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Evaluation of Redox Status in *Plasmodium berghei*-Infected Mice Treated with Captopril

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AMA and AIK designed the study and wrote the protocol. Author MA carried out laboratory analyses. Author SLP performed the statistical analysis. Author RKB wrote the first draft of the manuscript. Author SYB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Alteration of redox status is a fundamental process in the manifestation of many diseases including malaria. Studies have demonstrated that peptides of the renin-angiotensin-aldosterone system (RAAS), such as Angiotensin Converting Enzyme and Angiotensin II, play a significant role in the pathogenesis of malaria infection by inducing the generation of reactive oxygen species. The present study was aimed at determining the redox status of *Plasmodium berghei*-infected mice treated with captopril, an angiotensin-converting enzyme inhibitor.
Methodology: Five groups of eight mice each, categorised as control (not infected with *P. berghei*, not treated), malaria control (*P. berghei*-infected, not treated), Standard control (*P. berghei*-infected, treated with 0.03 mg/kg of standard drug, Lonart (Arthemeter 20 mg + Lumefantrine 120 mg), captopril low dose (*P. berghei*-infected, treated with 0.03 mg/kg captopril) and captopril high dose (*P. berghei*-infected, treated at with 0.09mg/kg captopril). The mice were treated for 14 days and were sacrificed on the 15th day. Blood samples were collected to determine the levels of reduced

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glutathione, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and total protein.

Results: Infection with *P. berghei* significantly ($p<0.05$) increased MDA, while CAT and SOD levels decreased significantly ($p<0.05$) compared to mice in the control group. However, MDA levels of mice treated with the standard drug (Artemether 20 mg, Lumefantrine 120 mg), 0.03 and 0.09 mg/kg captopril reduced significantly ($p<0.05$), while CAT and SOD levels significantly ($p<0.05$) increased when compared to the malaria control mice. Total protein and reduced glutathione were found to be significantly ($p<0.05$) high in the malaria control group, whereas a decreased was observed in mice treated with the standard drug and 0.03 mg/kg captopril. Treatment of mice with 0.09 mg/kg captopril significantly increased total protein compared to mice in 0.03 mg/kg captopril group and other groups.

Conclusion: In conclusion, this study has demonstrated that captopril at a low dose can effectively decrease oxidative stress.

Keywords: Redox status; malaria; Plasmodium berghei; captopril.

1. INTRODUCTION

Malaria is the third leading cause of death worldwide, with Africa having the highest number of cases and bearing over 90% of the world malaria record [1]. The disease is caused by the parasitic protozoan belonging to *Plasmodium species* and transmitted by infected female *Anopheles* mosquitoes. Five (5) species of *Plasmodium* infect and can be spread by humans [2], but most deaths are caused by *Plasmodium falciparum,* as it is associated with cerebral malaria, a serious complication that usually results to death or cognitive and visual impairment [3].

In tropical countries, malaria and hypertension are common diseases, and recent findings suggest a possible link because of their physiopathologic similarities such as endothelial dysfunction, and production of pro-inflammatory cytokines. During the erythrocytic phase of malaria, red blood cells (RBCs) lyse and release hemozoin, which consists primarily of ferriprotoporphyrin IX dimers and monomers (FP) and methemoglobin in plasmodial proteins. Hemozoin induces the release of cytokines (TNF-α and IL-1) through cells of the monocyte/macrophages system. Free heme is a powerful free radical generator, which can cause serious molecular damage to both host and parasite. The heme group contains Fe^{2+} atoms that can catalyse Fenton and Haber-Weiss reactions, generating free radicals. These heme groups can induce intravascular oxidative stress, causing changes in erythrocytes and endothelial cells and facilitating the internalisation of the parasite in tissues such as the liver and brain [4].

