasitemia, hematological and biochemical markers. The results showed that mefloquine/cotrimoxazole combination significantly ($p<0.05$) reduced percentage parasitemia when compared with single doses of cotrimoxazole and mefloquine. Curatively, cotrimoxazole, mefloquine and mefloquine /cotrimoxazole combination produced 84.90%, 98.95% and 96.96% respectively, when contrasted with the 90.84% delivered by chloroquine. Suppressively, cotrimoxazole, mefloquine, mefloquine / cotrimoxazole combination, delivered 84.62%, 90.20% and 97.55% respectively. The prophylactic test delivered cotrimoxazole (61.04%), mefloquine (75.23%), mefloquine /cotrimoxazole combination (89.94%) parasitamia inhibition for day 4. When compared to the individual doses of cotrimoxazole and mefloquine, the mefloquine/cotrimoxazole combo significantly ($p< 0.05$) prolonged the mean survival time in the curative, prophylactic, and suppressive tests. The combination also produced significant ($p<0.05$) reduction in anemia characterized by increased packed cell volume, hemoglobin, red blood cells and decrease in white blood cells, neutrophils lymphocytes and monocytes when compared with single doses of cotrimoxazole and mefloquine respectively. The result showed that the levels of aspertate transferase, alanine alanine transferase, alkaline phosphate total protein, total bilirubin, urea, creatinine and uric acid that were slightly altered was curtailed by mefloquine/cotrimoxazole. The study showed that cotrimoxazole can be utilized as an accomplice drug with mefloquine for the management of malaria.

Keywords: Cotrimoxazole, mefloquine, antiplasmodial, mice.

Introduction

The economic credibility of African nations is reinforced by malaria infection. According to forecast, *P. falciparum* decreases the rate of expansion of the continent's revenue by 1.3% on average annually and ends up costing the globe $12 billion in non-financial expenses each year (WHO, 2020). Majority of the burden is primarily transmitted by underprivileged, rural households with limited knowledge of contemporary deterrence and therapeutic interventions. Despite the devastation that malaria has caused, efforts are being made to save lives by increasing international funding for treatment and prevention programs (WHO, 2021). The incidence from malaria dropped significantly by 30% on average worldwide and by 34% in Africa between 2000 and 2013, as well as the risk of dying from malaria which dropped by 47% globally and also by 54% in Africa

(WHO, 2014). Family members, health-related processes, financial developments, and unforeseen circumstances are adversely affected by the financial impact associated with malaria. Initiatives for the financial evaluation of malaria infection prevention and control, as well as resource allocation, are quite effective but it is extremely important to obtain evidence of their profitability (Hailu *et al.*, 2017). The potential of *P. falciparum* burden to persevere and/or propagate notwithstanding the management as well as assimilation of medication at concentrations that seem to be comparable or greater than those commonly suggested is identified as antimalarial drug resistance (Reyburn, 2010). Although poor chemotherapy might indeed support and perpetrate impedance of *Plasmodium* to drugs that are now used in management of malaria, the mutagenesis incidence of the parasites, cumulative parasite bunch and inappropriate pharmacokinetics also contribute, to a large extend, to the resistivity of the parasite to many drugs formulated for the management of the disease in sub-Saharan Africa (Shibeshi *et al.,* 2020). Nevertheless, as the need for new antimalarial drugs expands, it is vital to investigate methods to avoid and at least slow down the development of resistance to therapeutic interventions (Fidock *et al.,* 2004).

Most commercially available antimalarial prescriptions can be categorized into three categories. Variations of aryl amino alcohol compounds include quinine, quinidine, halofantrine, lumefantrine, chloroquine, amodiaquine, mefloquine, and cycloquine. Antifolate treatments encompass proguanil, pyrimethamine, and trimethoprim. There are multiple constituents set of common and closely linked to artemisinin, such as artemisinin, dihydroartemisinin, artesunate, artemether, and arteether (Kumar *et al.,* 2018: Golenser *et al.,* 2006). Sizable portions of antimalarial prescribed medication shine the spotlight more on parasite erythrocytic asexual phases. Mefloquine, quinine, and chloroquine constitute a few of the incredibly quick antimalarial prescription drugs, and pyrimethamine, sulphonamides, and sulphone represent a few of the slower-acting alternatives. Contrary to gametocytocidal medications, which eliminate the parasite's erythrocytic types in the blood and stop mosquitoes from transmitting malaria, tissue schizonticidal medications focus on the hypnozoites in the liver. The growth of sporozoites and oocysts in mosquito is prevented or inhibited by sporotocides (Alam *et al.,* 2009).

