Scientific Research Publishing

Advances in Infectious Diseases, 2025, 15(4), 791-809
https://www.scirp.org/journal/aid
ISSN Online: 2164-2656
ISSN Print: 2164-2648

Therapeutic and Biochemical Effects of Chloroquine-Based Combination Therapies in Murine *Plasmodium berghei* Infection

Segun Afolabi Olomu*, Jane-Rose Ifunanyachi Oche, Yusuf Samuel Gazuwa, Titilayo Omolara Johnson

Department of Biochemistry, University of Jos, Jos, Nigeria
Email: *petlovekennels@gmail.com

How to cite this paper: Olomu, S.A., Oche, J.-R.I., Gazuwa, Y.S. and Johnson, T.O. (2025) Therapeutic and Biochemical Effects of Chloroquine-Based Combination Therapies in Murine *Plasmodium berghei* Infection. *Advances in Infectious Diseases*, **15**, 791-809.
https://doi.org/10.4236/aid.2025.154059

Received: December 8, 2025
Accepted: December 21, 2025
Published: December 24, 2025

Abstract

Background: Malaria remains a major global health burden, with increasing concerns about resistance to artemisinin-based combination therapies (ACTs). Chloroquine (CQ), once the mainstay of treatment, was abandoned due to widespread resistance but is regaining interest following reports of re-sensitivity in some endemic regions. This study evaluated the efficacy of CQ in combination with other standard antimalarials against *Plasmodium berghei* infection in mice. **Methods:** Wistar albino mice were inoculated intraperitoneally with *P. berghei* and randomly divided into groups treated with CQ, artemether-lumefantrine (AL), sulphadoxine-pyrimethamine (SP), dihydroartemisinin-piperaquine (DHAP), amodiaquine (AA), or CQ combined with each of these agents. Treatment was administered orally for four days, and parasitemia was monitored microscopically. On Day 4, blood samples were analyzed for oxidative stress markers (lipid peroxidation, nitric oxide), liver function tests (ALT, AST, ALP, total protein, albumin, bilirubin), and hematological parameters (RBC, Hb, PCV, WBC, platelets, leukocyte differentials). **Results:** CQ monotherapy produced moderate parasite suppression, whereas combinations—especially CQ + AL and CQ + DHAP—significantly reduced parasitemia compared to single-drug regimens ($p < 0.05$). CQ combinations also lowered lipid peroxidation, with CQ + AL (0.097 ± 0.011 mMol/mL) showing the strongest antioxidant effect. Nitric oxide levels, suppressed in infected controls, were restored in CQ + AL and CQ + SP groups. Liver enzymes elevated by infection were normalized by CQ + AL, while CQ + AA markedly improved hemoglobin (19.43 ± 1.29 g/dL) and RBC counts (10.93 ± 2.22 × 10^6/μL), reversing malaria-induced anemia. Platelet counts and lymphocyte levels were also improved in most CQ-based regimens. **Conclusion:** Chloroquine, when combined with other antimalarials, enhanced parasite clearance

and improved biochemical and hematological outcomes in murine malaria. These findings highlight the potential of CQ-based combinations as viable alternatives or adjuncts to ACTs, particularly in regions where CQ sensitivity has re-emerged. Further clinical validation is recommended.

Keywords

Malaria, *Plasmodium berghei,* Chloroquine, Combination Therapy, Oxidative Stress, Hematology, Liver Function

1. Introduction

In 2023, an estimated 263 million malaria cases were recorded across 83 countries, representing an increase from 252 million cases in 2022 [1]. Death rate remained stable at about 597,000, but children under five (5) accounted for 76% of deaths, with over 1,200 daily mostly in Africa [1]. The WHO African Region bore the heaviest burden, contributing 94% of cases and 95% of deaths globally. Furthermore, just over half of all malaria deaths occurred in four countries: Nigeria, the Democratic Republic of Congo, Niger, and the United Republic of Tanzania [2]. The global malaria control strategy depends on prompt diagnosis and effective chemotherapy, yet the widespread emergence of drug-resistance in *Plasmodium* parasites has significantly undermined the efficacy of available antimalarials. The resistance of the parasite to antimalarials is widely distributed globally, with different regions showing resistance to almost all antimalarials. For instance, partial artemisinin resistance has emerged independently in Southeast Asia, South America, and East Africa, including Rwanda, Uganda, Tanzania, and Ethiopia [3], with this resistance driven by mutations in the *PfKelch*13 gene [4]. These resistance have been associated with mutations in the parasite, which allows it to evade the activities of the drugs, thus developing the ability to adapt to its complex life cycle and the diverse environments it inhabits, ranging from the midgut and salivary glands of the mosquito to the human liver cells and red blood cells [4]. This increasing prevalence of drug-resistant malaria has necessitated the exploration of alternatives to enhance treatment efficacy and mitigate resistance development.

Combination therapy has proven to be a major strategy in modern malaria management, with artemisinin-based combination therapies (ACTs) currently endorsed as first-line treatment [5]. ACTs pair a fast-acting artemisinin derivative with a longer-acting partner drug, designed clear blood parasite and to ensure high efficacy with reduce the risk of resistance development [5]. However, partial resistance to artemisinins, characterized by delayed parasite clearance, has emerged in Southeast Asia and now threatens Africa [6]. Resistance to these drugs is generally less common worldwide, but multi-drug resistant malaria can have a significant impact in certain regions [3]. This alarming trend underscores the urgent need for innovative strategies to preserve existing antimalarials and develop new therapeu-

tic regimens.

