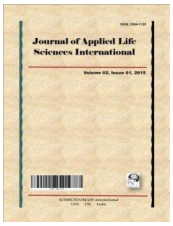

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Effect of Captopril on Some Haematological Parameters and DNA Fragmentation in *Plasmodium berghei*-Infected Mice

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Authors' contributions

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

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ABSTRACT

Background: Despite all efforts to establish the pathogenesis of malaria, it is far from being understood, and resistance to existing drugs remain a problem in several strains of *Plasmodium*. Different complications arise during malaria infection, and among these is the alteration in haematological parameters. This study was undertaken to evaluate the effect of captopril, an angiotensin converting enzyme (ACE) inhibitor, on some haematological parameters and DNA fragmentation in blood of *Plasmodium berghei*-infected mice.
Methodology: Forty (40) apparently healthy mice were divided into five groups of eight mice each: control (not infected, not treated), malaria control (*P. berghei*-infected, not treated), Standard control (*P. berghei*-infected, treated with 0.03 mg/kg Lonart: Artemether 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 0.09 mg/kg captopril). The mice were treated for 14 days, and parasitemia monitored every other day. The mice were sacrificed on the 15th day and blood

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obtained for biochemical analysis: plasma full blood count and DNA fragmentation.
Results: Induction of malaria significantly (P<0.05) decreased the red blood cell, haematocrit and platelet count, while haemoglobin level increased significantly (P<0.05) in malaria control mice compared to mice in the control group. Also, the plasma mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration decreased significantly (P<0.05) in malaria control group compared to the control group. Treatment with the standard drug, lonart and captopril improved all the haematological parameters, while haematological parameters of mice treated with 0.09 mg/kg of captopril were brought back to normal. With respect to DNA fragmentation, treatment of *P. berghei*-infected mice with lonart and captopril significantly (P<0.05) increased percentage DNA fragmentation compared to mice in control and malaria control groups.
Conclusion: This result shows that captopril, especially at high concentration, may play a protective role against malaria infection.

Keywords: Plasmodium berghei; haematological parameters; captopril; angiotensin converting enzyme.

1. INTRODUCTION

Malaria is a mosquito-borne infectious disease that affects humans and animals. It is caused by parasitic protozoans belonging to the *Plasmodium* family [1]. Malaria causes symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases, it causes yellow skin, seizures, coma or death [2]. Five (5) species of *Plasmodium* can infect and be spread by humans [3], but most deaths are caused by *P. falciparum* because *P. ovale, P. vivax* and *P. malariae* generally cause a milder form of the disease. The specie, *P. knowlesi* rarely cause disease in humans [1].

Studies have shown that the malaria parasite specifically targets red blood cells (RBCs) of its host, leading to complicated and harmful pathophysiologies causing oxidative stress. This leads to the oxidative damage of macromolecules such as proteins, nucleic acids and lipids, as well as hemolysis of the RBCs with concomitant extensive damage to endothelial cells and other vital organs within the system [4], resulting in haematological changes, known to be among the most common complications encountered in malaria. Haematological parameters are measurable indices of blood that serve as a marker for disease diagnosis [5]. Alterations in these parameters may be related to overt biochemical changes that occur during the asexual stage of the life cycle of the malaria parasite, because entry of *P. falciparum* into erythrocytes, usually leads to a marked secretion of inflammatory cytokines, endothelial cell activation, activation of coagulation cascade and sequestration of parasitized red blood cells. Also, alterations in these parameters are thought to have the capacity to act as adjuvant tool in strengthening the suspicion of malaria [6].

