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Full Length Research Paper

Changes in some biochemical parameters of kidney functions of *Plasmodium berghei* infected rats administered with some doses of artemether

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This study aimed at determining changes in urine concentrations of sodium (Na^+) and potassium (K^+) of *Plasmodium berghei* infected rats during a week of intramuscular administration of artemether (12.5 to 50.0 mg/kg/day) and one week thereafter. Their concentrations and that of creatinine and urea in the plasma were also determined at the end of the study. The observed changes were related to the effects of artemether on the kidneys of the rats. The urine levels of the two electrolytes decreased significantly during treatment ($P<0.05$). One week post-treatment with 12.5 mg/kg of artemether, the urine concentrations of the electrolytes increased to values that were not significantly different from that of day 0. At 25 and 50 mg/kg, their urine concentrations still remained significantly lower than day 0 values ($P<0.05$). Plasma concentrations of the electrolytes one week post-treatment increased, but they were only significant at 25 mg/kg for K^+. A significant increase in the plasma level of creatinine was observed at all the doses of the drug at one week post-treatment. A dose-dependent degeneration of the renal tissue of all the experimental rats was also observed. We concluded that high doses of artemether caused progressive degeneration of the renal tissue of *P. berghei* infected rats.

Key words: Artemether, electrolytes in urine, plasma creatinine concentration, *Plasmodium berghei*.

INTRODUCTION

Artemether is one of the derivatives of artemisinin. Its efficacy in the treatment of malaria, including those resulting from infection by chloroquine-resistant strains of *Plasmodium* is well documented (Qinghaosu Antimalarial Coordinating Research Group, 1979; China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials, 1982). It is being used worldwide in combination with other anti-malarials, as one of the first lines of treatment of cerebral malaria caused by chloroquine-resistant *Plasmodium* (Van Vugt et al., 1999; Nosten et al., 2000). Intramuscular administration of multiple doses of the drug to dogs, rats and rhesus monkeys has been reported to produce neurotoxic effects such as gait disturbances, loss of spinal and pain reflexes (Petras et al., 1997; Sumalee et al., 1997; Nontprasert et al., 1998, 2000; Xiao et al., 2002). High doses of artemether were also reported to have caused neuronal necrosis in the region of the brainstem of rats (Raymond et al., 1998; Xiao et al., 2002). Anorexia and a dose-dependent reduction in body weight have also been reported at these high doses (Qigui et al., 1998). Following one week of intramuscular administration of 12.5 to 50.0 mg/kg of artemether, we reported changes in some of the visceral functions of Wistar rats (Akomolafe et al., 2006). We reported a pattern of anorexia which manifested as a significant reduction in the food and

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water intake of all the treated rats. This was accompanied by significant increases in their urine output. These effects persisted until even one week after the stoppage of drug administration in those rats that received 50.0 mg/kg of the drug, whereas those that received lower doses had only their food intake restored during this period. We concluded that the significant increase in urine output without a corresponding increase in the water intake of the rats could exacerbate dehydration and lead to a deleterious effect on the ionic balance of the body fluid of the rats. We also postulated that high doses of artemether could cause impaired renal function of the treated rats and that the significant increase in urine output could be due to other effects of the drug on thirst, anti-diuretic hormone output and the osmotic pressure of their blood (Akomolafe et al., 2006).

The plasma levels of some electrolytes especially sodium (Na^+) and potassium (K^+), are very important for the proper function of the neuromuscular and cardiovascular systems (Guyton and Hall, 2001; Ganong, 2003). Excessive loss of these ions from the body through urine, stool or sweat could have serious deleterious effects on these two systems, likewise their excessive retention (Guyton and Hall, 2001; Ganong, 2003). Literature is scanty on the influence of artemisinin derivatives on the electrolyte balance of the body fluid of laboratory animals. Our recent study on the effects of seven days administration of some doses of artemether (12.5 to 50 mg/kg) on the plasma and urine levels of these electrolytes in uninfected rats revealed that their concentrations in urine decreased dose dependently during treatment (Akomolafe et al., 2011). The decrease was not reversed at 25 and 50 mg/kg even one week after treatment. A dose-dependent tissue degeneration was observed in the kidneys of the rats. We concluded that the doses of artemether used in the study caused impairment of renal functions in apparently healthy rats, leading to the inability of the kidneys to concentrate urine. Therefore, we carried out this study to determine changes in urine levels of Na^+ and K^+ during and after administration of artemether to rats that were infected with *Plasmodium berghei*. Plasma levels of the electrolytes, urea and creatinine were determined post-treatment with a view to shedding some light on its toxicity in the body fluid and renal tissue of rats treated for malaria.

MATERIALS AND METHODS

Eighty adult Wistar rats (200 to 250 g) were used for this study. The rats were obtained from the Animal Holding of the Department of Physiological Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. They were kept in the laboratory under natural light/dark cycle and were fed on normal mouse cubes (Ladokun feeds, Ibadan, Nigeria) and water *ad libitum*. The rats were divided into four groups labeled I, II, III and IV. Each of the groups consisted of 20 rats: ten males and ten females. Each rat was housed in a separate metabolic cage (Ohaus R Model; Ohaus, Pine Brook, NJ, USA) with free access to food and water. The rats were acclimatized for two weeks before the commencement of the experiments.

P. berghei (NK 65 strain) was used for this study. It was acquired from the Department of Pharmacology and Therapeutics, University of Ibadan, Nigeria.

Inoculum preparation

Donor rat with *P. berghei* strain with about 20 to 32% parasitaemia was used. The red blood cell (RBC) per unit volume was calculated from the inoculum size. The number of parasitized RBC in a volume of blood was then calculated by multiplying the percentage of parasitaemia by the number of RBC.

The desired volume of blood was obtained from the donor rat under chloroform anaesthesia by cardiac puncture using a heparnised sterile syringe. The blood was suitably diluted with sterile normal saline so that the final inoculum (0.2 ml) for each rat contained the required number of parasitized RBC (that is, 3.0×10^7 parasitized RBC) (a modification of the method of Shu-Hua et al., 2004).

Preparation of thin and thick films and staining technique

A small drop of blood from the tail of the rat was collected on clean non-greasy slide. Thin and thick blood films were made accordingly. The films were air-dried. The thin film only was then fixed using few drops of methanol and left to air-dry.

The malaria parasites in thick and thin blood films were stained with Giemsa stain. The stain was diluted with the sodium phosphate buffer at pH 7.2 in the ratio of 4:10, that is, 4 ml of the Giemsa stain to 10 ml of buffer solution. The stain was applied on the slide and allowed to stand for 10 to 12 min. The stain was poured off and the slide rinsed with buffer solution and allowed to air-dry.

Evaluation of parasitaemia

Each of the blood films prepared was mounted on a microscope and a drop of immersion oil was applied to the slide using 100x objective lens to locate the best field of view for counting both parasitized RBC and unparasitized RBC. In a particular field, the total number of red blood cells (TRBC) as well as the total number of parasitized RBC (PRBC) was counted. Percentage parasitaemia in each field was calculated as follows:

$$\text{Parasitaemia (\%)} = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} \times \frac{100}{1}$$

Ten fields were counted on each slide and the mean percentage parasitaemia was recorded for each rat. Rats with parasitemia level of not less than 25% were used for this study. This was observed four days after inoculation. Our pilot study revealed substantial parasite clearance (<1% parasitemia) on the 3rd and 13th day of treatment in experimental and control rats, respectively. Drug administration was commenced on the 5th day of inoculation, which was taken as day 0 of the experiment.

Drug administration

Injectable form of artemether (80 mg/ml) manufactured by Kunming Pharmaceutical Factory, Kunming, People's Republic of China was dispensed in 1 ml ampoules for intramuscular injection.

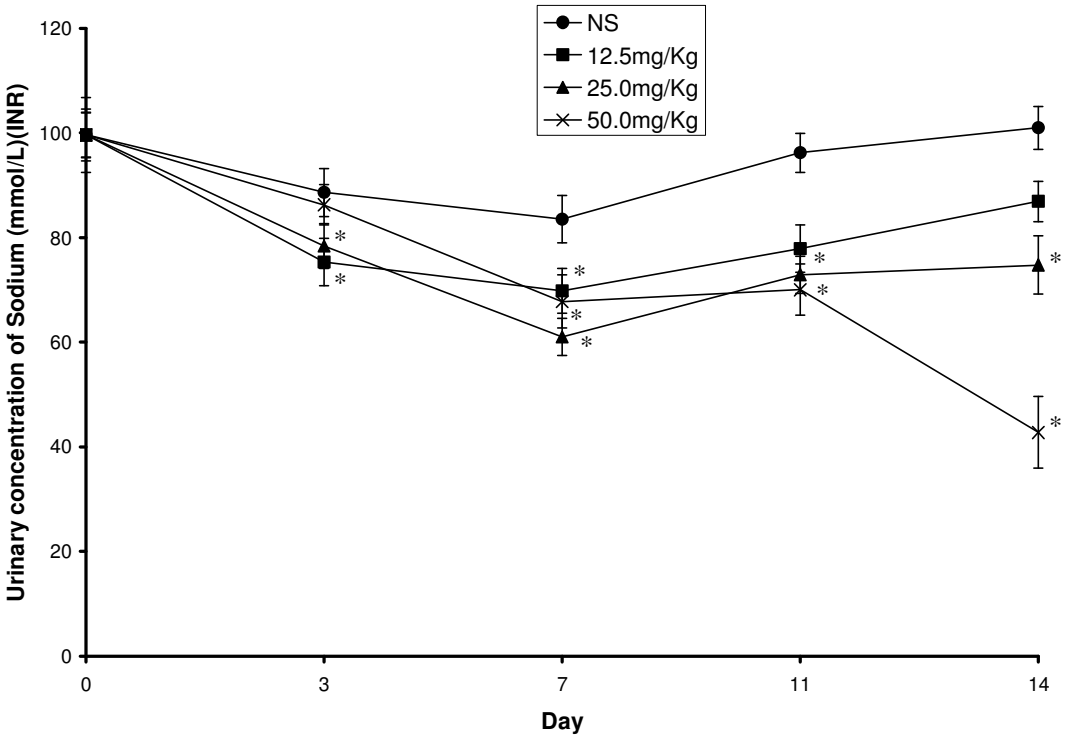

Figure 1. Variation in the urine concentration of sodium due to a week of intramuscular administration of artemether to *P. berghei* infected rats (NS = nomal saline). Each point is mean ±S.E.M (n = 20); *significantly different from the day 0 value ($p < 0.05$).

