Evaluation of the hepatic effect of concomitant administration of ciprofloxacin and some antimalarial drugs in *Plasmodium berghei* infected mice: An *in vivo* study

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Abstract: This study evaluated the hepatotoxic effects of artesunate (AS), artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) co-administration with ciprofloxacin (CIP) using animal model. Chloroquine sensitive *Plasmodium berghei* NK65 strain infected albino mice (120) were utilized for this study, carried out in three phases. Phase 1 comprised eleven groups treated with different doses of either AS, AL, ASAQ or CIP alone. Phase 2 consisted of nine groups treated with 7mg/kg of CIP combined with different doses of AS, AL, ASAQ. Phase 3 comprised ten groups treated with 14mg/kg of CIP (CIP2) with different doses of AS, AL, ASAQ. Seventy-two hours after administration of drugs, toxicity was determined by evaluating the effect of drugs on liver enzymes using spectrophotometer. Statistical analysis revealed that CIP alone significantly ($P<0.05$) reduced the levels of Aspartate Transaminase (AST) and Serum Alanine Transaminase (ALT) compared to AS, AL and ASAQ alone. Combination of different doses of AS, AL and ASAQ with 7mg/kg CIP significantly increased the level of AST and ALT while combination of AS, AL and ASAQ with 14mg/kg CIP significantly decreased AST and ALT levels. Care should be taken during the co-administration of low dose ciprofloxacin with artesunate, artemether-lumefantrine or artesunate-amodiaquine.

Keywords: Artemisinin combination therapy, Aspartate transaminase, Ciprofloxacin, Malaria, Serum alanine transaminase.

INTRODUCTION

Malaria has a negative effect on the development of Africa. More than half of outpatient visits to health facilities in Nigeria are due to malaria with the disease state also contributing to childhood and maternal deaths (NMCP, 2015). Loss of productivity and costs from malaria prophylaxis and therapy causes Nigeria billions of Naira annually (NMCP, 2015).

Artemisinin combination therapies (ACTs) are the only antimalarial drugs with very minimal resistance recorded (Noedl *et al.,* 2008). The ACTs reduce malaria parasite load and resolve malaria symptoms rapidly. Some previous studies have described artemisinin and its derivatives to be generally safe and well tolerated (Nosten *et al.,* 2000; Maiteki-Sebuguzi *et al.,* 2008).

Salmonella infection in Nigeria is on the rise, with the resultant illnesses leading to deaths in some cases (Akinyemi *et al.,* 2000). There is also a high incidence of malaria and enteric fever co-infection (Uneke, 2008). The frequent co-existence of these infections has led to the co-administration of an antimalarial and antibiotic(s). Ciprofloxacin is a broad spectrum fluoroquinolone antibacterial agent. It is structurally related to nalidixic acid. Ciprofloxacin inhibits the DNA gyrase activity, thereby causing a significant anti-parasitic effect against various strains of *Plasmodium falciparum* malaria (Mahmoudi *et al.,* 2003; Salmon *et al.,* 1990).

The co-administration of artemisinin/artemisinin derivatives and ciprofloxacin is a common practice in Nigeria in the treatment of malaria and enteric fever co-infection (Uwah *et al.,* 2014). The co-administration of two or more drugs is usually accompanied by a variety of therapeutic implications ranging from opposition, alteration, synergism, potentiating as well as physical and chemical antagonism (Adegbolagun and Uyelumo, 2013). Clinically significant interactions refer to a combination of therapeutic agents which have direct consequences on the patient's condition (Thanacoody, 2012). An *in-vivo* animal study showed that the effect of quinine is potentiated when administered in combination with ciprofloxacin (Ejikeme *et al.*, 2014).

Although liver enzymes are usually higher in acute liver toxicity they appear to decline with persistent intoxication (Obi *et al.,* 2004; Adegbesan *et al*., 2014). Induction of hepatic damage by some antimalarial agents has been reported in some studies (Farombi *et al.,* 2000; Pari and Amali, 2005; Adegbesan *et al*., 2014). The major site for the metabolism of the ACTs is the liver (Adegbesan *et al*.,

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2014. Some studies have reported that artemisinin caused an increase in hepatic enzyme levels (Tabassum and Agarwal, 2003; Aprioku and Obianime, 2011).

