Original Article

Kidney Toxicity Studies in Mice (BALB/C) Recurrently Infected with *Plasmodium berghei* and Treated With either Artemether plus Lumefantrine (AL) or Artesunate plus Amodiaquine (AA)

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ABSTRACT

Individuals residing in regions where malaria is endemic are frequently exposed to the disease and subsequently treated. The frequent exposure to malaria and its treatment could exert a deleterious effect on the kidneys, which are responsible for eliminating metabolites. This could potentially lead to oxidative stress and impair their function. Therefore, this study aimed to investigate the potential consequences of repeated exposure to malaria parasites and treatment with artemether plus lumefantrine (AL) or artesunate plus amodiaquine (AA) on kidney oxidative stress and functional markers. Three groups of male mice were randomly assigned for the study: the control group was administered distilled water, while the other two groups were infected with *Plasmodium berghei* and treated with either AL or AA for six consecutive periods. The study parameters were examined in the blood and kidney tissues following the initial, third, and sixth exposure intervals. The concentration of malondialdehyde (MDA) in the kidneys was significantly higher in mice exposed to *P. berghei* and treated with either AL ($p<0.001$) or AA ($p<0.01$) after the first, third, and sixth exposures than in the control group. Following the third and sixth exposures to *P. berghei* and AL or AA, there was a considerable increase ($p<0.001$) in the activities of kidney glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). The observed increases in MDA, GPx, SOD, and CAT levels did not follow a consistent upward trend. Furthermore, no statistically significant differences ($p>0.05$) were identified in the plasma levels of sodium, potassium, chloride, and creatinine between the groups exposed to *P. berghei* and treated with AL or AA and the control group following the sixth exposure. Histological analysis revealed the presence of glomerular edema in the kidney tissue of mice infected with *P. berghei* and treated with AL or AA during the initial, third, and sixth exposure periods. Mice that were repeatedly exposed to malarial parasites and treated with either AL or AA showed elevated levels of kidney lipid peroxidation during consecutive exposures. However, there was also evidence of elevated levels of GPx, SOD, and CAT activity in the kidneys, which may have protected against lipid peroxidation and preserved renal function. Nevertheless, the observed antioxidant activity proved to be insufficient for the prevention of glomerular edema.

Keywords: Kidney, Malaria, Antimalarial Drugs, Repeated Exposure, Oxidative Stress, Renal Function.

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1. Introduction

Malaria represents a substantial global health risk, predominantly affecting communities situated in tropical and subtropical regions. The causative agent, *Plasmodium* parasites, primarily infect humans through the bites of female *Anopheles* mosquitoes. Artemisinin-based combination therapies (ACTs) are employed for the management of uncomplicated malaria and have had a substantial impact on the prevention of fatalities and severe cases of malaria (1). ACTs combine fast-acting artemisinin-based drugs with partner drugs, offering significant effectiveness, rapid action, and reduced resistance development compared to single drugs. The World Health Organization (WHO) has identified five ACTs as being of particular importance. The five ACTs are: artesunate combined with amodiaquine (AA), artesunate paired with mefloquine (AM), artesunate-sulfadoxine coupled with pyrimethamine (ASP), artemether joined with lumefantrine (AL), and dihydroartemisinin combined with piperaquine (DP) (1,2). The kidney is a vital organ that plays a crucial role in maintaining fluid balance, regulating electrolytes and acid-base balance, regulating blood pressure, and removing waste products. It can be affected by malaria and antimalarial drugs (3). Malaria infection can result in kidney damage through cytokine production, iron overload, and endothelial cell injury. This may lead to oxidative stress, as evidenced by elevated levels of reactive oxygen species (ROS), lipid peroxidation products, and oxidative stress markers in individuals with malaria (4,5). Antimalarial drugs, particularly artemisinin derivatives, quinine, and chloroquine, have been demonstrated to induce nephrotoxicity through a number of mechanisms. These include interference with mitochondrial activity, depletion of glutathione levels, and the induction of apoptosis, which generates ROS and causes mitochondrial dysfunction in renal tubular cells (2,3,5,6). The occurrence of renal toxicity associated with antimalarial drugs is typically low; however, the primary concern is their administration to individuals with pre-existing kidney conditions (3). Another potential issue is the frequent use of these drugs in malaria-endemic countries, where recurrent exposure to malaria infections and treatment is common due to the high prevalence of the disease in these areas (7,8). This could pose a significant challenge to kidney metabolic activities. Accordingly, the present study was undertaken to investigate the potential consequences of repeated exposure to malaria and subsequent treatment with ACTs on oxidative stress and functional markers of the kidney, employing a well-established rodent model (6,9). The mice were infected and treated individually and repeatedly with *Plasmodium berghei* and either AL or AA, which mimicked recurrent malaria infections and treatments.

