

Full Length Research Paper

Effects of artemether on biochemical markers of liver function in *Plasmodium berghei*-infected and non-infected rats

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This study aimed at determining changes in plasma activities of some enzymes and concentrations of plasma organic constituents which are often used in the assessment of liver functions in uninfected rats (UNR) and *Plasmodium berghei* infected rats (INR), following a week of intramuscular administration of artemether (12.5 to 50.0 mg/kg/day). The observed changes were related to the effects of artemether on the liver of the rats. At all the doses tested, the plasma concentrations of total and conjugated bilirubin increased significantly in both INR and UNR. A significant decrease in the plasma concentrations of glucose was also observed in UNR. The levels of cholesterol were significantly higher in INR than UNR. Plasma glutamate oxaloacetate transaminase (GOT) activity was significantly increased in both categories of rats, but more significantly in INR. The activity of plasma glutamate pyruvate transaminase (GPT) increased significantly at 12.5 and 25.0 mg/kg only in UNR, while a significant increase was observed at 50.0 mg/kg in the INR. Photomicrograph of the liver revealed progressive tissue damage which was more pronounced in INR than UNR. We concluded that high doses of artemether are toxic to the liver of both infected and uninfected rats.

Key words: Artemether, bilirubin, uric acid, plasma glutamate oxaloacetate transaminase, plasma glutamate pyruvate transaminase, *Plasmodium berghei*.

INTRODUCTION

Artemether is one of the antimalarial drugs derived from artemisinin. It is reputed for its efficacy in the treatment of

malaria, including those resulting from infection by chloroquine – resistant strains of *Plasmodium* and this has been reported (Qinghaosu Antimalarial Coordinating Research Group, 1979a; 1979b; China Cooperative Research Group, 1982). As one of the first lines of treatment of cerebral malaria caused by chloroquine-resistant *Plasmodium*, artemether is being used worldwide in combination with other anti-malarials (Van Vugt et al., 1999; Nosten et al., 2000). Administration of multiple doses of the drug to dogs, rats and rhesus

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Abbreviations: UNR, Uninfected rats; INR, infected rats; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase.

monkeys by intramuscular route 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). High doses of artemether were also reported to have caused neuronal necrosis in the region of the brainstem of rats (Genovese et al., 1998). 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 have 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 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 impairment of 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). Our recent study on the effects of seven days' administration of the same doses of artemether on the plasma and urine levels of some major electrolytes of the body fluid in uninfected rats revealed that their concentrations in urine decreased dose dependently during treatment (Akomolafe et al., 2011). The reduction was not reversed at 25 and 50 mg/kg even one week after treatment. 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. We have also repeated the same experiment with *Plasmodium berghei* infected rats (Akomolafe et al., 2012).

The urine levels of sodium (Na^+) and potassium (K^+) decreased significantly during treatment. One week post-treatment with 12.5 mg/kg of artemether, the urine concentrations of the electrolytes rose back to values that were not significantly different from pre-treatment. At 25 and 50 mg/kg, their urine concentrations still remained significantly lower than pre-treatment levels. 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 doses of the drug one week post-treatment. 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. Since literature is scanty on the influence of artemether on the liver function of both healthy and infected rats, this study was carried out to investigate if artemether provokes alterations in blood enzyme activities and organic compounds levels often used in the assessment of liver functions.

MATERIALS AND METHODS

Uninfected experimental rats

Eighty (80) 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 clean water. The care and use of the animals and the experimental protocol of the study were in accordance with Experimental Animal Care and Use Regulation of the Animal House, College of Health Sciences, Obafemi Awolowo University, Ile Ife. They were divided into four groups labeled I, II, III and IV. Each of the groups consisted of 20 rats, 10 males and 10 females. Each of the rats was housed in a separate metabolic cage (Ohaus R Model; Ohaus, Pine Brook, NJ, USA) with access to food and water *ad libitum*. The rats were acclimatized for two weeks before the commencement of the experiments.

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.

Dose regimens

Each rat in group I (uninfected control, UNFC) that weighed 250 g was given 0.16 mL normal saline (equivalent to the volume of the drug that was administered to each 250 g rat that received 50.0 mg/kg/day of artemether) for one week. 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.

Infected experimental rats

Another 80 adult Wistar rats (200 to 250 g) were used for this study. They were obtained from the same source, fed and housed as the uninfected rats. The rats were divided into four groups labeled I, II, III and IV like the uninfected rats. Each group consisted of 10 males and 10 female rats.

Parasite used

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

A donor rat infected with *P. berghei* with approximately 20 to 32% of parasitaemia was used. After determining total red blood cell (RBC) number, blood was obtained from the animal under chloroform anaesthesia by cardiac puncture and diluted in normal saline for a final inoculum of 3.0×10^7 parasitized RBC (pRBC) in 0.2 mL of blood (a modification of the method described by Xiao et al., 2002).

Preparation of thin and thick films and staining technique

Small drops of blood from the tails of the infected rats were collected on clean non-greasy slides for thin and thick blood films. The films were air-dried and the thin film only was further fixed with a few drops of methanol, and left again to air-dry. The slides (thin and thick films) were stained with Giemsa stain, diluted (1:2.5) in a sodium phosphate buffer pH 7.2 and left to stand for 10 to 12 min. After pouring off the stain, the slides were rinsed with the buffer solution and allowed to air-dry.

Evaluation of parasitaemia

Evaluation of parasitaemia; percentage parasitaemia was determined (total number of pRBC / total number of RBC * 100) in ten fields on each slide for each infected rat. Parasitaemias as high as 25% were achieved on post inoculation day 3 (PID3). The animals with parasitaemias at least equal to 25% were treated with artemether on post-infection day 5 (PID5).

Drug administration

This was carried out as for the uninfected rats.

Dose regimens

The same dose regimen described for the uninfected rats was adopted for the infected animals. The Group I rats of this study were the control (infected control INFC). On treatment day 14 (TD14), that is one week after drug administration, the rats (both the Control and experimental) were sacrificed under chloroform anaesthesia. Blood was collected into a lithium heparinized specimen bottle by cardiac puncture, immediately centrifuged at 3000 rpm for 20 min and sent to biochemical analysis. Otherwise, plasma samples were frozen at -4°C until further use. Plasma levels of liver transaminases and other organic constituents were measured with spectrophotometer (Model SP 310, Biosystems Limited, Spain). Levels of plasma glutamate oxaloacetate transaminase, plasma glutamate pyruvate transaminase, glucose, uric acid and cholesterol were determined by the use of appropriate biochemical kits purchased from Randox Laboratories, UK. Total bilirubin, and conjugated bilirubin were measured using the diazo method of Jendrassik and Grof (1938), creatinine by Jaffe's reaction (Jaffe, 1886) and urea by diacetyl monoamine methods of Veniamin and Vakortzi-Lemonies (1970). The livers of the rats were taken out and kept in a 10% formalin solution until tissue sections were dyed with hematoxylin eosin staining for histological studies. Photomicrographs of the tissues were taken using Lect₃ Dialux Microscope (Bright Field 400 x).

Statistical analysis

The results were expressed as mean \pm standard error of mean (S.E.M) and subjected to one-way analysis of variance (ANOVA).

Significant differences were further tested by the Duncans Multiple Range and Student Neuman Keuls tests. Differences with probability values of $p < 0.05$ were considered significant.

RESULTS

Effects of artemether on plasma organic compounds

Total bilirubin

In the uninfected rats (UNR), there was a slight but significant increase in the plasma level of total bilirubin (Figure 1). In the INR, the increase in plasma total bilirubin was significantly higher than that of the UNR at any particular dose. In the INR, those rats that received normal saline had the least plasma level of total bilirubin.

Conjugated bilirubin

The pattern of artemether-induced increase in the plasma level of conjugated bilirubin in the INR was the same as that of total bilirubin, that is, it was significantly higher than the UNR at any particular dose (Figure 2).

Cholesterol

In the UNR, a significant increase in cholesterol level of the plasma was observed only in those rats that received 50.0 mg/kg of artemether (Figure 3). Infected rats had a significantly higher cholesterol level at any of the doses of artemether used, including NS, than the UNR.

