African Journal of Biotechnology Vol. 10(61), pp. 13238-13243, 10 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.716
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effect of artemether on rat hepatocytes during acute damage

Oguntibeju, O. O.[1]*, Akinola, F. F.[2] and Okonkwo, K. G.[3]

[1]Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, South Africa.
[2]Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[3]Department of Biomedical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Accepted 24 August, 2011

The aim of this study was to investigate the hepatocellular regeneration ability of artemether in experimental CCl_4-induced acute damage in rat hepatocytes. 20 Wistar rats were equally allocated to 4 groups. The first group was designated as the distilled water control group (group 1); the second group was CCl_4 toxic control (group 2) and received oral administration of CCl_4 (50 mg/kg body weight diluted 1:1 in Tween 80) for three days. The third group (group 3) was treated with CCl_4 followed by a subsequent administration of artemether and the fourth group (group 4) received artemether (50 mg/kg body weight) for 3 days. The body weight was recorded before and after the experiment and the plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkanine phosphatase (ALP) and album (ALB) levels were determined as markers of hepatocellular damage. Plasma AST and ALT levels were significantly higher in group 2, in comparison with group 1 ($P < 0.05$). Also, increase in ALP was observed in group 2 when compared to group 1. Observed improvement in plasma AST, ALT and ALP was recorded in group 3. In conclusion, artemether may have a possible regenerating effect on hepatocellular damage as it showed inhibitory effect of CCl_4 and could thus enhance normal liver functions.

Keywords: Uncomplicated malaria, artemether, antimalarial agent, liver enzymes.

INTRODUCTION

Artemether is a semi-synthetic drug used to treat malaria, especially chloroquine resistant malaria in Nigeria. Malaria has continued to be a major health problem in some parts of the world, especially in tropical countries (WHO, 2003). In sub-Saharan Africa, over a million persons die annually from malaria infection. This infection is caused by protozoan in the genus plasmodium which is transmitted by female anopheles mosquitoes. The plasmodium species that infect humans are *Plasmodium falciparum, Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium ovale*. Characteristically, the infection causes recurring attacks of severe chills, high fever and sweating (Ayyub et al., 2000; WHO, 2003; Falade et al., 2005). Delay in treating malaria may result in rapid deterioration in the patients' conditions, together with the development of a number of life threatening complications. One of such complications includes acute liver damage especially due to *P. falciparum* (Ayyub et al., 2000).

Consequently, chemotherapy is initiated as using antimalarial drugs. Chloroquine and quinine have been generally used as antimalarial drugs (Lehne et al., 1990; Falade et al., 2005). They are active against erythrocytic phases of the parasites, sporozoites and the exo-erythrocytic phases. However, the chloroquine resistant strains of the malaria parasites are much more prevalent in tropical countries such as Nigeria. WHO reported that chloroquine resistance is now virtually global and recommended as an effective treatment for patients with uncomplicated or severe malaria using quinine or where appropriate, an artemisinin derivative is used (WHO, 2000).

*Corresponding author. E-mail: oguntibejuo@cput.ac.za or bejufemi@yahoo.co.uk. Tel: +27219538495. Fax: +27219538490.

Artemisinins are derived from leaves of a plant called sweet wormwood or sweet annie (*Artemisia annua*) by Chinese scientists. Artemisinins" extracts have antipyretic properties. Since this initial discovery, a wide range of semi-synthetic, oil and water soluble derivatives of artemisinin have been developed with variety of formulations (Meshnick et al., 2002). Artemisinin derivatives have impressive parasiticidal properties *in vivo* and *in vitro* (White, 1997; Haynes et al., 2003). It rapidly arrest parasite metabolism and kill parasites more quickly than other antimalaria drugs (White, 1994).

Prospective clinical studies of over 10,000 patients, including post marketing surveillance of 4,600 patients in Thailand, have shown that this class of drugs reduces parasitemia and malaria-related symptoms more promptly than any previously known antimalarial agent and without drug-related adverse effects (WHO, 1998). Oral, rectal and intra-muscular regimens are generally rapidly effective and well tolerated treatments for both severe and uncomplicated *falciparum* malaria (Murphy et al., 1997). As a fast-acting antimalarial agent, artemether starts acting within 12 h of administration and makes it very useful in managing severe and complicated *P. falciparum* malaria and also effective in treating chloroquine-resistant strains of *P. falciparum* (Murphy et al., 1997; Falade et al., 2005).