Dose regimens

Each rat in Group I that weighed 250 g was given 0.16 ml of normal saline (equivalent to the volume of the drug that was administered to each rat of the same weight that received 50.0 mg/kg/day) for 1 week. This group served as the control. Each of the rats in Groups II, III and IV received 12.5, 25.0 and 50.0 mg/kg/day of artemether, respectively via the intramuscular route for one week.

Urine samples were collected into clean specimen bottles for 24 h on the day before the commencement of drug administration and this was taken as the day 0 urine for each of the rats. This procedure was repeated for days 3, 7, 11 and 14 of the study, that is, one week of drug administration and another one week later. The concentrations of Na^+ and K^+ in the samples were measured by Flame Photometry using Flame Photometer Model 410C Manufactured by Sherwood Instruments Cambridge UK.

On day 14, the rats were sacrificed under chloroform anaesthesia. A midline incision was made with a surgical blade to expose the abdominal organs. Blood was collected from their hearts by cardiac puncture and delivered into lithium heparinized specimen bottle. A new syringe was used for the collection of blood from each rat. The blood was immediately centrifuged at 3000 revolutions per minutes for 20 min. The plasma was thereafter separated into a specimen bottle in readiness for analysis. The concentrations of Na^+ and K^+ in the samples were determined using the same methods that were used in the analysis of urine.

The rats kidneys were dissected out and kept inside 10% formalin until their sections were cut and stained with eosin and hematoxylin for histological studies. Photomicrograph of the tissues was taken using $Lect_3$ Dialux Microscope (Bright Field) at 400x magnification.

Statistical analysis

The results were expressed as mean ± S.E.M and subjected to one-way ANOVA. Significant differences were further tested by the Duncan's multiple range and Student Neuman Keuls tests. Student t-test was used to compare the urine concentration of the electrolytes for each day with the day 0 value for each group. Differences with probability values of $p<0.05$ were considered significant.

RESULTS

Effect of artemether on urinary concentration/level of electrolytes

Sodium (Na^+)

There was no significant alteration in the urine Na^+ concentration of the control rats during and after treatment (Figure 1). During treatment, a significant reduction in the urine concentration of Na was observed at all the doses of artemether used in this study. After treatment, the concentration of this electrolyte remained significantly reduced at 25 and 50 mg/kg, while it was restored to values that were not significantly different from the pre-treatment value in rats that received 12.5 mg/kg of the drug.

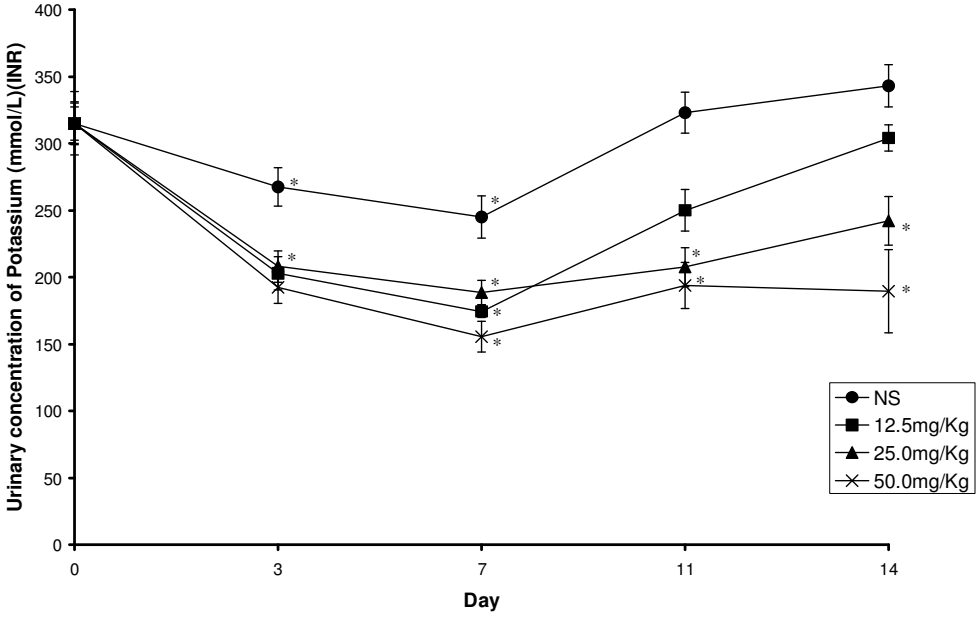

Figure 2. Variation in the urine concentration of potassium due to a week of intramuscular administration of artemether to *P. berghei* infected rats (NS = nomal saline). Each point is mean ± S.E.M (n = 20); * significantly different from the day 0 value ($p < 0.05$).

Potassium (K^+)

During treatment, a significant reduction in the concentration of K^+ in urine was observed in the control as well as the experimental rats (Figure 2). After treatment, the urine concentration of this electrolyte remained significantly lower than the day 0 value in rats that received 25 and 50 mg/kg, while that of the control and rats that received 12.5 mg/kg of the drug rose to values that were not significantly different from the day 0 concentrations.