Hypertension, traditionally considered a disease of western countries, has reached epidemic level in Africa, even presenting higher prevalence than in wealthier countries. The idea of a causative connection between hypertension and malaria has fascinated researchers, as the prevalence of hypertension is higher in populations that have been exposed to malaria for long periods [7,8]. As a consequence, a significant number of Africans are currently taking anti-hypertensive medication, and this number is expected to increase exponentially in the coming years [8]. Although hypertension and malaria do not have an obviously direct cross influence, evidence in literature show that Angiotensin II (Ang II) and other peptides of the renin angiotensin aldosterone system (RAAS) are associated with the pathogenesis of malaria infection [9]. Ang II is produced from Ang I by the action of ACE in the RAAS. It has been demonstrated that Ang II has beneficial effects on malaria by decreasing the invasion of human erythrocytes by *P. falciparum* and delaying the development of cerebral malaria. Other studies have shown that inhibitors of Ang II and Ang II receptors provide protection against malaria [10]. However, there is paucity of information on the effect of these drugs on haematological parameters and DNA fragmentation, which are among the most common complications encountered in malaria [11,12]. Thus, this study was aimed at determining the effect of captopril on some haematological parameters and DNA fragmentation in *Plasmodium berghei*-infected mice.

2. MATERIALS AND METHODS

2.1 Reagents

Diphenylamine solution (containing diphenylamine powder, glacial acetic acid, acetaldehyde and sulfuric acid), Phosphate buffer (pH 7.4), TTE solution (Tris EDTA buffer at pH 7.4 with 0.2% Triton X-100 stored at 4°C), Trichloroacetic acid (TCA), Methanol, Giemsa's stain and Leishman's stain were obtained from Sigma Aldrich (U.S.A). All other reagents used were of analytical grade.

2.2 Experimental Animals

A total of forty (40) apparently healthy male mice weighing between 24 g to 34 g obtained from the animal breeding unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, were used for the study. All animals were maintained 8 per cage and were fed standard animal feeds and maintained under standard conditions. They were allowed to acclimatize for two weeks before commencement of experiment.

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 parasite was inoculated into the experimental animals intraperitoneally on day zero, with 0.2 ml containing about 1×10^7 of parasitized red blood cells. In addition, the newly inoculated animals were monitored daily to determine the blood level of parasites.

2.4 Experimental Design

A pilot study was conducted to determine the effective dose of captopril that would significantly reduce parasitemia and decrease mortality before the commencement of the experiment. The drugs were prepared by obtaining the average weight of the mice and comparing it with the standard dosage given to human.

Drug dosage (in mg) =

$$\frac{\text{Weight of mice} \times \text{Standard dosage administered}}{\text{Average weight of human}}$$

Weight of mice was between 24-34 g, hence the average weight of 30 g was used, while 70 Kg was used for human.

70 Kg (70,000 g) =75 mg captopril
30 g = 0.032 mg/kg captopril

A higher dose of 0.09 mg/kg was also chosen after the pilot study.

The mice were randomly divided into five groups of 8 mice each as follows; group 1 were not infected with malaria 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/ml of Lonart (standard drug group), groups 4 and 5 were infected with *P. berghei* and treated with 0.03 and 0.09 mg/kg captopril, respectively. The mice were treated for 14 days and parasitemia level monitored every other day. On the 15th day, mice were sacrificed and blood was obtained for biochemical analysis.

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.

2.5 Determination of Full Blood Count

This was determined based on the Beckman Coulter method of counting and sizing, in combination with automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scattergram plots the cells based upon the measurements of these three parameters [13].

2.6 Determination of DNA Fragmentation

DNA Fragmentation was determined using Diphenylamine method as described by Sellins and Cohen [14]. Exactly 1:1 dilution of blood samples was done using phosphate buffer (pH 7.4) in well-labelled test tubes B. The cells were centrifuged at 200xg at 4°C for 10 minutes and the supernatants carefully transferred into new tubes labelled S. About 100 µl TTE solution was added to the pellets in tubes B and vortexed vigorously to allow the release of fragmented chromatin for nuclei, after cell lysis (due to the presence of Triton X-100 in the TTE solution) and disruption of nuclear structure (following Mg++ chelating by EDTA in the TTE solution). Tubes B were centrifuged at 20,000xg for 10