Chloroquine was once a cornerstone of malaria treatment due to its potency and ease of use, but the rapid spread of *P. falciparum* resistance, driven mainly by *Pfcrt K*76*T* and *Pfmdr*1 *N*86*Y* mutations, led to a sharp decline in efficacy. By the late 1990s, resistance was widespread, particularly in sub-Saharan Africa, where it contributed to a significant rise in malaria mortality. Consequently, chloroquine was removed from treatment protocols and replaced with artemisinin-based combination therapies (ACTs), which remain the standard first-line treatment [7]. However, following its global withdrawal due to widespread *P. falciparum* resistance, chloroquine has begun to regain sensitivity in several African countries where selective pressure was lifted. In Malawi for instance, cessation of chloroquine in 1993 led to a dramatic resurgence of parasite sensitivity: prevalence of the chloroquine-resistant *Pfcrt*-76*T* mutation dropped from approximately 85% in 1992 to just 13% by 2000, and by 2001, clinical efficacy had returned to 100% in treated individuals [8] [9]. Meanwhile, regions such as Central America west of Panama Canal, Haiti, and the Dominican Republic did not experience any form of chloroquine resistance [3]. Recent studies show a re-emergence of chloroquine-sensitive *P. falciparum* in several districts in the Central region of Ghana, with over 77% of samples showing the presence of chloroquine-sensitive markers [10] [11]. In countries such as Sudan, Nigeria, and Senegal, chloroquine sensitivity has returned after years of discontinued use [12]. These have prompted discussions about reintroducing chloroquine, either alone or in combination therapies, especially as ACT resistance rises, with ongoing studies evaluating their use in combination therapies.

Reversing resistance using drug combinations is one potential strategy that is explored widely. Previous in vitro studies have shown that certain compounds can reverse CQ resistance by inhibiting efflux transporters like P-glycoprotein in the parasite [13]. We propose that combining CQ with established antimalarials, particularly ACTs, could leverage similar synergistic or additive effects to restore CQ efficacy and address multidrug resistance. Combining CQ with other antimalarial agents such as artemether-lumefantrine (AL), sulphadoxine-pyrimethamine (SP), dihydroartemisinin-piperaquine (DHAP), and amodiaquine (AA) provides a rational approach, as these drugs possess distinct mechanisms of action. Such combinations may enhance parasite clearance, modulate oxidative stress, and protect against organ damage that often accompanies severe infection. The impact of these combinations on the pathophysiology of the host state, including oxidative stress, liver function, and hematological recovery, is critical for evaluating their overall therapeutic value and safety [14] [15].

Artemether-lumefantrine (AL) combines a rapidly acting artemisinin derivative with a longer-acting partner drug; artemether induces rapid parasite clearance through heme-activated free radical formation, while lumefantrine inhibits heme detoxification within the parasite food vacuole. Sulphadoxine-pyrimethamine (SP) exerts its antimalarial activity through sequential inhibition of folate biosynthesis,

with pyrimethamine targeting dihydrofolate reductase and sulphadoxine inhibiting dihydropteroate synthase, thereby impairing parasite DNA synthesis. Dihydroartemisinin-piperaquine (DHAP) similarly combines the fast parasiticidal action of dihydroartemisinin with piperaquine, a bisquinoline that interferes with heme polymerization and provides prolonged antimalarial activity. Amodiaquine (AA), a 4-aminoquinoline structurally related to chloroquine, disrupts heme detoxification within the parasite food vacuole. These distinct but complementary mechanisms provide a pharmacological rationale for combining these agents with chloroquine to enhance therapeutic efficacy and potentially limit resistance development [16].

This study was therefore designed to evaluate the efficacy of CQ alone and in combination with other antimalarials (AL, SP, DHAP, and AA) in *P. berghei*-infected mice. Parasitemia levels were monitored over the course of infection, while biochemical, liver function, and hematological indices were assessed to determine the broader physiological impact of the treatments. By examining both parasite suppression and host responses, this study seeks to provide insight into the therapeutic potential of CQ-based combinations as alternative strategies in malaria chemotherapy.

2. Materials and Methods

2.1. Experimental Animals

Healthy wistar albino mice of either sex, weighing 19 - 25 g, were used for this study. The animals were housed under standard laboratory conditions (temperature 22˚C ± 2˚C, relative humidity 50% - 60%, and a 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum. All experimental procedures were carried out in accordance with institutional ethical guidelines for animal care and use, and approval was obtained from the Animal Research Ethics Committee, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos.

2.2. Parasite Inoculation

A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) obtained from Institute for Advance Medical Research and Training (IAMRAT), University of Ibadan, Oyo State was used for infection. Donor mice with a parasitemia level of 20% - 30% were sacrificed, and blood containing the parasitized erythrocytes was collected by cardiac puncture into heparinized syringes. Each experimental mouse was inoculated intraperitoneally with 1×10^7 infected erythrocytes suspended in 0.2 mL of phosphate-buffered saline (PBS).

2.3. Experimental Design and Treatment Groups

Following infection, the mice were randomly divided into ten groups (n = 5 per group) in Table 1.

Table 1. Treatment groups.

Treatment	Group
Negative control (NC—infected, untreated)	1
Normal group (PC—uninfected, untreated)	2
Chloroquine (CQ)	3
Artemether-lumefantrine (AL)	4
Sulphadoxine-pyrimethamine (SP)	5
Dihydroartemisinin-piperaquine (DHAP)	6
Artesunate-Amodiaquine (AA)	7
CQ + AL	8
CQ + SP	9
CQ + DHAP	10
CQ + AA	11

All drugs were freshly prepared in normal saline and administered orally once daily for four consecutive days (Day 0 to Day 3 post-infection) at the following doses: 10 mg/kg for chloroquine (CQ), 10 mg/kg artemether + 60 mg/kg lumefantrine (ratio 1:6) daily, SP was given as sulfadoxine 25 mg/kg + pyrimethamine 1.25 mg/kg as a single curative dose, DHAP was dosed as dihydroartemisinin 4 mg/kg + piperaquine 18 mg/kg once daily for three days, and Amodiaquine (AA) was given at 10 mg/kg daily for three days, following established standard protocols.

2.4. Monitoring of Parasitemia

Parasitemia was monitored daily by preparing thin blood smears from the tail vein of each mouse. Smears were fixed in methanol, stained with 10% Giemsa, and examined microscopically under oil immersion at 100 × magnification. Parasitemia (%) was calculated as:

$$\%\ \text{Parasitemia} = \frac{\text{Number of infected RBCs}}{\text{Total Number of RBCs counted}} \times 100$$

The mean parasitemia levels of each group were recorded from Day 0 to Day 4 post-treatment.