2. Materials and Methods

2.1. Animals' Procurement and Management

A total of 36 male BALB/c mice with an average age of 8 weeks and a mean weight of 25 g were obtained from the Institute of Advanced Medical Research and Training at the University College Hospital in Ibadan, Nigeria. The rodents were housed in enclosures lined with wood shavings and provided with feed produced by Ladokun Feed Limited, a company based in Ibadan, Oyo State, Nigeria. The mice were provided with water and maintained on a 12-hour light/dark cycle throughout the course of the study.

2.2. Antimalaria Drugs

The antimalarial drugs Lumartem and Camosunate were procured from Cipla Pharmaceuticals Limited and Geneith Pharmaceuticals Limited, respectively. Lumartem contains artemether plus lumefantrine (20 mg/120 mg) and was administered in six doses of 1.14/6.84 mg/kg/day at intervals of 0, 8, 24, 36 48, and 60 hours. Camosunate comprises artesunate and amodiaquine in a 100 mg/300 mg ratio, and it was administered in three doses of 2.86/8.58 mg/kg/day for three consecutive days (6,9).

2.3. Parasite

The *P. berghei* NK65 strain employed in this study was obtained from the Institute of Advanced Medical Research and Training at the University College Hospital (UCH) in Ibadan, Nigeria. Microscopic slides were prepared from donor mouse tail blood and examined using a 100x oil immersion objective lens. The calculation of parasitemia entails dividing the number of infected red blood cells by the total, and subsequently multiplying the result by 100. The mice were intraperitoneally infected with 0.1 ml of a solution containing parasitized blood and normal saline, with a concentration of 10^4 parasites. Tail blood samples from infected mice were stained and examined under a microscope at two-day intervals to monitor the infection. The parasitaemia levels were less than 10% on day 7 in each consecutive infection, and no parasitaemia was observed on day 7 following either AL or AA therapy.

2.4. Research Design

A total of 36 male mice were randomly assigned to three distinct groups, as previously described in the literature (6, 9). Each group comprised 12 mice, housed in three cages of four mice each.

Group 1: The control group was provided with only distilled water.

Group 2: Six consecutive exposures to *P. berghei* were followed by theadministration of artemether plus lumefantrine (AL).

Group 3: Six consecutive exposures to *P. berghei* were followed by the administration of a combination of artesunate and amodiaquine (AA).

Mice were administered AL and AA for three consecutive days, commencing on the seventh day post-infection with *P. berghei*. The infection and treatment cycles were repeated six times, with a one-week interval break between each cycle. The daily weight was meticulously monitored , and blood and kidney tissue samples were obtained for oxidative stress and kidney function marker analyses at the completion of the first, third, and sixth cycles.

2.5. Oxidative Stress Markers Test
The level of malondialdehyde (MDA) was evaluated through a thiobarbituric acid-reactive substances (TBARS) assay (10). The concentration of reduced glutathione (GSH) was determined in accordance with the methodology outlined by Ilman (1959). The determination of glutathione peroxidase (GPx) activity was conducted in accordance with the methodology outlined by Rotruck et al. (11). The activity of superoxide dismutase (SOD) was evaluated in accordance with the methodology elucidated by Marklund and Marklund (12). The evaluation of catalase activity was conducted in accordance with the methodology delineated by Shangari and O'Brien (13).

2.6. Kidney Function Test
The method described by Murray (14) was utilized to quantify the concentration of creatinine in plasma and renal homogenates, as described in the Fortress diagnostic kit manual. Sodium concentrations in plasma and kidney homogenates were determined in accordance with the procedure outlined in the Teco Diagnostic Kit Manual(15). Potassium concentrations in the plasma and kidney homogenates were determined using the methodology described by Terry and Sesin (16), as outlines in the Teco Diagnostic Kit Manual. The chloride concentrations in the plasma and kidney homogenates were determined using the methodology described by Burtis *et al*. (17), as outlined in the Cypress diagnostic kit manual.