Glucose

The plasma glucose level of the UNR decreased significantly and dose dependently (Figure 4). The INR had lower plasma glucose levels than the UNR at 12.5 and 25.0 mg/kg of artemether and even than the control rats that received NS (Figure 4). At 50.0 mg/kg the plasma glucose level of INR was significantly higher than that of the UNR.

Urea

In the UNR, the plasma level of urea was significantly reduced in rats that received 12.5 and 25.0 mg/kg of artemether (Figure 5). The plasma urea level was higher in the INR than in the UNR at 25 mg/kg.

Uric acid

Artemether caused a significant increase in the plasma level of uric acid in the UNR, the highest level occurring at 50.0 mg/kg of artemether (Figure 6). In the INR that received normal saline (control 2), the plasma uric acid level was significantly higher than that of the UNR. In the

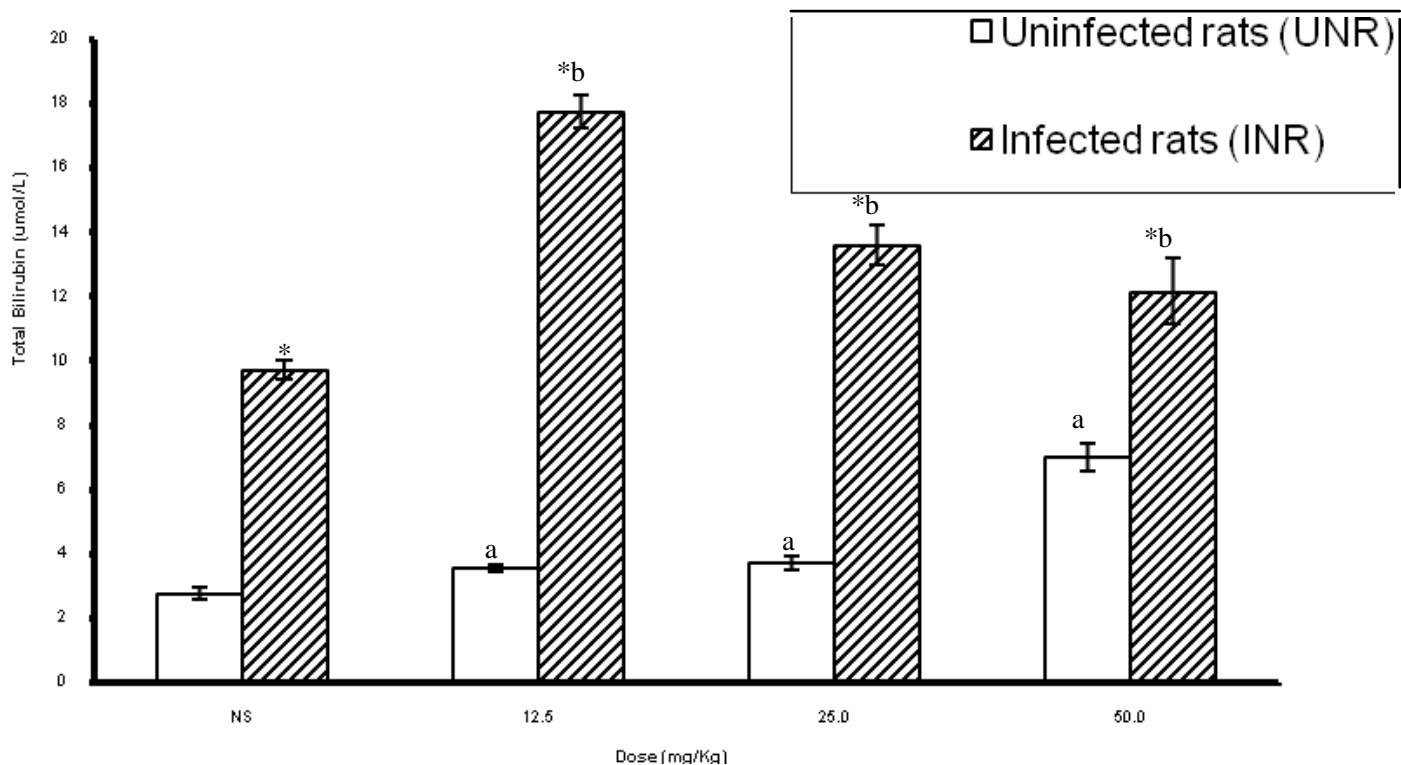


Figure 1. Effect of artemether on the total bilirubin concentration of the plasma of UNR and INR. Each bar is mean ± S.E.M. (n = 20). *Significant difference between INR and UNR. ^aSignificant difference between control and UNR. ^bSignificant difference between control and INR.

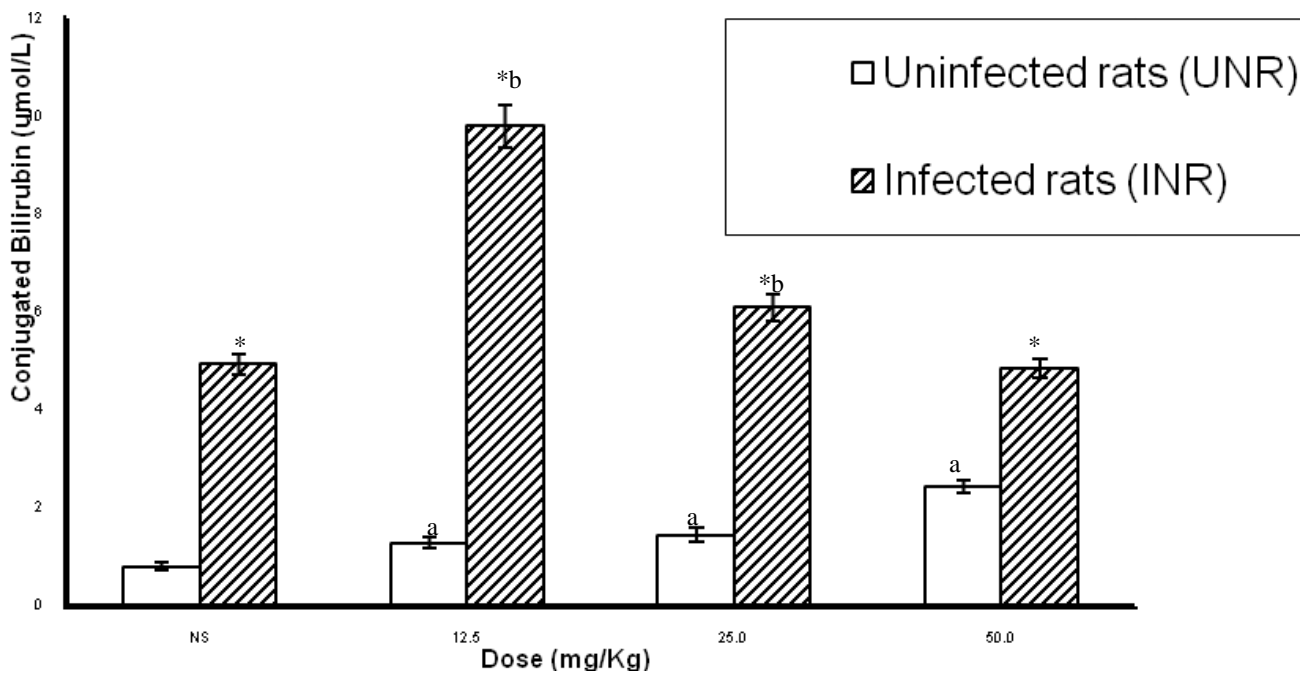


Figure 2. Effect of artemether on the conjugated bilirubin concentration of the plasma of UNR and INR. Each bar is mean ± S.E.M. (n = 20). *Significant difference between INR and UNR (p < 0.05). ^aSignificant difference between control and UNR (p < 0.05). ^bSignificant difference between control and INR (p < 0.05).

