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Effects of Zinc, Vitamin C and Folic Acid Co-Administration with Artemether-Lumefantrine on Parasitaemia and Oxido-Inflammatory Responses in *Plasmodium Berghei*-Infected Mice

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ABSTRACT
Malaria pathogenesis is characterised by severe oxidative stress, systemic inflammation, and profound anaemia, which are often exacerbated by concurrent malnutrition in endemic regions. While artemether-lumefantrine (AL) is highly efficacious in clearing parasites, it does not directly address this collateral damage to the host. This exploratory study evaluated the effects of co-administering Vitamins B9, C, and Zinc with standard AL therapy on oxido-inflammatory responses in *Plasmodium berghei*-infected Swiss albino mice. Following randomisation (per group), mice were treated with AL alone or in combination with the micronutrients for 4 days. Total parasite burden (Area Under the Curve, AUC), haematological profiles, hepatic antioxidant enzyme activities (Superoxide Dismutase [SOD] and Catalase [CAT]), and systemic C-Reactive Protein (CRP) levels were evaluated. The Kruskal-Wallis analysis of the AUC did not detect a statistically significant difference in parasite clearance kinetics among the treatment groups (). However, micronutrient co-administration significantly improved host biochemical recovery. Zinc-containing regimens markedly elevated hepatic SOD and CAT activities compared to untreated controls, while combinations incorporating Vitamins B9 and C improved Haemoglobin and Red Blood Cell counts and suppressed acute-phase CRP spikes. While the small sample size limits broad clinical inferences, the data suggests these micronutrients may ameliorate malaria-induced oxido-inflammatory damage. Larger pharmacological studies are required to determine specific synergistic mechanisms and clinical safety.

Keywords: Plasmodium berghei, Artemether-Lumefantrine, Oxidative Stress, Host-Directed Therapy, Zinc, Malaria Pathogenesis

INTRODUCTION

Malaria, caused by protozoan parasites of the genus *Plasmodium*, remains a formidable global health challenge. In 2023, the World Health Organisation reported an estimated 263 million cases and nearly 597,000 deaths worldwide (WHO, 2024). The disease burden is disproportionately concentrated in sub-Saharan Africa, with Nigeria alone accounting for approximately 27% of global cases and 31% of malaria-related mortality (Ajayi et al., 2023). Artemisinin-based combination therapies (ACTs), particularly artemether-lumefantrine (AL), remain the recommended first-line pharmacological intervention due to their rapid parasiticidal activity and historically high cure rates in endemic regions (Oyebola et al., 2024). However, the ultimate recovery of the patient depends not only on successful parasite clearance but also on the host's ability to survive the profound physiological damage inflicted by the infection.

The pathogenesis of malaria is inextricably linked to severe oxidative stress and systemic inflammation (Ogundeyi et al., 2025). During the intraerythrocytic stage of infection, *Plasmodium* parasites extensively degrade host haemoglobin, releasing toxic free haem and generating massive quantities of reactive oxygen species (ROS) (Salcedo-Sora et al., 2011). While artemisinin derivatives leverage this highly oxidative environment within the parasite's food vacuole to induce parasiticidal toxicity, the systemic spillover of ROS could overwhelm the host (Barua et al., 2018). This marked depletion of endogenous antioxidant defence, such as superoxide dismutase (SOD) and catalase (CAT), leads to widespread lipid peroxidation, severe acute-phase inflammatory responses, and profound anaemia, leaving the host vulnerable to severe morbidities even after the parasite has been cleared (Palmer et al., 2023). In highly endemic areas in Nigeria, this profound malaria-induced anaemia is a major contributor to adverse outcomes in vulnerable populations and is often aggravated by poor socioeconomic and nutritional circumstances (Sarki et al., 2019).