Effects of artemether on plasma electrolyte concentration

Plasma Na^+ concentration was significantly lower than the control value only at 12.5 mg/kg (Figure 3). Plasma K^+ level increased significantly at 25 mg/kg of artemether (Figure 4).

DISCUSSION

The periodic assay of Na^+ and K^+ in urine showed that, the concentrations of the electrolytes in rats treated with artemether fell during treatment (Figures 1 and 2). Their concentrations increased to the day 0 values at 12.5 mg/kg. At 25.0 and 50.0 mg/kg however, the concentrations of the electrolytes fell irreversibly and significantly. At these two doses, marked polyuria was reported in our previous studies (Akomolafe et al., 2006). Plates 1 to 4 also show progressive renal tissue damage with increasing dose of artemether. Inability of the kidney to concentrate or dilute the urine, usually occurs in case of damage to most of the nephrons (Guyton, 2001; Dunn, 2003). There is rapid tubular flow of the glomerular filtrate in the remaining functional nephrons, making reabsorption of water and electrolytes impossible. The kidney is therefore unable to concentrate or dilute the urine. This study indicates that the doses of artemether used were dose-dependently toxic to the renal tissue of the infected rats, as evidenced by the photomicrographs of the kidneys. The changes in plasma concentration of urea of experimental rats were not dose dependent (Figure 5). At 12.5 mg/kg, it was not significantly different from the control. It was significantly higher than the control at 25 mg/kg and significantly lower at 50 mg/kg. However, the plasma creatinine levels in all the experimental rats were significantly higher than that of the control (Figure 6). Plasma urea, creatinine and electrolytes are the most sensitive biochemical markers used in the assessment of renal tissue damage, because urea and creatinine are excreted through the kidneys, while the electrolytes are reabsorbed in the tubules. So, in cellular damage, there is retention of urea and creatinine in the blood, low reabsorption and much excretion of electrolytes by the tubules. The excessive fluid loss of rats that received these doses of artemether could lead to dehydration and a severe depletion of the major electrolytes of the extracellular fluid of the rats, however, there was an associated decrease in food intake (Akomolafe et al.,

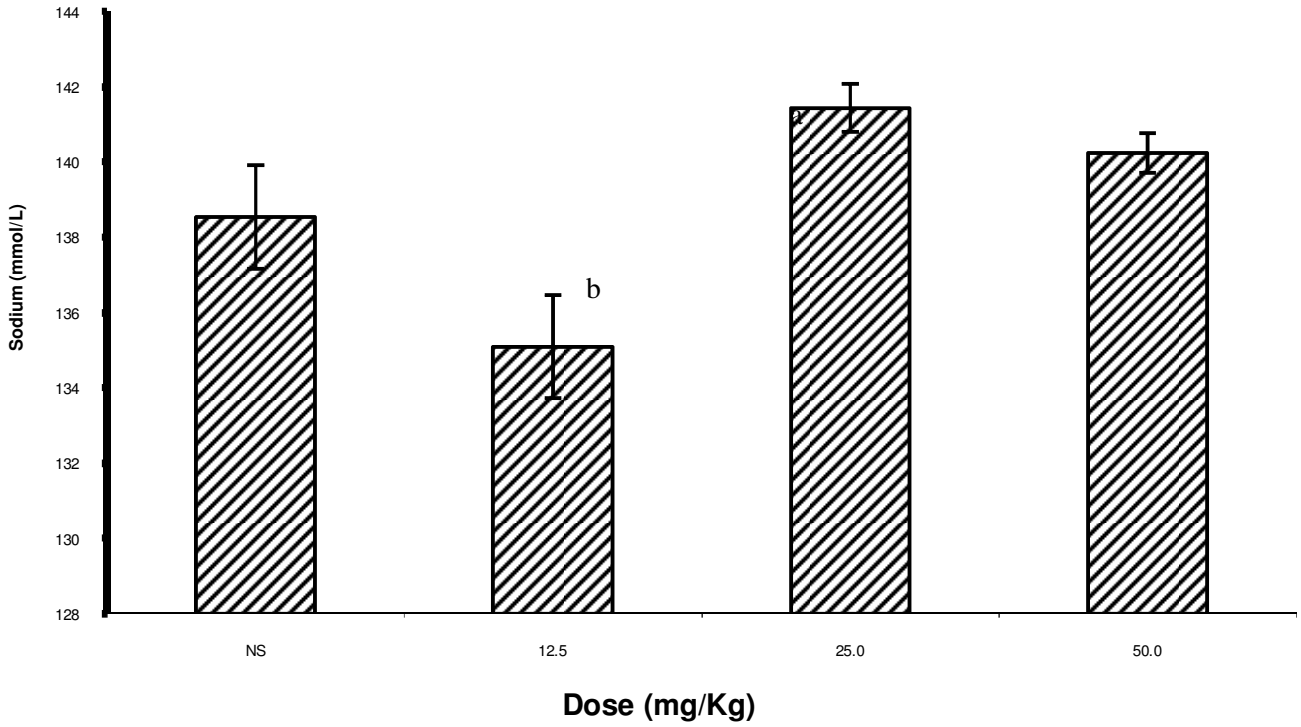

Figure 3. Effect of artemether on the sodium concentration of the plasma of *P. berghei* infected rats. Each bar is mean ± S.E.M (n = 20); [b] Significantly different from control ($p < 0.05$).