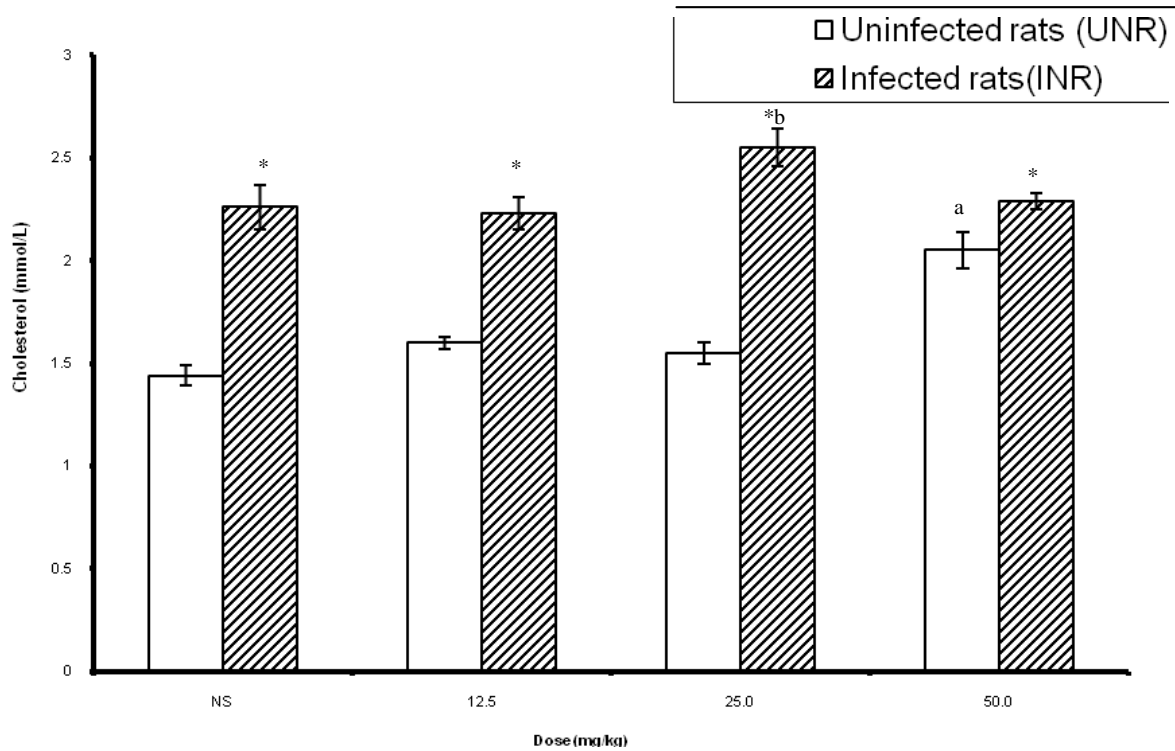


Figure 3. Effect of artemether on the cholesterol concentration of the plasma of UNR and INR. Each bar is mean \pm S.E.M. (n = 20). *Significant difference between INR and UNR. ^aSignificant difference between control and UNR. ^bSignificant difference between control and INR.

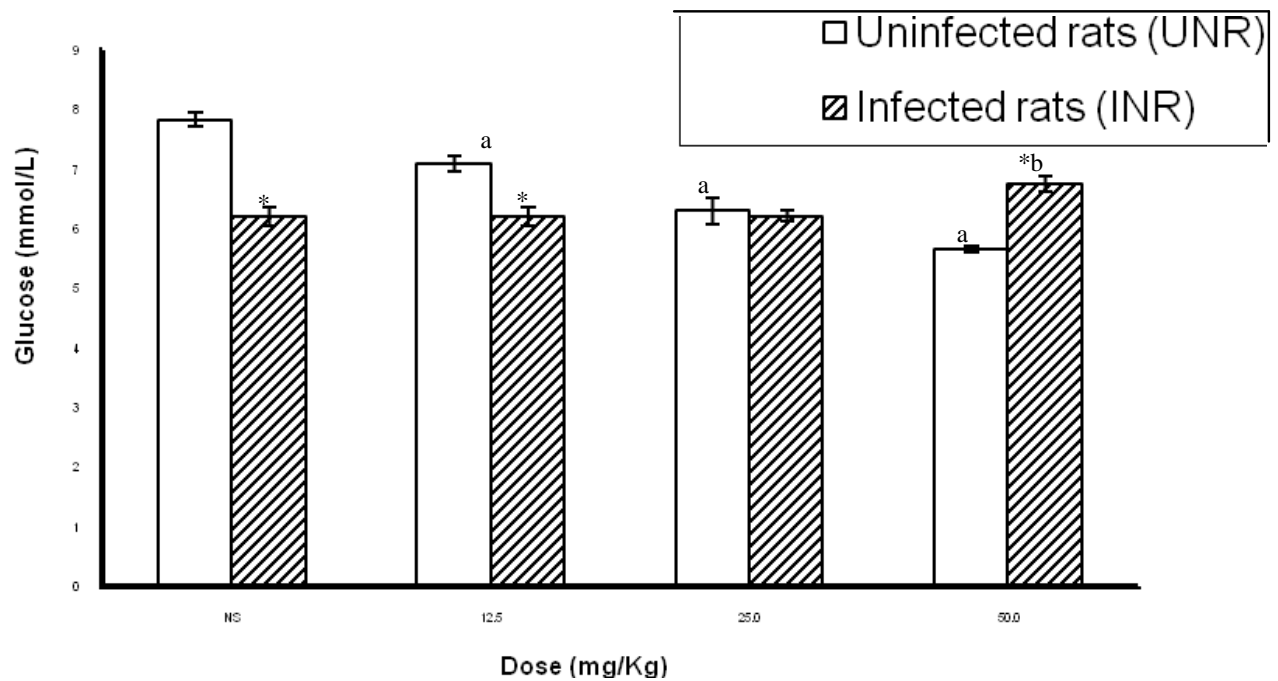


Figure 4. Effect of artemether on the glucose concentration of the plasma of uninfected and *P. berghei* infected rats. Each bar is mean \pm S.E.M. (n = 20). *Significant difference between INR and UNR. ^aSignificant difference between control and UNR. ^bSignificant difference between control and INR.

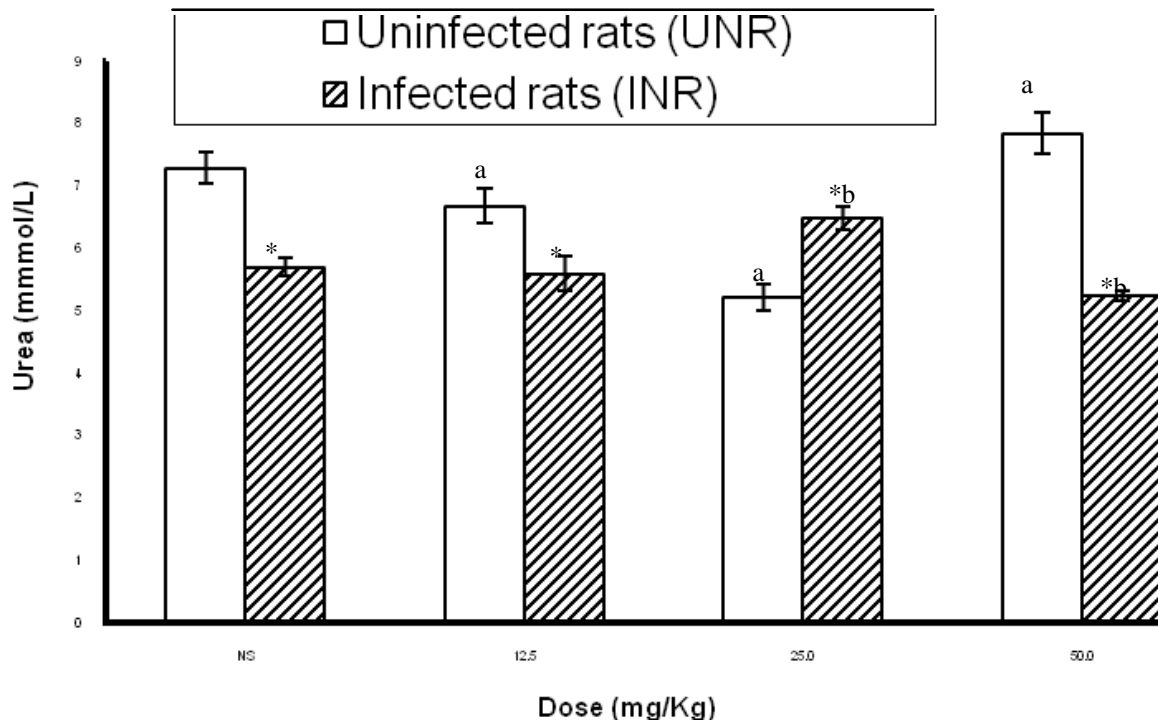


Figure 5. Effect of artemether on the urea concentration of the plasma of uninfected and *P. berghei* infected rats. Each bar is mean \pm S.E.M. (n = 20). *Significant difference between INR and UNR. ^aSignificant difference between control and UNR. ^bSignificant difference between control and INR.