Oxidative stress is a common pathogenic mechanism underlying the development of many diseases and conditions, including malaria infection where both the host and the parasite are affected. Various studies have shown that malaria parasite specifically targets and exerts oxidative stress on the RBCs of its host, which leads to oxidative damage on macromolecules such as proteins, nucleic acids and lipids, as well as hemolysis of the RBC with concomitant extensive damage to endothelial cells and other vital organs of the system [5]. The host's response to malaria is by the oxidative attack on intraerythrocytic parasites by activated macrophages thereby generating a large amount of reactive oxygen species (ROS) [6], leading to the death of the malaria parasite as a result of an imbalance between the formation of oxidising species and the activity of antioxidant. Studies have shown high levels of oxidative stress markers in infected humans and rats compared to uninfected groups [7,8]. *Plasmodium berghei* has developed an elaborate reduction-oxidation (redox) system to maintain adequate antioxidant defence mechanisms against any oxidative stress exerted by the host immune response during malaria infection throughout its lifecycle [9].

The role of angiotensin II (Ang II) in the development of oxidative stress has been reported [10]. Studies have demonstrated that Ang II is a pro-inflammatory and pro-fibrotic agent, which contributes to organ damage in disease, by inducing ROS production, cell growth, apoptosis, cell migration and differentiation disease [11]. Thus, inhibition of Ang II prevents the development of several non-infectious diseases. However, the effect of Ang II and other RAS peptides on the pathogenesis of malaria and other parasitic diseases is only just evolving, as these peptides have been shown to protect against malaria, by inducing impairment

of the erythrocytic cycle of *Plasmodium*, and reducing parasite growth [12]. There have been several scenarios of drug reactions and complaints from hypertensive patients having malaria. Therefore, understanding the effects of these antihypertensive drugs on relevant malaria-induced pathological processes is pertinent to further understand the biochemical basis for the antihypertensive-associated problems. Thus, this study aims to investigate the effect of inhibition of ACE on the redox status of *Plasmodium berghei* infected mice.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of forty (40) apparently healthy mice weighing between 24 g-34 g obtained from the animal breeding unit of the Department of Biological Science, Ahmadu Bello University, Zaria, Kaduna State, were used in the study. All animals were maintained at 8 per cage and maintained under standard conditions. They were allowed to acclimatise for two weeks before commencement of the experiment. The animals were allowed access to food and water *ad libitum* throughout the experiment.

2.2 Experimental Design

The effect of captopril on *P. berghei*-infected mice was investigated randomly by dividing mice into five groups of 8 mice each as follows; group 1 were not infected with *P. berghei* and not treated (normal control group), group 2 were infected with *P. berghei* and were not treated (Malaria control group), group 3 were infected with *P. berghei* and administered 0.03 mg/kg of Lonart (standard drug), groups 4 and 5 were infected with *P. berghei* and treated with 0.03 mg/kg and 0.09 mg/kg captopril, respectively. The mice were treated for 14 days, and on the 15^{th} day they were sacrificed and blood samples collected.

Doses were chosen after carrying out a pilot study to determine the effective oral dose of captopril that would significantly reduce parasitemia and decrease mortality.

All experimental protocols were approved and conducted with strict adherence to guidelines and procedures of the Institutional Animal Care and Use Committee of Bayero University, Kano.

2.3 Parasite Inoculation

The parasite, *Plasmodium berghei* (ANKA strain) was obtained from the Department of Parasitology, Faculty of Veterinary medicine Ahmadu Bello University, Zaria, Kaduna state. The experimental animals were given inoculums, which consists of *Plasmodium berghei*-parasitised red blood cells. Each experimental rat was intraperitoneally infected with 0.1ml of infected blood containing *Plasmodium berghe*i parasitised red blood cells. Also, the parasite level in the inoculated animals was monitored by microscopic test using thick and thin blood smears stained with Giemsa and Leishman stains respectively.

2.4 Determination of Catalase Activity

Catalase activity was determined according to the method of Aebi et al. [13]. Briefly, 0.1 ml of the blood sample was added to a cuvette containing 1.9 ml of 50 mM phosphate Buffer (pH 7.0). 1.0 ml of freshly prepared 30 mM H_2O_2 was then added to start up the reaction. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. The activity of catalase was expressed as units/mg protein [13].

2.5 Determination of Superoxide Dismutase Activity

SOD enzyme activity was determined according to the method describe by Sun and Zigman [14]. Briefly, 3.0 ml of 50 mM Na_2CO_3 buffer was added to 2 different test tubes, and 0.02 ml of serum sample was added. Then 0.03ml of the epinephrine stock solution was added to the tubes. Finally, absorbance was read at 480 nm.