Cases of hepatic dysfunction are being increasingly reported in patients with *P. falciparum* infection, from different parts of the world. The extent of hepatocellular dysfunction varies from mild abnormalities in liver function tests to hepatic failure. Patients with hepatocellular dysfunction in malaria are more prone to develop complications, but have a favorable outcome if hepatic involvement is recognized early, reversed and managed properly (John, 1995).

However, it is important to examine for hepatic dysfunction in patients with severe malaria, distinguish it from fulminant hepatic failure and manage it aggressively and this has increased the importance of the design and discovery of antimalarial drugs that will also confer significant protective and regenerative effect on the liver (John, 1995).

A study of such significant regenerative effect will be possible by exploring the fact that there is increase in hepatic enzymes activities indicated to be liver damage which has been observed in carbon tetrachloride induced liver damage using apparently healthy rats as experimental model (John, 1995).

Previous work has been done on artemether in areas such as potency and hepatotoxicity. However, considering the fact that it is one of the most active antimalarial drug recommended by World Health Organization in cases of uncomplicated malaria, especially in *P. falciparum* infection, this study is serves as a post-market non-clinical study of the drug in Nigeria and is intended to explore the possibilities that this novel antimalarial drug could have regenerative effect on acute liver damage which are associated with severe falciparum malaria which is common in Nigeria. The study aimed to investigate the possible hepatic regenerative effect of artemether on carbon tetrachloride induced acute liver damage and its significance using a rat model.

MATERIALS AND METHODS

Study design

This was an experimental laboratory-based study.

Animal care

The experiment was carried out at the Animal House, Department of Biomedical Science, Faculty of Basic Medical Science, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria and the study lasted for a period of 3 weeks. Twenty (20) Wistar rats weighing 120 to 220 g each were acquired from the animal house of the University. They were housed in cages and kept in the Animal House of the Department of Biomedical Science, Osogbo with an average ambient temperature of 28 to 32°C with a 12 h light-dark cycle and received rat feed (rat pellet) and water *ad libitium* during an acclimatization period of two weeks and also throughout the period of the experiment. The animals were then divided into groups as described in the experimental design.

Experimental design and protocol

20 Wistar rats were weighed and divided into four (4) groups of 5 (five) animals in each group as follows: Group 1: Untreated control: fed with standard rat pellet and water only throughout the period of experimentation. Group 2 (positive control): treated with carbon tetrachloride (diluted 1:1 in Tween 80) with a dose of 50 mg/kg body weight orally, once daily for 3 days to induce acute liver damage. Group 3 (test): Liver damage induced rats treated with artemether solution orally with dose of 50 mg/kg body weight, once daily for 3 days. Group 4 (drug control): Treated with artemether with an oral dose of 50 mg/kg body weight dissolved in Tween 80, once daily for 3 days.

Specimen collection

At the end of the experiment, blood samples (2 ml) were collected from each rat by tail bleeding and subsequent cardiac puncture using 2 ml syringes, into separate lithium heparinized containers. The plasma was extracted from each sample by centrifugation at 4000 rpm for 10 min. Plasma was stored at -20°C. The effect of carbon tetrachloride and artemether and their combined effect were assessed by comparing biochemical liver function tests for each animal.

Biochemical analysis

Estimation of plasma transaminases

Plasma transaminases (alanine aminotransferase, ALT and aspartate amino transferase, AST) were determined by method of Reitman and Frankel (1957).