Since ciprofloxacin is known to potentiate antimalarial activity of antimalarial agents, there is possibility of it enhancing their toxicity. Hence this study evaluates the hepatotoxic effect of co-administration of ciprofloxacin with artesunate, artemether-lumefantrine and artesunate-amodiaquine using AST and ALT levels in animal model.

MATERIALS AND METHODS

Drugs

Tablets Artesunate 50mg (Artesunat®; Mekophar Chemical Pharmaceutical Joint-Stock Company, Vietnam), Artesunate and amodiaquine 100/270 mg Winthrop® (Sanofi Aventis), Artemether/lumefantrine 20/120mg Coartem® (Norvatis) and ciprofloxacin 500 mg Ciprotab® (Fidson Healthcare Plc, Nigeria). All the drugs were purchased from a registered pharmacy in Nsukka, Enugu State, Nigeria.

Equipment and reagents

UV spectrophotometer (PEC Medicals, USA) and microscope. Reagents used include 0.9% normal saline, oil immersion, sodium hydroxide, Giemsa stain solution (pH 7.2), chloroform and methanol; all of analytical grade. Randox Laboratories Limited, United Kingdom was the source for the ALT and AST kits.

Animals

Adult male and female Swiss albino mice (120) weighing 18-25g were used for this study. The Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State provided and maintained the mice. The mice were observed under light/dark cycle in metabolic, well ventilated, rodent cubicle, fed with pelleted feed and provided with access to clean drinking water as desired. The recommendations from the Declaration of Helsinki as recorded by the Institute for Laboratory Animal Research, Washington, DC (1996) were observed in handling the animals used in this study.

Parasite

The Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University College Hospital, Ibadan, Nigeria was the source for the mouse-infective Chloroquine-sensitive strain of *Plasmodium berghei* NK-65

Parasite inoculation

Cryopreserved chloroquine-sensitive parasite was used to infect a donor mouse to develop parasitaemia. Microscopic examination of a thin blood film was used to establish the presence of parasitaemia (Olalubi *et al.,* 2011). The parasite-infested blood (1ml) was diluted with normal saline to 25mL, then 0.2mL of the dilution was injected intra-peritoneally into each of the healthy mice (Olalubi *et al.,* 2011).

Treatment of experimental animals

Treatment of animals started 72 hours after inoculation. The infected albino mice (120) were randomized into thirty groups (n = 4). This study was carried out in three phases.

Phase 1: Eleven groups were used in this phase. Two groups were treated with ciprofloxacin (CIP) alone at 7 mg/kg (CIP 1) and 14 mg/kg (CIP 2) doses respectively; three groups were treated with 3, 6 and 12mg/kg Artesunate (AS) doses respectively; three groups received 16, 32 and 64mg/kg doses of Artemether-lumefantrine (AL) alone respectively and the last three groups were treated with 11, 22 and 44mg/kg of artesunate-amodiaquine (ASAQ) alone respectively.

Phase 2: Nine groups of infected animals were used in this phase. Three groups received 3mg/kg, 6mg/kg, 12 mg/kg AS co-administered with 7mg/kg ciprofloxacin (CIP1) respectively. Three other groups received 16, 32 and 64 mg/kg of AL with CIP1 respectively. The last three groups respectively received 11, 22 and 44mg/kg of ASAQ with CIP1.

Phase 3: Ten groups of animals were used in this phase. Three groups were given 3, 6 and 12mg/kg AS co-administered with 14mg/kg of ciprofloxacin (CIP2) respectively. Another three groups also received 16, 32 and 64 mg/kg of AL with CIP2 respectively. Three other groups received 11, 22 and 44 mg/kg of ASAQ with CIP2 respectively. The last group received 0.1ml/kg of distilled water only and served as the negative control. All drugs administered were done by oral route once daily for three days based on the animal's body weight.

Toxicological evaluation

Chloroform anesthesia was used to sacrifice all the infected animals. Cardiac puncture using syringe and needle was used to collect about 2mL of blood into non-heparinized bottles from each animal. The serum used for ALT and AST activities assessments using Reitman-Frankel method were obtained from the blood samples centrifuged at 3000 rpm (Nwanjo *et al.,* 2007)

STATISTICAL ANALYSIS

Mean ± standard deviation (SD) was used to express the results. The Student t-test and one way analysis of variance (ANOVA) was used to analyze differences among the treated groups. Data were analyzed at 95% confidence interval with statistical significance set at $p< 0.05$.

