Arch. Bas. App. Med.13 (2025) 62-67
www.archivesbamui.com
www.ojshostng.com/index.php/abam

Research Article

Clearance of *Plasmodium berghei* in Mice Treated with Artesunate-Amodiaquine-Iron and Artesunate-Amodiaquine Combinations

Ebisike C.[1], Idachaba I.[1], Akinmuleya T.[1], Adebayo M[1],
Onyiagha H[1], Badejo J.[1,2], *Michael O[1,2]
[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Ibadan, Nigeria*
[2]*Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences,*
College of Medicine, University of Ibadan, Nigeria

Accepted: February 24, 2025

Abstract

Malaria remains a significant health burden in malaria-endemic regions, particularly in sub-Saharan Africa. Artemisinin-based combination therapies (ACTs) effectively clear malaria parasites and prevent anemia. Iron supplementation supports red blood cell production, but its impact on early malaria parasite dynamics when combined with ACTs remains underexplored. This study investigated the effect of iron supplementation on early parasite clearance in a murine model. Fourteen male Swiss mice were infected with *Plasmodium berghei* and randomized into two groups. One group received artesunate-amodiaquine (AS-AQ) and iron supplementation, while the other received AS-AQ only. Parasite densities were monitored six-hourly for 72 hours using Giemsa-stained thin blood films. Packed cell volume (PCV) and weight were evaluated at baseline and every 24 hours. Data was analyzed using SPSS version 22 and GraphPad Prism version 5. Parasite clearance was more rapid in the AS-AQ-Fe group, with a half-life of 12.3 hours compared to 40.8 hours in the AS-AQ group ($P < 0.001$). The AS-AQ group exhibited an early rise in parasitemia (parasite density), which was absent in the AS-AQ-Fe group. PCV increased significantly in the AS-AQ-Fe group (63.6 ± 2.6%) compared to the AS-AQ group (24.0 ± 4.9%; $P < 0.001$). Weight gain was also significantly higher in the AS-AQ-Fe group ($P = 0.013$). Iron supplementation with ACT significantly enhanced parasite clearance and improved hematological and weight parameters in *P. berghei* infected mice. Further research is needed to elucidate the underlying mechanisms to support this observation.

Key Words: *Malaria, Iron supplementation, Malaria parasite clearance, Artemisinin-based combination therapy, Murine model of malaria, Artesunate-Amodiaquine*

INTRODUCTION

Malaria parasites, when untreated, multiply rapidly in the host leading to rising parasite densities and decreasing red blood cells (White, 2017). Parasite induced destruction of red cells is a cause of anemia malaria endemic countries, especially in sub-Saharan Africa (Menendez *et al*., 2000). Anemia may cause impaired physical growth, cognitive impairment, behavioral and emotional problems, decreased immunity, and increased risk of morbidity and mortality. The artemisinin-based combination therapies (ACTs) are currently the first line treatment of malaria globally (Bosman and Mendis, 2007). They rapidly clear parasites from the host and prevent the development of anemia. Their ability to destroy malaria parasites while preserving the red cells have been reported (Price *et al*, 1987; White, 2008; and Dondorp *et al*, 2009)

Adequate iron levels are essential for supporting the cellular processes involved in cell division, including DNA synthesis, energy metabolism, cell cycle regulation, oxygen transport, and cellular signaling (Zhang, 2014). Iron deficiency can impair these processes and compromise cell division, leading to adverse effects on tissue growth, development, and function (Beard 2001). In malaria endemic countries, the co-existence of malaria parasites and iron deficiency is not uncommon (Kassebaum *et al* 2014). It has been reported that iron supplementation without antimalarial therapy may be deleterious to the host as this may result in a more rapid multiplication of malaria parasites (Hurrell, 2010). It is also known that iron supplementation improves red blood cell counts during malaria episodes.