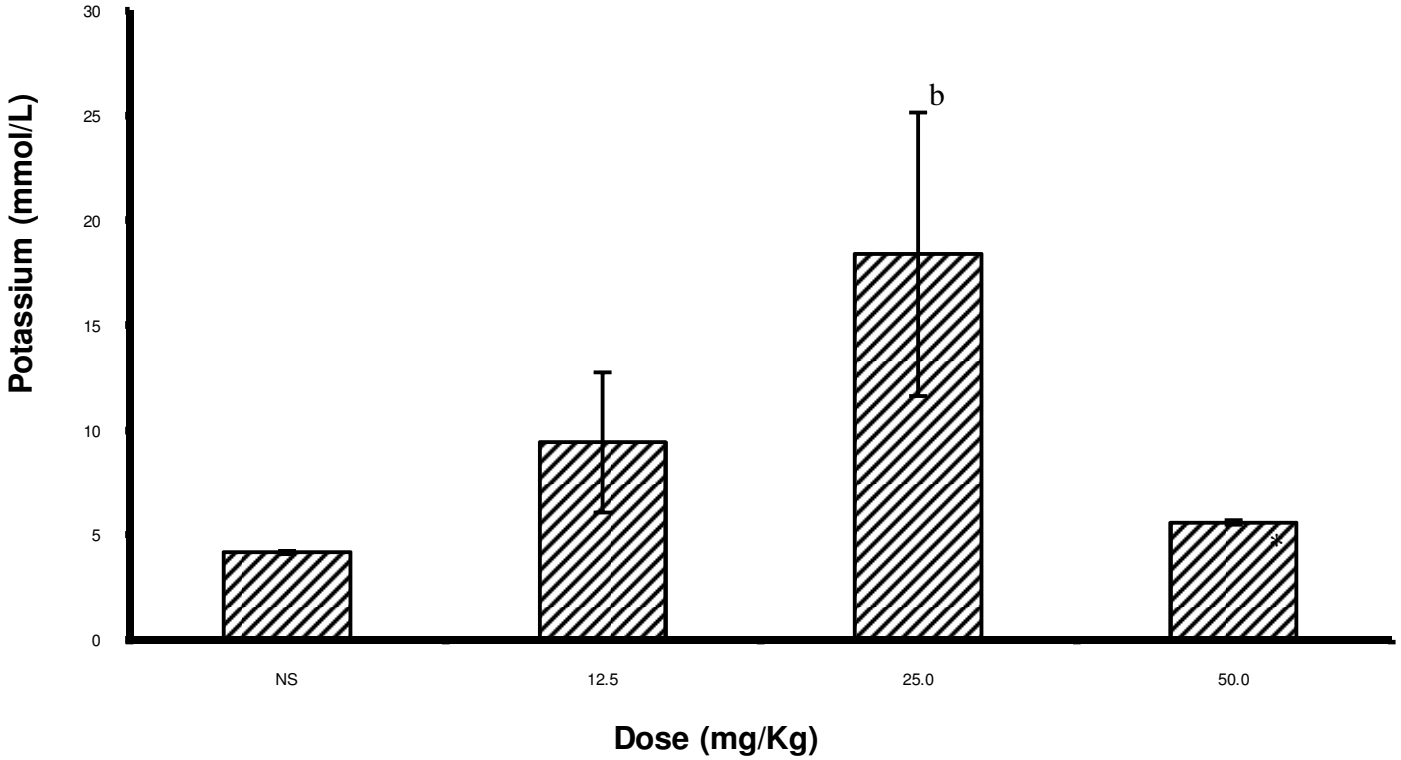

Figure 4. Effect of artemether on the potassium concentration of the plasma of *P. berghei* infected rats. Each bar is mean ± S.E.M (n = 20); [b] significantly different from control ($p < 0.05$).