In malaria-endemic regions, this pathology is frequently compounded by concurrent malnutrition. Deficiencies in essential micronutrients such as zinc, vitamin C (ascorbic acid), and vitamin B9 (folic acid) are prevalent and severely impair host immune function, antioxidant capacity, and erythropoiesis. While zinc is known to bolster general host immunity and serve as a critical structural cofactor for endogenous antioxidant enzymes, recent meta-analyses indicate that zinc supplementation alone does not significantly reduce the risk of parasitaemia (Kotepui et al., 2023). Similarly, laboratory models have demonstrated that the co-administration of vitamin C with AL can ameliorate oxido-inflammatory responses and improve murine survival, though its direct impact on accelerating parasite clearance remains contested (Ajayi et al., 2023; Ogundeyi et al., 2025). Furthermore, while folic acid is essential for red blood cell production and host recovery from anaemia, its involvement

in purine synthesis has historically raised concerns regarding potential antagonism with certain antimalarial mechanisms (Dawet et al., 2023).

Despite these fragmented insights, the complex pharmacological and biochemical interactions between this specific matrix of micronutrients and standard AL therapy remain insufficiently characterised. A critical knowledge gap persists regarding whether these supplements can be safely integrated to rescue the host without inadvertently antagonising the primary drug mechanism. Therefore, this study aimed to systematically evaluate the therapeutic efficacy of co-administering Vitamins B9, C, and Zinc with artemether-lumefantrine in a *Plasmodium berghei*-infected murine model. By quantifying parasite clearance kinetics, haematological recovery, and the modulation of oxidative stress and systemic inflammation, this research seeks to determine the viability of micronutrients as host-directed adjunct therapies in malaria management.

MATERIALS AND METHODS

Drugs, Chemicals, and Equipment

All chemicals, solvents, and reagents utilised in this study were of analytical grade. Artemether/Lumefantrine (Lonart® 20/120 powder) was used as the standard antimalarial drug. Micronutrient supplements included Folic acid (Vitamin B9) syrup, Vitamin C syrup, and Zinc tablets. Key laboratory consumables included Giemsa stain and capillary tubes. Major equipment utilised included a LEICA ICC50 HD microscope, a Finecare™ FIA Meter Plus (FS-113) for immunoassay detection, and an automated haematology analyser for blood parameter quantification.

Study Area and Ethical Approval

The animal experiments were conducted at the Animal House of the Department of Physiology, Gombe State University, Nigeria. Ethical clearance for the study was officially granted by the Animal Care and Use Research Ethics Committee of the Gombe State Ministry of Agriculture, Animal Husbandry and Cooperatives (Approval Number: MAAH/HQ/S/GEN/423/V.I/00061). All experimental protocols and animal handling procedures rigorously adhered to the established institutional guidelines for the care and use of laboratory animals.

Experimental Animals and Parasite Strain

Thirty (30) healthy Swiss Albino Mice (*Mus musculus*), weighing between 18 and 30 g, were procured and acclimatised under standard environmental conditions. The animals were housed in adequately ventilated cages and provided with a standard dry pellet diet and water *ad libitum*. The chloroquine-sensitive *Plasmodium berghei berghei* (NK 65 strain) was obtained from the National Malaria Research Institute (NMRI), Lagos, Nigeria, and maintained via serial passage.

Sample Size and Randomisation

To comply with ethical constraints regarding the reduction of animal use in exploratory experimental models, a sample size of $n = 3$ mice per group was selected. While this limits the statistical power required for equivalence testing, it provides sufficient baseline data for identifying broad oxido-inflammatory trends. Animals were randomly allocated to treatment groups to minimise selection bias, and outcome assessments were conducted by personnel blinded to the group assignments.

Inoculation and Experimental Design

To induce malaria infection, mice were inoculated intraperitoneally with 0.2 mL of diluted blood containing approximately 1.2×10^7 parasitised red blood cells. The injection sites were sterilised with alcohol-moistened cotton prior to and following inoculation.