Iron supplementation given simultaneously with antimalarial therapy may result in the prevention of anemia. Ekvall, Premji, and Björkman (Ekvall 2000) investigated the combined effects of micronutrient and iron supplementation together with effective antimalarial treatment on childhood anaemia in a malaria-endemic region. The study demonstrated that children who received both iron/micronutrient supplements and appropriate antimalarial therapy experienced significant improvements in haemoglobin levels, greater correction of anaemia, and better overall hematologic recovery compared to those receiving either intervention alone. The findings highlight a synergistic relationship: malaria treatment reduces

---

**Author for Correspondence:* Obaro Michael, Tel: +2348056642410, E-mail: *micobaro@gmail.com*

parasite-induced hemolysis and inflammation, while iron supplementation restores iron-dependent erythropoiesis. In another study (Kannan *et al*., 2019), Researchers enhanced the antimalarial drug artesunate by conjugating it with iron oxide nanoparticles, creating a formulation called ferromagnetic artesunate (FA). Using an external magnetic field to target and retain FA in the spleen, a major site of infection, dramatically improved drug delivery and efficacy in mice, clearing both drug-sensitive and drug-resistant malaria parasites. This targeted approach provided complete protection and prevented cerebral malaria without signs of toxicity, offering a promising strategy for more efficient treatment. Clark and colleagues in 2014 (Clark *et al* 2014), using a mouse model, demonstrated that host iron deficiency limits parasite growth but also compromises the effectiveness of a frontline ACT by restricting the production of the red blood cell compartment in which the drug is most potent. This finding underscores the complex interplay between host nutrition, anemia, and antimalarial drug efficacy. The effects of iron supplementation on early parasite dynamic is still under-evaluated. Using a Murine model of malaria, we studied the effects of iron supplementation on early malaria parasite dynamics. The goal was to compare malaria parasite elimination half-life after treatment with ACT, in mice that received concomitant iron supplementation and those that did not.

MATERIALS AND METHODS

Study location: The work was carried out at the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, located inside the University College Hospital, Ibadan, Oyo State, Nigeria. The study was conducted in 2018. All the animals used for the study were acquired from the animal house of the Institute.

Ethical care of animals and experimental design: Fourteen male Swiss mice weighing between 16 g and 20 g were obtained from the animal house of the College of Medicine, University of Ibadan. Animals were housed in cages and allowed to acclimatized for one week and had access to feed and clean water ad libitum. All the mice were kept at room temperature in a 12-hour light / dark cycle. Experiments were conducted in accordance with the internationally accepted laboratory animal use, care and guidelines (American Psychological Association, 1986). A donor mouse infected with chloroquine-sensitive *Plasmodium berghei* (ANKA) was also obtained from the Institute.

The parasite levels of the donor mouse were evaluated using blood taken from a 0.5–1mm section of the mice's tails. To infect the experimental mice, the donor mouse with a parasite of about 30–35% was sacrificed and blood was collected through a cardiac puncture into a 5 mL bottle containing 3.8% trisodium citrate (manufactured by BDH chemicals, England) added as an anticoagulant. The blood was then diluted in normal saline so that the final suspension contained about 1 × 107 infected red blood cells (RBCs) in every 0.2 ml suspension. The dilution was made based on donor mice parasite and RBC count of the normal mice in such a way that one ml blood contained 5 × 107 infected erythrocytes. Finally, each of the 16 mice was infected intraperitoneally with 0.2 ml of infected blood containing approximately 1 × 107 *P. berghei* parasitized RBCs.

A curative model as described by Ryley and Peters 1970 was used (Ryley and Peters, 1970). After infection with *Plasmodium berghei,* the fourteen mice were allocated into two groups of seven animals each. They were then fed for three days. On the fourth day, parasite density was evaluated using blood taken from the 0.5–1mm section of the mice's tails. This was the day zero (D0) parasite density. The infected mice were subsequently treated on the same day. The first group of seven mice was treated with artesunate-amodiaquine [manufactured by Front Pharmaceutical Company, China] and ferrous sulfate [manufactured by Therapeutic Laboratories, Nigeria]. The second group of seven mice was treated with artesunate-amodiaquine without ferrous sulfate.

