

EFFECT OF ARTESUNATE AND AMODIAQUINE ON RAT HEXOBARBITAL SLEEPING TIME AND SOME HEPATIC BIOCHEMICAL PARAMETERS

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ABSTRACT

Changes in malondialdehyde concentration, activities of some hepatic enzymes (alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase) and hexobarbital sleeping time following administration of rats with therapeutic doses of artesunate and /or amodiaquine twice daily for three days were investigated. The experimental animals were randomly divided into four groups: those administered distilled water (control) for 3 hours before the administration of 120 mg/kg body weight (b.w.) hexobarbital sodium intraperitoneally, those administered artesunate (2 mg/kg b.w.) for 3 hours before administration of hexobarbitone, those administered amodiaquine (6.12 mg/kg b.w.) for 3 hours before administration of hexobarbitone and those co-administered artesunate (2 mg/kg b.w.) and amodiaquine (6.12 mg/kg b.w.) for 3 hours before administration of hexobarbitone. The activities of alkaline phosphatase and aspartate aminotransferase were significantly reduced ($p < 0.05$) following the single and combined administration of artesunate and amodiaquine. There was no significant change ($p > 0.05$) in the activity of alanine aminotransferase following the single and combined administration of both drugs. No significant changes ($p > 0.05$) occurred in MDA content in the rats administered artesunate and/or amodiaquine. However, hexobarbital sleeping time was significantly prolonged ($p < 0.05$) in rats administered with artesunate and amodiaquine while co-administration of both drugs significantly shortened ($p < 0.05$) their hexobarbital sleeping time. The results therefore revealed that the single or combined repeated administration of both drugs may implicate negatively on membrane functions and integrity, and consequently distort hepatic drug biotransformation mechanism.

Key words: Artesunate, amodiaquine, hexobarbital sleeping time, liver and alkaline phosphatase

INTRODUCTION

Malaria remains a major public health threat especially among children and pregnant women in Africa. There are over 500 million cases of malaria annually among the world's poorest population (Snow *et al.*, 2005). Malaria claims the lives of nearly a million children each year in Africa alone (WHO, 2008). There have been reported increased resistance to conventional antimalarial drugs such as chloroquine, sulfadoxine, pyrimethamine and mefloquine. As a result, neurotherapeutic approaches are being developed. Prominent among them is artemisinin based combination therapy (ACT), for example, World Health Organization has endorsed ACT as the 'policy standard' for all malaria

infection in areas where *Plasmodium falciparum* is the prominent infectious species (WHO, 2003). Studies have shown that combining artesunate with existing antimalarial drugs may improve cure rates, delay emergency of resistance and reduce parasite tolerance time. Moreover, both artesunate and amodiaquine are highly efficacious for the treatment of malaria (Nosten *et al.*, 2000; Adjuik *et al.*, 2002). Artesunate is one of the artemisinin groups of drugs that treat malaria. It is a semi-synthetic derivative of artemisinin that is water soluble with a molecular mass of 384.421g/mol. Artesunate is prepared from dihydroartemisinin (DHA) by reacting it with succinic acid anhydride in basic medium. Pyridine as base/solvent, sodium bicarbonate in chloroform and catalyst *N,N*-dimethylamino pyridine (DMAP) and triethylamine in 1,2-dichloromethane have been used with yields up to 100% (Whyte, 2008). Artesunate is a potent blood schizonticide agent of *Plasmodium falciparum* as release of active oxygen species from this bond kills the parasite if accumulated in the erythrocyte cells and also suppresses the production or activity of antioxidant enzymes in the erythrocytes, causing lysis of parasite cells and also reduce gametocyte carriage rate (Mayne, 1994).

Amodiaquine (AQ) is a 4-aminoquinoline, similar to chloroquine (CQ) that has been used widely to treat and prevent malaria. Amodiaquine was first added to the World Health Organization (WHO) Essential Drugs List (EDL) in 1977. In 1979, the committee decided to delete it from the List due to its similarity with chloroquine. However, it was quickly reinstated in the same year (WHO, 2002). In the mid 1980s, fatal adverse drug reactions were described in travellers using amodiaquine for prophylaxis (Hatton *et al.*, 1986; Nefstel *et al.*, 1986). As a result, the manufacturer (Parke-Davis) modified the labelling and withdrew prophylaxis as an indication, while, in 1988, the WHO deleted it from the EDL and prevented its use in malaria control programmes (WHO, 1990). The 1996 version of Cochrane Review concluded that AQ was a valuable drug and supported its continued use for the treatment of uncomplicated malaria with the proviso that, due to the partial cross-resistance with CQ, research must continue into both its effectiveness and safety. These findings led the WHO to modify its recommendations and reinstate AQ as an option for treating falciparum malaria (WHO, 1997). Although licensed, this drug is not marketed in the United States but is widely available in Africa.

The recommended use of artemisinin-based combination therapy (ACT) by the World Health Organization for the treatment of uncomplicated *P. falciparum* malaria is believed to provide effective treatment against *P. falciparum* malaria and slow down the spread of drug resistance (WHO, 2001). Some African countries plagued with resistant forms of *P. falciparum* have instituted the ACTs as first line malaria treatment (World Malaria Report, 2005). Artesunate plus amodiaquine combination is one ACT recommended by the World Health Organization for use in malaria control programmes and a first line treatment for African children with uncomplicated malaria (WHO, 2004). