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Effect of an Angiotensin Converting Enzyme Inhibitor (Captopril) on Liver and Kidney Function Parameters 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 NSA and BAA carried out the laboratory work. Author RKB performed the statistical analysis and wrote the first draft of the manuscript. Author AIK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to investigate the effect of captopril on serum liver enzymes (AST, ALT, and ALP), total serum protein and kidney function parameters such as electrolytes (HCO_3^-, Cl^-, Na^+, and K^+), urea and creatinine in *Plasmodium berghei*-infected mice.
Methodology: A total of 40 apparently healthy mice were randomly divided into five groups of 8 mice each; normal control group (not infected, not treated), malaria control (malaria-infected, not treated), standard control (malaria-infected, treated with 0.03 mg/kg of standard drug, Arthemeter 20 mg + Lumefantrine 120 mg), group 4 (malaria-infected, treated with 0.03 mg/kg of captopril) and group 5 (malaria-infected, treated with 0.09 mg/kg of captopril). The mice were treated for 14 days and parasitemia level monitored every other day. On the 15th day, mice were sacrificed and blood obtained to determine serum liver enzymes (AST, ALT, and ALP), total protein, serum electrolyte (HCO_3^-, Cl^-, Na^+, and K^+), urea and creatinine levels.
Results: There was a significant increase (P<.05) in the % parasitemia, serum liver enzymes,

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urea, creatinine, and K^+ after induction of malaria. This decreased significantly (P<.05) when mice were treated with 0.03 mg/kg of standard drug and 0.09 mg/kg of captopril compared to mice treated with 0.03 mg/kg of captopril and malaria control mice. However, serum total protein, Na^+, K^+, and Cl^- decreased significantly (P<.05) in the malaria control group but increased significantly (P<.05) when treated with 0.03 mg/kg of standard drug and 0.09 mg/kg captopril compared to mice treated with 0.03 mg/kg of captopril.

Conclusion: This study has shown that captopril, at high concentration may be beneficial against malaria infection and renal disorders.

Keywords: Angiotensin converting enzyme; captopril; Plasmodium berghei; parasitemia; malaria.

1. INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan belonging to the *Plasmodium* family [1]. The disease is widely spread in tropical and sub-tropical regions, including much of sub-Saharan Africa, Asia and Latin America [2]. In 2015, there were 214 million cases of malaria worldwide [3]. This resulted in an estimated 438,000 deaths, 90% of which occurred in Africa [4]. The rates of the disease decreased from 2000 to 2015 by 37% [3]. Five species of *Plasmodium* infect humans. *Plasmodium falciparum* causes most deaths, *Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae* cause a milder form of malaria [2], while *Plasmodium knowlesi* rarely causes disease in humans. The malaria parasites usually affect the kidney, liver and brain. Both renal and hepatic malfunction can determine the level of severity of malaria infection. The clinical manifestation of renal involvement is associated with disease by *P. falciparum* and *P. malariae* [5]. The exoerythrocytic form of the malarial parasite invades the liver causing an increase in the activities of liver enzymes in serum; an evidence of liver dysfunction and compromise in membrane integrity [6]. Electrolytes (sodium, potassium, chloride, bicarbonates, etc.) are minerals present in blood and other body fluids, whose optimum range is essential for proper physiological activities. Thus, electrolyte imbalances and mineral disturbances are common clinical manifestations in several infectious diseases including malaria [7]. Sodium (Na^+) is the major cation of extracellular fluid, that regulates the normal distribution of water and osmotic pressure in various body fluids. Various health problems occur due to Na^+ disturbance [8], for example, hyponatremia, considered an important clinical manifestation of malaria, exaggerates the disease symptoms and results in severe malaria [9]. Potassium is identified as a crucial electrolyte for the accurate functioning of all body cells, tissues and organs. It is particularly important in skeletal and smooth muscle contraction. A low level of Potassium, called hypokalaemia is a common complication of severe malaria [10]. Antimalarials are the medications used for the treatment of malaria. Resistance among parasites has developed to several antimalarial medications [4]. *Plasmodium berghei* is one of four species of *Plasmodium* that infect murine rodents in Africa. *P. berghei* is widely used as model organism for the study of human malaria and is transmitted by *Anopheles* mosquitoes. *P. berghei* infects the liver after being injected into the bloodstream by a bite of an infected female mosquito. After a short period (a few days) of development and multiplication, these parasites leave the liver and invade erythrocytes (red blood cells). The multiplication of the parasite in the blood causes the pathology such as anaemia and damage of essential organs of the host such as lungs, liver and spleen. *P. berghei* infections may also affect the brain and can be the cause of cerebral complications in laboratory mice. These symptoms are to a certain degree comparable to symptoms of cerebral malaria in patients infected with the human malaria parasite *Plasmodium falciparum* [11].