2.7. Histopathological Analysis of the Kidney
The kidney tissues were placed in labeled bottles containing 10% formalin for subsequent analysis. Subsequently, the organs were dehydrated for one hour each through a series of ethanol concentrations, with the concentration increasing from 50% to 100%. Subsequently, the organs were immersed three times in xylene. The organs were then encased in melted paraffin wax, which solidified into blocks. Subsequently, 5µm sections were obtained from the aforementioned blocks by mounting them on a microtome. The tissue samples were examined microscopically to identify any changes, and images of the microscopic images were captured.

2.8. Statistical Analysis
A one-way analysis of variance (ANOVA) was employed to ascertain the significance of any discrepancies between the treatment and control groups. The generation of the corresponding graphs was accomplished with the use of GraphPad Prism 9.0. The data are presented as mean ± standard error of the mean (SEM) (n=3). The initial step in determining whether the data followed a normal distribution was to employ the Shapiro-Wilk test. The symbols *, **, and *** indicate statistically significant differences relative to the relevant control groups at the 0.05, 0.01, and 0.001 levels of significance, respectively.

3. Results
3.1. Effects of repeated *P. berghei* exposure and corresponding artemether plus lumefantrine (AL) or artesunate plus amodiaquine (AA) treatment on mouse body and kidney organ weights
Mice that were repeatedly exposed to *P. berghei* and treated with either AL or AA demonstrated a notable increase in the percentage change in body weight following the initial, third, and sixth exposure periods. This increase followed a pattern that commenced with the initial exposure period and continued to grow until the third and sixth periods (Table 1). Nevertheless, following the initial, third, and sixth exposures, no statistically significant difference was observed in kidney organ weight when compared to the control mice (Table 1).

3.2. Effects of repeated *P. berghei* exposure and corresponding AL or AA treatment on kidney tissue oxidative stress markers in mice
Following the initial, third, and sixth exposure periods, a statistically significant increase in malondialdehyde (MDA) levels was observed in both *P. berghei*-infected groups that received AL ($p<0.001$) and AA ($p<0.01$) compared to their respective control groups. It is important to note, however, that this increase did not follow an upward trend (Figure 1A). Following the third and sixth exposure periods, both the *P. berghei*-infected and AL or AA treatment groups exhibited a notable elevation ($p<0.001$) in kidney GPx, SOD, and CAT activities relative to the control group. The *P. berghei*-infected and AL treatment groups demonstrated the most pronounced activities. Similarly to the observed increase in MDA levels, the elevations in GPx, SOD, and CAT levels did not exhibit a consistent upward trajectory (Figure 1 C, D, and E). Following the conclusion of the initial exposure period, mice infected with *P. berghei* and administered AL exhibited a statistically significant ($p<0.01$) increase in CAT activity compared to the control group. (Figure 1 E).

3.3. Impact of repeated *P. berghei* exposure and corresponding AL or AA treatment on kidney function parameters in mice plasma
Following the initial exposure period, a marked increase ($p<0.001$) in plasma sodium concentration was observed in *P. berghei*-infected mice that had been administered either AL or AA treatment, in comparison to the control group (as illustrated in Figure 2A). As a consequence of the third exposure period, only the group infected with *P. berghei* and treated with AA exhibited a statistically significant increase ($p<0.001$) in plasma sodium levels. Following the third exposure to *P. berghei* and administration of AL, a statistically significant increase ($p<0.01$) in plasma potassium concentration was observed in comparison to the control group (Figure 2B). The plasma chloride concentration was found to be significantly lower following the initial exposure to *P. berghei* and treatment with AA ($p<0.001$) and following the third exposure period to *P. berghei* and treatment with AL ($p<0.01$) when compared to the control group (Figure 2C). Following the initial and third exposures to *P. berghei* infection and AL and AA treatments, no significant alterations in plasma creatinine levels were observed in comparison to the control group

(Figure 2D). Upon the conclusion of the sixth exposure period, no appreciable variations were observed in the plasma levels of sodium, potassium, chloride, and creatinine between mice infected with *P. berghei* that received either AL or AA treatment and the control group (Figure 2A. B. C. and D).