Dose of drugs used: Ferrous sulfate was administered at a dose of 18 mg/kg of elemental iron daily for three days. Amodiaquine was administered at a dose of 10 mg/kg daily for three days and Artesunate at a dose of 4 mg/kg daily for three days. The drugs were crushed to fine powder and mixed in normal saline such that 0.2 mL of the mixture contained the required amounts. The drugs were administered orally using an oral cannula attached to a 1 mL syringe.

Determination of Parasite Density: Pre-dosing and six hourly parasites were evaluated using blood taken from a 0.5–1mm section of the mice's tails. Parasitemia estimation was done for 72 hours. During the same period the animals received the drugs daily for three days (72 hours). Six hourly parasite densities were determined at time points 0 (pre-treatment), 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, and 72 hours.

Thin blood films were prepared, fixed with methanol, and air dried before being stained with buffered 10 % Giemsa stain. The parasitized red blood cells were identified by parasites presence within the cells. The parasite count was estimated by microscopic examination of the Giemsa-stained thin blood films using a magnification of 100X (oil immersion light microscope objective). Ten randomly selected high power fields were used to determine number of parasitized red blood cells and unparasitized red blood cells. Parasite density was calculated as the number of asexual parasites relative to a microliter of blood (assumed to contain 5,000,000 RBCs). The parasite density was estimated by using the formula:

Parasite Density (Parasites / μL) = (Number of Parasitized RBCs / Total Number of RBCs counted) × 5,000,000

The parasite densities were converted to geometric means using SPSS version 22.0.

Determination of early parasite dynamics: Early parasite dynamics was defined as changes in parasite density over 72 hours of treatment. An XY data table of time, parasite densities, and geometric means was developed. Time was entered into X, and geometric mean was entered into Y axes respectively. The geometric means were plotted using Graphpad prism version 8.0. The resulting plots were analyzed using a nonlinear regression curve, a one phase decay exponential model. In one-phase decay kinetics, the process being studied assumes a single exponential decay pattern. This is often encountered in situations such as the decay of a radioactive isotope, the clearance of a drug from the body, or the relaxation of a system towards equilibrium. The rate constant in this context quantifies the rate at which the process decays or diminishes over time. The half-life is defined as the time it takes for the quantity of the substance being studied (in this case the parasite density) to decrease by half from its initial value.

The rate constant and half-lives of the fitted curves were used to compare the rate of clearance of parasites from the blood of the experimental mice. Early rise in parasite density was defined as a rise in parasite density to levels above zero-hour parasite density during the period after the first dose of antimalarial, within the 72-hour period. Wilcoxon rank-sum test (SPSS version 22.0) was used to determine the difference in mean parasite density at 0, 24, 48. and 72 hours. An alpha value less than 0.05 was considered significant.

Determination of Weight: The weights of the mice were determined by the use of a small weighing balance. Each mouse was placed on an already-weighed small plastic cup before the weight, in grams, was determined by taking the readings on the weighing balance to two decimal places. The weight of each mouse was evaluated 24 hourly for 72 hours. The mean ± SD and statistical differences in weight were computed using SPSS version 22.

Determination of packed cell volume (PCV): PCV is the measure of the ratio of the volume occupied by red blood cells to the volume of whole blood. Blood samples were taken from the tip of the tail of the mice. The blood samples were put into hematocrit tubes, after which they were carefully sealed and placed on a centrifuge. They were spun in the centrifuge at 3000 RPM (rotations per minute) for five minutes. The height of the column of packed cells was then read from the hematocrit tube by means of a hematocrit reader after centrifugation. The result was expressed as a percentage. The PCV was evaluated 24 hourly for 72 hours. The mean ± SD and statistical differences in PCV were computed using SPSS version 22.