Following a 72-hour post-inoculation period to allow for the establishment of parasitaemia, the 30 mice were randomised into ten (10) experimental groups (per group) as follows:

Group 1: Normal control (Uninfected, untreated)
Group 2: Negative control (Infected, untreated)
Group 3: Standard control (Infected, treated with Artemether/Lumefantrine [AL])
Group 4: Infected + AL + Vitamin B9
Group 5: Infected + AL + Vitamin C
Group 6: Infected + AL + Zinc (Zn)
Group 7: Infected + AL + Vitamins B9 & C
Group 8: Infected + AL + Vitamin B9 & Zn
Group 9: Infected + AL + Vitamin C & Zn
Group 10: Infected + AL + Vitamins B9, C & Zn

Treatment Protocol

Therapeutic intervention was conducted utilising the standard curative antimalarial test (4-day suppressive protocol). Artemether-lumefantrine (AL), Vitamin B9, and Vitamin C were each administered at a dose of 70 mg/kg body weight, with dose volumes capped at 0.2 mL. Zinc was administered at a dose of 25 mg/kg body weight, with a maximum dose volume of 0.08 mL. All treatments were administered orally via gavage once daily at 9:00 am for four consecutive days. The uninfected normal control (Group 1) and infected negative control (Group 2) received equivalent volumes of the distilled water vehicle under the exact same dosing schedule.

Determination of Parasite Density

To account for polyparasitism (multiple ring-stage infections within individual erythrocytes) characteristic of aggressive *P. berghei* models, parasite load was expressed as Parasite Density (Parasites per 100 RBCs). Blood samples were collected from the tail vein daily (Day 0 to Day 4 post-treatment commencement). Thin blood smears were prepared on glass slides, fixed, and stained with Giemsa solution for 10 minutes.

The stained thin smears were examined under oil immersion (100x magnification). The total number of individual parasites observed was recorded against a fixed baseline of 1,000 red blood cells (RBCs) per slide. The final parasite density was calculated using the following formula:

$$Parasite\ Density\ (Parasite/100\ RBCs) = \left(\frac{Total\ Number\ of\ Parasites\ Counted}{Total\ Number\ of\ RBCs\ Observed\ (1{,}000)}\right) \times 100 \quad (1)$$

Given the constant denominator of 1,000 RBCs, this simplifies operationally to:

$$Parasite\ Density = \frac{Raw\ Parasite\ Count}{10} \quad (2)$$

To evaluate the overall efficacy of the therapeutic regimens over the complete observation period, the total parasite burden for each subject was subsequently calculated as the Area under the Curve (AUC) using the trapezoidal rule across the time-series data

$$AUC = \frac{y_0}{2} + y_1 + y_2 + y_3 + \frac{y_4}{2} \quad (3)$$

(Where y_0 is Day 0 density, y_1 is Day 1 density, etc.)

Haematological Assays

Following the conclusion of the 4-day monitoring period, the experimental animals were humanely sacrificed. Blood

samples were collected into EDTA containers and immediately processed using a Swelab Lumi automated haematology analyser (REF: 1620040; Boule Medical AB, Spånga, Sweden). The analyser utilised Coulter's Principle and flow cytometry (hydrodynamic focusing and laser scattering) to quantify the following parameters: Haematocrit (HCT; L/L), Haemoglobin (Hb; g/dL), Red Blood Cells (RBC; $x10^6/\mu L$), White Blood Cells (WBC; $x10^3/\mu L$), and Platelets (PLT; $x10^3/\mu L$).

Biochemical and Antioxidant Assays

C - Reactive Protein (CRP)

Serum CRP levels (mg/L) were quantified using assay kits sourced from Gentein Biotec, Inc. (Catalogue number: CLIA3079) and analysed via the Finecare™ FIA Meter Plus based on fluorescence sandwich immunodetection technology. A 75 µL volume of the buffered serum mixture was loaded into the test cartridge, and fluorescence signal intensity, reflecting the amount of captured CRP antigen, was measured.

Antioxidant Enzymes

Liver tissues were excised, washed in ice-cold saline, and homogenised to evaluate oxidative stress markers. Superoxide Dismutase (SOD) and Catalase (CAT) assay kits were sourced from Merck and Sigma-Aldrich.

Catalase (CAT) Activity

Assayed colourimetrically by measuring the degradation rate of a standard solution at 405 nm following a 10-minute incubation at 25°C. Activity was calculated based on sample mass and expressed as U/g tissue, where one unit catalyses the degradation of 1 µmol of substrate per minute.

