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Review

Antioxidant Role of Vitamins in Malaria and the Implications of Antioxidants as Adjuvant in Malaria Therapy: A Review

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Abstract

The prooxidant role of malaria parasites and chloroquine an antimalaria is well established. The antioxidant role of ascorbic acid and folate administered alone or as adjuvant therapy during malaria treatment in a research series is highlighted in this review. It was observed that oxidative stress is implicated in the pathogenesis of malaria and the oxidative assault may be aggravated by chloroquine, which was at some point a frontline antimalarial drug. Owing to the established antioxidant roles of vitamins, the administration either as mono, combined or as adjuvant therapy ameliorated malaria-induced oxidative assault. Supplementation with antioxidants represents a promising and enhanced therapeutic approach in malaria treatment. This review emphasized the beneficial roles of ascorbic acid and folate as adjuvants in malaria therapy.

Keywords: Antioxidant, ascorbic acid, folate, malaria.

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INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by the genus *Plasmodium*. It begins with a bite from an infected female anopheles mosquito, which introduces the protists via its saliva into the circulatory system, and ultimately to the liver where they mature and reproduce (Siciliano and Alano, 2015). The disease causes symptoms that typically include fever and headache, which can progress to coma or death in severe cases. Malaria is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas. The clinical consequences of malaria are diverse, and the prevalence of malaria-induced anaemia depends on the intensity of malaria transmission (White, 2018; Wroczynska *et al.*, 2005).

Significant progress has been recorded in the fight against malaria following the intensive use of control interventions, such as artemisinin-based combination therapy (ACT), development and use of rapid diagnostic tests (RDTs), long-lasting insecticidal nets (LLINs), and increased coverage of

indoor insecticide spraying. Even though malaria is preventable, it remains the leading cause of under-five morbidity and mortality in low and middle-income countries (Eba *et al.*, 2023; Seyoum *et al.*, 2023).

The ground-breaking recommendation of a cost-effective malaria vaccine for children at risk by the World Health Organization in 2021 is expected to significantly reduce malaria's impact. The RTS,S vaccine presents certain challenges because of its very ephemeral immunity, and it is recommended for short-term protection during the transmission season in regions of Africa with highly seasonal malaria. The research on malaria remains very active as there is a need to develop new strategies and tools to augment control and treatment (Rosenthal, 2022). The other approaches that have been developed and remodeled over the years focus on malaria vector control, early diagnosis and treatment with antimalarial drugs. However, treatment with antimalarial drugs is multifaceted and is faced with the challenge of drug resistance (Petersen *et al.*, 2011; Wicht *et al.*, 2020).

The role of oxidative stress during malaria infection has been reported to be either protective or involved in the pathophysiology of the disease. The generation of free radicals has been linked with the development of systemic complications caused by malaria parasites. Erythrocytes infected with *Plasmodium falciparum* were observed to produce more •OH radical and H₂O₂ compared to normal erythrocytes. The release of haem from the haemoglobin of the host during parasite nutrition at the erythrocytic stage of the disease has also been reported as a potential source of free radicals (Percário et al., 2012). The phagocytes in the host produce reactive oxygen species as an immune response to Plasmodium infection and this has been reported to contribute to the clearance of the parasite as they are highly susceptible to alteration in the redox equilibrium. Although this may offer a therapeutic potential, the excessive production of these free radicals can mediate inflammation and cause extensive damage to host cells and tissues and probably contribute to the severity of pathologies.

The relationship between the redox status of malaria parasites and that of their host is complex, and supplementation of antimalarial therapy with antioxidants have been studied extensively. This review highlights research conducted on the impact of antioxidants on antimalarial therapy using ANKA strain of chloroquine-sensitive *Plasmodium berghei*. The studies were conducted in the Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma.

Study 1: Impact of *Plasmodium berghei* and chloroquine on haematological and antioxidants indices in mice

The study investigated effect of chloroquine treatment and parasitaemia on haematological and antioxidant indices in mice. Three groups of ten mice each categorized as; Group I (control), Group II (non-parasitized chloroquine treated; NPcqT) and Group III (parasitized non-treated; PnT) were used in the study. Chloroquine sensitive ANKA strain of *Plasmodium berghei* was used for the malaria infection and chloroquine was administered at 25 mg/kg BW. The results of the study showed that parasitaemia (Group III) significantly increased oxidative stress parameters, the plasma total protein, globulin, erythrocyte fragility, total bilirubin and glucose-6-phosphate dehydrogenase (G6PD) activity. The liver superoxide dismutase and catalase activities were also significantly high compared to the other groups but the PCV was significantly lower in this group. In Group II, the erythrocyte fragility, plasma total bilirubin, PCV, plasma SOD, CAT, G6PD, GSH were significantly low in comparison with control, while liver SOD, CAT increased, and GSH concentration reduced significantly. Results from this study suggest that both malaria parasites and chloroquine treatment induced oxidative stress in mice (Iyawe and Onigbinde, 2009a).

Study 2: The chloroquine and folic acid interactions in respiration induced oxidative stress

The research assessed the influence of chloroquine and folic

acid on respiration-induced oxidative stress in healthy mice. A total of forty mice comprising twenty males and twenty females were used in the study. Each sex category was divided into four groups of five mice; Group I (control), Group II (chloroquine-treated), Group III (folic acid-treated) and Group IV (chloroquine-folic acid-treated). The drugs were administered at 25 mg/kg BW and treatment lasted for three days. In the male category, the ALT, AST, GGT activities and MDA concentration were lowest in Group III. There was no significant difference between CAT activities in Groups I and III. The activity of SOD was higher in Group IV. In the female category, the ALT, AST and GGT activities as well as the MDA concentration followed the pattern observed in the males. There were no significant differences but the activities and concentration were lower in Group III. The CAT and SOD activities were lower in Group III and highest in Group IV respectively. The result of this study corroborated the earlier observation on the antioxidant capacity of folic acid when administered in combination with an antimalarial. Owing to the observed reduction in the antioxidant activity of folic acid when administered in combination with chloroquine compared to when it was administered alone, it was suggested that chloroquine suppressed the antioxidant activity of folic acid (Iyawe and Onigbinde, 2006).

Study 3: The role of ascorbic acid in the treatment of *Plasmodium berghei* infected mice

This work aimed at examining the role of ascorbic acid in oxidative stress induced by malaria parasites. Three experimental groups; Group I (control; non-parasitized-nontreated), Group II (parasitized-nontreated; PnT) and Group III; parasitized, ascorbic acid treated; P+asc), were used for the study. The vitamin treatment lasted for three days after parasitaemia was established in the mice. The result revealed that ascorbic acid treatment ameliorated the oxidative assault and reduced the impact of parasitism on biomarkers. The erythrocyte fragility PCV, total bilirubin, direct bilirubin, indirect bilirubin and total protein in Group III were lower compared to Group II. This result aligned with the established roles of vitamins in the host during malaria infection and treatment (Iyawe and Onigbinde, 2009b).

Study 4: Ascorbic and folic acids intervention in *Plasmodium berghei* induced oxidative stress in mice

This research studied the effect of ascorbic and folic acids on the haematological and antioxidants status of malaria parasite infected mice. Three experimental groups; Group I (control; non-parasitized-nontreated), Group II (parasitized-nontreated; PnT) and Group III; parasitized, ascorbic and folic acid treated; P+as+faT), were used for the study. The vitamin treatment lasted for three days after parasitaemia was established in the mice. The result obtained from this study revealed that ascorbic and folic acid intervention during malaria significantly reduced ($p < 0.05$) total protein and erythrocyte fragility, and increased

($P < 0.05$) packed cell volume (PCV) in Group III when compared to Group II. Furthermore, the vitamins inhibited lipid peroxidation in serum, and reduced the superoxide dismutase and catalase activities, and lowered the concentration of reduced glutathione. The degree of lipid peroxidation in kidney tissue was also inhibited by the administration of the vitamins. In liver tissue, SOD, CAT and glucose-6-phosphate dehydrogenase (G6PD) activities significantly reduced following the administration of the vitamins. The result of this study suggested that the co-administration of ascorbic acid and folic acid can ameliorate the oxidative assault on the host during malaria parasite infection (Iyawe and Onigbinde, 2011).

Study 5: Effects of *Plasmodium berghei* infection and folic acid treatment on biochemical and antioxidant indicators in mice.