RESULTS

Plasmodial infection in the mice was noticeable in the blood after 72 hours of inoculation. The results are displayed in the figs. below.

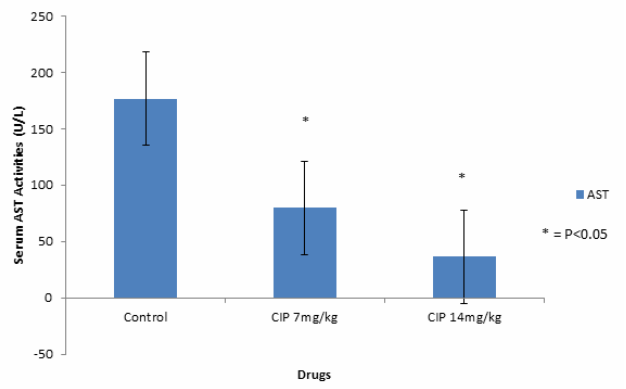

Fig. 1: Effect of Ciprofloxacin on the mean serum concentration ± SE of Aspartate transaminase (AST) in *P.berghei* infected mice.

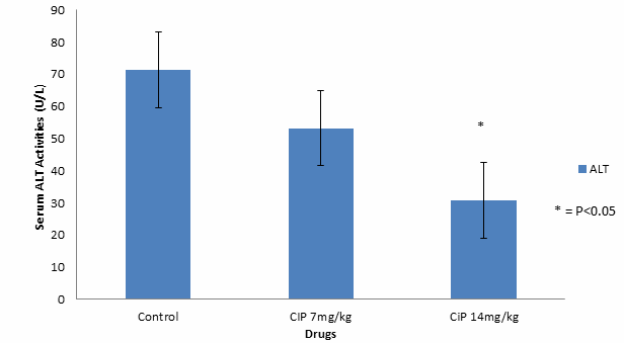

Fig. 2: Effect of Ciprofloxacin on the serum alanine aminotransferase (ALT) ± SE in *P. berghei* infected mice.

In fig. 1, the AST level decreased from 176.50±13.44 U/L in the control group to 80.00±21.09 U/L in the group treated with ciprofloxacin 7mg/kg (CIP1) and 36.50± 17.90U/L in group treated with ciprofloxacin 14 mg/kg (CIP2), respectively. The treated groups showed statistically significant decreases over the control group and in a dose dependent manner.

Fig. 2 depicts a decrease in ALT level from 71.50± 4.95U/L in the control group to 53.25±22.51 U/L in group treated with CIP1 and 30.75±16.62 U/L in group treated with CIP2. The decrease was significant ($p<0.05$) in the CIP2 group over the control and in a dose dependent manner.

Also, the AST activities decreased from 176.50±13.44U/L in the control group to 108.75±42.44 U/L in 3mg/kg treated group, 110±60.65U/L in 6mg/kg treated group and 147±11.02 U/L in groups treated with 12mg/kg Artesunate alone at the end of the 72h treatment; but the groups treated with Artesunate co-administered with 7mg/kg of ciprofloxacin caused increase in AST activity at 3mg/kg and 6mg/kg of Artesunate beyond the control group. The groups treated with Artesunate co-administered with 14mg/kg ciprofloxacin caused decrease in AST activity at 3mg/kg, 6mg/kg and 12mg/kg of Artesunate as thus 109.25±19.31U/L, 147.75±60.75 and 157±25.53 U/L respectively (fig. 3).

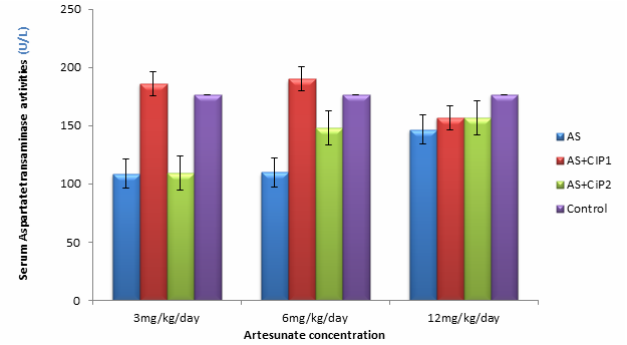

Fig. 3: Effect of Artesunate (AS) alone and in combination with Ciprofloxacin (CIP) on the serum AST activities in *P.berghei* infected mice.