Mefloquine (MFQ), which was first introduced in the 1970s (Ravina, 2011) is a 4-quinoline methanol particle with a half-life of 2-3 weeks (Lee *et al.,* 2017). The emergence of many drug-resistant strains of the parasite might have resulted from these malaria treatment therapeutic interventions (WHO, 2019). Mefloquine is a blood schizonticide that works well against intraerythrocytic stages of the vivax and ovale *Plasmodium* species as well as their gametocidal stages. The parasite ability to utilize and process erythrocyte hemoglobin appears to be interfered with by mefloquine (Shibeshi *et al.,* 2020). In an effort to prevent protein union and with schizonticidal negative effects, it specifically targets the *P. falciparum* 80S ribosome (ASHS, 2014). In addition to cooperating with proteins that are involved in parasite film fatty acid dealing as well as basic needs usage, mefloquine layer adhesion precludes merozoite assassination attempt. It depends on hemoglobin as well as generates a substance that could potentially be detrimental towards the parasite (Wong *et al.,* 2017).

The second generation of cephalosporin-based antibiotics includes cotrimoxazole (CMX). CMX works by concurrently constraining folate sequence metabolites. Trimethoprim is a synthesized antibacterial agent which also relates to the diaminopyrimidine category which results in a broad spectrum of adverse effects on pathogenic microbes through impeding the dihydrofolate reductase catalyst (Tiphaine *et al.,* 2016; Wrobe *et al.,* 2020, Pessanha de Carvalho *et al.,* 2021). A sulfonamide substance termed sulfamethoxazole interacts with p-aminobenzoic acid to hamper the union of folic acid in microbes (WHO, 2021). The development of thymidine and purines encompasses the binding proteins folic acid, which is readily available inside the CMX block microbial blend (Co-Goldberg *et al.,* 2012). In addition to chloroquine or perhaps other widely accepted antimalarial medications, children who struggle with malaria infection as well as microbial respiratory infection are treated with cotrimoxazole (Fehintola *et al.,* 2006). This present study aimed at assessing whether CMX can be repurposed as a partner drug with MFQ in mice infected with *plasmodium berghei.*

MATERIALS AND METHODS

Acquisition of mice

For this experiment, 30 adult male and female Swiss albino mice within the weight range of 18 and 30grams were obtained from the creature office research center of pharmacology Department, University of Port Harcourt in Rivers State, Nigeria. They were provided with a standard pellet diet and preserved in pervasive compartments with uninterrupted water availability. The National Institutes of Health (NIH) rule for the consideration and utilization of laboratory animals was followed when the mice underwent an approximately fourteen-day adaptive capacity time frame before the trial began and were kept in a conventional research laboratory (NIH, 2011).

Acquisition of malaria parasite strain

Donor mice, infected with *Plasmodium berghei*, were obtained from the Nigerian Institute of Medical Research in Yaba, Lagos State (NK65). To verify the parasite, tail blood specimens were collected. In mouse model with persistent blood sections, the parasites were still prominent. By saline-weakening the blood, a standard inoculum of parasitized erythrocytes (1 x 10^7) was prepared. Each experimental animal received an intravenous infusion intravenous injection of erythrocytes containing 1x10^7 *P. berghei.*

Acquisition of drugs

Mefloquine (MQ) designed and produced by Artepharm Co., Ltd., China, Cotrimoxazole(CMX),and Chloroquine (CQ) produced by Evans Pharm, Nigeria were utilized. The portions that were utilized were: MFQ (15mg/kg) (Les *et al.*, 2017), (CQ (10mg/kg) (Somsak *et al.*, 2018) and CMX (10mg/kg) (Teldandi *et al.*, 2009).

Assessment of curative effect of mefloquine-cotrimoxazole in parasitized mice.

Experimental Design

The adjusted strategy demonstrated by Okon *et al.* (2014) was utilized. Thirty albino mice were isolated into five (5) cluster, infected with *P. berghei* (1x10^7) intraperitoneally, and left untreated for three days during which the mice exhibited malaria symptoms. The groups were designated 1,2,3,4 and 5. While group one was not administered with any drug, groups 2,3,4 and 5 were administered with 10mg/kg Chloroquine-CQ, 10mg/kg Cotrimoxazole-CMX, 10mg/kg Mefloquine-MFQ and 10mg/kg combined dose of Cotrimoxazole-Mefloquine- MFQ-CMX respectively. The routine treatment with all the drugs was done orally. The rodents in each group had blood samples taken from their tails on day 5 of treatment in order to make thin and thick blood smears. The prepared blood smears were observed carefully and checked for the presence of malaria parasites using the light microscope

Percentage parasitemia and inhibition were determined using the formula of Gboeloh *et al.* (2014) and Nworgu *et al.* (2022).

$$PP = \frac{RBCp}{RBCt} \ X\ 100$$

Where:

PP = Percentage Parasitemia

RBCp = Number of parasitized red blood cells
RBCt = Total number of RBC count

$$Ip = \frac{Pnc - Ptg}{Pnc} \ X100$$

Where:

Ip = Percentage Inhibition

Pnc= Percentage of negative control

Ptg = Percentage of treated groups

Assessment of suppressive effect of mefloquine-cotrimoxazole in parasitized mice.