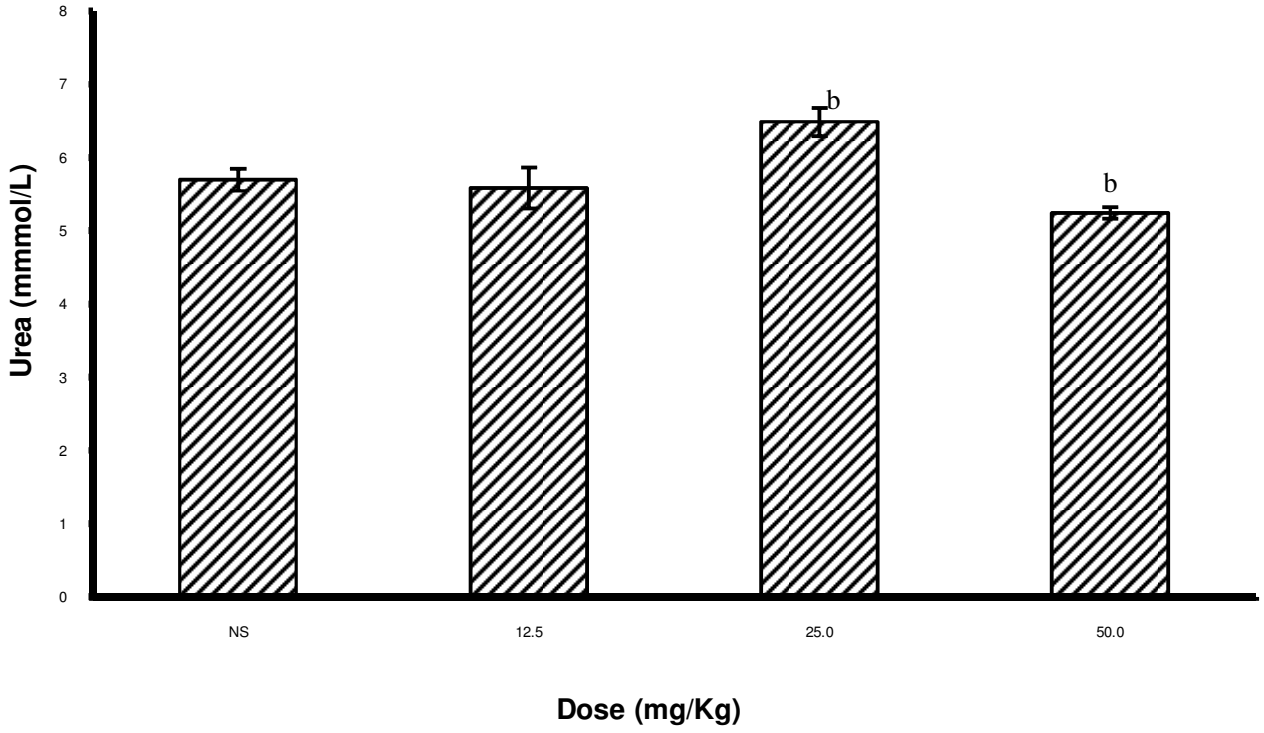

Figure 5. Effect of artemether on the urea concentration of the plasma of *P. berghei* infected rats. Each bar is mean ± S.E.M (n = 20); [b]significantly different from control ($p < 0.05$).

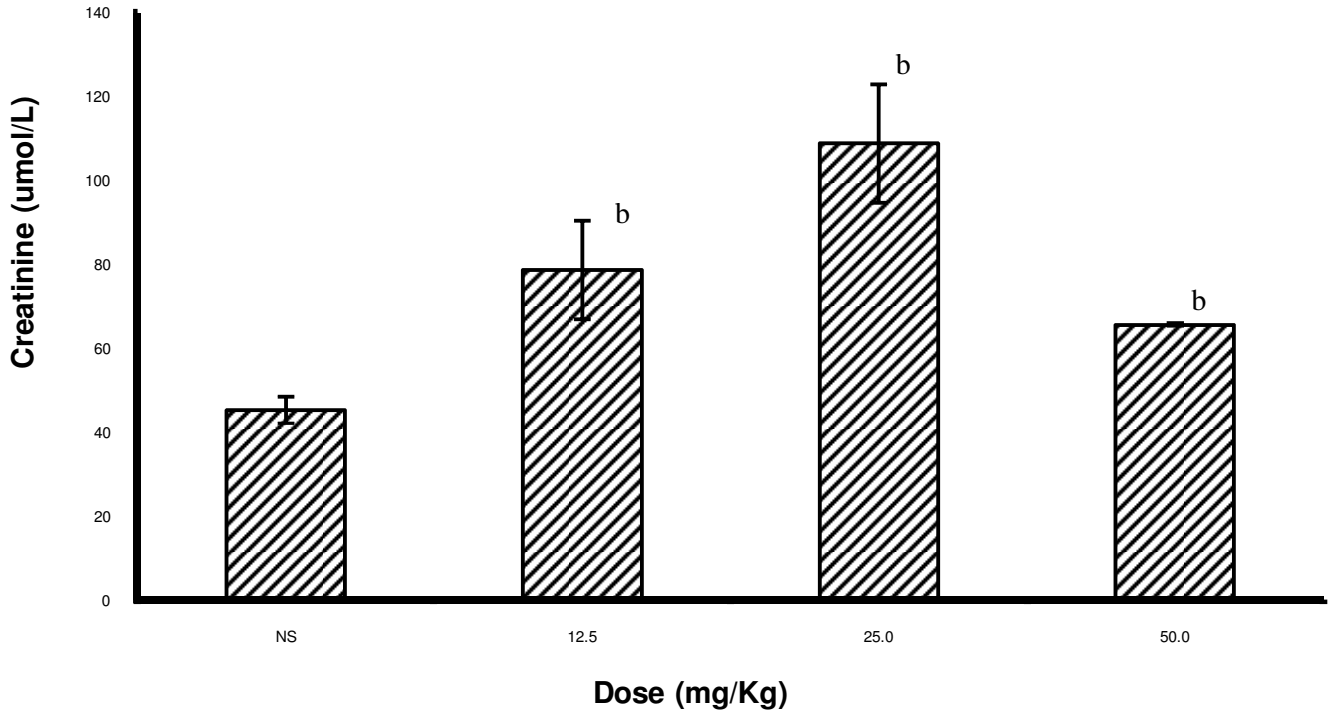

Figure 6. Effect of artemether on the creatinine concentration of the plasma of *P. berghei* infected rats. Each bar is mean ± S.E.M (n = 20); [b] Significantly different from control ($p < 0.05$).