The renin-angiotensin-aldosterone system is one of the most important regulatory systems for blood volume, arterial blood pressure, and cardiovascular homeostasis. Angiotensin II is the principal effector hormone of renin-angiotensin-aldosterone system in vascular biology mediating effects through two receptors: Angiotensin receptor type I and angiotensin receptor type II [12]. New findings highlight the importance of host-specific signaling pathways that can control parasite invasion and development [13]. Some reports have shown that Angiotensin peptides can induce impairment of erythrocytic cycle of *Plasmodium*, reducing parasite growth *in vitro* [13,14]. It has been reported that treatment of *Plasmodium* infected mice with captopril, known

to reduce angiotensin II level decreases parasitemia level and protects against experimental cerebral malaria [15]. Other studies have reported the protective effect of angiotensin II and other peptides of Renin-Angiotensin-Aldosterone System (RAAS) in malaria outcome. However, there is paucity of information on the effect of these treatments on kidney and liver function, and ultimately, malaria outcome.

2. MATERIALS AND METHODS

2.1 Reagents

Reagents used were commercially obtained from reputable manufacturers. For alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine amino transeferase (ALT), urea, and creatinine, reagent sets obtained from RANDOX laboratories, Atrim, UK, was used. For total protein, Spectrum reagent kit was used. While reagent set from Teco diagnostic laboratories was used to assay serum chloride, potassium, sodium, and carbon dioxide. Distilled water, Normal saline, Giemsa's stain, Leishman's stain used were of analytical grade.

2.2 Animals

A total of forty (40) apparently healthy 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.

2.3 Experimental Design

A pilot study was conducted to determine the effective oral 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 were between 24-34 g, hence 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.

All animals were maintained 8 per cage, fed standard animal feeds and water and maintained under standard conditions. They were allowed to acclimatize for two weeks before commencement of experiment. The mice were randomly divided into five groups of 8 mice each as follows;

Group I: Normal control (not infected, not treated)

Group II: Malarial control (infected with *P.berghei,* not treated)

Group III: Malarial and treated with 0.03 mg/kg of standard drug (Arthemeter 20 mg + Lumefantrine 120 mg)

Group IV: Malarial and treated with Captopril (0.03 mg/kg)

Group V: Malarial and treated with Captopril (0.09 mg/kg).

2.4 Parasite Inoculation

ANKA strain of *Plasmodium berghei* was obtained from Department of Parasitology, Faculty of veterinary medicine, Ahmadu Bello University Zaria, Kaduna State. Parasite was inoculated into the experimental animals. Each mouse was inoculated intraperitoneally on day zero, with 0.2 ml containing about 1×10^7 of parasitized red blood cells of infected blood containing *P. berghei* parasitized red blood cells. In addition, the newly inoculated animals were monitored daily to determine the blood level of parasites. The mice were treated for 14 days and parasitemia level monitored every other day. On the 15^{th} day, mice were sacrificed and blood was obtained for biochemical analysis.

2.5 Estimation of Parasitemia

Malaria parasite was estimated by microscopic test using thick and thin blood smears stained with Giemsa and Leishman stains. Thick and thin films were carried out as described by Monica [16]. Blood was collected from the tail of the mice and the relative malaria parasite count in each blood sample was determined from Giemsa

stained thick blood film and Leishman stained thin blood film. Malaria parasite was confirmed by microscopic examination using x100 objective lens (oil immersion lens). Percentage parasitemia was calculated as follows after staining for 30 minutes and viewed under the microscope:

> % parasitemia = (Number of parasitized red blood cell / Total number of normal red blood cells counted) ×100.