A modified method by Nworgu *et al.* (2022) was used to resolve the suppressive test. Thirty Swiss albino mice (n=6) received *P. berghei* (1x10^7) ip and given two hours to become infected prior to actually initiation of treatment. The normal saline (0.2mL) was used to treat the negative control (group 1) . Groups 2,3, 4 and 5 were given daily oral doses of CQ (10 mg/kg), MQ (10 mg/kg), CMX (10 mg/kg), and CMX/MFQ (10mg/kg) respectively for 4 days. Blood samples from the mice tail were taken on day 5 after the course of treatment and stained on slides with 10% Giemsa stain. The percentage suppressive effect was calculated using the aforementioned formula.

Assessment of prophylactic effect of mefloquine-cotrimoxazole in parasitized mice.

The modified method by Adikwu *et al.* (2022) was adopted for prophylactic test. Thirty mice (n=6) were grouped into 5. For four days, normal saline was given to the control while the test mice were given daily oral doses of CQ (10 mg/kg), CMX (10 mg/kg), MQ (10 mg/kg) and CMX/MF (10mg/kg) respectively. On the fifth day, *P. berghei* (1x10^7) ip was administered to all the mice, and they were then allowed a 72-hour waiting period. Blood samples were drawn from the tail of each mouse and smeared on well labeled slides and 10% Giemsa stain was used for staining, and percentage prophylactic effect was calculated.

Assessment of Mean Survival Time (MST)

The mortality rates were tracked for 4 days in all the groups and mean survival time determined using the method of Adikwu *et al.* (2023).

$$MST = \frac{a}{b}$$

Where:

MST= Mean Survival Time
a= Combined duration that each mouse in a group has survived (days)
b= Total number of mice in that group

Assessment of hematological indices

Mice in the curative group had their blood drawn through a cardiac puncture and placed in tubes containing anticoagulant. The blood tests were measured for red blood cells (RBCs), packed cell volume (PVC), hemoglobin (Hb), and white blood cells (WBCs) using a Cell-Dyn Model 331 430 autoanalyzer.

Assessment of Biochemical indices

Mice from the curative group were tested using an auto analyser for aspartate transaminase (AST), alanine transaminase
(ALT), total bilirubin (TB) and albumin (ALB), Urea, Creatinine and Uric acid.

RESULTS

Curative effect of mefloquine-cotrimoxazole in parasitized mice

The parasitized mice showed a steady increase in the level of parasitemia before treatment. Similar trend was observed in group 1(untreated) where the level of parasitemia was 41.07±4.27, 55.08±8.85 and 66.31±9.10 for days one, two and three respectively. After treatment of groups 2, 3, 4 and 5 with CQ, CMX, MFQ and MFQ/CMX, there was a significant($p<0.05$) decrease in level of parasitemia of the treated groups when compared with group 1. The parasitemia level in mice treated with CQ significantly decreased from 24.45±0.58 to 6.07±0.58 three days after treatment, showing a curative effect of 90.84% while those treated with CMX had a decreased parasitemia from 37.63±1.76 to 10.01±0.43 representing 84.90% curative effect, three days after administration. Similar trend was also recorded in mice treated with MFQ where parasitemia level progressively reduced from 25.68±0.47 to 6.66±0.71 at the end of third day of treatment. This represents 89.95% curative effect (Table 1). The curative effect of combined MFQ/CMX showed a curative effect of 96.96%. There was a significant decrease in parasitemia from 19.85±0.72 on day one to 2.01±0.41 on day three. Comparing the curative effects of CQ, CMX, and MFQ on days one, two, and three, respectively, revealed that the curative impact of MFQ/CMX was statistically significant ($p<0.05$).

Table 1: Curative effect of mefloquine-cotrimoxazole in parasitized mice

Group	Treatment	Day1	Day2	Day3	Curative effect (%)	MST
		% Parasitaemia				
$1_{(untreated)}$	NEG	41.07±4.27	55.08±8.85	66.31±9.10	0.00	88.92±0.94
2	CQ	24.45±0.58[a]	15.31±0.69[a]	6.07±0.58[a]	90.84	25.14±0.05[a]
3	CMX	37.63±1.76[b]	20.85±0.32[b]	10.01±0.43[b]	84.90	20.32±0.45[b]
4	MFQ	25.68±0.47[a]	15.69±0.69[a]	6.66±0.71[a]	89.95	25.09±0.05[a]
5	MFQ-CMX	19.85±0.72[c]	5.85±0.48[d]	2.01±0.41[d]	96.96	36.56±0.49[d]

Mean and ± SEM (Standard Error of Mean) were used to present data. When compared to the negative control, the values with the superscript "a" showed a significant difference ($p< 0.05$); similarly, the values with the superscripts "b" and "c" showed a significant difference when compared to the positive control; and the values with the superscripts "d" showed a significant difference between the control groups and the mefloquine-treated group.