Changes in weight and PCV: The weight and PCV data were entered into spreadsheets and analyzed using SPSS version 22. Simple descriptive statistics was used. Results were expressed as mean and standard deviation. Alpha values less than 0.05 were considered significant. Graphs showing the changes in weight and PCV were plotted using Graphpad Prism version 8.0.

RESULTS

The first group received artesunate-amodiaquine-iron (AS-AQ-Fe), while the second group received artesunate-amodiaquine (AS-AQ) without iron. The geometric means of the parasite densities of the two groups are shown in Table 1. At the initiation of the experiment, both groups displayed comparable levels of parasitemia, indicating equivalent initial infection parasite densities. The geometric mean of parasite density declined after commencement of treatment (Figure 1). The parasite density at 36 hours was significantly lower in the AS-AQ-Fe group compared to the AS-AQ group ($P < 0.001$). The rate constant for AS-AQ-Fe and AS-AQ was 0.06 Hour-1 and 0.02 Hour-1 respectively. The half-lives for AS-AQ-Fe and AS-AQ were 12.3 Hours and 40.8 Hours respectively. The AS-AQ treated mice recorded early rise in parasite density, with the recorded peaks at 12 and 18 hours after treatment. The AS-AQ-Fe treated group did not record an early rise in parasitemia post treatment.

Other parameters evaluated were changes in packed cell volume (PCV) and weight during the 72-hour period. At baseline, PCV was 30.7 ± 2.7 for AS-AQ-Fe and 32 ± 3.8 for AS-AQ (ρ-value 0.485). After 72 hours, mean PCV was 63.6 ± 2.6 for AS-AQ-Fe compared to 24.0 ± 4.9 for AS-AQ ($\rho < 0.001$). Figure 2 shows the changes in PCV over the 72-hour period in the two groups of mice. Baseline weights were 20.02 ± 2.1 (AS-AQ-Fe) and 17.22 ± 2.8 (AS-AQ), with no significant difference (ρ-value 0.053). At 72 hours, mean weights were 23.20 ± 1.5 for AS-AQ-Fe and 17.75 ± 4.2 for AS-AQ ($\rho = 0.013$). The temporal changes are depicted in Figure 3.

Table 1.

Geometric Means of parasite densities of the two groups of mice

Hour	AS-AQ-Fe	AS-AQ	ρ-Value
0	250520	311245	0.421
6	137054	223998	0.241
12	166326	325527	0.054
18	80809	325527	0.004*
24	43046	165068	0.007*
30	39152	73163	0.274
36	4395	95777	0.000*
42	4556	3080	0/266
48	16	669	0.478
54	4	214	0.242
60	0	38	0.110
66	0	71	0.337
72	0	3	0.377

* p-value < 0.05

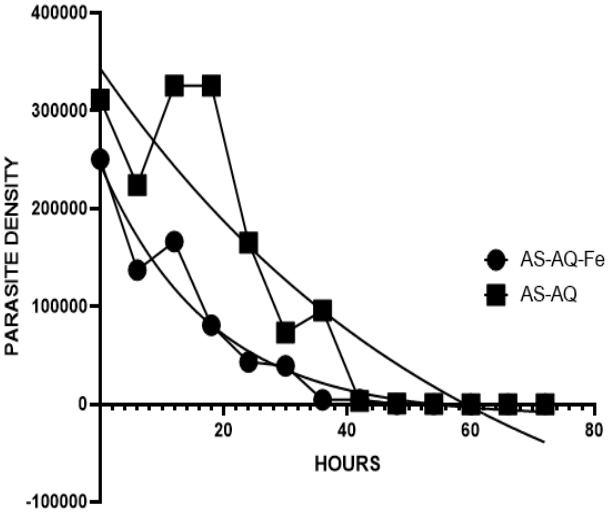

Figure 1:

Plot of geometric means of parasite densities of the two groups of mice with non-linear regression (one phase decay) curve fitted to the data

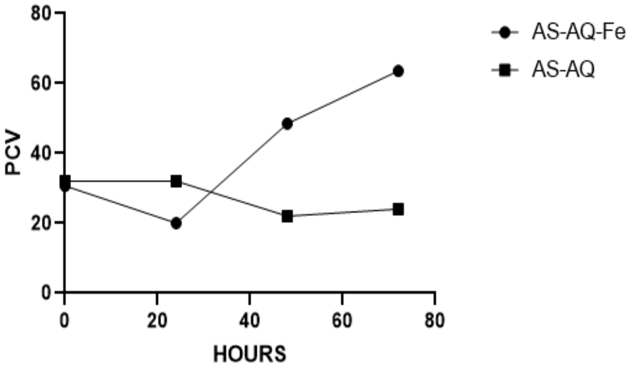

Figure 2:
Changes in PCV over the 72-hour period in the two groups of mice

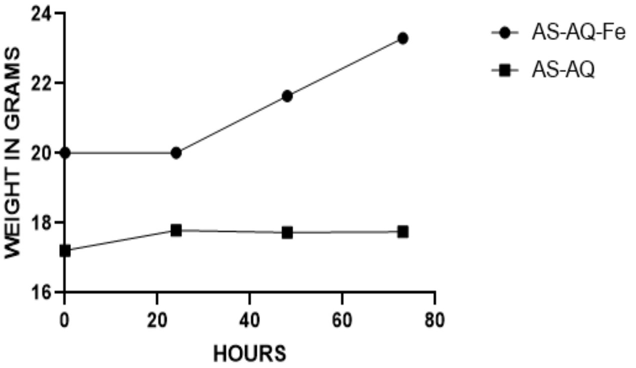

Figure 3:
Changes in weight over the 72-hour period in the two groups of mice

DISCUSSION

The results of this study suggest that iron supplementation in combination with standard antimalarial therapy (amodiaquine and artesunate) improved parasite clearance rates in mice infected with *Plasmodium berghei*. Specifically, the iron-supplemented group exhibited faster malaria parasite clearance at 24, 36, and 48 hours compared to the group that did not receive iron. In addition, by 72 hours after treatment, the hematocrit and weight gain were also significantly higher in the iron-supplemented group, indicating enhanced recovery from the infection.

The faster clearance of malaria parasites in the iron-supplemented group aligns with some existing research showing that iron is critical for erythropoiesis and immune function. Cordoso and co-authors reported that experimental mice with iron deficiency had higher parasite densities and lower parasite clearance rates (Cardoso *et al.*, 1993). However, in 1996, they observed that the development of *P. berghei* was neither suppressed by iron deficiency nor enhanced by iron supplementation in a rat model of malaria in contrast to their findings in mice, They also observed that the time course of parasitemia was similar in all treatment groups (Cardoso *et al.*, 1996). Castberg and colleagues (Castberg *et al.*, 2018), reported that nutritional iron deficiency was associated with increased mortality from *P. chabaudi* malaria in experimental mice. The researchers also found that administering brief, well-timed iron supplements alongside the primary treatment partially reduced the increased mortality. In explaining potential reasons for the observation, they noted that the moribund animals were characterized by low levels of hepcidin and high levels of fibroblast growth factor 23 and that all the infected mice had extramedullary splenic haematopoiesis. In addition, iron-supplemented mice had visually detectable intracellular iron stores. They, therefore concluded that the iron supplementation may have contributed to the more rapid replacement of infected red blood cells (RBCs) with healthy ones, thus reducing the availability of viable RBCs for parasite replication (Castberg *et al.*, 2018).