The research studied the specific effect of folic acid in the treatment of *P. berghei* infection in the absence of an antimalarial. A total of 30 mice divided randomly into three groups; Group I (control), Group II (parasitized-non treated) and Group III (parasitized + folic acid treated) comprising 10 mice each were used for the study. The result of the study revealed that erythrocyte fragility was highest in Group II, and in the same group, the lowest percentage PCV was recorded. The total, direct and indirect bilirubin concentrations in serum were highest in Group II. These parameters were also elevated in Group III but was not significantly different from Group I. The liver function enzymes and plasma protein parameters; AST, GGT, ALT, total protein and globulin followed the pattern observed for bilirubin concentration. However, the albumin concentration was not significantly different in Groups II and III. The results obtained from this study suggested that folic acid exhibits antioxidant capacity under parasitized condition and may effectively reduce oxidative stress (Iyawe and Onigbinde, 2010).

Study 6: Effect of chloroquine and ascorbic acid interactions on the oxidative stress status of *Plasmodium berghei* infected Mice

The aim of this study was to determine if ascorbic acid will exhibit antioxidant property during chloroquine treatment of parasitized mice. Forty mice (20 males and 20 females) randomly divided into four groups; Group I (control), Group II (parasitized-non-treated; PnT), Group III (chloroquine treated), and Group IV (chloroquine-ascorbic acid treated). The test groups were infected with ANKA strain of chloroquine sensitive *P. berghei* and treatment lasted for 3 days at 25 mg/kg BW.

In the male category, MDA concentration was significantly low in Group IV compared to the other groups. The SOD activity was highest in Group III but was not significantly different when Group IV was compared to group IV. The CAT, AST, ALT, GGT activities were not significantly different when Groups III and IV were compared to Group I. In the

female category, MDA concentration was significantly low in Group IV while the concentration observed in Group I and III were not significantly different. Unlike the male category, SOD activity was highest in Group IV but was not significantly different from the other groups. There was no significant difference in the CAT and GGT activities in Groups I, III and IV. The AST and ALT activities were significantly higher in Group IV. This study confirmed the antioxidant role of vitamins during malaria treatment and the addition of vitamins as adjuvant therapy did not induce hepatotoxicity (Iyawe *et al.*, 2006).

Study 7: Chloroquine and vitamin combination effects on *Plasmodium. berghei* induced oxidative stress

The study examined the combined effects of vitamins in combination with antimalarial (chloroquine) to determine if different types of vitamins have varying effect on the antimalarial capacity of chloroquine. In this study, the effect of chloroquine, folic and ascorbic acids on malaria parasite induced oxidative stress was determined. Five groups of experimental animals comprising 10 animals each; Group I = control (non-parasitized-nontreated; nPnT), Group II = parasitized nontreated (PnT), Group III = parasitized chloroquine and ascorbic acid treated (Pcq+asT), Group IV = parasitized chloroquine and folic acid treated (Pcq+faT) and Group V = parasitized chloroquine, ascorbic and folic acid treated (Pcq+asT+faT), were used for the study. The dose regimens were administered at 25 mg/kgBW and lasted for three days after parasitaemia was established with Gemsa stain. Biochemical and haematological parameters were assayed using standard procedures. The combination of chloroquine and vitamins significantly ($p \leq 0.05$) reduced erythrocyte fragility (EF) compared with Group II. The total bilirubin concentration was highest and significantly higher in the Group II. The difference was not significant in Groups III, IV and V but was slightly higher in Group III. The direct and indirect bilirubin components followed a similar pattern but indirect bilirubin was significantly lower in Group IV. The packed cell volume (PCV) was significantly lower in Group II and there was no significant change in Groups III, IV and V compared to Group I. The total serum protein, albumin and globulin concentrations in Groups III, IV and V were not significantly different but were higher than the concentrations observed in Group I. The AST, ALT and GGT activities were significantly higher in Group II.

The result of the liver tissue antioxidant status revealed that there was no significant difference between the groups treated with the antimalaria-antioxidant combination therapy. There was no significant difference in the MDA and GSH concentrations in Groups III, IV and V. The SOD and CAT activities were significantly lower in Group II but there was no significant difference between Groups I and IV as well as Groups III and V respectively. The activity of glucose-6-phosphate dehydrogenase was significantly higher in Group II and there was no significant difference between Groups III, IV and V. The results obtained revealed that chloroquine + folic

acid treatment was more effective compared to the other combinations used in the study (Iyawe and Onigbinde, 2012).