minutes to separate fragmented DNA from intact chromatin and supernatants were carefully transferred into new tubes labelled T. Another 100 µl TTE solution was added to the small pellets in tubes B, while 100 µl of 25% of TCA was added to tubes T, B and S and vortexed vigorously. Precipitation was allowed to proceed overnight at 4°C and precipitated DNA was recovered by pelleting for 10 minutes at 20,000xg at 4°C. The supernatants were discarded by aspiration and DNA was hydrolyzed by adding 160 µl of 5% TCA and heated for 15 minutes at 90°C in a heating block. A blank was prepared with 160 µl of 5% TCA alone. About 320 µl of freshly prepared DPA solution was added to each tube and vortexed. The colour was allowed to develop for about 4 hours at 37°C. Two 200 µl aliquots of coloured solution were transferred (ignoring dark particles) from each tube to a glass tube (cuvette), and absorbance was read at 600nm using a spectrophotometer.

The percentage of fragmented DNA was calculated using the formula;

$$\%\ \text{fragmented DNA} = \frac{T \times 100}{T + B}$$

2.7 Statistical Analysis

All experiments were carried out in triplicate and the results were expressed as mean $\pm$ standard deviation. Differences between the groups were analyzed 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 renin-angiotensin-aldosterone system (RAAS) is one of the most important regulatory systems of blood volume, arterial pressure and cardiovascular homeostasis. Angiotensin II (Ang II) is a principal effector hormone of the RAAS in vascular biology. It mediates its effects via two main receptors; Type I (AT1) and Type II (AT2) [15]. When Ang II binds to AT1 on vascular smooth muscles cells, it mobilizes intracellular Ca^{2+}, leading to cellular contraction, which if sustained increases peripheral vascular resistance, resulting in high blood pressure [16]. Recent reports have shown that Angiotensin peptides can induce impairments of the erythrocytic cycle of *Plasmodium*, reducing the parasite growth *in vitro* [17,18]. Reports have shown that the development of Cerebral Malaria (CM) depends on the initial levels of parasitaemia in mice and possibly in humans [19,20], hence, this could be an explanation for Angiotensin II protection from CM.

From the results of the plasma full blood count analysed (Table 1), it was observed that the full blood count parameters of the mice were altered after infection with *P. berghei*. The RBC, haematocrit (HCT) and platelet (PLT) levels decreased significantly ($p<0.05$) in mice within the malaria control group than the normal control, standard control and group treated with 0.09 mg/kg captopril. The RBC decreased ($p<0.05$) significantly in the malaria control group (6.1±0.18 $x10^3$ U/L), when compared to the normal control group (9.32±0.20 $x10^3$ U/L), standard control (8.12±0.28 $x10^3$ U/L) and group treated with 0.09 mg/kg captopril (9.14±0.19 $x10^3$ U/L). There was a significant ($p<0.05$) decrease in the levels of HCT in the malaria control group (40.1±0.66%) when compared to the normal control (46.0±0.64%), standard control (42.76±0.76%) as well as group treated with 0.09 mg/kg captopril (42.22±2.26%). Also, HGB significantly ($p<0.05$) increased in the malaria control group (15.2±0.30 g/dl). There was no significant ($p>0.05$) difference between the normal control group (14.1±0.18 g/dl) and the group treated with captopril at 0.09 mg/kg (13.6±0.48 g/dl). The levels of PLT decreased significantly ($p<0.05$) in the malaria control group (1250.4±4.27 $x10^3$ U/L), when compared to the normal control group (1350.4±4.27 $x10^3$ U/L), standard control (1319.6±8.82 $x10^3$ U/L) and 0.03 mg/kg captopril (1325±15.3 $x10^3$U/L). The findings of this study correlate with reports of previous findings, which shows that abnormalities such as anemia and thrombocytopenia are observed in patients with malaria [21]. The observed decrease in the levels of RBCs in the plasma in the malaria control group could be due to the lysis of RBCs by the malaria parasite [12].

The results for the plasma concentration of white blood cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) are presented in Table 2. From the results, there was no significant ($p>0.05$) difference in WBC of mice in the normal control group (8.53±0.04 $x10^3$ U/L), standard control group (8.48±0.03 $x10^3$ U/L), and mice

given 0.09 mg/kg (8.51±0.01 x10^3 U/L) and 0.03 mg/kg (8.46±0.03 x10^3U/L) captopril, while that of mice in the malaria control group (8.81±0.06 x10^3 U/L) was significantly ($p<0.05$) higher than all other groups. The MCV values in malaria control group significantly ($p<0.05$) decreased when compared to the normal control group (from 49.2±0.13 to 42.1±0.27 fl), while the MCH level in malaria control mice though significantly ($p<0.05$) lower than the normal control group (15.0±0.18 pg), increased significantly after treatment with the 0.09 mg/kg captopril (15.1±0.19 pg). With respect to the levels of MCHC, there was a significant ($p<0.05$) decrease in the malaria control group compared to normal control (from 31.22±0.82 to 24.1±0.52 g/L). These results show evidence of hemolysis on induction with *P. berghei*. This has been attributed to either the accumulation of the drugs within neutral lipids causing parasite and/or host membrane damage or generation of endoperoxides radicals that can negatively affect the three-dimensional structure of the membrane proteins and lipid bilayer leading to haemolysis [22].

It has been established that malaria parasite basically targets RBCs, however, it is not nucleated but other lineages like lymphocytes, monocytes, macrophages and neutrophils are nucleated having DNA as part of their genetic material [23,24]. Hence, damage to this DNA can be evaluated using DNA fragmentation assay which allows for determination of the amount of DNA that is degraded upon treatment of cells with certain agents as a function of staining deoxyribose with diphenylamine [13]. The plasma DNA fragmentation results of treated and untreated mice (Fig. 1) shows a significant ($p<0.05$) increase in the levels of fragmented DNA in malaria control group (1.73±0.06%) compared to the normal control group (0.27±0.00%). This further increased significantly ($p<0.05$) in mice treated with the standard drug, lonart (3.23±0.11%). Treatment with 0.09 mg/kg captopril (2.26±0.02) and 0.03 mg/kg captopril (2.68±0-02%) significantly ($p<0.05$) increased the levels of fragmented DNA in a dose-dependent manner when compared to the standard control group. The considerably higher percentage DNA Fragmentation in the blood of mice treated with the standard drug is evident that antimalarial drugs increase DNA fragmentation. This agrees with studies by Muhammad et al. [4] who demonstrated that administration of antimalarial drugs to patients suffering from malaria, especially at a high dose increases DNA fragmentation [4]. This has been attributed to the generation of ROS during infection or by the reactive intermediates after the biotransformation of drugs. This signifies that most of the antimalarial drugs used may be genotoxic as fragmented DNA is one of the basic markers of toxicities which might be due to apoptotic or necrotic form of cell death. The effect of Fenton and Haber Weiss reactions

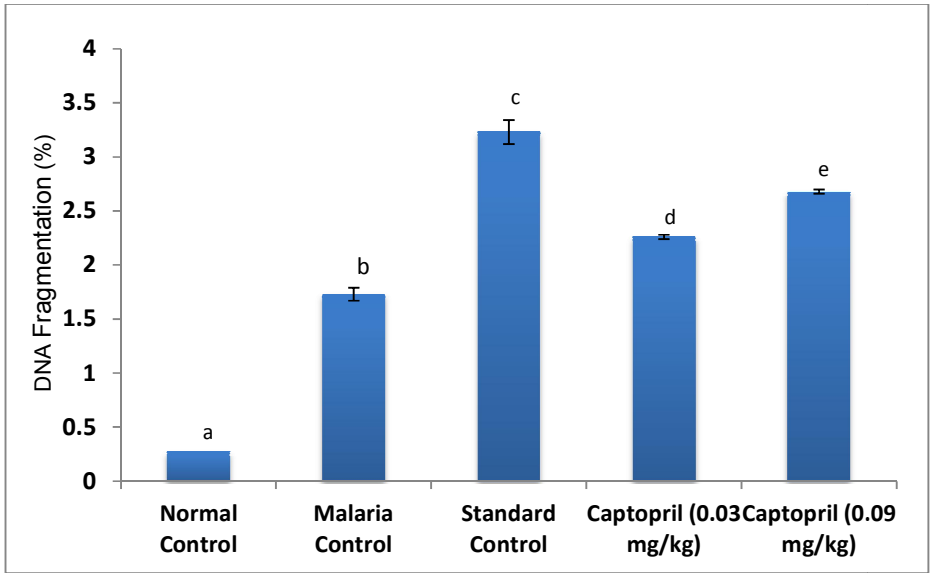

Fig. 1. Percentage (%) DNA fragmentation in plasma of treated and untreated mice infected with *Plasmodium berghei*

Values are Mean ±standard deviation Values with different letters are significantly different at p<0.05

Table 1. Plasma concentration of RBC, HCT, PLT and HGB in treated and control mice

Parameters	Normal control	Malarial control	Standard drug	Captopril (0.03 mg/kg)	Captopril (0.09 mg/kg)
RBC(10^3U/L)	9.32±0.20[a]	6.1±0.18[b]	8.12±0.28[c]	9.14±0.19[a]	8.12±0.10[d]
HCT (%)	46.0±0.64[a]	40.1±0.66[b]	42.76±0.76[c]	46.12±0.29[a]	42.22±2.62[d]
PLT(10^3U/L)	1350.4±4.27[a]	1250.4±4.27[b]	1319±8.82[c]	1349±3.20[a]	1325±15.30[c]
HGB (g/L)	14.1±0.18[a]	15.2±0.30[b]	13.36±0.35[c]	13.6±0.48[a]	14.1±0.23[a]

Values are Mean ±standard deviation
Values with different superscript along a row are significantly different at p<0.05.
RBC= Red Blood Cells, HCT= Haematocrit, PLT= Platelet, HGB= Haemoglobin

Table 2. Plasma concentration of WBC, MCV, MCH and MCHC in treated and control mice

Parameters	Normal control	Malarial control	Standard control	Captopril (0.03 mg/kg)	Captopril (0.09 mg/kg)
WBC(10^3U/L)	8.53±0.04[a]	8.81±0.06[b]	8.48±0.03[a]	8.51±0.01[a]	8.46±0.03[a]
MCV(fL)	49.2±0.13[a]	42.1±0.27[b]	48.5±0.25[b]	49.3±0.12[a]	48.4±0.28[b]
MCH(pg)	15.0±0.18[a]	14.0±0.34[b]	14.4±0.33[b]	15.1±0.19[a]	14.4±0.37[c]
MCHC(g/L)	31.22±0.82[a]	24.1±0.52[b]	30.9±0.55[a]	32.0±1.00[a]	30.9±0.55[a]

Values are Mean ±standard deviation
Values with different superscript along a row are significantly different at p<0.05.
WBC= White Blood Cells, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration

as a result of haemolysis, released haemoglobin and subsequently iron due to heme catabolism is the release of hydroxyl radical which can attack guanine at its C-8 position to yield an oxidation product, 8-hydroxydeoxyguanosin [25]. It can also attack other bases like adenine to yield 8 (or 4-, 5-)-hydroxyadenine. Other products are the results of interactions between pyrimidines and hydroxyl radicals leading to the formation of thymine peroxide, thymine glycols, 5-(hydroxymethyl) uracyl, and other such products that can easily cause DNA damage [25].

4. CONCLUSION

In conclusion, this study has shown that hematological parameters are altered in *P. berghei*-infected mice, and treatment with captopril stabilized these parameters. Also, Lonart (Artemether 20 mg, Lumefantrine 120 mg) and captopril induced DNA fragmentation as a consequence of DNA damage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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