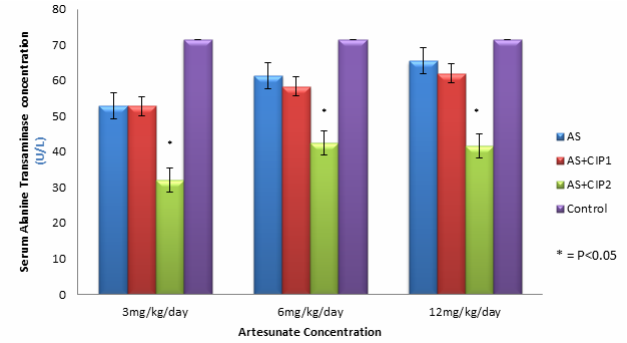

Fig. 4: Effect of Artesunate (AS) alone and in combination with Ciprofloxacin (CIP) on the serum ALT activities in *P.berghei* infected mice.

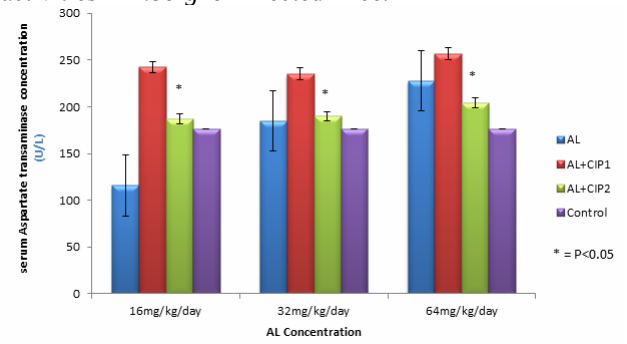

Fig. 5: Effect of Artemether- Lumefantrine (AL) alone and in combination with Ciprofloxacin (CIP) on the serum AST in *P. berghei* infected mice.

In fig. 4, there was a dose dependent decrease in the ALT concentration in all the treated groups. The groups treated with Artesunate co-administered with 14mg/kg of ciprofloxacin produced a statistically significant ($p<0.05$) decrease in AST activities when compared to the control group.

Fig. 5 shows that the AL alone steadily increased the AST activity in a dose dependent pattern though not statistically significant. The AST activity in groups treated with lower dose of ciprofloxacin (7mg/kg, CIP1) with different doses of AL were higher than AL groups alone. The AL doses with low dose of ciprofloxacin (14mg/kg, CIP2) showed significantly lower AST activity than those combined with lower dose of ciprofloxacin but all the doses AL combined with 14mg/kg ciprofloxacin (CIP2) caused increase in AST activity than the control groups treated with distilled water.

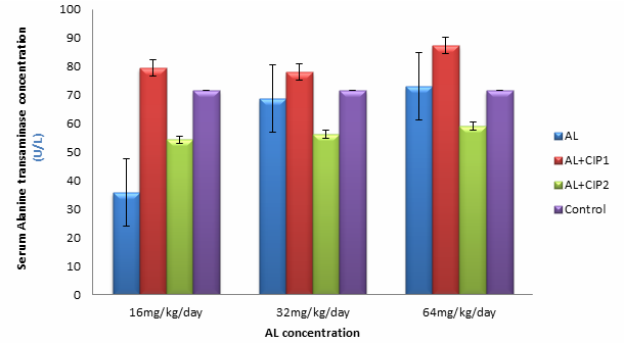

Fig. 6: Effect of Artemether-lumefantrine (AL) alone and in combination with Ciprofloxacin (CIP) on the serum ALT activities in *P.berghei* infected mice.

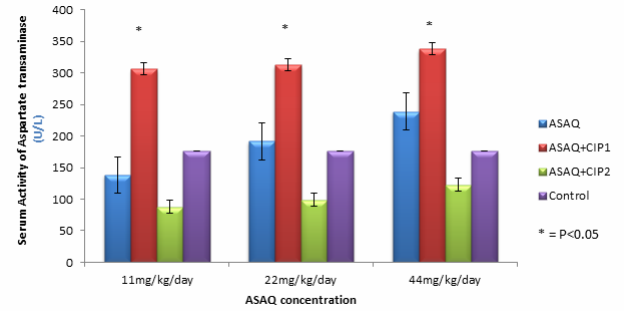

Fig. 7: Effect of Artesunate-amodiaquine (ASAQ) alone and in combination with Ciprofloxacin (CIP) on the mean serum ± SE AST activities in *P.berghei* infected mice.