3.4. Impact of repeated *P. berghei* exposure and corresponding AL or AA treatment on kidney functional parameters and histopathology of kidney tissues

The findings of this study demonstrated a notable elevation in renal tissue sodium concentration following the third and sixth exposures to *P. berghei* and the corresponding treatment with AL ($p<0.01$) in comparison to the control group (Figure 3A). However, no significant alterations in potassium and chlorine concentrations in the kidneys were observed following the first, third, and sixth exposures to *P. berghei* and the corresponding treatments with either AL or AA, when compared to the control group (Figure 3B and 3C). The concentration of creatinine in kidney tissue exhibited a notable increase ($p<0.001$) in both the *P. berghei*-infected and AL-treated groups following the initial exposure period and AA treatment after the third period, in comparison to the control group (Figure 3D). Nevertheless, no notable alterations were discerned in creatinine levels following the sixth exposure to *P. berghei* and AL or AA treatment, when compared to the control group (Figure 3D). The histopathological changes observed in mice infected with *P. berghei* and treated with either AL or AA after the first, third, and sixth exposure periods were characterized by edema in the glomerulus (Figure 4). Conversely,congestion of blood vessels in the interstitium was observed only in mice infected with *P. berghei* and treated with AA after the first and third exposure periods (Figure 4).

Table 1: Body and kidney organ weight of mice infected with *P. berghei* and treated with either artemether/lumefantrine or artesunate/amodiaquine after the first, third, and sixth exposure periods.

Exposure Periods	Groups	Initial Body Weight (g)	Final Body Weight (g)	Change in Body weight (%)	Kidney Organ Weight (g)
First	CTL	23.57±0.28	25.53±0.38	8.39±2.39	0.37±0.05
	INF+AL	24.50±0.31	26.87±0.55	9.75±3.54	0.40±0.03
	INF+AA	24.17±0.18	24.80±0.47	3.04±2.42	0.32±0.01
Third	CTL	25.30±0.25	30.10±1.04	18.92±3.01	0.41±0.06
	INF+AL	24.20±0.44	29.07±0.15	20.17±1.57	0.36±0.04
	INF+AA	24.67±0.07	30.67±0.88	24.31±3.33	0.43±0.04
Sixth	CTL	25.27±0.27	31.60±0.83	29.22±4.14	0.44±0.04
	INF+AL	24.70±0.21	31.37±0.35	27.03±2.35	0.37±0.01
	INF+AA	24.53±0.29	30.83±0.44	25.73±2.82	0.47±0.08

CTL= Control, INF+AL= infected with *P.berghei* and treated with Artemether plus lumefantrine, INF+AA = Infected with *P.berghei* and treated with Artesunate plus Amodiaquine