Is MST at the end of the table necessary?

KEYS:
NEG=Parasitized untreated control
CQ= Chloroquine (Positive control)
CMX= Cotrimoxazole
MFQ= Mefloquine

Suppressive effect of mefloquine-cotrimoxazole in parasitized mice

The suppressive test findings showed that groups 1,2,3,4 and 5 that received treatment exhibit significant difference ($P<0.05$) in the percentage suppression of the parasite when contrasted with the untreated group 1, (negative control). On day four, the percentage suppression for CQ, CMX, MFQ and CMX/MFQ were 90.30%, 84.62% 90.20% and 97.55% respectively (Table 2). MFQ-CMX also significantly ($p<0.05$) prolonged MST (Table 2).

Table 2: Suppressive effect of mefloquine-cotrimoxazole in parasitized mice

Group	Treatment	Day1	Day2	Day4	% suppression	MST
			% Parasitaemia			
$1_{(untreated)}$	NEG	25.68±0.47	38.50±1.16	42.07±3.90	**0.00**	9.66±1.23
2	CQ	10.75±0.43[a]	6.09±0.57[a]	4.08±0.37[a]	**90.30**	30.31±0.37[a]
3	CMX	17.01±0.43[b]	10.04±0.41[b]	6.47±0.42[b]	**84.62**	25.42±0.38[b]
4	MFQ	10.77±0.43[a]	6.14±0.56[a]	4.12±0.37[a]	**90.20**	30.28±0.38[a]
5	MFQ-CMX	4.07±0.37[c]	2.07±0.45[d]	1.03±0.37[d]	**97.55**	42.69±10.57[d]

Mean and ± SEM(Standard Error of Mean) are used to describe data. When compared to Group 1, values with the superscript "a" demonstrated a significant difference ($p< 0.05$). Superscript "d" values demonstrated a significant difference when compared to Groups 1, 2, 3, and 4, (NEG, CQ, CMX and MFQ) while superscript "b" and "c" values demonstrated a significant difference when compared to Group 2 (positive control).

KEYS: KEYS:
NEG=Parasitized untreated control
CQ= Chloroquine (Positive control)
CMX= Cotrimoxazole
MFQ= Mefloquine
MST?

Prophylactic effect of mefloquine-cotrimoxazole in parasitized mice

The result of the prophylactic indicated that mice in the treated mice groups showed a significant ($p<0.05$) reduction in parasitemia when contrasted with group 1. The CQ group demonstrated inhibition on day four (4) of 75.25% for CMX, 61.04% for CMX/MFQ, 75.23% for MFQ, and 89.94% for CMX/MFQ, respectively. MFQ-CMX prolonged MST significantly ($p<0.05$) when compared to individual doses of MFQ and CMX (Table 3).

Table 3: Prophylactic effect of mefloquine-cotrimoxazole in parasitized mice.

Group	Treatment	Day1	Day2	Day4	% prophylatic	**MST**
			% Parasitaemia			
1	NEG	29.98±0.27	38.50±1.16	50.44±0.08	**0.00**	9.82±2.31
2	CQ	5.76±0.47[a]	8.42±0.04[a]	12.48±0.25[a]	**75.25**	32.55±0.37[a]
3	CMX	9.49±0.20[b]	13.71±0.96[b]	19.65±0.48[b]	**61.04**	27.43±0.52[b]
4	MFQ	5.83±0.47[a]	8.48±0.04[a]	12.49±0.24[a]	**75.23**	32.53±0.38[a]
5	MFQ-CMX	0.56±0.27[d]	2.14±0.29[d]	5.07±0.28[d]	**89.94**	44.43±0.80[d]

Mean and ± SEM (Standard Error of Mean) are used to describe data. When compared to the negative control, the values with the superscript "a" showed a significant difference ($p< 0.05$); when compared to the positive control, the values with the superscript "b" showed a significant difference ($p<0.05$); and when compared to the control groups and the mefloquine-treated group, the values with the superscript "d" showed a significant difference ($p<0.05$).

KEYS:
NEG = Parasitized untreated control
CQ= Chloroquine
CMX= Cotrimoxazole
MFQ = Mefloquine

Effect of mefloquine and cotrimoxazole on hematological indices of treated mice

Five days after starting treatment, mice in all groups were sacrificed, and blood was obtained by cardiac puncture to measure haematological and biochemical parameters.