2.5. Biochemical Assays

2.5.1. Oxidative Stress Markers

At the end of the experiment (Day 4), mice were euthanized, and blood samples were collected via cardiac puncture into heparinized tubes. Plasma was separated by centrifugation at 3000 rpm for 10 min and analyzed for markers of oxidative stress. Lipid peroxidation was estimated by measuring malondialdehyde (MDA) concentration using the thiobarbituric acid reactive substances (TBARS) method, as described by [17] while nitric oxide (NO) levels were quantified using the Griess

reagent method.

2.5.2. Liver Function Tests

Serum was analyzed for liver function markers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), and total bilirubin (TBiL). All assays were performed using COBAS CIII clinical chemistry analyzer and commercial diagnostic kits were according to the instructions of the manufacturers.

2.5.3. Hematological Analysis

Whole blood samples were analyzed using an automated hematology analyzer to determine white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), platelet count (PLT), and differential leukocyte counts (lymphocytes, monocytes, and neutrophils).

2.6. Statistical Analysis

All values were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. P-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Parasitemia Suppression

Parasitemia levels were monitored over five days across various treatment groups, as observed in Figure 1. The negative control group showed a progressive increase in parasitemia from 1.1% on day 0 to 3.12% on day 4. In contrast, all treatment groups exhibited varying degrees of parasitemia suppression. CQ (Chloroquine) showed moderate suppression, peaking at 1.66% on day 2 and dropping to 0.7% by day 4. AL (Artemether-Lumefantrine) and SP (Sulfadoxine-Pyrimethamine) demonstrated better control, with AL reducing parasitemia to 0.54% and SP to

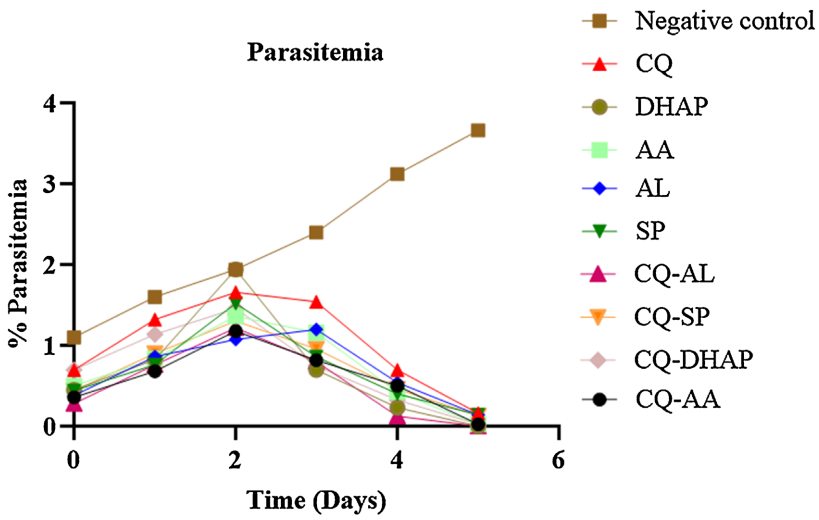

Figure 1. Parasitemia.

0.4% by day 4. DHAP (Dihydroartemisinin-Piperaquine) was the most effective monotherapy, reducing parasitemia to 0.23% by day 4. AA (Amodiaquine-Artesunate) also showed significant suppression (0.425% on day 4). Combination therapies, particularly CQ-AL and CQ-DHAP, were highly effective, with CQ-AL reducing parasitemia to 0.125% and CQ-DHAP to 0.325% by day 4.

3.2. Oxidative Stress Parameters

Lipid peroxidation, measured as malondialdehyde (MDA) concentration, varied across treatment groups, reflecting differential oxidative stress responses. The negative control group (0.126 ± 0.003 mMol/mL) served as the baseline for comparison. The untreated- uninfected group (0.116 ± 0.004 mMol/mL) showed a slight but significant reduction in MDA levels relative to the negative control (p = 0.028), likely reflecting oxidative imbalance associated with infection. Notably, several treatment groups demonstrated lipid peroxidation levels lower than the negative control, suggesting an enhanced protective or antioxidant effect. Among monotherapies, amodiaquine (AA) recorded a significant reduction (0.101 ± 0.02 mMol/mL) with p = 0.022, while chloroquine (0.126 ± 0.017 mMol/mL) and sulphadoxine-pyrimethamine (0.133 ± 0.006 mMol/mL) maintained values comparable to control (p > 0.05). The most pronounced effects of lipid peroxidation were observed in combination regimens. CQ + AL produced the lowest MDA concentration (0.097 ± 0.011 mMol/mL), indicating a strong suppression of lipid peroxidation relative to both negative (p = 0.007) and positive (p = 0.034) controls. CQ + DHAP also reduced MDA (0.114 ± 0.013 mMol/mL), suggesting antioxidant synergy between the partner drugs. Collectively, these findings indicate that combinations involving chloroquine, particularly CQ + AL and CQ + DHAP, exert higher protection against oxidative stress compared to either drug administered alone. This antioxidant effect may contribute to the improved therapeutic profile observed in combination regimens.

Data presented in Table 2 shows that: Nitric oxide (NO) levels in the negative control (infected, untreated) group were significantly higher than those of the normal control, reflecting infection-induced upregulation of host nitric oxide production as part of the innate immune response to *Plasmodium berghei*. This elevation is consistent with macrophage activation and inflammatory signaling commonly associated with malaria infection. Among the monotherapy groups, artemether-lumefantrine (AL) treatment resulted in a significant reduction in NO levels compared to the negative control, suggesting a suppression of excessive inflammatory responses following parasite clearance. In contrast, SP and DHAP monotherapies produced NO levels comparable to the negative control, indicating persistence of infection-related inflammatory stimulation. Although AL and DHAP significantly reduced parasitemia, both treatments were associated with increased lipid peroxidation, suggesting residual oxidative stress. In the combination therapy groups, particularly CQ + AL, NO levels were maintained at values comparable to the negative control, indicating a balanced immune modulation in which

parasite suppression occurred without excessive attenuation or overactivation of nitric oxide-mediated host defenses.