Superoxide Dismutase (SOD) Activity

Determined by the reduction of blue tetrazole to blue formazan. Absorbance was detected at 540 nm in a 96-well microplate following a 30-minute incubation at 37°C. Results were calculated based on the percentage of inhibition and sample weight, expressed as U/g tissue.

Data Analysis

Data analyses and data visualisations were conducted using JASP and Microsoft Excel software. To determine the appropriate inferential framework, exploratory data diagnostics were performed on all dependent variables. The Shapiro-Wilk test was utilised to assess the normality of data distribution, and Levene's test was applied to evaluate the homogeneity of variance. Diagnostic results indicated significant violations of parametric assumptions ($p < 0.05$) across the majority of the dataset, primarily driven by intrinsic biological variance and the non-normal distribution of parasite densities.

To maintain methodological consistency across the study, a uniform non-parametric approach was adopted. Continuous data are expressed as medians with interquartile ranges (IQR). The Kruskal-Wallis H test was employed to evaluate statistical differences among the ten experimental groups. Where omnibus significance was detected, Dunn's post hoc test, adjusted with the Bonferroni correction for multiple comparisons, was applied to identify specific pairwise differences. Statistical significance was defined *a priori* at $p < 0.05$.

RESULTS AND DISCUSSION

Parasite Density and Clearance Kinetics

The progression of *Plasmodium berghei* infection and the subsequent clearance kinetics following therapeutic interventions were monitored over 4 days. As expected, the infected, untreated negative control (Group 2) demonstrated a rapid escalation in parasite density, peaking at a median of 61.0 parasites/100 RBCs (IQR: 8.50) by Day 4.

To evaluate the overall therapeutic efficacy and ensure the micronutrient supplements did not antagonise the primary antimalarial agent, the total parasite burden was quantified by calculating the Area under the Curve (AUC) for each subject. A Kruskal-Wallis H test indicated a general trend of variance across the groups, though the omnibus model did not reach statistical significance at the *a priori* threshold ($H(9) = 15.38$, $p = 0.081$). However, pairwise comparisons utilising Dunn's test with a Bonferroni correction confirmed a significant difference between the uninfected normal control and the infected negative control ($p = 0.027$), validating the successful induction of the murine infection model. Crucially, the addition of Vitamins B9, C, and Zinc, either individually and in combination, resulted in median AUC values comparable to the standard Artemether-Lumefantrine (AL) treatment, indicating that micronutrient co-administration did not interfere with or antagonise the primary parasitic clearance kinetics of AL.