Our study suggests that the faster parasite clearance observed may be partly due to the mechanisms of action of artesunate-amodiaquine (ASAQ) combination against the malaria parasite. The two drugs act on the parasite in complementary ways. Artesunate acts rapidly by generating toxic radicals that damage the parasite's internal structures. Amodiaquine works by blocking the parasite's ability to safely neutralize the toxic heme released when it digests hemoglobin. This leads to a buildup of toxic heme inside the parasite. Together, they mediate a synergistic effect that rapidly and effectively clears malaria parasites. It is possible that this synergy could be further enhanced by iron supplementation. The parasite's heme production (targeted by amodiaquine) and the radical-generating process of artesunate are both iron-dependent. Supplemental iron may increase the availability of this crucial element within the infected red blood cell, potentially intensifying the very metabolic disruptions that ASAQ causes. Essentially, more iron could "fuel" the mechanisms that these drugs exploit, making them even more effective at killing parasites and accelerating clearance. In short, we propose that iron supplementation might not just correct anemia but could actively augment the pharmacodynamic effects of ASAQ on the parasite. There is a need to conduct more studies on pharmacodynamic responses, especially in respect to plasma iron concentrations. There is a complex interplay between the nutritional status, inflammatory responses, hormonal factors and the regulation of iron homeostasis, in health and in infectious conditions like malaria.

A key factor in the interplay, is hepcidin which has been described as the principal regulator of systemic iron homeostasis. In a systematic review (Nemeth and Ganz, 2006), it was noted that hepcidin controls plasma iron concentration and tissue distribution of iron by inhibiting intestinal iron absorption, iron recycling by macrophages, and iron mobilization from hepatic stores. In addition, it was also noted that hepcidin acts by inhibiting cellular iron efflux through binding to and inducing the degradation of ferroportin, the sole known cellular iron exporter. The report also stated that the synthesis of hepcidin is homeostatically increased by iron loading and decreased by anemia and hypoxia. In relation to acute infection, hepcidin has been shown to be elevated causing a decrease in serum iron levels and contributing to the development of anemia of inflammation, and that this may be a host defense mechanism to limit the availability of iron to invading microorganisms. Molecular studies on the role of iron in infections continue to suggest a double-edged sword effect, in which the evidence remains conflicting. In some studies, iron is found to be beneficial while in others, it is deleterious to the host and the pathogen (Nemeth and Ganz, 2006).

While iron supports red blood cell regeneration and immune function, it has also been reported to increase susceptibility to infection in certain settings by promoting pathogen growth. In a 2021 systematic review and meta-analysis by Shah and colleagues, they found that the administration of intravenous iron is associated with an increased risk of infection compared to oral iron or no iron. The meta-analysis reported a relative risk of 1.33 (95% confidence interval, 1.10 to 1.64), indicating a 33% higher risk of infection in patients receiving intravenous iron compared to those who did not (Shah *et al*., 2021). This observation has been reported in some other human studies where excess iron supplementation in malaria-endemic regions led to an increased risk of malaria and other infections. A large trial by Sazawal and colleagues (Sazawal *et al* 2006) was terminated early on account of the observation that children who received iron and folic acid supplements had a significantly increased risk of serious illness (including malaria) and death compared to those who did not. The evidence was a direct and influential and to revised World Health Organization (WHO) guidelines on iron supplementation. However, in our study, which was conducted over 72 hours, the findings were in support of beneficial effects of iron supplementation. Thus, while iron supplementation within therapeutic limits may improve outcomes without exacerbating the infection, we are hesitant in stating that the observed effects in mice infected with *Plasmodium berghei* are conclusive, and by no means are we extrapolating the findings beyond the limits of our experimental conditions.

On the gain in weight and rapid increase in hematocrit in mice that received iron supplementation, these may be indicative of improved erythropoiesis. Malaria-induced anemia is a common complication due to the destruction of infected RBCs and the suppression of erythropoiesis (Chang, 2004). By restoring iron levels, the process of erythropoiesis is enhanced, leading to a quicker recovery of the RBC population, as evidenced by higher hematocrit levels. This restoration of RBCs may have contributed to improved oxygen transport, which in turn could support metabolism and physical recovery, as reflected by the higher weight gain in the iron-supplemented group. The relationship between plasma iron levels and malaria parasite survival remains debated.