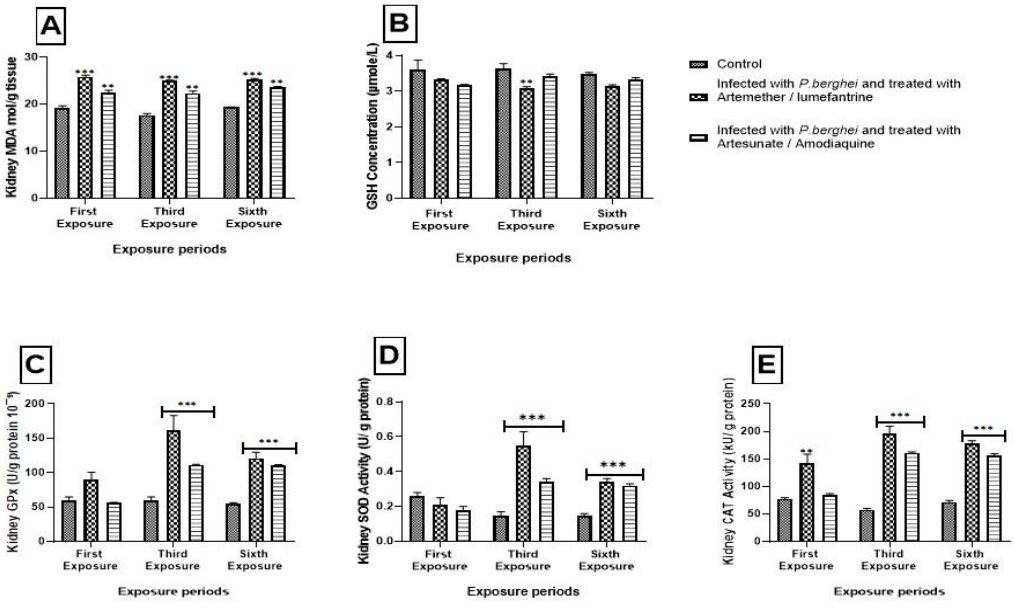

Figure 1. Kidney tissue (A) Malondialdehyde (MDA) concentration, (B) Glutathione (GSH) concentration, (C) Glutathione peroxidase (GPx) activities (D) Superoxide dismutase (SOD) activities (E) Catalase (CAT) activities of mice after the 1st, 3rd, and 6th Infection with *P. berghei* and treatment with artemether plus lumefantrine (AL) or artesunate plus amodiaquine (AA).

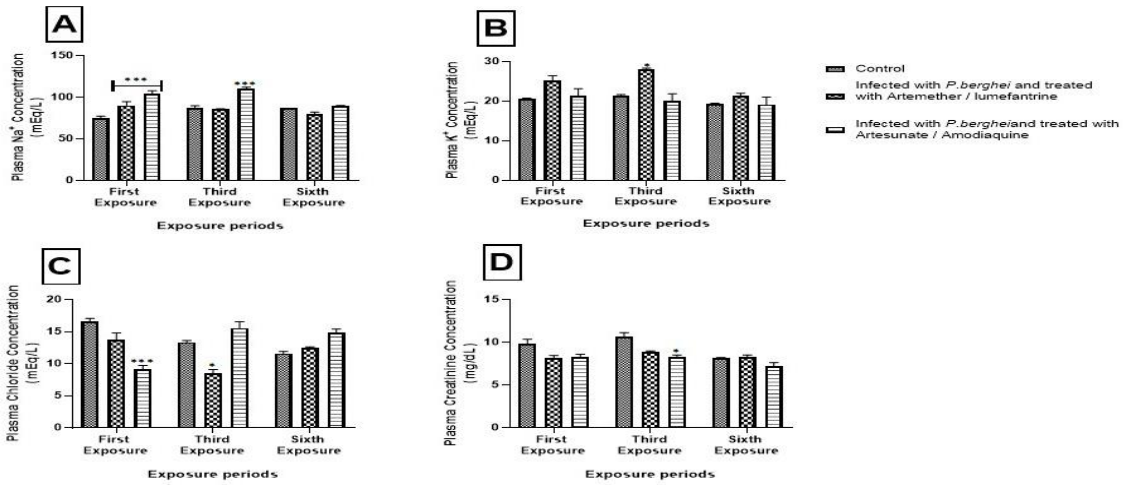

Figure 2. Plasma (A) Sodium (Na^+) concentration, (B) potassium (K^+) concentration, (C) chloride (Cl^-) concentration (D) Creatinine concentration of mice after the 1st, 3rd, and 6th Infection with *P. berghei* and treatment with artemether plus lumefantrine (AL) or artesunate plus amodiaquine (AA).