2.6 Determination of Reduced Glutathione Concentration

The estimation of reduced glutathione (GSH) level was determined as described by Eilman [15]. About 0.1 ml of the blood sample was diluted in 0.1 M phosphate buffer (pH 7.4) and added to an equal volume of 20% trichloroacetic acid (TCA) containing 1mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5min before centrifugation for 10min at 200 rpm. The supernatant (200 µl) was then transferred to new test tubes and 1.8 ml of the Eilman's reagent (5, 5'-dithio bis-2-nitrobenzoic acid) added. All the test tubes were made up to 2

ml volume and absorbance measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from the standard curve of known GSH.

2.7 Determination of Malondialdehyde Concentration

Malondialdehyde (MDA) concentration was determined as an index to monitor lipid peroxidation, and this was done according to the method described by Ohkawa et al. [16]. Briefly, 0.2 ml of normal saline was pipetted into test tubes labelled as sample test and sample blank before adding 0.2 ml of serum into the sample test tubes only. About 0.5 ml of TCA solution was added to each of the sample tests and sample blank tubes, followed by 0.1 ml of TBA solution. The mixture in each tube was heated for 60 min in a water bath at 95℃ and then allowed to cool to room temperature on an ice bath, before adding 3 ml of n-butanol and then mixed vigorously. The absorbance of the pink supernatant (butanol phase) was read against the absorbance of the sample blank at 532 nm. MDA formed was then calculated using the molar extinction coefficient of 1.56 X 10^5 cm^{-1} M^{-1}.

2.8 Determination of Total Protein

The total protein was determined by Biuret method [17]. Briefly, 1.0 ml of Biuret reagent was added to three test tubes set as blank, standard and sample and 20 µl of standard and serum sample was pipette to the standard and sample test tube, respectively. Each test tube was mixed and incubated for 10 minutes at room temperature, and absorbance was read against reagent blank within 30 minutes at 546 nm.

2.9 Statistical Analysis

All experiments were carried out in triplicates, and the results were expressed as a mean ± standard deviation. Differences between the groups were analysed by one-way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) software (SPSS Inc., Chicago, Standard Version 20.0). P-values <0.05 were considered significant for differences in mean using the least of significance difference (LSD).

3. RESULTS AND DISCUSSION

The antioxidant system represents the main pathway to detoxify free radicals produced during haemoglobin metabolism in infected erythrocytes [18]. Detoxification of reactive oxygen species is one of the prerequisites of aerobic life and the antioxidant enzymes involved in these are the superoxide dismutase enzymes, glutathione S-transferases, and catalases [19]. Superoxide dismutase (SOD) plays a unique role in the survival of aerobic organisms, in that it protects the biological integrity of cells and tissues against the harmful effects of superoxide free radicals [20]. It is an important isoenzyme functioning as superoxide radicals' scavengers in the living organisms [21]. Its activity can be induced by stress [22], SOD is an important enzyme family in living cells for maintaining normal physiological conditions and coping with stress. Catalase is also a free radical-metabolising enzyme that protects the cell membrane from damage by the highly reactive free radicals [23]. The high level of SOD and catalase observed in control (50.00±5.25 U/ml and 2.22±0.02 U/mg) group as compared to the malaria control (33.33±4.43 U/ml and 0.93±0.01 U/mg) can be attributed to the absence or low level of oxidative stress, while the low SOD and catalase activities in malaria control mice signifies a diminished first line of antioxidant defence due to the infection. This event may be responsible for the presence of oxidative stress in the infected cells and thus contribute to pathophysiological effects of the disease. On the other hand, the high level of SOD and catalase activity in mice treated with the standard drug, Lonart (75.00±5.01 U/ml and 1.62±0.73 U/mg) and 0.03 mg/kg captopril (62.50±12.50 U/ml and 1.37±0.44 U/mg) shows that the drugs prevented oxidative stress probably by preventing pathological conditions in the affected organs. This confirms the effect of Angiotensin II inhibition by captopril as Angiotensin II has been shown to induce ROS generation [18].