The results of the hematological analysis indicated that there was a significant ($p<0.05$) increase in the pack cell volume (PCV) of groups 2(40.80±0.03), 3 (32.58±0.15), 4(40.65±0.05) and 5(58.16±0.06) when compared with group 1 (20.33±0.30). Similarly, there was also a significant improvement in the performance of haemoglobin (Hb) in all the treated groups when compared to the infected but untreated group. The performance of haemoglobin (Hb) in the treated groups were 14.35±0.05, 10.40±0.07, 14.32±0.12 and 18.33±0.18 for groups 2, 3.4 and 5 respectively, while that of group 1 was 5.21±0.24. Red blood cells (RBC) levels statistically increased ($p<0.005$) in all the treated groups compared to group 1. The recorded values were 3.68±0.07, 2.28±0.09, 3.56±0.12 and 6.27±0.08 respectively while group 1 had 1.06±0.02 (Table 4). Decreased values were observed in the level of white blood cell (WBC) in all the treated groups compared to the infected but untreated group. While group 1 recorded 25.17±0.38 in the level of WBC, groups 2, 3, 4 and 5 had 10.44±0.19, 14.18±0.15, 10.48±0.18 and 6.03±0.02 respectively (Table 4).

Table 4: Effect of mefloquine-cotrimozaxole on packed cell volume, haemoglobin, red blood cell and white blood cell of parasitized mice after five days

Group	Treatment	PCV (%)	HB (g/dL)	RBCs ($x10^6$)	WBCs (cells/L)
1	NEG	$20.33±0.3^{0}a$	$5.21±0.2^{4}a$	$1.06±0.02^{a}$	$25.17±0.38^{a}$
2	CQ	$40.80±0.0^{3}b$	$14.35±0.05^{b}$	$3.68±0.07^{b}$	$10.44±0.19^{cb}$
3	CMX	$32.58±0.15^{b}$	$10.40±0.07^{b}$	$2.28±0.09^{b}$	$14.18±0.15^{c}$
4	MFQ	$40.65±0.05^{b}$	$14.32±0.12^{b}$	$3.56±0.12^{b}$	$10.48±0.18^{c}$
5	MFQ-CMX	$58.16±0.06^{e}$	$18.33±0.18^{be}$	$6.27±0.08^{be}$	$6.03±0.02^{ce}$

Mean and ±SEM (Standard Error of Mean) are used to describe data. When compared to the normal control group, the values with the superscript "a" demonstrated significant difference ($p< 0.05$); when compared to the negative control group, the values with the superscript "b" also demonstrated significant difference; and when compared to the positive control group and the mefloquine-treated group, the values with the superscript "c"; and the values with the superscript "e" showed significant difference.

KEYS:
NEG= Parasitized untreated control
CQ= Chloroquine
CMX= Cotrimoxazole
MFQ= Mefloquine

Effect of mefloquine-cotrimozaxole on neutrophils, lymphocytes and monocytes

When compared to the group 1, the level of neutrophil (NEU), lymphocyte (LYMP), and monocyte (MONO) revealed a significant (P0.05) decrease. The value of NEU in group 1 was 220.5±0.6 while the values for group 2, 3, 4, and 5 were 86.8±0.3, 133.0±0.4, 87.5±0.6 and 73.5±0.3 respectively (Table 5). Similarly, the level of lymphocyte in group 1 was 82.75±0.25 while the values for groups 2, 3, 4 and 5 were 27.00±0.41, 35.75±0.48, 27.75±0.63 and 21.50±0.65 respectively. Monocytes (MONO) had a higher level in group 1 as against 1.51±0.08 in group 2, 2.10±0.01 in group 3, 1.52±0.08 in group 4 and 0.37±0.00 in group 5 (Table 5)

Table 5: Effect of mefloquine-cotrimozaxole on neutrophils, lymphocytes and monocyte of parasitized mice

Group	Treatment	NEU(%)	LYMP(%)	MONO(%)
1	NEG	220.5±0.6	82.75±0.25	5.47±0.09
2	CQ	$86.8±0.3^{b}$	$27.00±0.41^{b}$	$1.51±0.08^{b}$
3	CMX	$133.0±0.4^{b}$	$35.75±0.48^{b}$	2.10±0.01b
4	MFQ	$87.5±0.6^{bc}$	$27.75±0.63^{c}$	$1.52±0.08^{c}$
5	MFQ-CMX	$73.5±0.3^{bc}$	$21.50±0.65^{bc}$	$0.37±0.00^{b}$

Mean and ±SEM (Standard Error of Mean) were used to describe data. When values with superscript "a" were compared to the negative control, there was a significant difference, whereas when values with superscript "c" were compared to the group 2, there was no significant difference ($p>0.05$).