Table 2. Biochemical markers of oxidative stress.

Treatent groups	Lipid peroxidation (mMol/mL)	Nitric oxide (mMol/mL)
NC	0.126 ± 0.003	45.383 ± 0.41
PC	0.116 ± 0.004[a]	40.227 ± 3.03[a]
CQ	0.126 ± 0.017	45.501 ± 8.44
AL	0.197 ± 0.02[a,b]	38.470 ± 5.12[a]
SP	0.133 ± 0.006[b]	44.094 ± 1.73
DHAP	0.190 ± 0.02[a,b]	45.032 ± 0.54
AA	0.101 ± 0.02[a]	42.923 ± 2.93
CQ + AL	0.097 ± 0.011[a,b,d]	45.383 ± 0.20[b,d]
CQ + SP	0.126 ± 0.017	45.266 ± 0.35[b]
CQ + DHAP	0.114 ± 0.013[f]	44.563 ± 0.35[b]
CQ + AA	0.160 ± 0.006[a,b,g]	44.798 ± 0.20[b]

3.3. Liver Function Tests (LFTs)

Table 3 shows LFTs indicating that infected untreated mice exhibited elevated ALT (18.67 ± 6.11 U/L), AST (24.00 ± 5.20 U/L), and TBil (134.67 ± 14.29 μmol/L), indicating hepatic injury. Treatment with CQ produced a significant increase in ALT (20.67 ± 4.16 U/L; $p = 0.04$) and albumin (28.00 ± 1.73 g/dL; $p = 0.05$) compared to the normal group while ALP (114.67 ± 22.81 U/L; $p = 0.001$) was significantly reduced relative to normal group. The AL group showed significant reduction in ALT (9.33 ± 5.13 U/L; $p = 0.05$) compared with negative

Table 3. Liver function tests.

	ALT	AST	ALP	TP	ALB	TBiL
NC	18.67 ± 6.11	24.00 ± 5.20	150.67 ± 34.53	53.33 ± 3.21	24.00 ± 1.00	134.67 ± 14.29
PC	14.33 ± 2.31	18.33 ± 0.58	212.33 ± 8.08[a]	52.00 ± 2.65	24.67 ± 2.08	128.67 ± 19.66
CQ	20.67 ± 4.16[b]	19.67 ± 5.13	114.67 ± 22.81[b]	55.33 ± 1.15[b]	28.00 ± 1.73[a,b]	111.67 ± 1.53[a]
AL	9.33 ± 5.13[a,b]	17.67 ± 8.62	152.33 ± 35.36	55.50 ± 6.36	24.00 ± 1.73	116.00 ± 26.85
SP	20.00 ± 4.36[b]	21.33 ± 4.04	131.67 ± 26.65	56.33 ± 0.58[b]	27.33 ± 1.15	108.00 ± 1.41
DHAP	18.33 ± 2.08	33.50 ± 8.74[a,b]	91.33 ± 0.58[a,b]	54.75 ± 4.92	24.00 ± 1.00	119.00 ± 38.12
AA	23.33 ± 4.04[a,b]	27.00 ± 4.76[a]	117.33 ± 19.55[a]	47.33 ± 2.08[ab]	22.67 ± 1.53	134.33 ± 7.23
CQ-AL	12.00 ± 6.08[a,b,c]	16.67 ± 7.21	100.67 ± 21.03[a,b]	56.33 ± 4.04	32.00 ± 3.46[a,b,d]	139.33 ± 26.10
CQ-SP	49.33 ± 4.16[a,b,c,e]	89.00 ± 10.58[a,b,c,e]	116.33 ± 27.30[b,]	58.33 ± 0.58[b,c,e]	26.33 ± 1.53[a]	166.33 ± 19.43[a,b,c,e]
CQ-DHAP	17.33 ± 8.08	26.00 ± 7.00	119.33 ± 37.75[b]	51.00 ± 3.46[c]	26.00 ± 4.00	166.50 ± 13.44[c]
CQ-AA	18.00 ± 7.00	16.33 ± 0.58[a,b,g]	185.67 ± 41.14[c,g]	57.33 ± 1.15[b,c,g]	27.33 ± 0.58[a,b,g]	191.00 ± 12.12[a,b,c,g]

control. The SP group demonstrated significantly elevated ALT (20.00 ± 4.36 U/L; $p = 0.05$) and TP (56.33 ± 0.58 g/dL; $p = 0.025$) compared with the normal group. The AA group showed significant increases in ALT (23.33 ± 4.04 U/L) compared with the normal group ($p = 0.001$) and AST (27.00 ± 4.76 U/L; $p = 0.02$) with significant reduction in ALP (117.33 ± 19.55 U/L; $p = 0.007$), TP (47.33 ± 2.08 g/dL; $p = 0.03$). Combination therapy of CQ-AL resulted in a significant decrease in ALT (12.00 ± 6.08 U/L; $p < 0.05$) with significant increase in ALB (32.00 ± 3.46 g/dL; $p = 0.009$), compared with negative control. The CQ-SP combination produced the most pronounced changes, with significant elevations in ALT (49.33 ± 4.16 U/L; $p = 0.001$) compared with negative control and normal group ($p = 0.0001$), AST (89.00 ± 10.58 U/L) increased significantly compared with negative control ($p = 0.003$) and normal group ($p = 0.0001$), significant increase in TP (58.33 ± 0.58 g/dL; $p = 0.03$ with negative control; $p = 0.008$ with normal group), and TBiL (166.33 ± 19.43 μmol/L) $p = 0.04$ compared to NC and $p = 0.003$ compared with normal group. The CQ-DHAP group exhibited significantly elevated ALP (166.50 ± 13.44 μmol/L; $p = 0.007$) compared to the normal group. The CQ-AA group showed significant increases in ALP (185.67 ± 41.14 U/L; $p = 0.05$), TP (57.33 ± 1.15 g/dL; $p = 0.01$, compared with normal group), ALB (27.33 ± 0.58 g/dL; $p = 0.05$), and TBiL (191.00 ± 12.12 μmol/L; $p = 0.005$) relative to normal group.