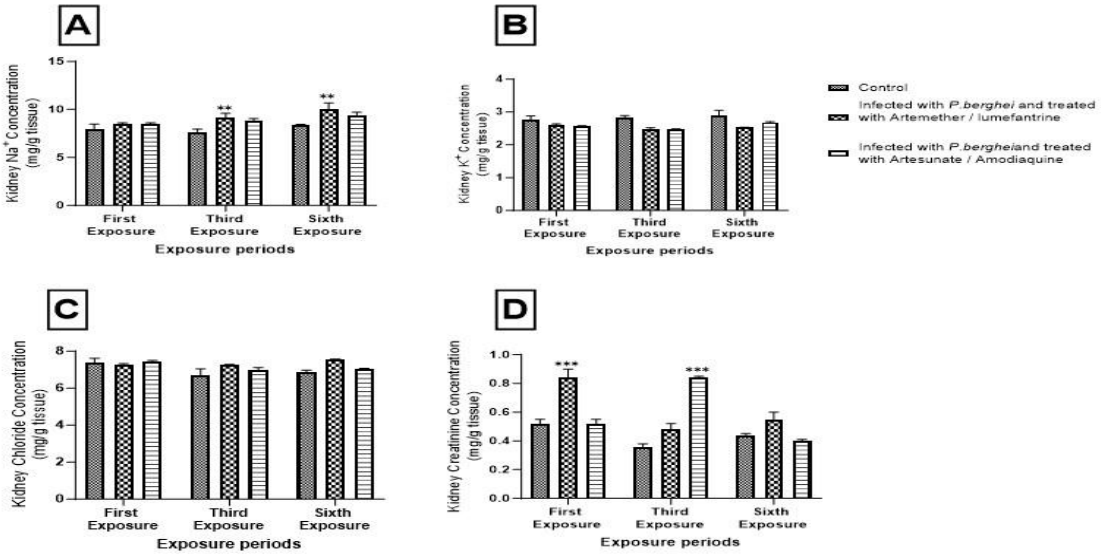

Figure 3. Kidney (A) Sodium (Na^+) concentration, (B) potassium (K^+) concentration, (C) chloride (Cl^-) concentration (D) Creatinine concentration of mice after the 1st, 3rd, and 6th Infection with *P. berghei* and treatment with artemether plus lumefantrine (AL) or artesunate plus amodiaquine (AA).

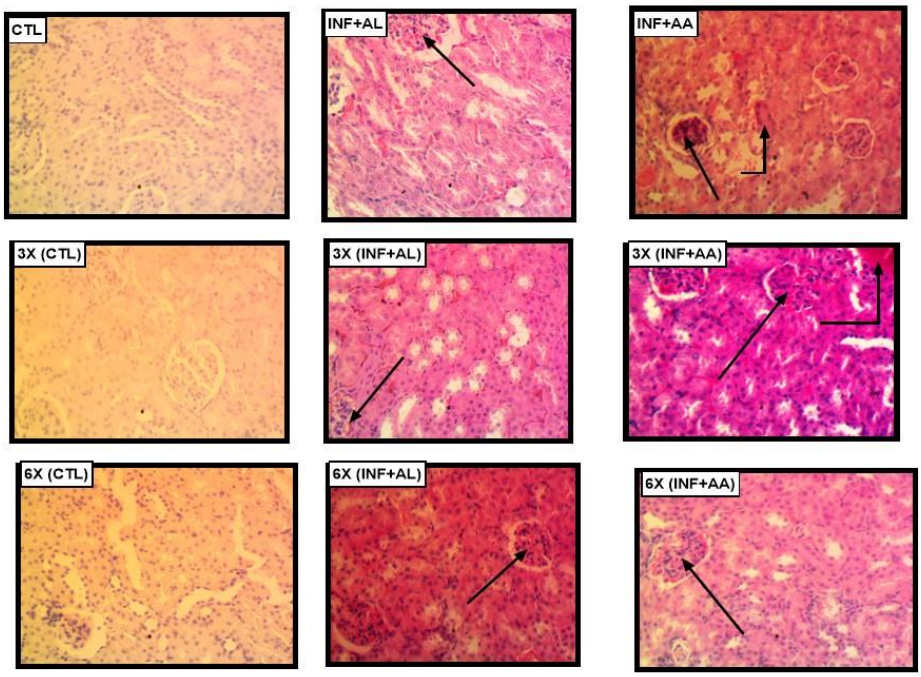

Figure 4. Kidney Histology section (x400; H & E) after the first, third, and sixth infection with *P. berghei* and treatment with either artemether plus lumefantrine (AL) or artesunate plus amodiaquine (AA): Arrow: Oedema in the glomerulus; Bent Arrow: Congestion of blood vessels in the interstitium.