KEYS:
NEG= Parasitized untreated control
CQ= Chloroquine
CMX= Cotrimoxazole
MFQ= Mefloquine

Effect of mefloquine/cotrimoxazole on biochemical of parasitized mice

There was a significant ($p<0.05$) rise in the amount of biochemical indices (Aspertate Transferase (AST), Alanine Transferase (ALT), Alkaline phosphate (ALP),Total bilirubin(TB), and Albumin (ALB) when compared to group 1. The values for AST were 13.50±0.65, 13.75±0.48, 13.50±0.50 and 13.25±0.25 in group 2, 3, 4 and 5 respectively while the recorded value for group 1 was 14.75±0.25 (Table 6).

The values for ALT in group 1 was 16.75±0.25 while 15.75±0.63, 16.00±0.41, 15.75±0.63 and 15.25±0.48 were recorded in groups 2, 3, 4 and 5 respectively. Again 192.5±0.50 was recorded for ALP in group 1 while 190.7±0.48, 191.0±0.41, 191.0±0.41 and 190.5±0.29 was recorded in groups 2, 3, 4, and 5 respectively. The values for TB in group 1 was 7.91±0.29 . However, there was a relatively gradual and significant increase in the value of TB in groups 2(6.78±0.45), 3(6.82±0.43), 4(6.80±0.46) and 5 (6.60±0.34) respectively (Table 6). The value of ALB also reduced in group 1 from 4.28±0.34 to 3.36±0.32, 3.49±0.35, 3.31±0.30, and 3.21±0.38 in groups 2,3,4 and 5 respectively (Table 6)

Table 6: Effect of mefloquine-cotrimoxazole on biochemical indices of parasitized mice

Treatment	AST(u/l)	ALT(u/l)	ALP(u/l)	TB(g/dl)	ALB (g/dl)
NEG	14.75±0.25[a]	16.75±0.25	192.5±0.50	7.91±0.29	4.28±0.34
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]
CMX	13.75±0.48[b]	16.00±0.41[b]	191.0±0.41[b]	6.82±0.43[b]	3.49±0.35[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-CMX	13.25±0.25[b]	15.25±0.48[b]	190.5±0.29[b]	6.60±0.34[b]	3.21±0.38[b]

Mean and ±SEM (Standard Error of Mean).are used to describe data. Values with superscript "b" did not differ significantly from the group 2 (positive control).

KEYS:
NEG= Parasitized untreated control
CQ= Chloroquine
CMX= Cotrimoxazole
MFQ = Mefloquine
AST= Aspertate Transferase
ALT= Alanine Transferase
ALP= Alkaline phosphate
TB=Total bilirubin
ALB= Albumin

Effect of mefloquine-cotrimoxazole on Creatinine, Total protein, Urea, Uric acid of parasitized mice

The treatment of the mice in all the groups with the various drugs showed no significance difference ($p>0.05$) in the performance of creatinine (CRE), total protein (TP), urea (UR) and uric acid (UA) when contrasted with group 1 (Table 7). The value of creatinine, total protein, urea and uric acid in mice that were infected but not treated (Group 1) were 59.50±0.29, 83.50±0.96, 4.29±0.07 and 4.4±00.10 respectively. Group 2 had 58.25±0.25, 82.75±0.48, 2.41±0.49 and 3.15±0.17 for creatinine, total protein, urea and uric acid respectively while group 3 had 58.50±0.29, 82.75±0.48, 2.71±0.50 and 3.38±0.20 for creatinine, total protein, urea and uric acid respectively. The groups treated with MFQ had 58.25±0.25, 82.75±0.48, 2.42±0.49 and 3.16±0.17 for creatinine, total protein, urea and uric acid respectively while Group 5 treated with the MFQ-CMX had 58.00±0.00, 82.50±0.50, 2.37±0.46 and 3.10±0.15 for creatinine, total protein, urea and uric acid respectively (Table 7).

Table 7: Effect of mefloquine-cotrimoxazole on Creatinine, Total protein, Urea, Uric acid of parasitized mice

Group	Treatment	CRE(mg/dl)	TP(g/dL)	UR(mg/dl)	UA(mg/dL)
1	NEG	59.50±0.29[a]	83.50±0.96[a]	4.29±0.07[a]	4.4±00.10[a]
2	CQ	58.25±0.25[b]	82.75±0.48[b]	2.41±0.49[b]	3.15±0.17[b]
3	CMX	58.50±0.29[b]	82.75±0.48[b]	2.71±0.50[b]	3.38±0.20[b]
4	MFQ	58.25±0.25[b]	82.75±0.48[b]	2.42±0.49[b]	3.16±0.17[b]
5	MFQ-CMX	58.00±0.00[b]	82.50±0.50[b]	2.37±0.46[b]	3.10±0.15[b]

Mean and ±SEM (Standard Error of Mean) are used to describe data. When values with the superscript "a" were compared to the group 1, there was no discernible change ($p>0.05$).