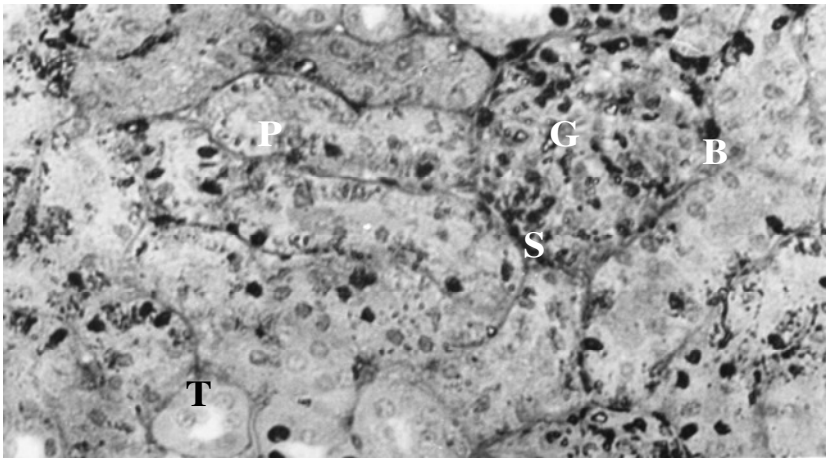

Plate 1. Photomicrograph of the kidney of infected rats that received normal saline i.m. for 7 days (control 2). Magnification 400x. The Bowman's capsule (B) appears distinct, but the capsular space (S) is closed by inflamed glomerulus (G). The tubular cells (C) are densely stained and the inter-tubular spaces (P) are almost closed. The tubular architecture (T) is still fairly preserved. There is mesangial cell (M) proliferation.

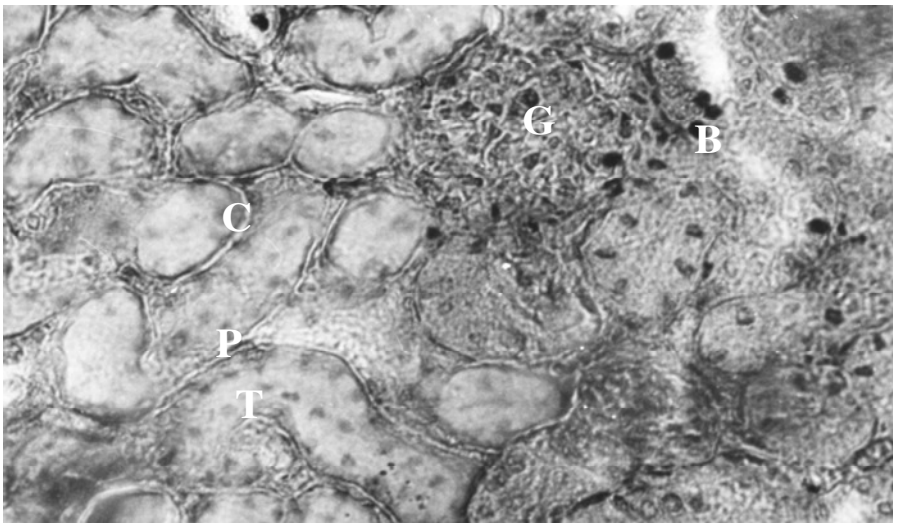

Plate 2. Photomicrograph of the kidney of rats that received 12.5 mg/kg of artemether i.m. for 7 days. Magnification 400x. Bowman's capsule (B) has started degeneration. The glomerulus (G) has broken down. The tubules (T) are inflamed and have distorted shapes. Their cells (C) are densely stained and the spaces between them (P) are almost closed completely.

2006).

Only the experimental rats that received 25 and 50 mg/kg of artemether had insignificantly higher plasma concentrations of Na^+ than the control rats. However, all the experimental rats had higher concentrations of K^+ than the control, the difference being significant at 25 mg/kg only. Sodium is the major cation of the extracellular fluid, while potassium is the major cation of the intracellular fluid (Guyton, 2001; Gannong, 2003). Saroj et al. (2002) reported an increase in the plasma level of K^+ (hyperkalemia) in patients with acute renal failure resulting from *Plasmodium falciparum* infection. This according to them was due to the rapid lysis of blood cells by the parasites. They also reported that the inflammation that is associated with malarial infection could lead to leakage of fluid from the intravenous compartment due to increased vascular permeability, thereby leading to hypovolemia and haemo-concentration. The increase in the plasma level of Na^+ and K^+ observed at 25 and 50 mg/kg in this study (Figures 4 and 5) is in conformity with the report of the researchers. A significant polyuria had been reported in these rats (Akomolafe et al., 2006).