Generally, normal group had normal liver enzyme levels, while NC showed mild elevation. CQ-SP and CQ-AA groups exhibited significant increases in ALT, AST, and TBiL, indicating hepatic stress. AL, CQ-AL, and DHAP groups maintained near-normal liver profiles, suggesting hepatoprotective effects. CQ-AL showed the highest Albumin and Total Protein, indicating improved liver synthetic function.

3.4. Hematological Parameters

The negative control (NC) group, representing infected and untreated subjects, showed reduced haematological indices, including RBC (7.30 ± 1.15 × 10^{12}/L), Hb (14.70 ± 2.31 g/dL), and PCV (34.07% ± 6.05%), indicating malaria-induced anemia. Platelet count was 1168.00 ± 53.74 × 10^9/L, and WBC was 4.40 ± 1.40 × 10^9/L. The normal group had significantly higher PCV (47.10 ± 0.52%; $p = 0.01$), PLT (1399.67 ± 51.54 × 10^9/L; $p = 0.002$), LYM (57.67% ± 5.46%; $p = 0.008$), and lower MON (4.67% ± 0.12%, $p = 0.013$) and NEUT (32.10% ± 3.65%; $p = 0.014$) compared to NC, indicating normal haematological status. CQ treatment significantly improved Hb (18.20 ± 1.55 g/dL, $p = 0.05$ compared with negative control), PCV (58.27% ± 1.90%; $p = 0.001$; $p = 0.01$ respectively), and PLT (1623.33 ± 158.99 × 10^9/L, $p = 0.004$; $p = 0.002$ respectively) compared to NC and normal control. It also increased NEUT (48.80 ± 2.31%; $p = 0.001$) and MON (7.27% ± 0.99%, $p = 0.004$), compared to the normal control, suggesting immune activation. Artemether-Lumefantrine (AL) showed significantly higher PCV (47.90% ± 7.59%, $p = 0.035$), PLT (1381.67 ± 178.23 × 10^9/L, $p = 0.05$), and LYM (52.40% ± 8.86%, $p =$

0.04) compared to NC. MON was significantly lower (3.67% ± 1.0%, $p = 0.009$), indicating reduced inflammation. Sulfadoxine-Pyrimethamine (SP) significantly increased WBC (8.27 ± 0.65 × 10^9/L, $p = 0.006$ compared with negative control; $p = 0.0001$ compared with the normal group) and LYM (63.70% ± 4.77%, $p = 0.003$), while reducing NEUT (28.70% ± 3.64%, $p = 0.008$) and PLT (1067.33 ± 52.20 × 10^9/L, $p = 0.05$ compared to negative control; $p = 0.001$ compared to normal group) compared to PC. DHAP treatment resulted in significantly lower RBC (7.23 ± 0.67 × 10^{12}/L, $p = 0.042$ compared to normal group) and MON (5.20% ± 0.69%, $p = 0.027$ compared to negative control), with reduced NEUT (29.40% ± 3.08%; $p = 0.008$ compared to negative control), suggesting mild suppression of haematological recovery. AA significantly elevated RBC (18.17 ± 0.32 × 10^{12}/L, $p = 0.0001$ compared to NC; $p = 0.001$ compared to normal group), Hb (18.40 ± 0.10 g/dL, $p = 0.025$ compared to negative control; $p = 0.0001$ compared to normal group), PCV (56.10 ± 0.89%, $p = 0.002$ compared to negative control; $p = 0.0001$ compared to normal group), and PLT (1369.67 ± 46.88 × 10^9/L, $p = 0.002$ compared to negative control) compared to NC and normal group, indicating strong recovery. LYM was also significantly higher (46.77% ± 1.25%, $p = 0.041$ compared to negative control; $p = 0.014$ compared to normal group).

Among Combination Therapies

CQ-AL significantly improved WBC (7.50+1.32 × 10^9/L; $p = 0.025$ compared to the negative control), Hb (17.60 ± 0.62 g/dL; $p = 0.006$ compared to AL; $p = 0.052$ compared to negative control), LYM (58.30% ± 7.17%; $p = 0.011$ compared with the negative control), MON (6.73% ± 1.89%; $p = 0.004$ compared with CQ; $p = 0.03$ compared to AL), PLT (1432.67 ± 192.62 × 10^9/L; $p = 0.035$ compared to negative control) and PCV (46.17% ± 3.33%; $p = 0.019$ compared to negative control). CQ-SP showed significantly higher RBC (11.23 ± 2.54 × 10^{12}/L, $p = 0.036$ compared to negative control; $p = 0.05$ compared to normal group), PCV (55.83% ± 6.52%, $p = 0.007$ compared to negative control), and NEUT (50.80% ± 6.54%; $p = 0.003$ compared to SP). CQ-DHAP had the highest WBC (13.47 ± 4.35 × 10^9/L; $p = 0.013$ compared to negative control; $p = 0.011$ compared to positive control; $p = 0.02$ compared to chloroquine; $p = 0.03$ compared to DHAP), but significantly lower RBC (6.53 ± 0.42 × 10^{12}/L; $p = 0.002$ compared to the normal control group), Hb (14.07 ± 1.59 g/dL, $p = 0.02$ compared to both normal group and CQ), and MON (3.87% ± 0.31%, $p = 0.006$ compared to negative control; $p = 0.007$ compared to normal group; $p = 0.002$ compared to CQ; $p = 0.02$ compared to DHAP). CQ-AA showed the highest Hb (19.43 ± 1.29 g/dL, $p = 0.018$ compared to negative control; $p = 0.008$ compared to normal group), LYM (62.53% ± 5.80%, $p = 0.04$ compared to negative control; $p = 0.005$ compared to CQ; $p = 0.005$ compared to AA), and significantly lower MON (3.33% ± 0.81%, $p = 0.006$ compared to negative control, $p = 0.024$ compare to positive control; $p = 0.003$ compared to CQ), indicating ameliorative effect on haematological parameters as indicated in **Table 4**.