KEYS:
NEG= Parasitized untreated control
NEG= Parasitized untreated control
CQ= Chloroquine
CMX= Cotrimoxazole
MFQ = Mefloquine
CRE= Creatinine
TP=Total protein
UR= Urea
UA= Uric acid

DSCUSSION

Plasmodium falciparum has established antimalarial drug resistance over time and has previously been confirmed to develop resistance to widely available antimalarial prescription medicines (Mita *et al.,* 1988; Gupta *et al.,* 2019; Toshihiro *et al.,* 2002). The world faces an important public health demand for additional safe and efficacious antimalarial, and in recent time, there is report and overwhelming evidence that *Plasmodium falciparum* is sensitive to artemisinin derivative (Olasehinde, 2014). Hence, researches have conducted studies into other molecules and compounds with the intension of resolving the problem of malaria resistivity, such researches involve the use of antibiotics (Gingras and Jensen, 1993; Ubulom *et al.,* 2015 Gaillard *et al.,* 2015; Gaillard *et al.,* 2016;).
This study assessed whether cotrimoxazole, Mefloquine and combination of the two antibiotics could be repurposed as antiplasmodial agents for the management of malaria in a mice model infected with *plasmodium berghei*. The study makes use of an animal model for the reason that it has been widely employed to shed light on the root cause of disease mechanism, assess the effectiveness of novel therapeutics, and forecast sequence responses (Seok *et al.,* 2013). The Rane suppressive model was employed in this study in view of the fact that it is frequently utilized in preliminary evaluation of the efficacy of antimalarial compounds (Kretti *et al.,* 2009). *Plasmodium berghei* was used to induce malaria in mice, as it is frequently employed for preclinical antimalarial investigations of new chemical substances with quantifiable therapeutic effects (Willcox *et al.,* 2004). The curative test revealed that mice treated with a combination of cotrimoxazole and mefloquine (MFQ/CMX) had decreased level of parasitemia that are comparable to chloroquine (CQ). The outcomes of the suppressive and prophylactic test showed that the treated groups had lower percentage parasitemia levels, particularly the MFQ-CMX combination, which was at parity with (CQ). This supports the findings of Adikwu *et al.* (2022) and Tan *et al. (*2011). Tan *et al.* (2011) recorded that doxycycline, an antibiotic, is an efficacious and prophylactic antimalarial agent especially against schizonts at the liver stage while Adikwu *et al.* (2022) reported that an antibiotic, Azithromycin has anti-plasmodial property and produced best result when used in combination with Amodiaquine. The potency of these drugs may be attributed to their high lipophilicity which enables them to penetrate into the cells (Sande and Marshal, 1980; Pradines *et al.,* 2001). Remarkably, the capabilities of the individual drugs to prolong mean survival time (MST) was monitored, with the underlying principle that MST is used for the assessment of prospective antimalarial agents to combat or minimize fatalities (Fidock *et al.,* 2004). In the current study, the drugs impacted positively on MST in the treated groups when contrasted to group 1 and was regarded to be highly active (Duplessis *et al.,* 2015). MFQ-CMX combination recorded the highest MST prolongation, which was at par with CQ, in the curative, suppressive, and prophylactic tests.

Hematological parameters such as PCV, HB, RBCs, WBCs, Neutrophils, Lymphocytes, and Monocytes levels are distinctive malaria biomarkers and are frequently utilized to ascertain the degree to which a treatment is effective against *Plasmodium infection*. PCV is used to diagnose haemodilution, hemoconcentration, erythrocytosis, and weakness. Although an increase in PCV tends to suggest parchedness or an unusual expansion in red blood cell, a reduction in PCV denotes red blood cell destruction and poor bone marrow formation (Isacc *et al.,* 2013). The