Oxidative stress is associated with peroxidation of cellular lipids, which is determined by measurement of TBA-reactive substances. Measurement of lipid peroxidation end products such as TBA-reactive substances provides a good index of cell destruction because cells and tissues damaged by any mechanism tend to peroxidise more rapidly than normal [24]. Therefore, the concentration of lipid peroxidation products may reflect the degree of oxidative stress in malaria infection. The high level of MDA, an index of lipid peroxidation, conforms to studies by Siddiqi et al. [25] and has been attributed to the formation of hydroxyl radicals known to perturb the membrane [26]eventually

causing the increase observed in *P. berghei*-infected mice (Table 1). The elevation in lipid peroxidation in the plasma of infected mice may point to lipid peroxide level as a reflection of the severity of a disease process [27].

The concentration of reduced glutathione increased significantly ($p<0.05$) in the malaria control group compared to the normal group, whereas significant ($p<0.05$) decrease was observed after treatment with 0.03 mg/kg captopril and the standard drug (Figure 1). The glutathione cycle is one of the major systems for avoiding the deleterious effects of free radicals. Glutathione peroxidase (GPx) and glutathione S-transferase (GST) are known to use GSH as a substrate. The increase in GSH as observed in this study may be due to the infection, which increases radical metabolites, such as H_2O_2 and lipid peroxidation products, thus suppressing the ability of GPx and GST to utilise their substrate, GSH [28].

The result of total protein content is shown in Fig. 2. From the result, the induction of malaria significantly ($p<0.05$) increased total protein compared to the control group. This is similar to reports of previous studies where hyperproteinaemia and hyperglobulinaemia were observed in malaria-infected mice and rabbits respectively [20,21]. However, treatment with captopril (low dose) and lonart reduced total protein content. This may be attributed to the fact that both drugs increased lymphocyte

Table 1. Effect of captopril on some oxidative stress parameters in serum of *Plasmodium berghei*- infected mice

Groups	Normal control	Malaria control	Standard control	Captopril (0.03 mg/kg)	Captopril (0.09 mg/kg)
SOD (U/ml)	50.00 ± 5.25^{a}	33.33 ± 4.43^{b}	75.00 ± 5.01^{c}	62.50 ± 12.50^{c}	25.00 ± 0.01^{d}
CAT (U/mg)	2.22 ± 0.02^{a}	0.93 ± 0.01^{b}	1.62 ± 0.73^{a}	1.37 ± 0.44^{a}	0.89 ± 0.46^{b}
MDA(x10^{-6}moles/ml)	1.81 ± 1.73^{a}	3.41 ± 1.20^{b}	1.77 ± 1.04^{a}	1.99 ± 1.40^{a}	1.80 ± 1.32^{a}

MDA=Malondialdehyde, SOD=Superoxide dismutase, CAT=Catalase, Standard drug (Artemether 20mg, Lumefantrine 120mg)
Values are mean ± standard deviation
Values with different superscript along a row are significantly different ($p<0.05$)

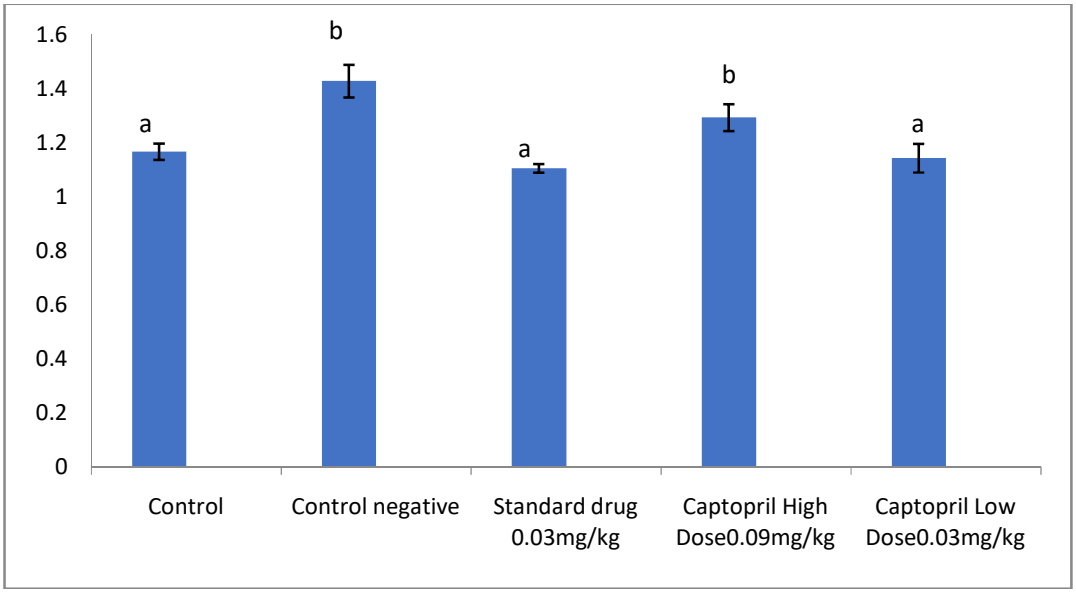

Fig. 1. Effect of captopril on GSH in serum of *Plasmodium berghei*- infected mice
Values are mean ± standard deviation
Values with different superscript letters are significantly different ($p<0.05$)

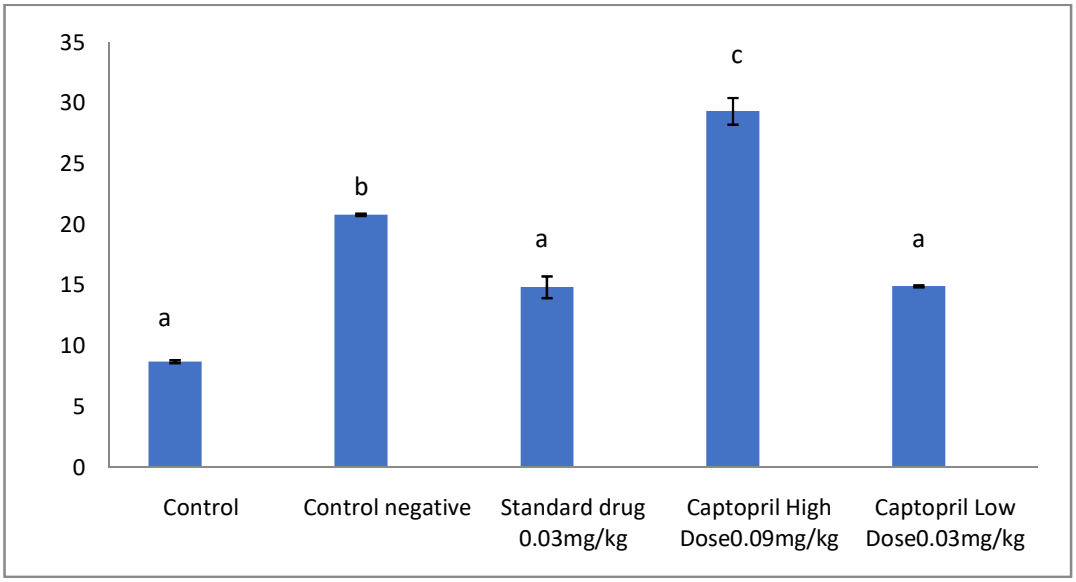

Fig. 2. Effect of captopril on total protein in serum of *Plasmodium berghei*- infected mice
Values are mean ± standard deviation
Values with different superscript letters are significantly different (p<0.05)

circulation and the activities of these cells to cause reductions in parasite density and consequently a reduction in protein secretion by lymphocytes [29].

4. CONCLUSION

This study shows that the standard antimalarial drug (Artemether 20 mg, Lumefantrine 120 mg) and captopril significantly reduced (P<0.05) oxidative stress associated with malaria. The reduction of oxidative stress parameters by captopril in *P. berghei*-infected mice shows that the renin-angiotensin system may play a significant role in the pathogenesis of malaria infection.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental protocols were approved and conducted with strict adherence to guidelines and procedures of the Institutional Animal Care and Use Committee of Bayero University, Kano.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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