2.6 Biochemical Analysis

Serum aspartate aminotransferase (AST), alanine transaminase (ALT) was determined by the method of Reitman and Frankel [17] which is based on transamination reaction. Serum alkaline phosphatase (ALP) was assayed by Rec [18] method, while serum total protein was assayed by Biuret method [19]. Serum creatinine was determined by colorimetric method described by Narayanan and Appleton [20]. Serum urea was determined by the Urease-Berthelot's method described by Barnett et al [21]. Chloride was determined by a direct method based on the modification of the colorimetric method of Skeggs and Hochstrasser [22]. Sodium was determined based on modifications of those first described by Maruna [23]. Serum bicarbonate was determined calorimetrically.

2.7 Statistical Analysis

One-way ANOVA was performed to depict statistical differences using GraphPad InsSat3 software (2000) version 3.05 by GraphPad Inc. The results were presented as Mean ± Standard deviation and a *P*-value < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

The present study was carried out to explore the hypothesis that captopril, an ACE inhibitor has a positive effect on malaria, by monitoring its effect on parasitemia, liver and kidney function parameters in *P. berghei*-infected mice. The investigation carried out to assess the percentage (%) level of parasitemia in the treated and control mice before and after treatment showed that induction with *P. berghei* increased parasitemia in all groups when compared to that of the normal control group. However, after 14 days of treatment, parasitemia level decreased significantly (*P*<.05) back to normal in mice treated with 0.09 mg/kg of captopril (0.03±0.01%) and 0.03 mg/kg of standard drug, lonart (0.02±0.00%). Lonart is a combination of 80 mg Artemether and 480 mg Lumefantrine. It acts within the malaria parasite causing morphological changes within the parasite membranes due to the presence of the endoperoxide bridge in artemether that causes release of free-radicals; while lumefantrine interferes in the polymerization processes. Both compounds also cause morphological changes in ribosomes as well as in the endoplasmic reticulum resulting in inhibition of protein synthesis [24].

The % parasitemia of mice treated with 0.03 mg/kg of captopril (4.26±0.11%), though lower than malaria control (6.17±0.06) was higher than that of mice given 0.09 mg/kg of captopril, showing a dose dependent effect (Table 1). This result correlates with studies by Silva-Filho et al. [15] who reported that losartan and captopril reduced parasitemia, promoted survival benefits and attenuated signals involved in the development of cerebral malaria. They attributed this to the inhibition of ACE by captopril, resulting in low levels of Angiotensin II, and accumulated Ang I being converted to Ang (1-7), which binds specifically to the Mas receptors decreasing the T-cell response and protein kinase A (PKA) activity and consequently reducing the parasite invasion.

Results of liver function parameters showed significantly (P<.05) higher levels of ALP, AST and ALT in malaria control mice than mice in all other groups. This observation agrees with the reports by Onyemakonor et al. [25] who demonstrated that *P. falciparum* malarial infection significantly increased the serum activities of AST, ALT, and ALP in a manner that positively correlates with the parasites' density and concluded that malarial infection confers a measure of hepatic compromise. Treatment with 0.03 mg/kg of standard drug and 0.09 mg/kg captopril significantly (*P*<.05) decreased the level of the enzymes. Captopril at 0.03 mg/kg also decreased the serum liver enzymes, but significantly (*P*<.05) higher than that of the group treated with 0.09 mg/kg. The serum protein level of malaria control mice was not significantly (P>0.05) different from normal control group but was significantly (*P*<.05) lower than those of mice treated with 0.03 mg/kg standard group and those treated with 0.09 mg/kg of captopril (Table 2).