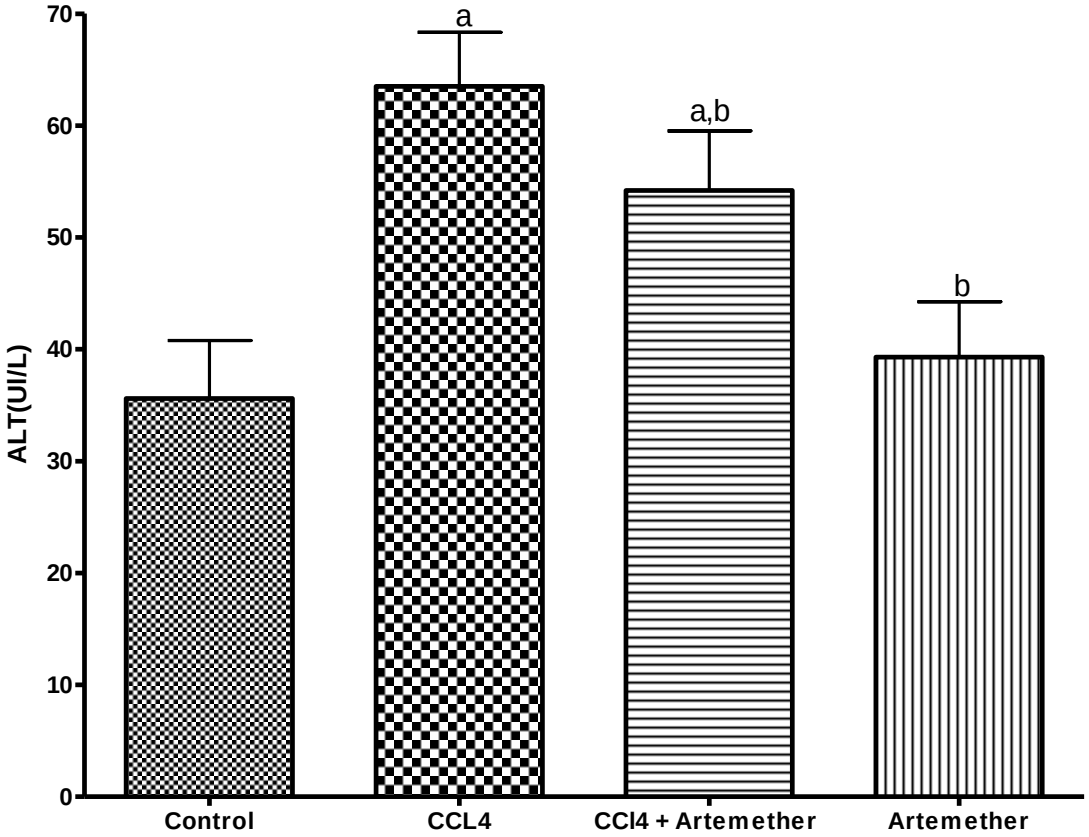

Figure 1. Effect of artemether on serum alanine aminotransferase (ALT) activity. Different superscripts represent mean±S.D (n = 5) which is considered significant at p<0.05. Superscripts (a) and (b) compared with CCl4, CCl4 + artemether and artemether, respectively.

Construction of calibration curve was done by plotting the corresponding absorbance of standards against their respective AST/ALT activities. The measurable ranges with these graphs are from 5 to 150 U/L for ALT and 5 to 190 U/L for AST.

Determination of plasma activity of alkaline phosphatase (ALP) in this study was done using spectrophotometric method as described elsewhere (Taiwo et al., 2003). The plasma activity of ALP was determined using this formula IU/L = 2760 × A of test in nm/min, where A is the absorbance at 405 nm and values of ALP were recorded for each sample.

Plasma albumin level was determined by a method described elsewhere (Tijani et al., 2009). A calibration curve was prepared using various dilutions of the reagent albumin standard ranging from albumin concentrations of 0 to 50.0 g/L. Corresponding concentrations to various optical densities of all the samples analyzed were obtained using the prepared calibration curve and values recorded accordingly in g/L.

Statistical analysis

Results were tested using ANOVA and complemented with Student's t-test and level of significance was set at P< 0.05.