Gordeuk and colleagues in their report that the iron chelator deferoxamine (which removes iron) improves recovery in cerebral malaria. This supports the principle that iron availability may be crucial for parasite survival and that manipulating iron has anti-parasitic effect (Gordeuk *et al* 1992). Wang and colleagues in a report observed that that artemisinin is activated by heme-iron (ferrous iron, Fe^{2+}) within the parasite, leading to the alkylation of numerous parasite proteins and subsequently parasite death. Their study elucidates the iron-dependent mechanism of artemisinin, the core component of artemisinin-based first-line therapies, showing how intra-parasitic iron directly activates a potent drug (Wang *et al* 2015). Pradel and Schlitzer in their review explained that the malaria parasite apicoplast houses an essential iron-sulfur cluster (Fe-S) biosynthesis pathway and that disruption of iron availability directly inhibits this pathway, killing the parasite by preventing the synthesis of vital molecules like isoprenoids (Pradel *et al* 2010). These and some other studies indicate that iron's role is dual and perhaps paradoxical in malaria. While host supplementation can sometimes increase parasite growth by providing a vital nutrient, strategic manipulation of iron at the parasite level (e.g., via chelation or exploiting it for drug activation) exerts direct lethal effects. The pathway to this dual effect of malaria is not a direct one. It is known that iron is essential for the growth and proliferation of malaria parasites, and alterations in iron availability can disrupt parasite metabolism and survival. It is also possible that supplementation with iron provides a favorable environment for the host while simultaneously limiting the replicative capacity of the parasite, thereby facilitating parasite clearance. There is a need for more mechanistic studies to clarify this dual relationship.

Our study has limitations that need to be stated. The findings are based on an animal model of malaria infection, and extrapolation to human populations is not a consideration. Additionally, measurements of iron levels and other molecular parameters were not done; thus, specific mechanisms underlying the observed effects of iron supplementation on parasite clearance cannot be firmly proposed.

In conclusion, our findings suggest that iron supplementation may enhance the efficacy of antimalarial therapies during the acute phase of treatment, accelerating parasite clearance and aiding in recovery from malaria-induced anemia. However, further studies are needed to evaluate in more detail molecular interactions behind the observations reported.

Acknowledgments

Authors acknowledge for care of and laboratory procedures with respect to the animals during the study.

Author contributions: *OM designed the study. OM, JB, EC, II, JB, AM, AT, and OH conducted the experimental study. All authors participated in the manuscript writing and approved the final version of the manuscript.*

Conflict of interest declaration: *No conflict of interest was declared.*

Funding: *This work was not supported by any funding agency.*

REFERENCES

American Psychological Association. 1986. Guidelines for ethical conduct in the care and use of animals. *Journal of the Experimental Analysis of Behavior*. 45(2):127.

Bosman A, Mendis KN. A major transition in malaria treatment: the adoption and deployment of artemisinin-based combination therapies. *Am J Trop Med Hyg*. 2007 Dec;77(6 Suppl):193-7.

Cardoso, M. A., C. Colli, P.B. Garcia, M.U. Ferreira, M.V. Penteado, and H.F. Andrade Júnior. 1993. Effect of dietary iron on the course of *Plasmodium berghei* malaria in young rats. *Brazilian Journal of Medical and Biological Research Revista Brasileira de Pesquisas Medicas e Biologicas*. 26(12):1297–1303.

Cardoso, M. A., M.U. Ferreira, G.S. Ribeiro, M.D. Penteado, and H.F. Andrade Júnior. 1996. Dietary iron supplementation does not aggravate experimental malaria in young rats. *The Journal of Nutrition*. 126(2):467–475. https://doi.org/10.1093/jn/126.2.467

Castberg, F.C., L. Maretty, T. Staalsoe, C. Hempel, E. Clasen-Linde, L. Hviid, and J.A. Kurtzhals. 2018. Increased Plasmodium chabaudi malaria mortality in mice with nutritional iron deficiency can be reduced by short-term adjunctive iron supplementation. *Malaria Journal*. 17:1-12.