Table 1. Percentage parasitemia level before and after administration of drugs in *Plasmodium berghei*-infected mice

Days	Normal control	Malaria control	Standard control	Captopril (0.03 mg/kg)	Captopril (0.09 mg/kg)
Before Induction	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
After Induction	0.00±0.00[a]	4.74±0.70[b]	4.79±0.01[b]	4.80±0.01[b]	4.77±0.03[b]
2	0.00±0.00[a]	4.82±0.04[c]	3.95±0.03[b]	4.81±0.06[c]	3.96±0.01[b]
4	0.00±0.00[a]	4.94±0.06[c]	2.76±0.13[b]	4.91±0.05[c]	2.78±0.11[b]
6	0.00±0.00[a]	5.01±0.01[c]	1.95±0.04 [b]	4.96±0.05[c]	1.96±0.04[b]
8	0.00±0.00[a]	5.22±0.01[d]	0.83±0.01[b]	4.95±0.04[c]	0.81±0.01[b]
10	0.00±0.00[a]	5.64±0.01[d]	0.18±0.05[b]	4.77±0.12[c]	0.19±0.06[b]
12	0.00±0.00[a]	5.87±0.01[d]	0.06±0.02[b]	4.69±0.05[c]	0.07±0.02[b]
14	0.00±0.00[a]	6.17±0.06[d]	0.02±0.00[a]	4.26±0.11[c]	0.03±0.01[a]

Values are Mean ±standard deviation

Values with different superscript along a row are significantly different at P<.05

Table 2. Effect of captopril on some liver function parameters in *Plasmodium berghei*-infected mice

Parameters	Normal control	Malaria control	Standard control	Captoprl (0.03 mg/kg)	Captopril (0.09 mg/kg)
ALP (U/L)	35.3 ± 1.52[a]	80.33 ± 2.51[b]	35.00 ± 2.00[a]	51.00 ± 4.00[c]	39.33±2.51[a]
AST (U/L)	87.33 ± 4.04[a]	200.0 ± 11.13[b]	106.67 ± 10.40[c]	132.33 ± 4.16[d]	104.33±4.04[c]
ALT (U/L)	29.00±3.00[a]	51.60±2.51[b]	31.00±3.00[a]	39.66±1.52[c]	33.00±4.35[c]
Total protein (mg/dl)	5.60±0.40[a]	5.40±0.25[a]	6.50±0.30[b]	6.10±0.20[b]	6.30±0.55[b]

Values are Mean ±standard deviation

Values with different superscript along a row are significantly different at P<.05

Table 3. Serum concentrations of creatinine and urea in treated and untreated *Plasmodium berghei*-infected mice

Parameters (mg/dl)	Normal control	Malaria control	Standard control	Captoprl (0.03 mg/kg)	Captopril (0.09 mg/kg)
Creatinine	0.70 ± 0.1[a]	7.14 ± 3.56[c]	0.70 ± 0.10[a]	3.86 ± 1.05[b]	0.73 ± 0.10[a]
Urea	21.25 ±7.62[a]	88.14 ± 7.36[c]	28.58 ±13.50[a]	50.05 ± 8.24[b]	29.26 ± 18.9[a]

Values are Mean ±standard deviation

Values with different superscript along a row are significantly different at P<.05

From the results of the study it was observed that the creatinine levels in the malaria control group (7.14±3.56 mg/dL) was significantly (*P*<.05) higher than the normal control and all treated groups, while there was no significant (*P*>0.05) difference in the level of creatinine of mice in the normal control group (0.70±0.1 mg/dL), standard control group (0.70±0.10 mg/dL) and those treated with 0.09 mg/kg of captopril (0.73±0.1 mg/dL). Among complications that are associated with malaria, renal dysfunctions are common in malaria endemic regions [26,27]. This study showed a significant increase in the level of urea and creatinine in the malaria control group when compared to that of the control group. The observed increase in creatinine and urea could be as a result of sequestration of the parasite into the renal microvasculature bed, which may lead to ischemia [28]. The level of urea increased significantly (*P*<.05) in the malaria control group (88.14 ± 7.36 mg/dL) compared to the normal control group (21.25 ±7.62 mg/dL). However, treatment with the standard drug (28.58 ±13.50 mg/dL), 0.03 (50.05 ± 8.24 mg/dL) and 0.09 mg/kg captopril (29.26 ± 18.9) decreased it significantly (*P*<.05). There was no significant (*P*<.05) difference in urea levels of mice given the standard drug, 0.09 mg/kg captopril and normal control (Table 3 above).