RESULTS AND DISCUSSION

ALT and AST levels significantly increased in group 2, relative to the distilled water control group 1 (P <0 .05) and non significantly lower in group 3, relative to group 2, and non significantly higher in group 4, relative to group 1 (Figures 1 and 2). ALP mean levels slightly increased in group 2 as compared to group 1 and reasonably decreased, though insignificantly, in group 3, relative to group 2 (Figure 3). For ALB, there was an observed decrease in group 2, relative to group 1 and no difference in mean level between groups 1 and 4, and also between groups 2 and 3 (Figure 4). There was no significance difference in the mean body weights between weights taken before and after the experiment.

The biochemical indices monitored in the liver were 'biomarkers' for assessing hepatic damage and their activities in the blood played a significant role in the investigation and diagnosis (Dada and Omokhodion, 2007) of effect on the liver cells and to a reasonable extent, the toxicity of the carbon tetrachloride (MacGregor and Lang, 1996) and the potential ameliorating effect of artemether.

Furthermore, biochemical analysis of the carbon tetrachloride (CCl_4) induced acute liver damage group revealed an observed increased but non-significant alkaline phosphatase level as compared to those of the distilled water control group indicating a possible assault

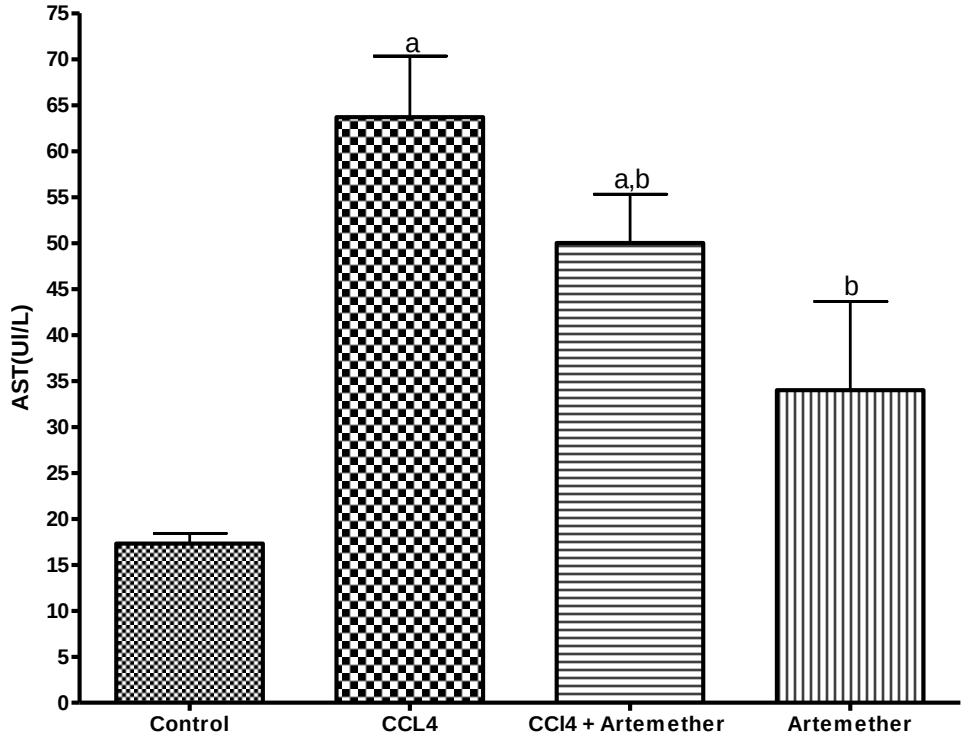

Figure 2. Effect of artemether on serum aspartate aminotransferase (AST) activity. Different superscripts represent mean±S.D (n = 5) which is considered significant at p<0.05. Superscripts (a) and (b) compared with CCl4, CCl4 + artemether and artemether, respectively.