Chang, K.H., and M.M. Stevenson. 2004. Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria. *International Journal for Parasitology*. 34(13-14):1501-16.

Clark MA, Goheen MM, Cerami C. Host iron status and iron supplementation mediate susceptibility to erythrocytic stage *Plasmodium berghei*. *Infect Immun*. 2014 Sep;82(9):3794-800.

Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, *et al*. Artemisinin resistance in Plasmodium falciparum malaria. *N Engl J Med*. 2009;361(5):455–67.

Ekvall, H., Z. Premji, and A. Björkman. 2000. Micronutrient and iron supplementation and effective antimalarial treatment synergistically improve childhood anaemia. *Tropical Medicine & International Health*. 5(10):696-705.

Gordeuk VR, Thuma PE, Brittenham GM, McLaren CE, Parry D, Backenstose AR, *et al*. Effect of iron chelation therapy on recovery from deep coma in children with cerebral malaria. *N Engl J Med*. 1992 Nov 12;327(20):1473–7.

Hurrell, R. 2010. Iron and malaria: absorption, efficacy and safety. *International Journal for Vitamin and Nutrition Research*. 80(4):279.

Kannan, D., N. Yadav, S. Ahmad, P. Namdev, S. Bhattacharjee, B. Lochab, and S. Singh. 2019. Pre-clinical study of iron oxide nanoparticles fortified artesunate for efficient targeting of malarial parasite. *EBioMedicine*. 45:261-77.

Kassebaum NJ, Jasrasaria R, Naghavi M, *et al*. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. 2014;123(5):615–24.

Menendez, C., A.F. Fleming, and P.L. Alonso. 2000. Malaria-related anaemia. *Parasitology Today*. 16(11):469-476.

Nemeth, E., and T. Ganz. 2006. Regulation of iron metabolism by hepcidin. *Annual Review of Nutrition*. 26(1):323–342.

Pradel G, Schlitzer M. Antibiotics in malaria therapy and their effect on the parasite apicoplast. *Curr Mol Med*. 2010 Jun;10(4):335-49.

Price RN, Nosten F, Luxemburger C, ter Kuile FO, Paiphun L, Chongsuphajaisiddhi T, White NJ. Effects of artemisinin derivatives on malaria parasite clearance and host haematological recovery. *Trans R Soc Trop Med Hyg*. 1998;92(4):412–7.

Ryley, J.F., and W. Peters. 1970. The antimalarial activity of some quinolone esters. *Annals of Tropical Medicine & Parasitology*. 64(2):209-22.

Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, *et al*. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet*. 2006 Jan 14;367(9505):133-43.

Shah, A.A., K. Donovan, C. Seeley, E.A. Dickson, A.J. Palmer, C. Doree, S. Brunskill, J. Reid, A.G. Acheson, A. Sugavanam, and E. Litton. 2021. Risk of infection associated with administration of intravenous iron: a systematic review and meta-analysis. *JAMA Network Open*. 4(11):e2133935.

Wang J, Zhang CJ, Chia WN, Loh CC, Li Z, Lee YM, *et al*. Haem-activated promiscuous targeting of artemisinin in Plasmodium falciparum. *Nat Commun*. 2015 Aug 26;6:10111.

White NJ. Qinghaosu (artemisinin): the price of success. *Science*. 2008;320(5874):330–4.

White, N.J. 2017. Malaria parasite clearance. *Malaria Journal.* 16(1):88.

Zhang, C. 2014. Essential functions of iron-requiring proteins in DNA replication, repair and cell cycle control. *Protein & Cell.* 5(10):750-60

.