The AL alone steadily increased the ALT activity in a dose dependent pattern though not statistically significant. The ALT activity in groups combined with AL and CIP1 were higher than the AL groups alone and control group. The AL doses with CIP1 dose of ciprofloxacin showed higher activity of ALT than those combined with CIP2 dose of ciprofloxacin (fig. 6).

In fig. 7, ASAQ alone steadily increased the AST activities in a dose dependent pattern though not statistically significant. The AST activities in the groups treated with lower dose of ciprofloxacin with the different doses of ASAQ were significantly higher than ASAQ groups alone and the control group. The ASAQ doses with low dose of ciprofloxacin showed significantly lower activities of AST than those combined with lower dose of ciprofloxacin.

From fig. 8, the ASAQ groups increased the activities of ALT in a dose dependent manner. The ASAQ doses with low dose of ciprofloxacin (CIP2) showed significantly lower activity of ALT than those combined with lower dose of ciprofloxacin (CIP1).

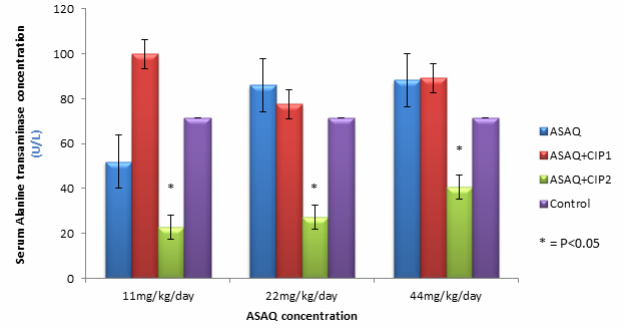

Fig. 8: Effect of Artesunate-amodiaquine (ASAQ) alone and in combination with Ciprofloxacin (CIP) on the mean serum ALT ± SE activities in *P.berghei* infected mice.

DISCUSSION

Various antimalarials have proved to influence biochemical environment within and around the *Plasmodium* infected erythrocytes with variable outcomes (Olayemi *et al.*, 2012). Malaria parasites can invade liver cells causing congestion, sinusoidal obstruction and inflammation of the cells (Jarikre *et al.*, 2001). These can cause the liver enzymes (transaminases and alkaline phosphatase) to leak into the circulatory system with a resultant enhanced enzyme activity (Burtis *et al.*, 2001). Aminotransferase (transaminase) levels indicate hepatocellular injury, damage to cytoplasmic and/or mitochondrial membranes (Mayne, 1994; Wynne and Edward, 2012). This is characterized by release of enzyme aspartate and alanine aminotransferases from damaged hepatocytes. These enzymes are found in many body tissues with the highest concentration in hepatocytes and muscle cells. In the liver, ALT is found only in the cytoplasm whereas AST is found in both the cytoplasm and mitochondrion (Soares *et al*., 2013).

Results from this study showed that the malaria parasite, *Plasmodium berghei* caused an increase in the activities of the serum liver enzymes (ALT and AST). This is in agreement with studies that reported an increase in liver aminotransferases and a minimal increase in alkaline phosphatase in human (Jarikre *et al.*, 2001; Maduka *et al.*, 2008). The increase in the liver enzymes may be as a result of damage to the hepatocytes by the parasite since merozoites infect and rupture the liver cells. Once the liver cells are damaged, these enzymes leak into the circulatory system (Onyesom, 2012). The results also showed that there was more AST activity than ALT activity probably because liver cells have a higher AST concentration than ALT (Hassan and Yousef, 2009). When the membranes of the cytoplasm or mitochondrion are

damaged, as occurs in infiltrative disorders, there is a lesser increase in plasma ALT than AST activities (Mayne, 1994).

Treatment with ciprofloxacin resulted in significantly reduced AST activities when compared to the control group of malarious animals. There was clinically significant reduction in liver enzyme when treated with CIP alone compared to treatment with AS, AL and ASAQ alone suggesting that ACTs induced increase in liver enzyme activity. This finding is in line with the previous reports that artemisinin caused enhanced hepatic enzyme serum levels (Tabassum and Agarwal, 2003; Aprioku and Obianime, 2011). However, the ability of ACTs to induce hepatic injuries has caused a lot of controversy. Rats administered with ACTs had more liver damage (Adaramoye *et al.,* 2008) and no liver damage (Georgewill and Ebong, 2012) in two different studies. Another study showed increased liver enzyme activities in rats treated with therapeutic doses of Lonart®, an ACT formulation (Adegbesan *et al.,* 2014).