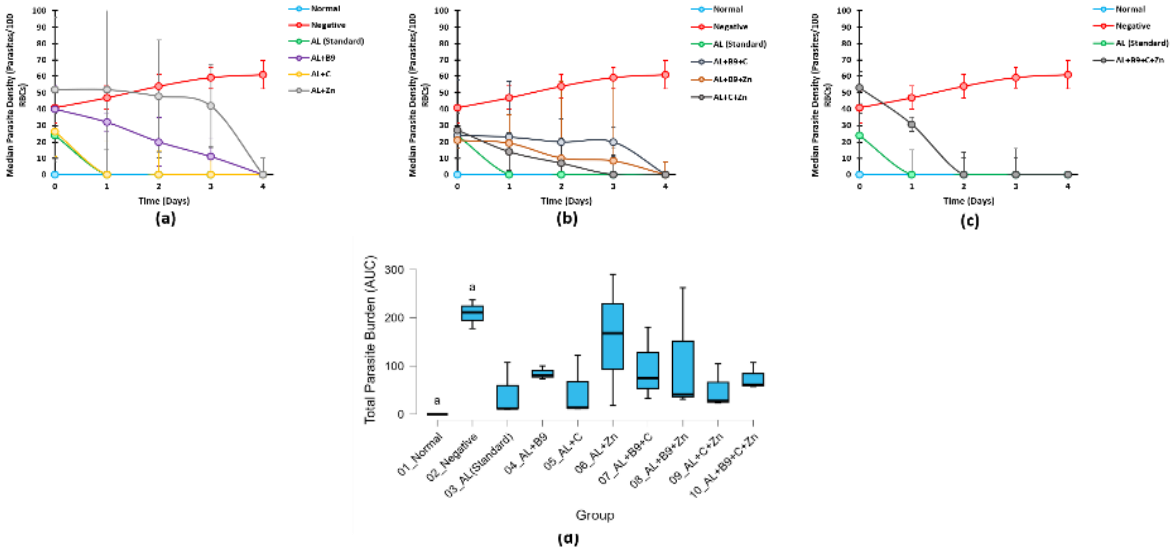


Figure 1: Parasite clearance kinetics and total parasite burden. (a – c)
Time-series progression of median parasite density (Parasites/100 RBCs) over the 4-day treatment period. Error bars represent the interquartile range (IQR). **(d)** Total parasite burden expressed as Area under the Curve (AUC). Data are presented as median and IQR boxplots with individual data points (per group). The omnibus Kruskal-Wallis analysis did not reach statistical significance across all treatment regimens (p = 0.081), indicating that micronutrient co-administration did not significantly antagonise the primary clearance efficacy of Artemether-Lumefantrine (AL).

Haematological Recovery Profile
Following the 4-day treatment period, haematological parameters were assessed to evaluate the impact of micronutrient co-administration on infection-induced anaemia and immune mobilisation. The negative control group exhibited hallmark signs of severe malaria-induced anaemia, characterised by depressed median Haemoglobin (10.1 g/dL) and Red Blood Cell counts (3.46 x10^6/µL), alongside marked leucocytosis (median WBC: 7.4 x10^3/µL). While the standard AL treatment facilitated partial haematological recovery, extreme intra-group variance was observed. Conversely, the co-administration of micronutrients generally stabilised the haematological profile. Notably, combinations involving Folic Acid (Vitamin B9) and Vitamin C (Groups 4, 7, 9, and 10) demonstrated robust restoration of Haemoglobin (ranging from 13.4 to 15.6 g/dL) and RBC counts (ranging from 9.22 to 10.8 x10^6/µL), closely mirroring the baseline profiles of the uninfected normal control group (median Hb: 13.2 g/dL; RBC: 8.04 x10^6/µL).