DISCUSSION

The debate on the role of oxidative stress in the pathogenesis of malaria and as an important strategy deployed by the host to combat the parasite has remained an active aspect of malaria research. As malaria parasites invade red blood cells and replicate, they generate reactive oxygen species (ROS) that can cause oxidative damage. Research evidence suggest that malaria-induced oxidative stress leads to the activation of proteins such as haem oxygenase which ultimately contributes to malaria pathology, including severe anaemia, severe cerebral malaria and death (Gomes *et al.*, 2022; Percário *et al.*, 2012).

In this review, the prooxidant role of malaria parasites and the antimalarial, chloroquine was established in Study 1. The observed prooxidant activity aligns with other research reports published by Becker *et al.* (2004), Percário *et al.* (2012) and Vasquez *et al.* (2021). Hydroxyl radicals (OH•) are generated in the liver during malaria infection, and infected erythrocytes release two-fold of the concentration of OH• and H₂O₂ compared to normal erythrocytes. The liberation of the haem during the erythrocytic stage is also associated with the consequent oxidative assault during malaria infection (Percário *et al.*, 2012).

In Study 2, the prooxidant capacity of chloroquine was confirmed. Several research evidences have shown that the quinolines including chloroquine, though having a good antimalarial capacity induce systemic oxidative stress. In vitro data have showed that chloroquine can form a complex with free-haem promoting the lipid peroxidation of phospholipid bilayers (Klouda and Stone, 2020). The capacity of free haem to promote peroxidative reactions was potentiated in presence of proteins while antioxidants decreased the degree of peroxidation (Herraiz *et al.*, 2019). In Studies 2, 3 and 5, the vitamins, folic acid and ascorbic acid demonstrated antioxidant capacity during malaria. Supplementation with antioxidants has been reported as a promising therapeutic approach in malaria to reduce the oxidative assault on the host by the parasites and the antimalarial. Ascorbic acid, alpha-tocopherol and folate are some of the most studied antioxidants in malaria therapy (Isah and Ibrahim, 2014; Klouda and Stone, 2020). Isah and Ibrahim (2014) published a detailed summary of the effect of vitamins as mono-, combination or adjuvant therapy to reduce the oxidative stress associated with malaria and its treatment. According to Isah and Ibrahim (2014), ascorbic acid demonstrates prooxidant activity in malaria by inhibiting the anti-plasmodial effects of cod liver oil that has been reported to exhibit antimalarial capacity based on the capacity to induce oxidative stress in the parasite. The prooxidant activity was attributed to the growth stage of the parasite and the presence of metal ions in the host cell. The co-administration of vitamin C and E ameliorated the malaria-induced changes in the oxidative stress markers as seen in the concentration of malondialdehyde, and activities of catalase and superoxide dismutase in the serum, liver and brain of the infected animals.

The decreased plasma concentrations of vitamins C and E in hosts during malaria has been attributed to their protective role during the infection. The observed additive antioxidant effects of the two vitamins has been attributed to the capacity of vitamin C to reduce α -tocopherol radicals in the oxidative chain reaction by vitamin E (Chen *et al.*, 2001; Ebhohimen *et al.*, 2021).

The free radical scavenging property of folate has been reported and its antioxidant properties in malaria studied in this research series revealed that it improved the efficacy of chloroquine and vitamin C treatment in *P. berghei*-infected mice. The role of folate during malaria has been suggested to be determined by the type of antimalarial treatment. The co-administration of folate with antifolates is not recommended except in areas with low resistance to these drugs (Isah and Ibrahim, 2014). The antioxidant effect of the vitamins co-administered during malaria infection and in combination with chloroquine reduced the oxidative assault compared to groups treated with antimalarial alone as evidenced (Iyawe *et al.*, 2006).

The World Health Organization (WHO) has projected that by 2030, the global prevalence rate of malaria in endemic regions will be reduced by at least 40%. This target is part of the WHO's Global Malaria Action Plan, which was launched in 2007 with the aim of reducing the global burden of malaria. However, the reduction in the prevalence of malaria is expected to vary from region to region. In the most affected regions, such as sub-Saharan Africa, the prevalence of malaria is expected to drop by more than 50%, while in other regions the reduction may be lower. Additionally, the WHO estimates that some countries may still face a higher burden of malaria in 2030 than in 2007. Understanding the role of adjuvant antimalarial therapies especially compounds with antioxidant capacity is crucial in the development of new drugs to treat malaria and the associated oxidative stress.

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