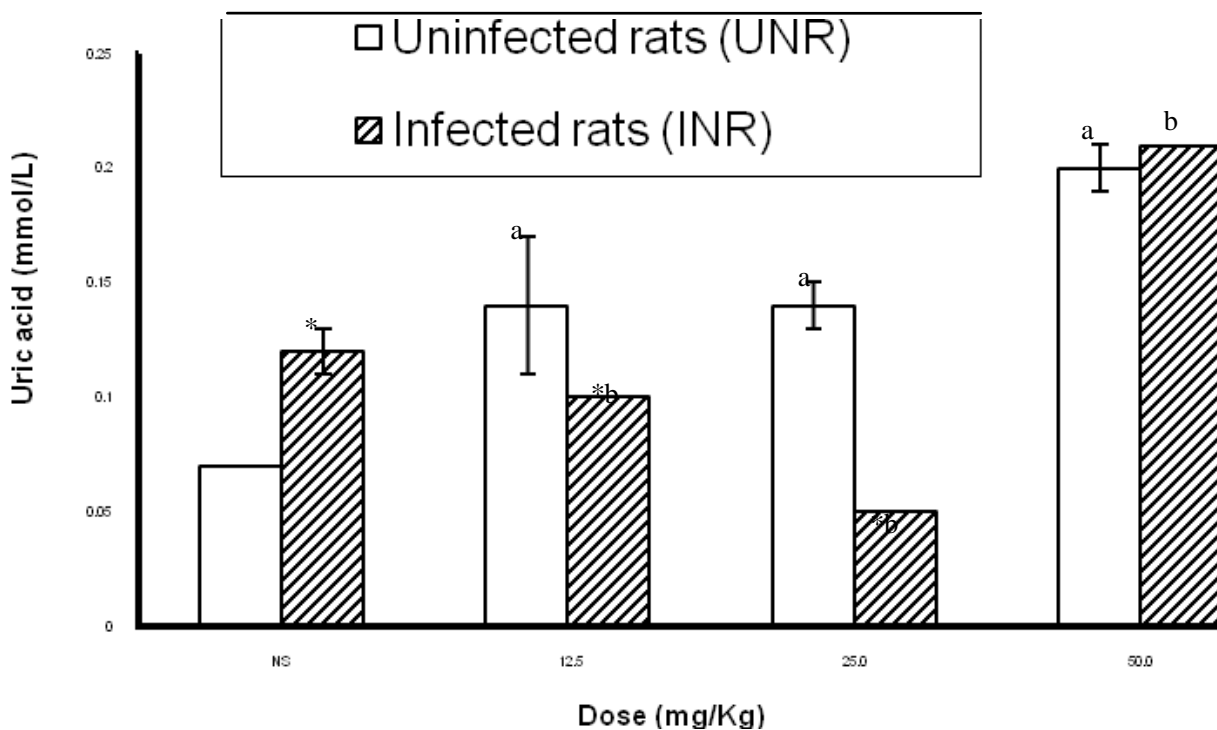


Figure 6. Effect of artemether on the uric acid concentration of the plasma of uninfected and *P. berghei* infected rats. Each bar is mean \pm S.E.M. (n = 20). *Significant difference between INR and UNR. ^aSignificant difference between control and UNR. ^bSignificant difference between control and INR.

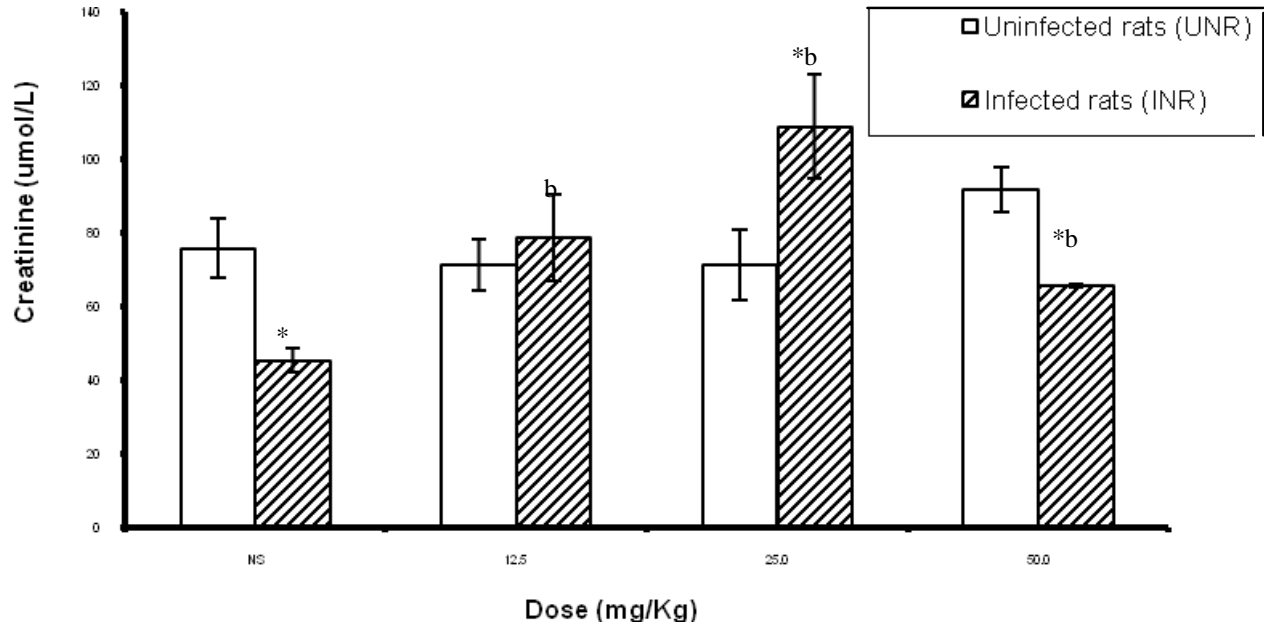


Figure 7. Effect of artemether on the creatinine concentration of the plasma of uninfected and *P. berghei* infected rats. Each bar is mean \pm S.E.M. (n = 20). *Significant difference between INR and UNR. ^Significant difference between control and UNR. ^Significant difference between control and INR.

INR that received 12.5 and 25.0 mg/kg of artemether, the plasma uric acid level was significantly lower than that of the UNR at the corresponding doses. At 50.0 mg/kg, the INR had a higher, though insignificant, plasma uric acid level than the UNR that got the same dose of artemether.

Creatinine

In the UNR, there was no significant change in the plasma creatinine level of the rats at all the doses tested (Figure 7). In the INR, the plasma creatinine levels of the treated rats were significantly higher than those of the control. INR that received NS had a significantly lower plasma creatinine level than the UNR that got the same fluid.

Effect of artemether on liver transaminases

Plasma glutamate oxaloacetate transaminase (GOT)

Among the UNR, the plasma activity of GOT in the treated rats was not significantly different from that of the control rats (Figure 8). Among the INR, the levels of GOT were significantly higher than those of the UNR at any given dose, including UNFC.

Plasma glutamate pyruvate transaminase (GPT)

Among the UNR, GPT activity was significantly higher at 12.5 and 25.0 mg/kg than that of the control rats, while at

50.0 mg/kg, it was not significantly different from that of the UNFC (Figure 9). Among the INR, there was no significant difference between GPT levels in treated and INFC, except at 25.0 mg/kg where it was significantly lower. GPT activity was significantly higher in INR than in UNR at 50.0 mg/kg of artemether. The liver cells are also degenerating. Tissue necrosis was evident in the slide; the cell boundaries are not well defined. The central vein (C) is still enlarged, but not completely well demarcated.