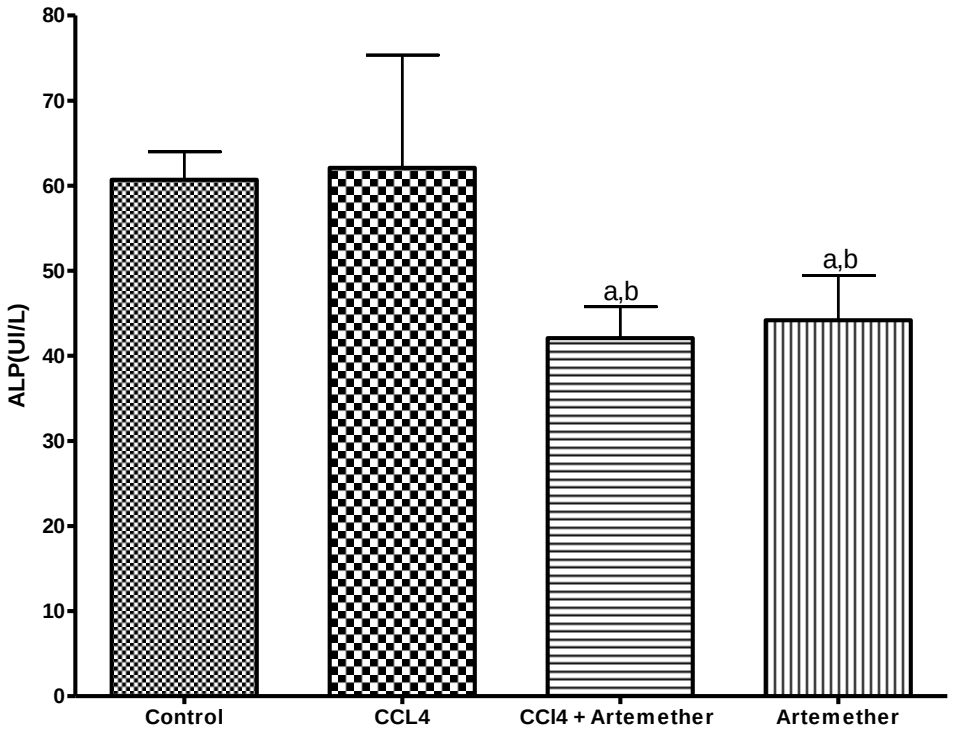

Figure 3. Effect of artemether on serum alkaline phosphatase (ALP) activity. Different superscripts represent mean±S.D (n = 5) which is considered significant at p<0.05. Superscripts (a) and (b) compared with CCl4, CCl4 + artemether and artemether, respectively.