CQ-AL and CQ-DHAP are the most effective combinations for parasitemia

Table 4. Haematology tests.

	WBC	RBC	Hb	PCV	PLT	LYM	MON	NEUT
NC	4.40 ± 1.40	7.30 ± 1.15	14.70 ± 2.31	34.07 ± 6.05	1168.00 ± 53.74	36.97 ± 7.22	7.93 ± 1.62	49.70 ± 8.27
PC	4.30 ± 0.17	8.13 ± 0.15	16.37 ± 0.38	47.10 ± 0.52^{a}	1399.67 ± 51.54^{a}	57.67 ± 5.46^{a}	4.67 ± 0.12^{a}	32.10 ± 3.65^{a}
CQ	5.20 ± 0.95	8.20 ± 1.81	18.20 ± 1.55^{b}	58.27 ± 1.90^{a,b}	1623.33 ± 158.99^{a,b}	43.80 ± 3.86^{b}	7.27 ± 0.99^{b}	48.80 ± 2.31^{b}
AL	6.87 ± 3.40	10.17 ± 4.31	14.93 ± 0.86^{a}	47.90 ± 7.59^{a}	1381.67 ± 178.23^{a}	52.40 ± 8.86^{a}	3.67 ± 1.0^{a}	52.53 ± 13.01^{b}
SP	8.27 ± 0.65^{a,b}	13.87 ± 5.28	15.30 ± 1.82	52.93 ± 6.29^{a}	1067.33 ± 52.20^{b}	63.70 ± 4.77^{a}	6.07 ± 2.04	28.70 ± 3.64^{a}
DHAP	6.27 ± 2.10	7.23 ± 0.67^{b}	16.00 ± 0.87	46.80 ± 1.73^{a}	1310.67 ± 152.22	64.87 ± 3.65^{a}	5.20 ± 0.69^{a}	29.40 ± 3.08^{a}
AA	9.27 ± 0.15^{a,b}	18.17 ± 0.32^{a,b}	18.40 ± 0.10^{a,b}	56.10 ± 0.89^{a,b}	1369.67 ± 46.88^{a}	46.77 ± 1.25^{a,b}	4.20 ± 0.40^{a}	46.83 ± 2.83^{b}
CQ-AL	7.50 ± 1.32^{a,b,c}	8.20 ± 0.52	17.60 ± 0.62^{b,d}	46.17 ± 3.33^{a,c}	1432.67 ± 192.62^{a}	58.30 ± 7.17^{a,c}	6.73 ± 1.89^{d}	34.70 ± 9.18^{c}
CQ-SP	5.23 ± 1.59^{e}	11.23 ± 2.54^{a,b}	18.00 ± 2.60	55.83 ± 6.52^{a,b}	1364.33 ± 459.71	45.67 ± 6.13^{b,e}	3.67 ± 0.81^{a,b,c}	50.80 ± 6.54^{b,e}
CQ-DHAP	13.47 ± 4.35^{a,b,c,f}	6.53 ± 0.42^{b}	14.07 ± 1.59^{b,c}	39.17 ± 4.08^{b,c,f}	1342.00 ± 159.51^{c}	57.87 ± 4.01^{a,c,f}	3.87 ± 0.31^{a,b,c,f}	34.30 ± 5.19^{a,c}
CQ-AA	10.93 ± 2.22^{a,b,c}	8.90 ± 0.53^{a,b,g}	19.43 ± 1.29^{a,b}	55.47 ± 2.97^{a,b}	1305.33 ± 14.01^{a,b,c,g}	62.53 ± 5.80^{a,c,g}	3.33 ± 0.81^{a,b,c}	34.13 ± 5.00^{a,c,g}

suppression and systemic recovery. AL and DHAP monotherapies are effective but may induce oxidative stress. CQ-SP and CQ-AA combinations, while reducing parasitemia, may pose risks of hepatic and haematological toxicity. CQ-AL offers the best balance of efficacy and safety, improving liver and haematological parameters while effectively controlling parasitemia.

4. Discussion

The present study evaluated the therapeutic efficacy and systemic impact of chloroquine (CQ) alone and in combination with various antimalarial agents namely, artemether-lumefantrine(AL), sulphadoxine-pyrimethamine (SP), dihydroartemisinin-piperaquine (DHAP), and amodiaquine (AA), against *Plasmodium berghei* infection in Wistar albino mice. The findings provide compelling evidence that CQ-based combination therapies, particularly CQ-AL and CQ-DHAP, could offer enhanced antimalarial efficacy and systemic protection compared to monotherapies.

4.1. Parasitemia Suppression and Synergistic Effects

CQ monotherapy demonstrated moderate parasite suppression, consistent with

its partial resurgence in efficacy following decades of discontinued use [9] [10]. However, its combination with AL and DHAP significantly enhanced parasitemia clearance, with CQ-AL achieving the lowest parasitemia levels by Day 4. This synergistic effect likely stems from the complementary pharmacodynamics of the partner drugs—the inhibition of heme detoxification by CQ and rapid parasite clearance of AL and DHAP prolonged half-life [6]. Chloroquine exerts its antimalarial effect by inhibiting the detoxification of free heme released during hemoglobin digestion in the parasite's digestive vacuole. Normally, *Plasmodium* converts toxic heme into inert hemozoin crystals using proteins like PfHRP-2. Chloroquine disrupts this process by binding to free heme with high affinity, forming a toxic complex that damages the parasite [18] [19]. On the other hand, artemisinin derivatives such as artemether and dihydroartemisinin act rapidly on the early ring stages of the parasite, causing swift parasite clearance. Their partner drugs, lumefantrine and piperaquine, have long half-lives, which help eliminate residual parasites and prevent recrudescence [20]. When combined, the heme-targeting mechanism of chloroquine complements the multi-stage action of ACTs. This dual approach not only enhances efficacy but also reduces the likelihood of resistance development, as parasites are exposed to multiple lethal mechanisms over an extended period [20]. These findings supports reports suggesting that combining fast-acting and long-acting agents can delay resistance development and improve treatment outcomes [21].