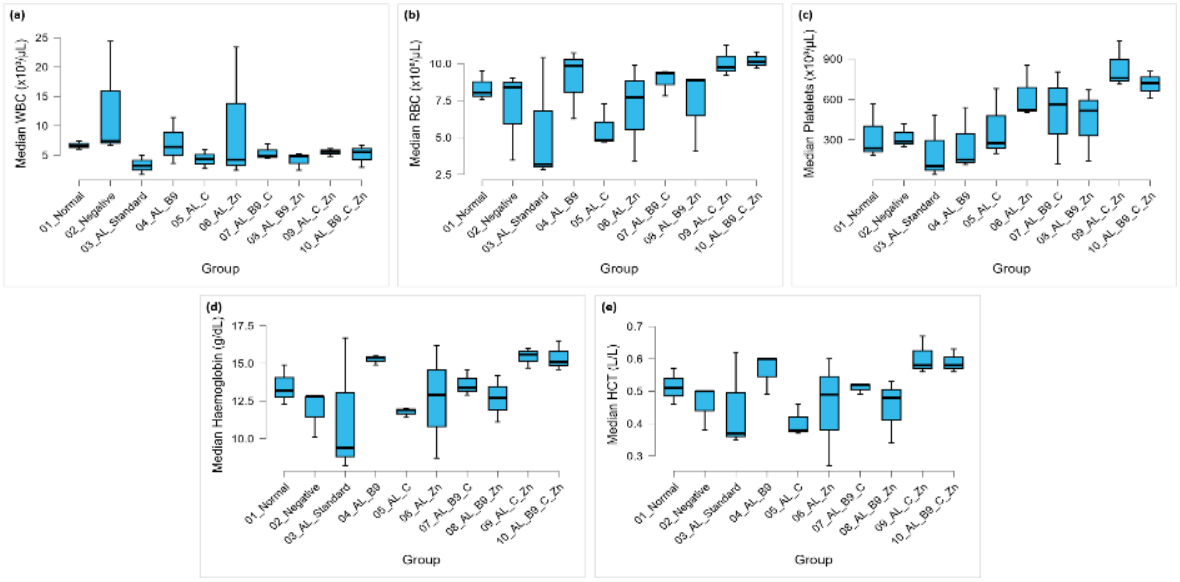


Figure 2: Haematological profiles following 4-day therapeutic intervention

Box-and-whisker plots depicting **(a)** White Blood Cells (x10^3/µL), **(b)** Red Blood Cells (x10^6/µL), **(c)** Platelets (x10^3/µL), **(d)** Haemoglobin (g/dL), and **(e)** Haematocrit (L/L). Boxes represent the interquartile range (IQR) with the median indicated by the central horizontal line; whiskers denote the data range. Individual biological replicates () are overlaid. Analysis was performed using the Kruskal-Wallis H test followed by Dunn's post hoc test with Bonferroni correction.

Modulation of Oxidative Stress (SOD and CAT)
The most profound treatment differentiations were observed in the host's oxidative stress biomarkers. *P. berghei* infection induced severe oxidative damage in the negative control group, evidenced by a catastrophic depletion of hepatic Superoxide Dismutase (SOD) activity (median: 23.3 U/g tissue) and Catalase (CAT) activity (median: 3.8 U/g tissue). Treatment with AL alone provided moderate antioxidant rescue (median SOD: 62.5 U/g; CAT: 4.1 U/g). However, co-administration of Zinc, either alone (Group 6) or in a complete combination matrix (Group 10), significantly improved the restoration of antioxidant defences. Mice in Group 10 (AL + B9 + C + Zn) exhibited a median SOD activity of 77.1 U/g and CAT activity of 5.1 U/g, effectively approaching the physiological baseline of the uninfected normal controls (median SOD: 76.6 U/g; CAT: 7.7 U/g).

Inflammatory Response (C - reactive protein)
Systemic inflammation was quantified via serum C - reactive protein (CRP) levels. The negative control group exhibited severe acute-phase inflammation, with CRP levels peaking at a median of 4.8 mg/L. Standard AL therapy halved this inflammatory marker (median: 2.4 mg/L).
Co-administration of single vitamin supplements, specifically Vitamin B9 (Group 4; median: 1.9 mg/L) and Vitamin C (Group 5; median: 1.5 mg/L), resulted in the most potent suppression of CRP, completely normalising the inflammatory profile against the uninfected baseline (median: 2.0 mg/L). Interestingly, while Zinc was the primary driver of oxidative stress rescue, groups receiving Zinc regimens exhibited slightly elevated CRP baselines relative to the B9/C groups, though they remained markedly lower than the untreated negative controls.