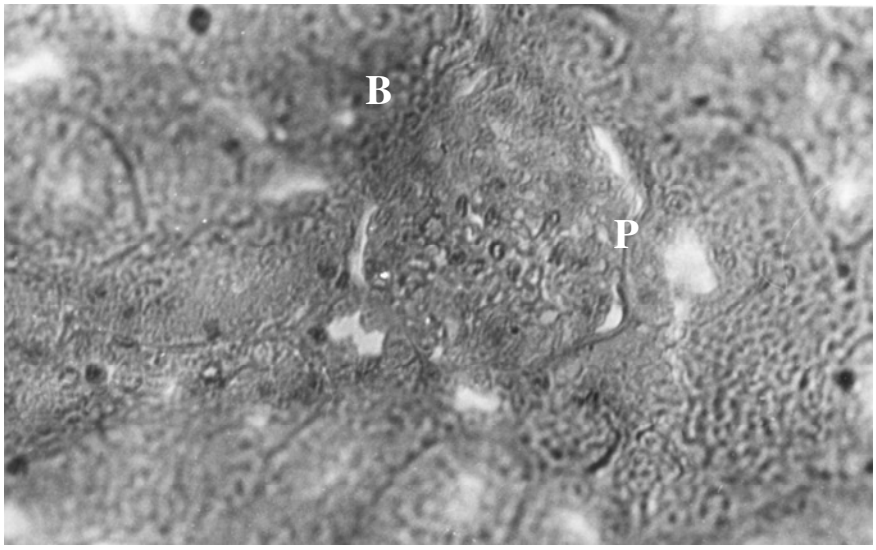

Plate 3. Photomicrograph of the kidney of rats that received 25.0 mg/kg of artemether i.m. for 7 days. Magnification 400x. The Bowman's capsule (B) has degenerated completely. The renal tubular architecture (T) is almost completely distorted, as most of the cell boundaries of the tubular cells are no more distinct. The inter-tubular spaces (P) are almost completely filled up by inflamed tubules.

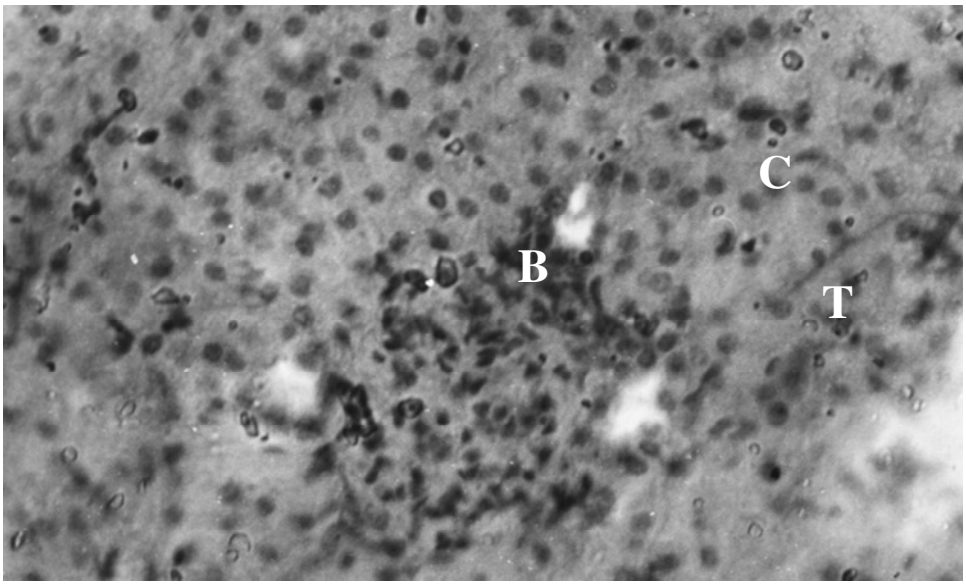

Plate 4. Photomicrograph of the kidney of rats that received 50.0 mg/kg of artemether i.m. for 7 days. Magnification 400x; The Bowman's capsule (B) has undergone complete degeneration, neither the glomerulus nor the capsular space can be seen again. The renal tubules (T) have lost their integrity to a very large extent. All the spaces between them have been filled up and their cells (C) are densely stained.

Photomicrographs of their kidneys revealed much tissue damage (Plates 2 to 4). The excessive water loss by these rats was responsible for the high levels of Na^+ and K^+ in their plasma. Hyperkalemia causes bradycardia or reduced heart rate (Guyton, 2001). The elevated value of K^+ in these rats could have a severe consequence on the functioning of their hearts. The significant increase in the plasma creatinine concentration of the experimental rats as revealed by Figure 6 was an indication of renal tissue damage.

The necrosis observed in the renal tissues of the experimental rats could not be attributed to malarial infection, since significant parasite clearance was achieved even before the completion of treatment. The control rats that achieved parasite clearance by their own immune system much later showed evidence of less tissue damage in their kidney. Artemether induced necrosis in the renal tissue of the rats (Plates 2 to 4) led to a breakdown in the renal handling of the major electrolytes of the body fluid: Na^+ and K^+, and the inability of their nephrons to retain water in the body. The resulting excessive loss of water also contributed to the raised plasma levels of Na^+ and K^+ in this study.

This study revealed that the doses of artemether used are toxic to the kidney of the infected rats. The toxicity manifested as renal tissue damage, elevated levels of plasma creatinine and urea resulting from impaired renal function, and electrolyte imbalance in their body fluid.

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