Our study revealed that the co-administration of different concentrations of AS, AL and ASAQ with 7 mg/kg ciprofloxacin resulted in a significant increase in both AST and ALT levels when compared to their combination with 14 mg/kg ciprofloxacin. This suggests that 14 mg/kg ciprofloxacin when co-administered with AS, AL and ASAQ may be hepatoprotective. This finding is in agreement with the report which showed that ciprofloxacin produced no significant liver changes (histology) after evaluation of 20 and 200mg/kg/day of ciprofloxacin (Başaran *et al.,* 1993).

The activities of ALT and AST when treated with artesunate alone were dose dependent but non-significantly reduced when compared to the control. Artesunate in laboratory animals have been reported to be devoid of hepatotoxic effects (Obianime and Aprioku, 2009; Utoh-Nedosa *et al.,* 2009), although, a high dose of 16mg/kg has been reported to result in significant alteration of liver enzymes (Nwanjo and Oze, 2007). The combination of artesunate with lower dose ciprofloxacin non-significantly increased the activities of AST. Compared to the control, the effect of this combination on the ALT activity is dose dependent with non-significant difference. The increase in liver enzyme activities may be as a result of the drug combination. The activities of ALT of the group treated with artesunate and higher dose of ciprofloxacin were dose dependently and significantly decreased but non-significantly decreased in AST activities when compared to the control group.

Assessment of the liver enzyme showed a difference (non-significant) in the ALT and AST values for different doses of artemether-lumefantrine and ciprofloxacin compared to the control. Increase in activities of ALT and AST by AL was dose- dependent. Previous studies have reported an increase in liver oxidative stress from oral artemether-lumefantrine combination causing a significant elevation of ALT and AST (Adaramoye *et al.,* 2008; Adegbesan *et al*., 2014). The combination of artemether-lumefantrine with lower dose of ciprofloxacin increased the activities of ALT and AST more than the combination with high dose ciprofloxacin.

This study also showed a dose-dependent increase (non-significant) in liver enzyme levels on administration of artesunate/amodiaquine alone. The ASAQ alone increased the levels of AST and ALT more than the levels observed with AS and AL. This increased liver enzyme activity observed with ASAQ could be as a result of amodiaquine action as artemisinin and its derivatives on their own have low toxicological effects and any toxicity observed in artemisinin combination treatment may be due to the partner agents such as amodiaquine, lumefantrine, mefloquine, and piperaquine (Nosten and White, 2007). Moreover, a study has reported that amodiaquine can induce hepatic damage (Farombi *et al.,* 2000). ALT and AST activities increased significantly in the treatment with artesunate-amodiaquine combination and lower dose ciprofloxacin when compared to the control group. However, combination of ASAQ with high dose 14 mg/kg ciprofloxacin appeared to be significantly hepato-protective as it gave a lower AST and ALT value than ASAQ combination with 7 mg/kg. Our study observations differed from another study (Olayemi *et al.,* 2009) which observed a higher liver enzyme value at higher concentration of ciprofloxacin compared to their lower concentrations.

The artemisinin derivatives have a characteristic endoperoxide bridge and generate free radicals which may distribute to other parts of the body, the liver inclusive, causing toxicity (Cumming *et al.,* 1997; Robert and Meunier, 1998). It is possible that artesunate, artemether-lumefantrine and artesunate-amodiaquine used in this study may have acted in a similar manner to induce toxicity through the generation of free radicals.

The study does not show the exact mechanisms of interactions of these drug combinations. Also, other parameters in liver function tests were not considered.

CONCLUSION

Our study has shown that concurrent administration of 7 kg/mg of ciprofloxacin with artesunate, artemether-lumefantrine or artesunate-amodiaquine exposed animal to hepatotoxic effect, while 14mg/kg ciprofloxacin exhibited a hepatoprotective effect. Therefore, it is suggested that caution be taken in administering low dose ciprofloxacin with ACTs in patients with impaired hepatic function. There is need for either dose adjustment or

adequate time interval be observed in order to avoid compromising therapeutic outcome.

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