Table 4. Serum electrolyte level in treated and untreated *Plasmodium berghei*-infected mice

Parameters (mmol/L)	Normal control	Malaria control	Standard control	Captoprl 0.03 mg/kg	Captopril 0.09 mg/kg
Sodium (Na^+)	151.68±17.78[a]	97.34±8.57[c]	149.41±11.40[a]	121.30±14.24[b]	148.52 ±6.19[a]
Potassium (K^+)	5.11±0.03[a]	15.50±0.51[c]	5.12±0.13[a]	10.37±0.52[b]	5.39±2.26[a]
Chloride (Cl^-)	103.24±11.31[a]	31.75±18.72[c]	101.23±22.32[a]	67.49±5.15[b]	100.67±22.27[a]
Bicarbonate (HCO_3)	17.65±1.10 [a]	8.51±1.36 [c]	17.37±1.35 [a]	14.04±0.87[b]	17.32±1.41 [a]

Values are Mean ±standard deviation
Values with different superscript along a row are significantly different at P<.05

The results of serum electrolytes (sodium, potassium, chloride, and bicarbonate) concentration in both treated and control mice showed that the serum level of Na^+, HCO_3^- and Cl^- in the normal control (151.68±17.78 mmol/L, 17.65±1.10 mmol/L and 103.24±11.31 mmol/L, respectively), standard control (149.41±11.40 mmol/L, 17.37±1.35 mmol/L and 101.23±22.32 mmol/L, respectively) and group treated with 0.09 mg/kg captopril (148.52±6.19 mmol/L, 17.32±1.41 mmol/L and 100.67±22.27 mmol/L, respectively) were not significantly (*P*>.05) different. In mice treated with 0.03 mg/kg captopril, Na^+, HCO_3^- and Cl^- levels (121.30±14.24 mmol/L, 14.04±0.87 mmol/L and 67.49±5.15 mmol/L, respectively) were significantly (*P*<.05) lower than all other groups, except the malaria control group (97.34±8.57 mmol/L, 8.51±1.36 mmol/L and 31.75±18.72 mmol/L, respectively) which decreased significantly (*P*<.05) upon induction of malaria. With respect to potassium (K^+), the mean value increased significantly (*P*<.05) in malaria control mice (15.50±0.51 mmol/L) than normal control mice (5.11±0.03 mmol/L), standard control mice (5.12±0.13 mmol/L), mice treated with 0.03 (10.37±0.52 mmol/L) and 0.09 mg/kg captopril (5.39±2.26 mmol/L). There was no significant (*P*>.05) difference in the level of K^+ between the normal control (5.11±0.03 mmol/L), standard control (5.12±0.13 mmol/L), and 0.09 mg/kg captopril (5.39±2.26 mmol/L) mice. Electrolyte imbalances in the body may also serve as an indicator of renal failure and this has been indicated in malaria-infected individuals [29]. In this study, there was significant decrease in the level of Na^+, Cl and HCO_3^- in the malaria control group when compared to that of the normal control. The level of Na^+, Cl and HCO_3^- increased significantly back to normal levels in mice treated with 0.09 mg/kg of captopril than those treated with 0.03 mg/kg of captopril. A significant increase in the level of K^+ was recorded in the malaria control group when compared to that of the normal control (Table 4). This result conforms to previous report indicating hyponatraemia and hyperkalemia during malaria infection [30,31]. It has been reported that impaired glomerular filtration as a result of malaria infection could be responsible for the reduction in the Na^+ available in the renal tubule for K^+ exchange. This may lead to the increase in the serum K^+ level [30,32], but on the administration of 0.09 mg/kg of captopril, the level of K^+ decreased significantly back to normal level when compared to the level of K^+ in the groups treated with 0.03 mg/kg of captopril. However, this study correlates with another study that reports that angiotensin-converting enzyme inhibitors (e.g. Captopril) can be used for the treatment of renal (kidney) related disorders [33].

4. CONCLUSION

This study has shown that captopril, an angiotensin-converting enzyme inhibitor at high concentration 0.09 mg/kg reduces kidney related disorders associated with malaria. It also possesses hepatoprotective effect, by decreasing serum levels of the enzymes with minimal effect at low concentration 0.03 mg/kg. Thus, captopril may be beneficial against malaria-induced pathology in mouse models, hence, may possess the potential therapeutic effect as an adjunctive treatment for malaria, although further studies are required to establish this.

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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