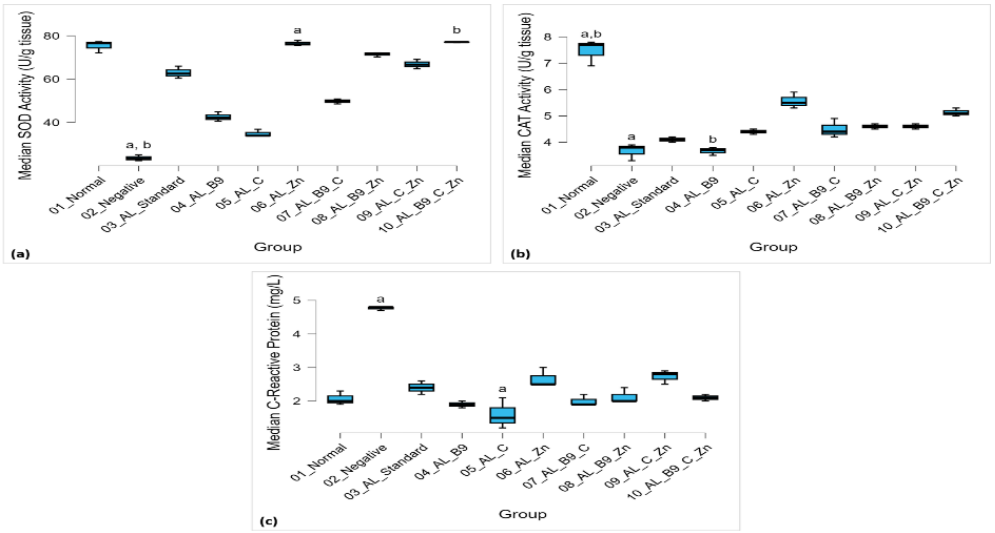


Figure 3: Micronutrient-mediated rescue of host oxidative stress and systemic inflammation

Assessment of hepatic antioxidant capacity via **(A)** Superoxide Dismutase (SOD; U/g tissue) and **(B)** Catalase (CAT; U/g tissue) activity, alongside **(C)** systemic C-Reactive Protein (CRP; mg/L) levels. Data are presented as median and IQR boxplots (). Zinc-containing regimens (Groups 6 and 10) demonstrated complete restoration of SOD and CAT activities to uninfected baseline levels. Significance markers indicate differences determined via Kruskal-Wallis and Dunn's post hoc pairwise comparisons (values with the same letter are significantly different from each other).

Discussion

The concurrent burden of malaria and micronutrient deficiency in endemic regions presents a compounded challenge to host survival and recovery. While Artemisinin-based Combination Therapies (ACTs) such as artemether-lumefantrine (AL) remain highly efficacious at eliminating the *Plasmodium* parasite, the resulting oxidative stress and systemic inflammation often leave the host physiologically compromised (Percário et al., 2012). This study evaluated the therapeutic efficacy of co-administering Vitamins B9, C, and Zinc with standard AL therapy in a *P. berghei*-infected murine model. The findings reveal a compelling dichotomy: while micronutrient supplementation does not significantly alter the primary parasite clearance kinetics of AL, it profoundly accelerates host biochemical and haematological recovery, primarily through the restoration of antioxidant enzyme activity and the suppression of acute-phase inflammation.

A major finding of this study is the demonstration of pharmacological safety and non-interference. The Kruskal-Wallis analysis of the total parasite burden (AUC) indicated that co-administering these micronutrients did not yield a statistically significant acceleration in parasite clearance (p = 0.081). From a clinical pharmacology perspective, this is a positive outcome. It confirms that the addition of antioxidant and hematopoietic supplements does not antagonise the primary mechanism of action of artemether-lumefantrine, which relies heavily on the generation of localised reactive oxygen species (ROS) and free radicals within the parasite's food vacuole to induce parasiticidal toxicity (Kavishe et al., 2017). It is important to acknowledge that the lack of statistical significance in clearance acceleration may represent a Type II error driven by the extreme biological variance and polyparasitism inherent to *in vivo P. berghei* models, exacerbated by the small sample size (). Nevertheless, the data clearly support the safety of these adjuncts, paving the way for their use in host rescue without risking drug failure.

The most striking therapeutic benefit observed was the rescue of the host's endogenous antioxidant defence system. Malaria pathogenesis is inextricably linked to severe oxidative stress, driven by host immune macrophage activation and the degradation of haemoglobin by the parasite (Buthelezi et al., 2025; Vasquez et al., 2021). This was distinctly reflected in the catastrophic depletion of hepatic Superoxide Dismutase (SOD) and Catalase (CAT) activities in the untreated negative control group (Figures 3(a) and 3(b)). While AL therapy alone provided partial recovery, co-administration of Zinc, both individually and synergistically with Vitamins B9 and C, significantly improved restoration of SOD and CAT activities to baseline uninfected levels. Zinc is an essential structural and functional catalytic cofactor for the Cu/Zn-SOD metalloenzyme (CDC, 2024). Its supplementation likely circumvents infection-induced deficiency, directly restoring the enzymatic capacity required to catalyse the dismutation of toxic superoxide radicals into molecular oxygen and hydrogen peroxide, thereby mitigating widespread tissue damage.