4. Discussion

This study examined the impact of repeated *Plasmodium berghei* infection and treatment with artemether plus lumefantrine (AL) or artesunate plus amodiaquine (AA) on kidney oxidative stress and function in mice. The findings provide insights into the kidney consequences of repeated malaria infection and treatment cycles. Malondialdehyde (MDA) levels in the kidneys of mice increased following single and repeated exposure to *P. berghei* and treatment with AA or AL, potentially influenced by both the infection and the antimalarial drugs. Prior research has demonstrated that *Plasmodium* infection elevates malondialdehyde (MDA) levels in the kidneys (18). Additionally, meta-analyses and reviews have documented increased MDA concentrations in the presence of *Plasmodium* (5, 19). Artemisinin-based combination therapies (ACTs) have been demonstrated to exacerbate lipid peroxidation and oxidative stress (2, 6, 20), which can lead to renal lipid peroxidation, increased oxidative stress, fibrosis, inflammation, apoptosis, and reduced glomerular filtration (21, 22). Additionally, the results demonstrated that the elevation in MDA concentration did not exhibit a linear upward trajectory following repeated exposure, a finding that is analogous to the observations made in a prior investigation of liver tissue (6). Following the third and sixth exposure periods, there was an observed increase in GPx, SOD, and CAT enzyme activities within the kidneys, which may have played a role in regulating lipid peroxidation within this organ (23). Apart from an increase in plasma sodium levels following the initial and third exposure periods to *P. berghei* and AL or AA treatments following the third exposure period to *P. berghei* and plasma potassium level after AL treatmnet following *P.berghei* exposure, no significant differences in plasma sodium, potassium, chloride, and creatinine concentrations were observed between the control and treatment groups following the first, third, and sixth consecutive exposure periods. This indicates that the kidney is capable of effectively regulating the excretion of sodium, chloride, potassium, and creatinine despite continuous exposure to malaria and antimalarial drugs for up to six consecutive exposure periods. This observation may be attributed to the low parasitemia level of the *P. berghei* infection and the three-day standard AL or AA doses employed in the present investigation. Severe malaria infections and prolonged or high-dose therapeutic treatment with antimalarial drugs have been demonstrated to interfere with transporters and channels involved in Na/Cl/K processing in the kidneys (3, 24). Similarly, in this study, the levels of potassium, chloride, and creatinine in kidney tissues were not significantly altered by repeated exposure to *P. berghei* and treatment with AL or AA after six consecutive exposures. This finding supports the conclusion that plasma renal function was unaffected by the drug and parasite after repeated exposure. While histological analysis of the kidney tissue revealed indications of glomerular edema following the initial, third, and sixth exposure periods, this may be attributed to factors such as inflammation, infection, or injury. These conditions have the potential to impair kidney function (25). However, this did not affect renal

function in the present study. The elevated creatinine levels observed in the kidneys following the initial exposure to AL and the third exposure to AA may indicate a reduction in glomerular filtration rate (GFR), suggesting impaired kidney function in the removal of blood waste. This may also be attributed to congestion of the renal tissue following the initial and third exposures to *P. berghei* and A/A treatment, indicating impaired blood flow or increased vascular permeability in the renal microcirculation during exposure. However, these alterations were restored after the sixth exposure, indicating that repeated exposure to malaria infection and antimalarial drugs may induce adaptive changes in kidney function, which could be involved in activating protective mechanisms or repairing damaged tissues. In conclusion, the findings of this study indicate that exposure to malaria parasites and treatment with AL or AA resulted in an increase in lipid peroxidation in the kidneys. The antioxidant activities of kidney GPx, SOD, and CAT helped to protect against peroxidation, consequently preserving the normal kidney function parameters (Na/CL/K and creatinine). However, these activities were not sufficient to prevent histological alterations, such as edema in the glomerulus. Further research is required to elucidate the influence of recurrent exposure to malaria and its concomitant treatments on renal function. This may facilitate the identification of potential risks for individuals with pre-existing renal conditions in malaria-endemic countries who are frequently exposed to malaria infections and are administered antimalarial drugs.

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Authors' Contribution
AD, OAI, and ABI developed the idea for the experiment. AD, OAI, ABI, and FMM created the research approach and conducted the study. The manuscript was written by AD. All authors have reviewed, modified, and approved the final version of the manuscript.

Ethics
This research was approved by the College of Veterinary Medicine Research Ethics Committee at the Federal University of Agriculture, Abeokuta (FUNAAB/COLVET/CREC/2019/07/01). This research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised in 1996).

Conflict of Interest
The authors certify that they have no conflicts of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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