DISCUSSION

In the UNR that were treated with artemether, there was a slight but significant increase in the total and conjugated bilirubin levels in the plasma (Figures 1 and 2); while significantly higher levels were observed in the INR. Scott et al. (1989) and Meshnick et al. (1991) reported that high concentrations of artemisinin caused oxidation of blood cell membrane proteins and decrease in red blood cell deformability; they suggested that the drug may affect the plasma membrane function in infected erythrocytes. Plasmodium causes rapid haemolysis or red blood cell break down, which results in increased plasma bilirubin level. When red blood cells are haemolyzed rapidly and the hepatic cells cannot excrete the bilirubin as rapidly as it is formed, there is an increase in the plasma level of bilirubin (Burtis and Ashwood, 1996; Guyton, 2001). When there is obstruction of the bile ducts as it occurred in gallstone or there is damage

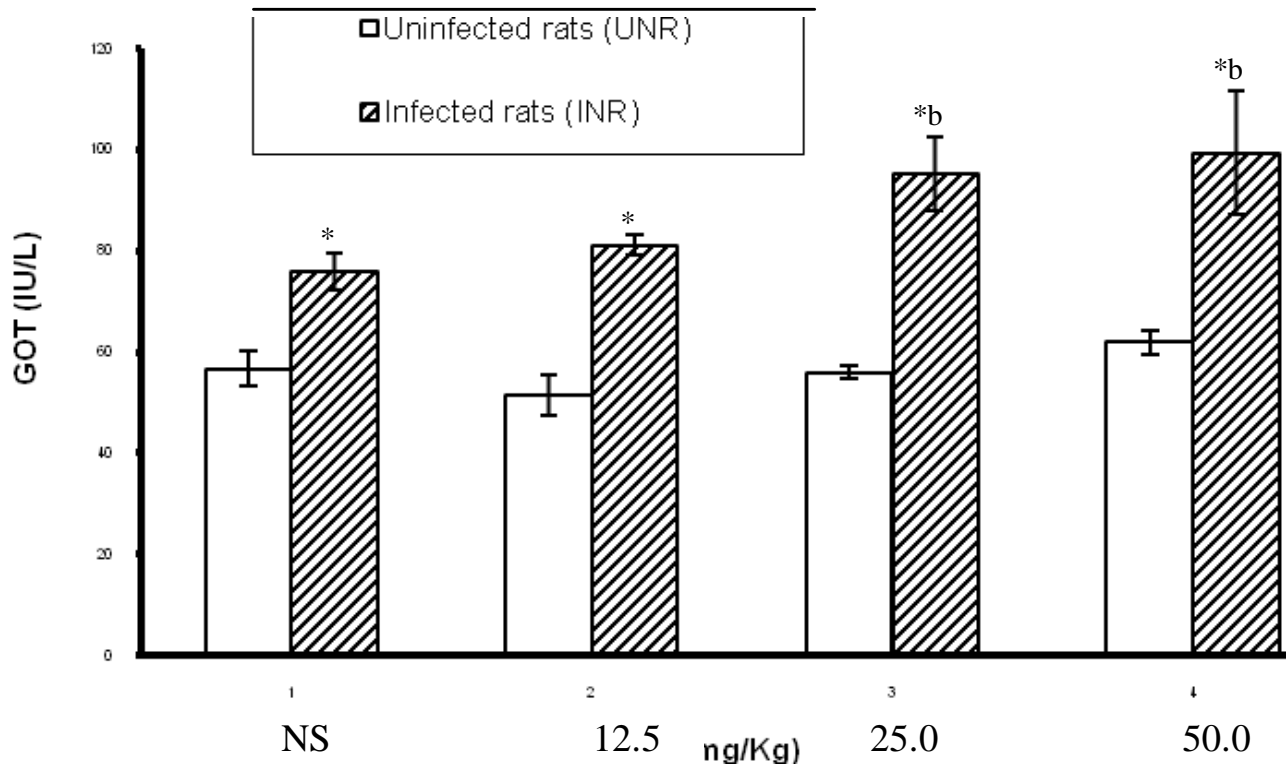


Figure 8. Effect of artemether on the GOT activity in the plasma of uninfected and *P. berghei* infected rats. Each bar is mean \pm S.E.M. (n = 20). *Significant difference between INR and UNR. ^bSignificant difference between control and INR.

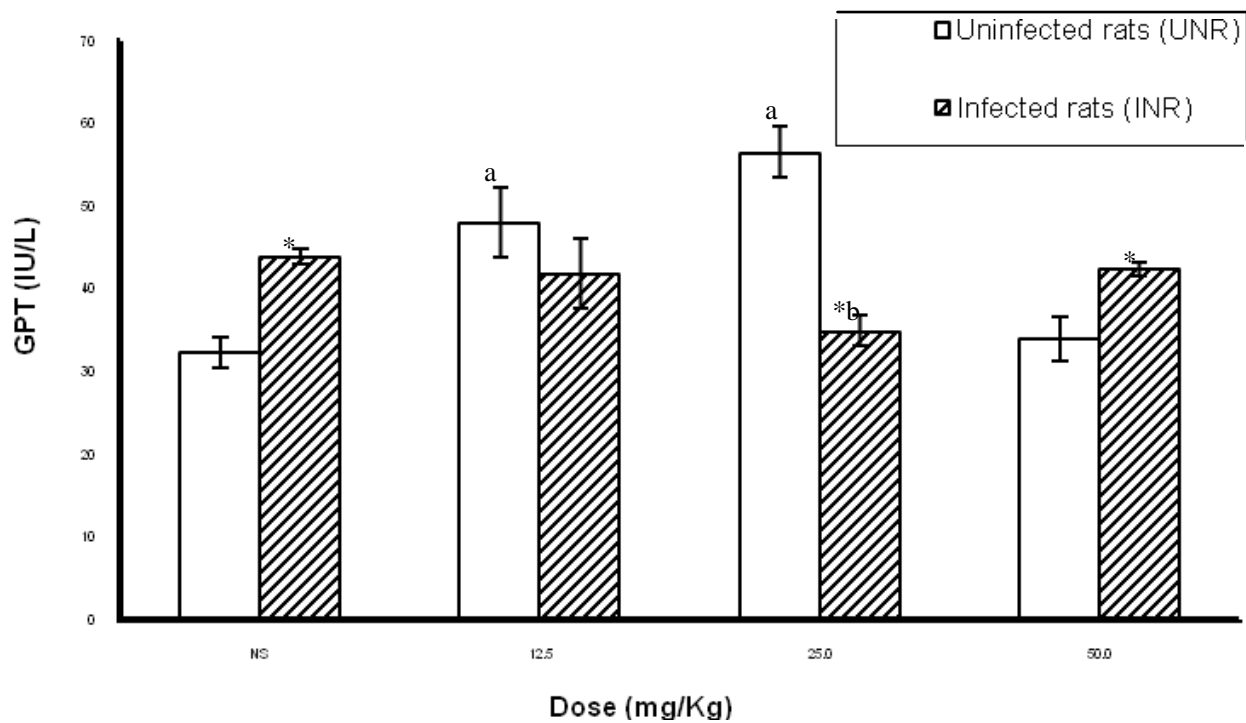


Figure 9. Effect of artemether on the GPT activity in the plasma of uninfected and *P. berghei* infected rats. Each bar is mean \pm S.E.M. (n = 20). *Significant difference between INR and UNR. ^aSignificant difference between control and UNR. ^bSignificant difference between control and INR.

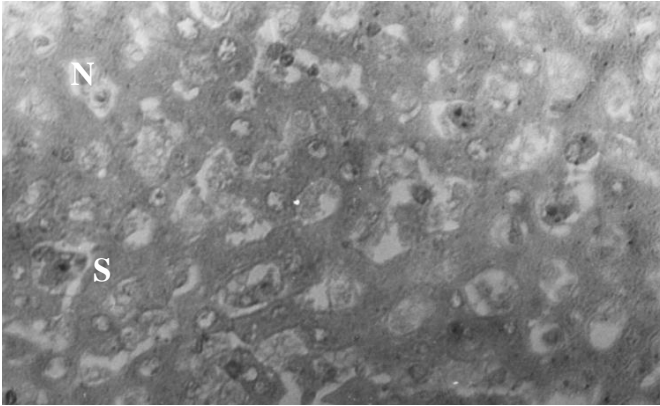


Plate 1. Photomicrograph of the liver of UNR that received normal saline i.m. for 7 days. Mag x400. The cells appear normal. The sinusoidal spaces (S) are distinct. The nuclei of the cells (N) also appear normal.

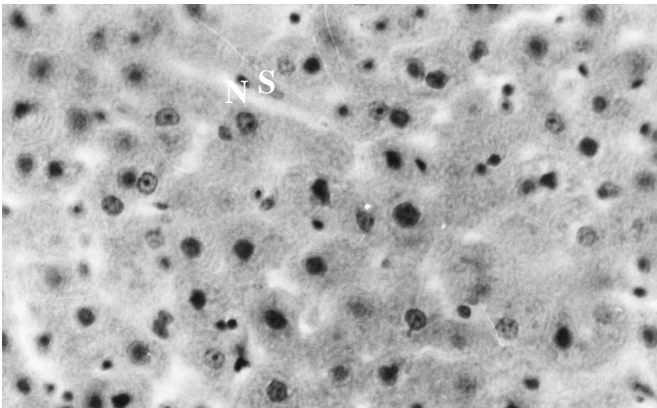


Plate 2. Photomicrograph of the liver of UNR that received 12.5 mg/kg of artemether i.m. for 7 days. Mag x400. The cells also appear normal except that their nuclei (N) are densely stained. The sinusoidal spaces (S) are also very distinct.

to the liver cells, as in hepatitis, the rate of bilirubin formation is normal, but the bilirubin formed cannot pass from the blood into the intestines (Guyton, 2001; Ganong, 2003). The free bilirubin usually still enters the liver cells and becomes conjugated. This conjugated bilirubin is then returned to the blood probably by rupture of the congested bile canaliculi and direct emptying of the bile into the lymph leaving the liver. Thus, most of the bilirubin in the plasma becomes the conjugated type rather than the free type (Guyton, 2001; Ganong, 2003). Plates 1 to 8 show marked liver cell damage in both the infected and uninfected rats that were treated with artemether. The liver cell necrosis and the effect of high concentration of artemisinin on blood cell membrane were responsible for the increased level of bilirubin in the plasma of the treated rats in this study. This study therefore reveals that high doses of artemether were not only neurotoxic as reported by previous researchers, but also hepatotoxic.

The plasma level of cholesterol increased significantly at 50.0 mg/kg in the UNR, but that of the INR was significantly higher than the UNR at all the doses tested (Figure 3). Increased plasma level of cholesterol is one of the features of nephrotic syndrome and obstructive jaundice (Burtis and Ashwood, 1996; 2001; Dunn, 2003). We have reported the nephrotoxic effects of these doses of artemether in healthy and *P. berghei* infected rats in our previous studies (Akamolafe et al., 2011; 2012). The observed increase in the plasma concentrations of cholesterol in the present study revealed that the high doses of artemether tested induced liver damage in both infected and uninfected rats, a fact that was also corroborated by the photomicrographs of the liver tissue (Plate 1 to 8).

There was a slight but significant reduction in the plasma glucose level of the UNR (Figure 4). That of the INR was lower than that of the UNR, except at 50.0 mg/kg (Figure 4). A reduction in food consumption or prolonged starvation reduces the plasma glucose level (Guyton, 2001; Ganong, 2003). The pattern of anorexia induced by artemether in both categories of rats has been reported in one of our previous studies (Akamolafe et al., 2006). In that study, we reported that anorexia was more pronounced in the INR than in the UNR. The lower plasma glucose level of INR was due to greater artemether induced anorexia in the rats. In that same study, we reported an increased urination without a corresponding increase in water intake in INR that received 50 mg/kg of artemether, leading to severe dehydration. The dehydration which occurred in INR that got 50.0 mg/kg of artemether could be responsible for their higher plasma level of glucose despite a significant reduction in food intake.

In the UNR, the plasma activity of GOT in the treated rats was not significantly different from that of the control rats (UNFC) (Figure 8). The activity of this enzyme in INR was significantly greater than in UNR at any particular dose of artemether, and even in the control. Wotton (1964) reported that the heart and the liver are particularly rich in GOT and that damage to these organs which involves necrosis of cells or increased cell permeability can be expected to raise activity of the enzyme. This study reveals extensive damage to the liver tissue caused by artemether. Plates 1 to 8, which was responsible for the increase in GOT activity observed in this study. The greater increase in the activity of GOT in the INR, was due to the inflammation of the liver cells induced by the malaria parasites. Protozoal infection (which includes malaria) evokes a form of inflammatory process in the body tissues and organs (Goodwin, 1976; Bowman and Rand, 1980). Acute inflammatory response in the host is usually triggered off by the injury of the body cells so that the normal intracellular constituents are released into the surroundings. The higher activity of GOT in the INR in this study is in conformity with the report of these workers.

The plasma GPT activity of the UNR increase signifi-

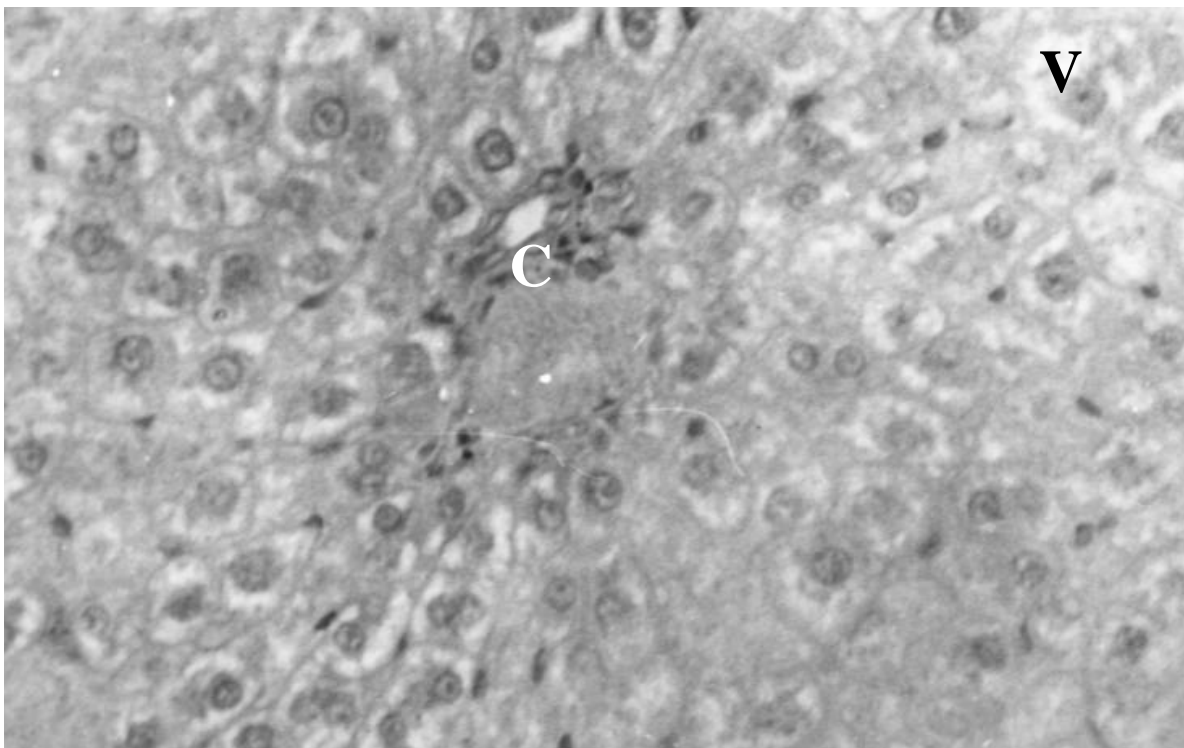


Plate 3. Photomicrograph of the liver of UNR that received 25.0 mg/kg of artemether i.m. for 7 days. Mag x400. There is much cytoplasmic vacuolation (V) which is an evidence of inflammation. The sinusoidal spaces are obliterated. The central vein (C) is highly inflamed.