4.2. Oxidative Stress and Antioxidant Protection

Oxidative stress, a hallmark of malaria pathophysiology, was assessed via lipid peroxidation (MDA) and nitric oxide (NO) levels.

Nitric oxide (NO) plays a dual role in malaria, contributing to parasite killing while also participating in inflammatory tissue damage when excessively produced. In the present study, elevated NO levels in the infected untreated group reflect infection-induced activation of host immune responses, particularly macrophage-derived inducible nitric oxide synthase activity. The reduction in NO observed following artemether-lumefantrine (AL) monotherapy suggests attenuation of inflammation secondary to parasite clearance, consistent with the known immunomodulatory effects of artemisinin derivatives. In contrast, sulphadoxine-pyrimethamine (SP) and dihydroartemisinin-piperaquine (DHAP) monotherapies maintained NO levels comparable to the infected control, indicating sustained inflammatory signaling despite treatment. Importantly, CQ-based combination therapies preserved NO levels within a physiological range, suggesting balanced immune modulation rather than excessive suppression or overstimulation of nitric oxide-mediated host defenses [22] [23].

A notable finding of this study is the paradoxical increase in lipid peroxidation observed with AL and DHAP monotherapies despite effective parasite suppression. Artemisinin derivatives exert their antimalarial activity through the generation of reactive oxygen species following heme-mediated activation, a mechanism

that, while parasiticidal, may also promote oxidative damage to host biomolecules. Similarly, piperaquine and lumefantrine have been associated with oxidative stress under certain experimental conditions. The elevated lipid peroxidation observed in these groups therefore likely reflects drug-induced oxidative burden rather than uncontrolled parasitemia.

In contrast, combination of these agents with chloroquine resulted in a marked reduction in lipid peroxidation, indicating a net antioxidant effect. This apparent reversal may be attributed to the ability of chloroquine to stabilize the parasite food vacuole and limit excessive heme-driven free radical generation, thereby indirectly reducing oxidative spillover to host tissues. Additionally, chloroquine has been reported to exert immunomodulatory and anti-inflammatory effects, including attenuation of pro-oxidant cytokine signaling and lysosomal stabilization, which may further contribute to reduced lipid peroxidation. These findings suggest that chloroquine does not merely act as an additional parasiticidal agent but may also mitigate oxidative stress induced by artemisinin-based partner drugs [14]. Overall, the observed biochemical outcomes indicate that chloroquine-based combinations achieve effective parasite clearance while simultaneously limiting oxidative tissue damage and maintaining regulated nitric oxide production. This dual benefit supports the potential re-evaluation of chloroquine as a rational partner drug in combination therapies, particularly in settings where chloroquine sensitivity has re-emerged.

4.3. Liver Function and Hepatotoxicity

Liver function tests revealed that untreated infection induced hepatic injury, evidenced by elevated ALT, AST, and total bilirubin. While CQ-SP and CQ-AA combinations exacerbated liver enzyme elevations—suggesting potential hepatotoxicity, CQ-AL and DHAP monotherapy maintained near-normal liver profiles. CQ-AL, in particular, improved albumin and total protein levels, indicating preserved hepatic activity. The elevated bilirubin observed in CQ + AA highlights the need for caution, as some combinations may exacerbate hemolysis or hepatic burden. These findings underscore the importance of evaluating not only parasitemia suppression but also organ-specific toxicity when assessing antimalarial regimens [15]. It is necessary for antimalarial drug assessment to go beyond measuring parasitemia suppression to include organ-specific toxicity, which affects treatment safety and long-term outcomes.

4.4. Hematological Recovery and Immune Modulation

Malaria-induced anemia and leukocyte alterations were evident in the negative control group. Treatment with CQ-AL and CQ-AA significantly improved hematological indices, including hemoglobin, packed cell volume, and platelet counts. CQ-AL also restored lymphocyte and monocyte levels, suggesting immune modulation and recovery. According to [24], a comparative observational study on patients with *Plasmodium vivax* malaria found that chloroquine treatment was as-

sociated with faster recovery of lymphocyte counts, although the difference was not statistically significant and Artemether-lumefantrine led to a more rapid recovery of platelet counts and total white blood cell counts, indicating a broader hematological benefit. These findings suggest that while AL may be more effective in general hematological recovery, chloroquine may have a specific immunomodulatory effect, particularly on lymphocytes, which are crucial for adaptive immune responses. Together, the CQ-AL combination may offer a synergistic benefit as chloroquine modulates immune responses and protects immune cells, while AL ensures rapid parasite clearance and supports overall hematological recovery. While the combination of chloroquine and dihydroartemisinin-piperaquine (CQ + DHAP) demonstrated superior parasite clearance and elicited a robust leukocytic response, our findings reveal a paradoxical suppression of key hematological indices, notably reduced red blood cell (RBC) count and hemoglobin (Hb) levels. This hematological suppression, despite effective parasitemia control, is a critical observation with potential clinical significance.

Malaria-induced anemia results primarily from hemolysis of infected and uninfected erythrocytes, bone marrow suppression, and immune-mediated destruction. The observed decline in RBC and Hb in the CQ + DHAP group may reflect drug-related hematotoxic effects or an exacerbation of bone marrow suppression beyond that caused by infection alone. Artemisinin derivatives, including piperaquine, have been associated in some studies with transient bone marrow suppression or delayed reticulocyte recovery, potentially impairing erythropoiesis. Furthermore, piperaquine's long half-life and accumulation may contribute to hematological toxicity in susceptible hosts.