findings of the current study indicated that the mice that were infected but not treated had decreased PCV which is a sign of anemia. Nevertheless, the constituent treatment with the drug increased the PCV in infected and treated groups. This is in consonance with the record of Adikwu *et al*. (2022) who reported a steady increase in haemotological indices of swiss albino mice infected with *Plasmodium* after treatment. MFQ/CMX curtailed the elevated HB and RBCs noticed in the study which was at par with CQ. The basic function of WBC is to safeguard the body against foreign bodies. This WBCs capability basically in body safeguard against unfamiliar bodies (Mojisola *et al.*, 2013). The WBCs of the untreated mice group actually increased, meanwhile the treated mice group greatly reduced the high increase in WBC count; however, the highest decrease was observed in MFQ/CMX. Extremely high number of neutrophils and lymphocytes were found in the group of infected but untreated mice. The constituent drug helps to reduce the mice NEU, LYM levels, especially in the MFQ-CMX treated groups. In the study, elevated Monocyte was reduced by MFQ-CMX, which was comparable to to CQ. The *P. berghei* parasitized, untreated mice in this research had higher levels of AST, ALT, ALP, TB, TP, UR, CRE, and UA than did the treated mice. Malaria-induced hepatocyte injury may manifest significantly elevated serum levels of these enzymes (Al-salaby *et al.,* 2016). With the assistance of parasitized erythrocyte adhesion, supportive provocative reaction, and oxidative pressure, malaria-related organ health hazards are therefore hypothesized to include serious kidney damage and a breakdown of liver capability (Wichapoon *et al.,* 2017). The utilization of hemoglobin by malaria parasites and the erythrocyte annihilation lead to harmful free heme that can incite oxidative pressure during *P. berghei* ANKA infection (Kumar, 2018).

The liver's importance to health and its role as a crucial component at the initial stages of plasmodial development may be the cause of the pathology that has been identified in the liver. This might alter the physiology and surface structure of the host hepatocytes (Gboeloh, 2016). The increased levels of AST, ALT and ALP observed was similar to what was recorded by Kochar *et al.* (2020; Woodford *et al.,* 2018). Due to hepatic disease and malaria infection, there were high levels of TB recorded in the micethat were decreased in the treatment group. Ugokwe *et al.* (2015) also reported high levels of TB.

Erythrocytes and other hemoproteins, including myoglobin and cytochromes, break down to produce TB. However, visible jaundice can result from extremely high TB in the plasma to even more than double of its normal count (Kausar *et al.,* 2010). While Oluwole *et al.* (2010) pointed out that higher levels of bilirubin bill in malaria patients is a confirmation of increased RBCs, elevated serum level is closely linked with damage, biliary tract obstruction, and neonatal jaundice.

Following treatment, as observed in the treated groups, the increased levels of ALB in the untreated mice diminished. Sukanya *et al.* (2017) observed that ALB levels were significantly decreased in a series of experiment on the interference of biochemical parameters due to *Plasmodium berghei* infection causing organs dysfunction. This study contrasts with their findings. In comparison to the control group, TP in untreated parasitized group significantly decreased.

The constituent drug used to treat the groups was effective in increasing the lowered TP level. High blood protein levels may be an early sign of internal organ damage from intensifying inflammation, such as liver or kidney damage (Klein *et al.*, 2002). The study results correspond with those reported by Abolaji *et al.* (2013), who discovered an increase in protein levels in all groups administered withthe antimalarial medication compared to the control. When compared to the control group, the results revealed that urea significantly increased in the untreated group.

The severity of the malaria infection may, however, have had a significant effect on the level of urea in infected individuals, as indicated by high levels of urea in the infected mice. In contrast to the untreated group of mice, the resultant treated mice groups were recorded reduced urea levels. Comparatively individual doses of CMX and MFQ and MFQ/CMX had the best reduction potency. This is in line with the studies by Bigoniya *et al.* (2015). The elevated levels of creatinine observed in the untreated mice group were effectively diminished in the treated groups. Moreover, a rise in plasma creatinine levels in malaria patients increases the likelihood of impaired renal function (Geraldo *et al.,* 2017).In contrast to the present findings, the study by Elbadawi *et al.* (2013) found that malaria patients had substantially lower levels of creatinine. The mice in the treated group had lower levels of uric acid than the mice in the untreated group, indicating a decrease from the elevated levels. This report finding corroborates that of Anigboro (2018). This present finding indicated that the liver stage of *Plasmodium* infection may be cured by MFQ-CMX. The ability of the compounds that make up MFQ-CMX to target *Plasmodium* parasites at various sites may offer an explanation for the antiplasmodial activity which was observed. MFQ treatmentexplicitly focuses on the 80S ribosome of the *P. falciparum*, repressing protein union and causing resulting schizonticidal impacts (ASHS, 2014). CMX works by progressively inhibiting folic acid pathway enzymes. Folic acid, an important and necessary co-factor in the production of thymidine and purines, is synthesized by microbes and is simultaneously obstructed by CMX (Co-Goldberg *et al.,* 2012).

CONCLUSION

The current study demonstrated that the management of malaria with CMX and MFQ administered singly is relatively effective but a combination of the two drugs produced a significant result especially on the liver stage parasites . It is therefore recommended that malaria should be treated with a combination of an antimalarial and antibiotics because of *Plasmodium* and bacterium coinfection which could occur during plasmodiasis. CMX enhances the anti-plasmodial activity of MFQ.

This study recommends using MFQ-CMX to treat malaria.
Conflict of interest: The authors declare no conflict of interest

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