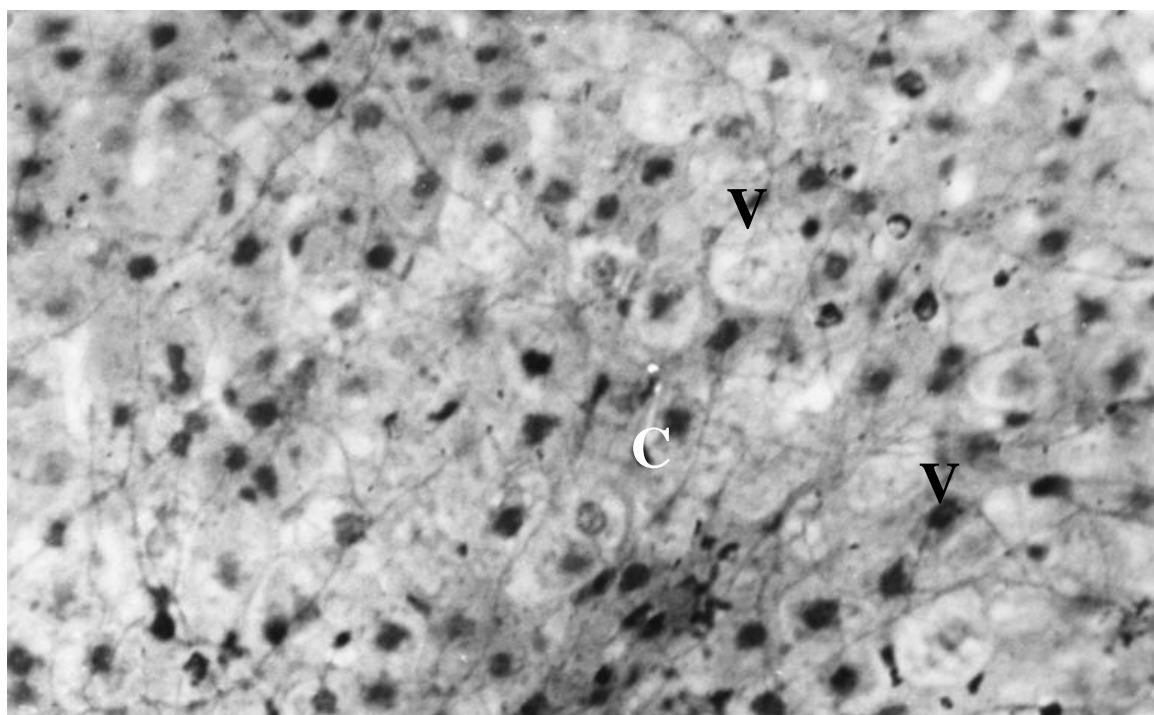


Plate 4. Photomicrograph of the liver of UNR that received 50.0 mg/kg of artemether i.m. for 7 days. Mag x400. There is marked cytoplasmic vacuolation (V). The cells are very big and plumpy as a result of advance stage of inflammation. The sinusoidal spaces are completely closed. The central vein (C) is also inflamed.

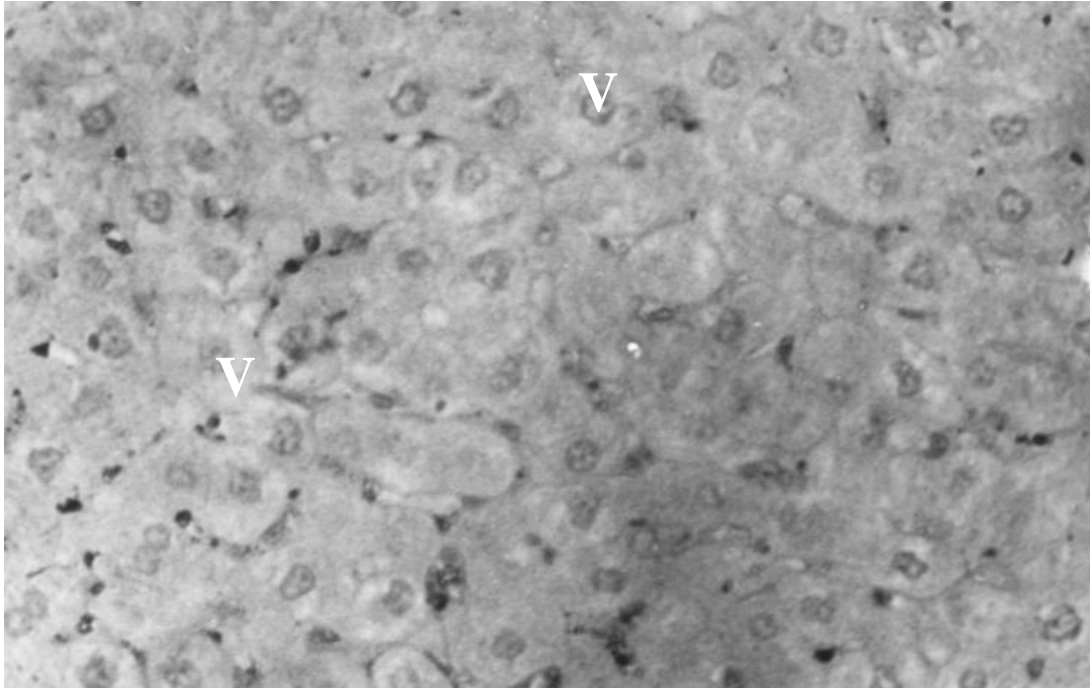


Plate 5. Photomicrograph of the liver of INR that received normal saline i.m. for 7 days. Mag x400. The liver cells are vacuolated (V) and plumpy. The sinusoids are not visible because they have been closed by the inflamed cells.

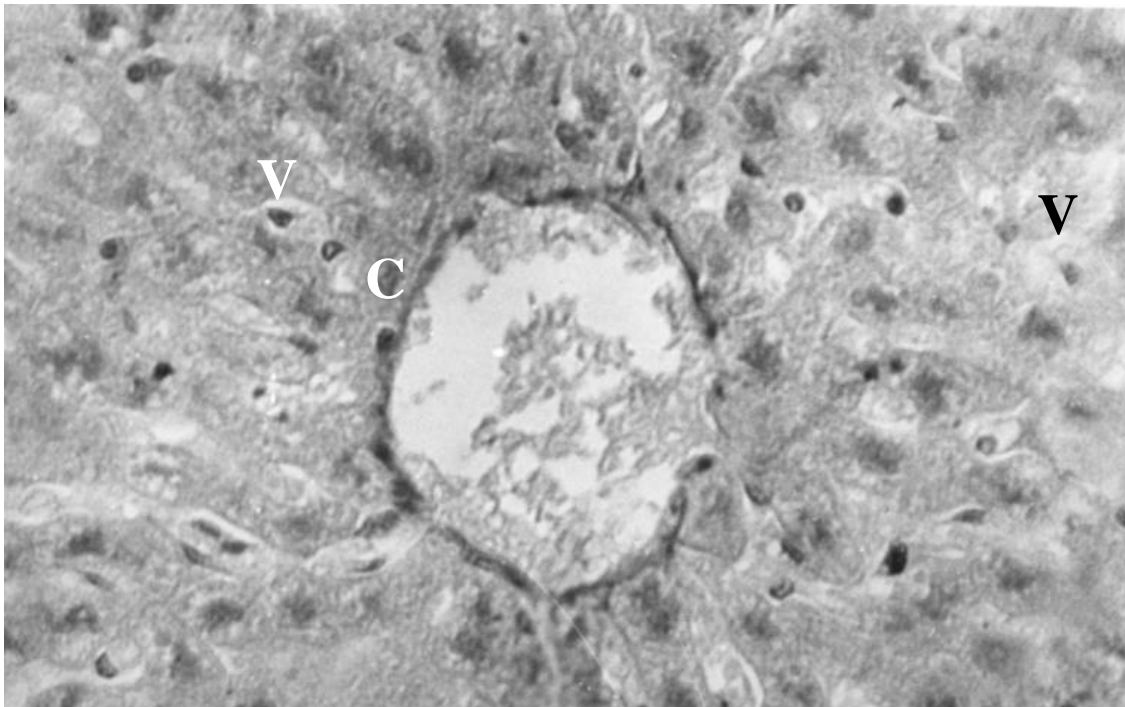


Plate 6. Photomicrograph of the liver of INR that received 12.5 mg/kg of artemether i.m. for 7 days. Mag x400. The liver cells are also vacuolated (V). The sinusoids (S) are closed. The central vein (C) is very large. These are also evidences of inflammation. Cell degeneration is also evident in this tissue.