Chloroquine is generally considered hematologically safe at therapeutic doses and may even mitigate malaria-associated anemia by reducing parasitic burden. However, when combined with DHAP, drug-drug interactions or additive toxicities could underlie the hematological deficits observed. This raises important safety considerations, especially for populations with preexisting anemia or other hematological vulnerabilities.

Clinically, these findings underscore the necessity for careful hematological monitoring during CQ + DHAP therapy. While this combination is efficacious in parasite suppression, the risk of exacerbating anemia may limit its utility or warrant adjunctive interventions, such as supportive care or dose adjustments. Future investigations should focus on mechanistic studies to delineate whether the hematological suppression is due to direct drug toxicity, immune modulation, or altered marrow response. Pharmacokinetic profiling and longer-term follow-up studies would further clarify the risk-benefit profile of this combination.

In summary, while the CQ + DHAP combination offers potent antimalarial efficacy, its association with hematological suppression warrants cautious consideration, emphasizing the need for routine monitoring of hematological parameters and further research to optimize safe dosing regimens in malaria treatment [25]-[27].

4.5. Therapeutic Implications and Future Directions

The resurgence of CQ sensitivity in parts of Africa, coupled with rising ACT resistance, necessitates innovative therapeutic strategies [11] [12]. This study supports the potential reintroduction of CQ in combination regimens, particularly with AL and DHAP, to enhance efficacy and mitigate resistance. However, combinations such as CQ-SP and CQ-AA warrant caution due to their hepatotoxic and hematological side effects. These findings show the therapeutic promise of CQ-based combinations in malaria management. The high parasite suppression, antioxidant benefits, hepatoprotection, and hematological recovery observed with CQ + AL, CQ + DHAP, and CQ + AA suggest that such regimens could be valuable alternatives or adjuncts to ACTs, particularly in regions where chloroquine sensitivity has re-emerged. Importantly, the reintroduction of chloroquine must be approached cautiously, with surveillance for resistance markers such as *pfcrt K*76*T* and *pfmdr*1 *N*86*Y*, to prevent rapid re-selection of resistant strains.

Future studies should explore the molecular mechanisms underlying the observed synergistic effects, including transporter inhibition, immune modulation, and redox balance [13]. Additionally, clinical trials in human populations are essential to validate these findings and assess pharmacokinetics, safety, and long-term efficacy.

5. Conclusions

This study demonstrates that combining chloroquine with other standard antimalarial drugs significantly enhances therapeutic efficacy against *Plasmodium berghei* infection in mice. While chloroquine monotherapy produced only moderate parasite suppression, its combinations—particularly with artemether-lumefantrine (CQ + AL), dihydroartemisinin-piperaquine (CQ + DHAP), and amodiaquine (CQ + AA)—achieved superior parasitemia clearance. Beyond antiparasitic activity, these regimens improved host biochemical and hematological parameters by reducing lipid peroxidation, restoring nitric oxide balance, normalizing liver function markers, and reversing malaria-induced anemia and thrombocytopenia. The hepatoprotective and antioxidant effects observed underscore the therapeutic advantage of combination therapy in mitigating malaria-associated systemic pathology.

Given the documented re-emergence of chloroquine sensitivity in several endemic regions and the rising concerns of artemisinin-based combination therapy (ACT) resistance, these findings provide experimental evidence supporting the reconsideration of chloroquine-based combinations as viable alternatives or adjuncts in malaria treatment protocols. Specifically, CQ + AL and CQ + AA emerge as the most promising candidates, offering effective parasite clearance coupled with favorable safety profiles. These combinations could be strategically positioned as second-line treatments or as frontline therapies in regions with confirmed chloroquine re-sensitivity and early signs of ACT resistance. This approach may help

extend the useful therapeutic lifespan of current antimalarials and provide valuable options for malaria control programs facing evolving drug resistance challenges.

Further clinical trials and molecular investigations are essential to validate the efficacy, safety, pharmacokinetics, and resistance dynamics of these combinations in human populations before widespread implementation.

Declarations

Study Limitation

This study is limited by the use of *Plasmodium berghei*-infected mice, a model that cannot fully replicate the biological complexity and clinical behavior of human *P. falciparum* infection, thereby limiting direct extrapolation to human settings. Moreover, the controlled laboratory conditions do not account for the genetic diversity, environmental influences, and immunological variability that characterize malaria in real-world populations.

Acknowledgements

We gratefully acknowledge the invaluable support and assistance of the staff of the Biochemistry Department and Animal Experimental Unit, Departments of Pharmacology, University of Jos, as well as the staff of the Chemical Pathology Department at Faith Alive Medical Center, Jos North, Plateau State. Their contributions were essential to the successful completion of this study.

Authors' Information

Dr. Olomu Segun Afolabi (PhD), Dr. Oche I. Jane-Rose (PhD)*, Prof. Titilayo Johnson (PhD), and Prof. Samuel Yusufu Gazuwa (PhD) are lecturers in the Department of Biochemistry, Faculty of Medical Sciences, College of Health Sciences, University of Jos, Plateau State, Nigeria.

Authors' Contribution

This study was conducted through the collaboration of all authors. Olomu A. Segun and Oche I. Jane-Rose conceived the study, designed the experiments, performed the laboratory investigations, and prepared the initial draft of the manuscript. Johnson Titilayo supervised the research, while Gazuwa Y. Samuel served as co-supervisor. Johnson Titilayo and Gazuwa Y. Samuel critically reviewed and revised the manuscript. All authors contributed to data analysis and interpretation, and all authors read and approved the final version of the manuscript.

Ethics Approval

The research protocol for this study was reviewed and approved by the Animal Experimental Unit Ethics Committee, Faculty of Pharmaceutical Sciences, University of Jos, under approval number: UJ/FPS/F17/-0039.

Consent for Publication

All authors consent to the submission of this manuscript to *Advances in Infectious Diseases* (*AID*). The authors confirm that the work is original and has not been published previously in any form.

Funding

TETFUND IBR Research Grant Number: 022.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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