Concurrently, the haematological and inflammatory profiles underline the distinct but complementary roles of Vitamins B9 (Folic Acid) and C (Ascorbic Acid). *Plasmodium* infection reliably induces profound anaemia and leucocytosis, alongside severe systemic inflammation (Akinosoglou et al., 2012; Lakkavaram et al., 2020; Sakzabre et al., 2020), as evidenced by the spike in C-Reactive Protein (CRP) (Figure 3(c)). Groups supplemented with Vitamins B9 and C demonstrated the most robust suppression of CRP, normalising the inflammatory profile more effectively than AL alone. Vitamin C is a potent aqueous-phase antioxidant capable of directly quenching circulating ROS (Ogundeyi et al., 2025), thereby dampening the inflammatory cytokine cascade that triggers acute-phase CRP hepatic synthesis. Furthermore, the recovery of Haemoglobin and Red Blood Cell counts in groups receiving Folic Acid highlights its critical role in nucleic acid synthesis and host erythropoiesis (Sakzabre et al., 2020; Tola, 2024). While *Plasmodium* can synthesise its own folates *de novo*, the data indicate that exogenous B9 supplementation may have disproportionately benefited the host's bone marrow recovery rather than exacerbating parasitaemia.

Interestingly, while Zinc drove the oxidative stress rescue, groups receiving Zinc exhibited a slightly elevated CRP baseline compared to those receiving exclusively B9 and C. This nuanced finding suggests that while Zinc robustly defends against intracellular oxidative damage (Lee, 2018; Wong et al., 2025), its immunomodulatory properties may sustain a mild, protective acute-phase response necessary for complete tissue repair.

Despite the biochemical findings, this study has several limitations that warrant consideration. Foremost is the restricted sample size (per group), which inherently limits the statistical power of the inferential models. This constraint necessitated the use of non-parametric analytical frameworks and likely contributed to a Type II error regarding the overarching parasite clearance kinetics, where subtle but potentially biologically relevant accelerations in clearance may not have achieved statistical significance. Secondly, while the *Plasmodium berghei* murine model is an established proxy for human malaria (Ajayi et al., 2023; Dawet et al., 2023; Lakkavaram et al., 2020; Ogundeyi et al., 2025), inherent inter-species differences in micronutrient metabolism and immunological responses necessitate caution when extrapolating these findings directly to human *P. falciparum* infections. Furthermore, the evaluation of oxidative stress was localised to hepatic tissue homogenates (SOD and CAT); future studies incorporating a broader panel of organ-specific markers (e.g., splenic or cerebral tissue) alongside a dose-response optimisation curve for the micronutrients would provide a more comprehensive understanding of the systemic rescue dynamics and potential toxicity thresholds.

Thus, the co-administration of Zinc, Vitamin C, and Folic Acid with artemether-lumefantrine provides a highly synergistic therapeutic advantage. Rather than acting as direct parasiticidal agents, these micronutrients function as potent host-directed therapies. They secure the physiological integrity of the host, neutralise infection-induced and drug-induced oxidative collateral damage, and accelerate haematological recovery without compromising the established efficacy of standard ACTs.

Conclusion

The co-administration of Vitamins B9, C, and Zinc alongside artemether-lumefantrine (AL) therapy offers observable biochemical benefits in a *P. berghei* murine model. While the small sample size precludes definitive claims of non-antagonism or pharmacological safety, the adjuncts did not result in a statistically significant delay in parasite clearance. Furthermore, the micronutrients supported host recovery; Zinc contributed to the elevation of hepatic antioxidant enzymes (SOD and CAT), while Folic Acid and Vitamin C improved haematological indices and suppressed acute-phase systemic inflammation (CRP). These findings highlight the potential of host-directed nutritional adjuncts during malaria treatment. However, larger animal studies, robust pharmacokinetic interaction assessments, and controlled human clinical trials are required before any clinical recommendations can be established.

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