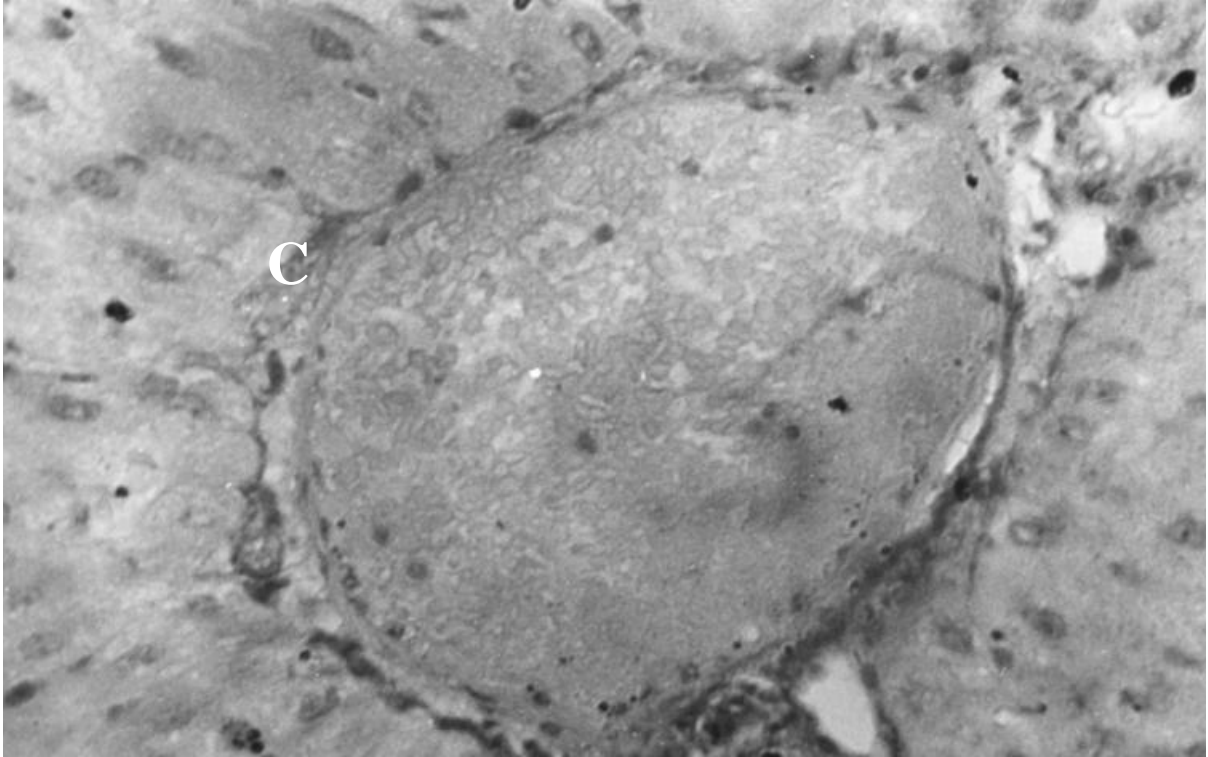


Plate 7. Photomicrograph of the liver of INR that received 25.0 mg/kg of artemether i.m. for 7 days. Mag x400. Some of the liver cells are either degenerating or have degenerated completely. The central vein (C) is enlarged as a result of inflammation.

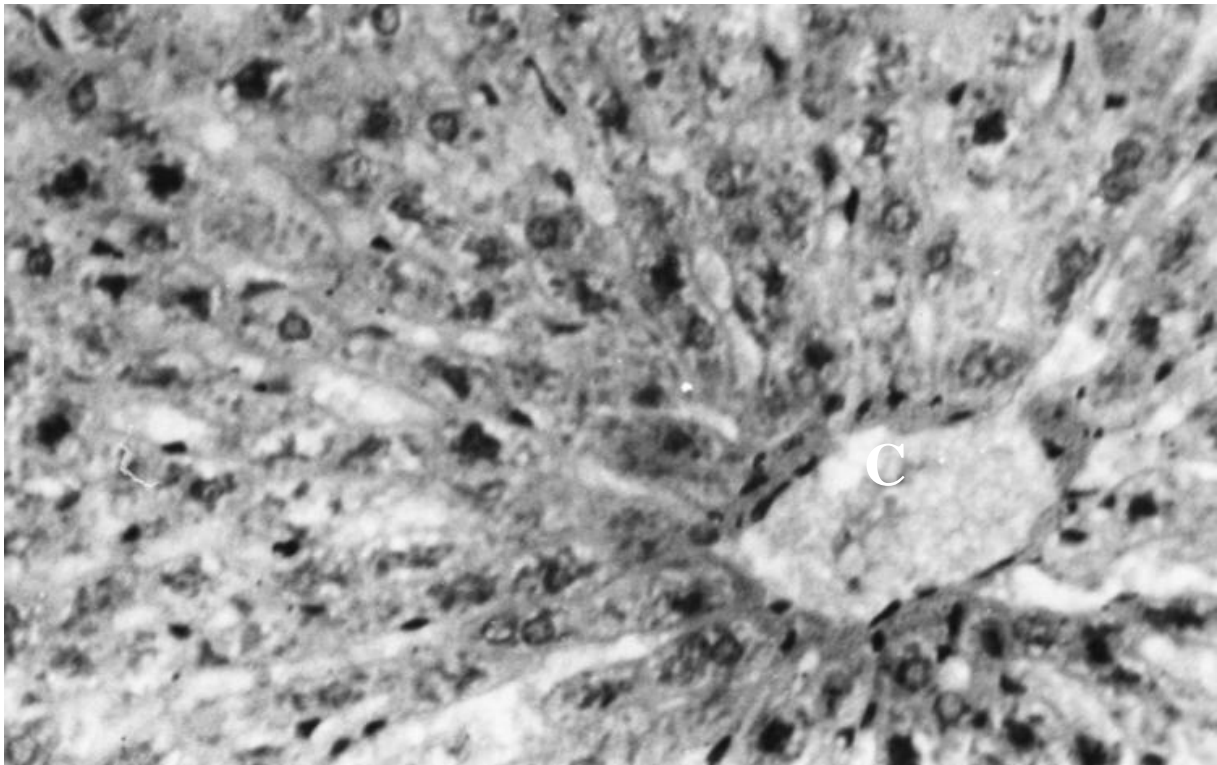


Plate 8. Photomicrograph of the liver of INR that received 50.0 mg/kg of artemether i.m. for 7 days. Mag x400.

cantly at 12.5 and 25.0 mg/kg only, while there was no significant increase in the level of the enzyme in the INR (Figure 9), despite the observed damage to the liver of these rats (Plate 1 to 8). GPT activity rises rapidly after myocardial infarction, peak values are reached within 24 to 48 h, and normal levels are regained typically within three to five days. GPT activity is not usually elevated in myocardial infarction unless the lesion is a large one, or there is associated liver damage (Wotton, 1964; Burtis et al., 1996). The observed reduction in the activity of GPT among the INR in this study, despite the liver damage could be due to the time lag between the onset of liver tissue damage and the assay of the enzyme. From the results of this study, it is concluded that high doses of artemether is toxic to the liver of both infected and uninfected rats, resulting in the impairment of liver functions which manifested as tissue necrosis, accumulation of metabolic wastes and elevation of the activity of liver enzymes in the blood.

ACKNOWLEDGEMENTS

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