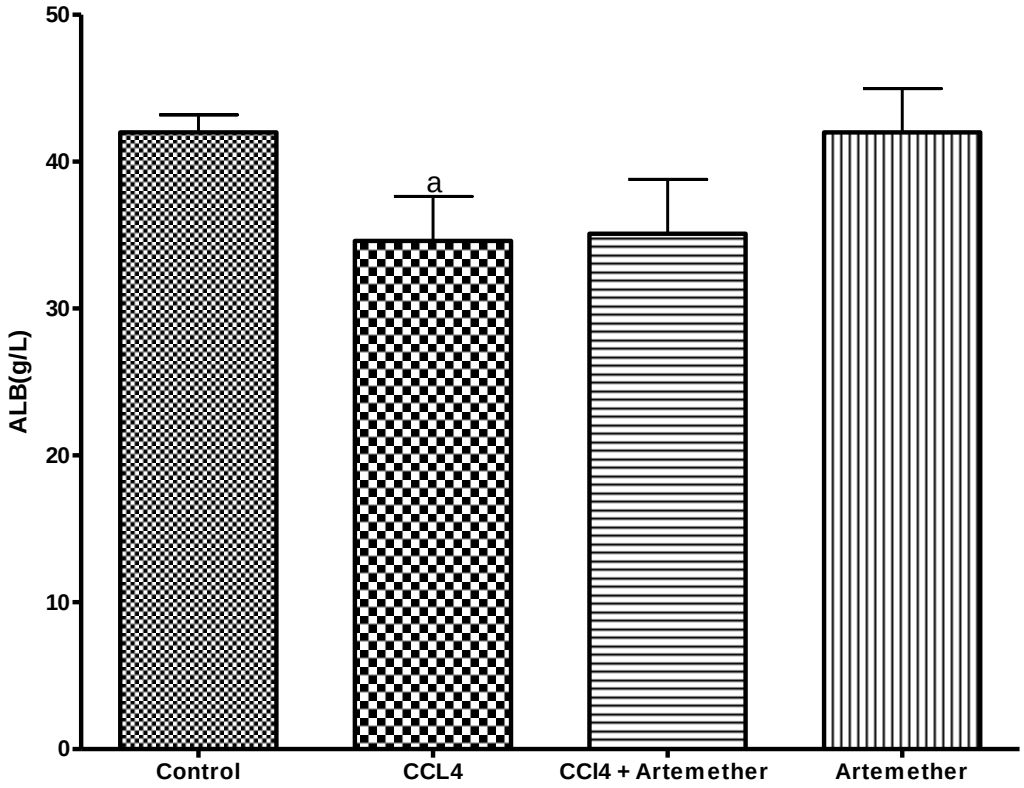

Figure 4. Effect of artemether on serum albumin (ALB). Different superscripts represent mean±S.D (n = 5) which is considered significant at $p<0.05$. Superscripts (a) and (b) compared with CCl4, CCl4 + artemether and artemether respectively.

on the liver cells in line with the findings of Morakinyo et al. (2009). However, the non-significant change in the serum alkaline phosphatase activities (Figure 3) is an indication that there was no significant leakage of the enzyme into the serum. This suggests that CCl_4 at the administered dose and period of time may be inducing the synthesis of the alkaline phosphatase in the tissues but possibly at a slow rate.

Similarly, there was significant increase in enzyme activities of AST and ALT in CCl_4 group, relative to the distilled water group. This may have resulted from increased functional activity of the tissues caused by CCl_4 in line with the findings of Hayes et al. (1986) indicating a possible hepatic injury.

The observed reduction in albumin concentrations is a sign of liver damage, arising from the administration of the CCl_4. This may be an indication of diminished synthetic function of the liver which may consequently lead to enhanced retention of fluid in the tissues spaces (Naganna, 1989).

All these are in line with already documented toxic effect of CCl_4 as a result of peroxidation of the membrane lipids by trichloromethyl radicals. These radicals initiate lipid peroxidation chain reactions that start with taking hydrogen ions from polyunsaturated fatty acids (PUFA). Peroxidation of lipids containing polyunsaturated fatty acids, in particular, impairs the structure of biological membranes, causing severe cell damage (Gasso et al., 1996) which in this case is believed to be mild considering the low dosage and the short period of CCl_4 administration.

However, the decrease in enzyme activities of the liver transaminases in CCl_4^+ artemether treated groups, relative to the CCl_4 group is believed to have been due to a suspected free radical scavenger activity of artemether which is thought to be inhibitors of lipid peroxidation, and this confirms the findings of Xie et al. (2005). Decrease in the plasma alkaline phosphatase in the artemether treated groups may have been as a result of the inhibitory effect of the drug on the CCl_4 induced synthesis of alkaline phosphatase within the hepatocells. This suggests that artemether may have a possible repair effect on hepatocellular damage as it has a suspected inhibitory effect on CCl_4 and may also enhance normal liver cell functions. There was no significant difference in the body weight of the animals in the different groups.

Conclusion

Analysis of the result of this experimental study showed that artemether may have a possible regenerating effect

on hepato-cellular damage as it has a suspected inhibitory effect on CCl_4 and may also enhance normal liver cell functions.

During the course of this study, the major limiting factor was the time frame of the study, which did not allow for the long term study of the effect of the drug on the liver. Chronic carbon tetrachloride induced liver damage would have been more ideal for the study of hepatocellular repair and repair mechanism. Other constraints such as the inability to analyze more specific liver biomarkers is envisaged.

The study of this novel antimalarial drug has also revealed other interesting prospects of artemether, especially in its possible regenerating activity. Further study to investigate specific enzyme makers, growth factors and mitogens, and their activity profiles is recommended to provide further insight and understanding of the mechanism of action of artemether.

REFERENCES

Ayyub M, Barlas S, Lubbad E (2000). Usefulness of exchange transfusion in acute liver failure due to severe falciparum malaria. Am. J. Gastroenterol. 95: 802-804.

Dada OA, Omokhodion FO (2007). Home management of malaria by mothers of children under-five in Abeokuta, southwest Nigeria. Trop. Doc. 37: 217-219.

Falade C, Makanga M, Premji Z (2005). Efficacy and safety of artemether/lumefantrine tablets (six-dose regime) in African infants and children with acute, uncomplicated faliparum malaria. Trans. R. Soc. Med. Hyp. 99: 459-467.

Gassó M, Rubio M, Varela G (1996). Effects of S-adenosylmethionine on lipid peroxidation and liver fibrogenesis in carbon tetrachloride-induced cirrhosis. J. Hepatol. 25: 200-205.

Hayes J, Condie L, Borzelleca J (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice. Fund. Appl. Toxicol. 7:454-63.

Haynes RK, Monti D, Taramelli D, Basilico N, Parapini S, Olliaro P (2003). Artemisinin Antimalarials Do Not Inhibit Hemozoin Formation. Antimicrob Agents Chemother. 47: p. 1175.

John FM (1995). Malaria: In Colliers Encyclopedia. Colliers: New York. 15: 259-260.

Lehne RA, Leanne C, Diane H (1990). Pharmacology for nursing care. W.B. Saunders Company, Philadelphia. pp. 964-965.

MacGregor D, Lang M (1996). Carbon tetrachloride: Genetic effects and other modes of action. Mut. Res. 366: 181-195.

Meshnick SR, Taylor TE, Kamchongwongpaisan S(2002). Artemisinin and the antimalarial endoperoxides: From herbal remedy to targeted chemother. Microbiol. Rev. 60: 301-315.

Morakinyo AO, Oludare GO, Ojulari S, Afolabi AO (2009). Effects of short term administration of artemether-lumefantrine on testicular functions and antioxidant defence in the rat. Res. J. Med. Med. Sci. 4: 165-170.

Murphy SA, Mberu E, Muhia D, English M, Crawley J, Waruiru C. (1997). The disposition of intramuscular artemether in children with cerebral malaria: a preliminary study. Trans. R. Soc. Trop. Med. Hyg. 91: 331-334.

Naganna B (1989). Plasma proteins. In: Textbook of biochemistry and human biology. Talwar GP, Srivastava LM, Moudgil KD, Prentice-Hall of India Private Ltd New- Delhi, 2nd ed pp. 59-61.

Reitman S, Frank S (1957). Transaminases. Am. J. Clin. Pathol. 28: p. 56.

Taiwo VO, Olaniyi MO, Ogunsanmi AO (2003). Comparative plasma biochemical changes and susceptibility of erythrocytes to *in vitro* peroxidation during experimental trypanosome Congolese and T bucei infections in sheep. Israel J. Vet. Med. 40: 345-348.

Tijani AY, Uguru MO, Salawu OA, Abubakar A, Onyekwelu NO, Akingbasote JA (2009). Effect of Faidherbia albida on some biochemical parameters of rats infected with Trypanosoma brucei brucei. Afr. J. Pharm. Pharmcol. 3: 26-30.

White NJ (1994). Clinical pharmacokinetice and pharmacodynamics of artesunate and derivatives. Trans. R. Soc Trop. Med. Hyg. 88: S41-S43.

White NJ (1997). Assessment of the pharmacodynamic properties of anti-malaria drugs *in vivo*. Antimicrob Agents Chemother. 41: 1413-1422.

WHO (1998). Malaria chemotherapy. Tech. Reg. Ser. WHO Geneva.

WHO (2000). Management of severe malaria. A practical handbook.

WHO (2003). Position of WHO roll back malaria department on malaria treatment policy, http://www.who.int/malaria/docs/who_atp_position.pdf.

Xie LH, Johnson TO, Weina PJ, Si Y, Haeberle A, Upadhyay R, Wong E, Li Q (2005). Risk Assessment and therapeutic indices of artemether in Plasmodium berghei-infected and uninfected rats. Int. J. Toxicol. 24: 251-264.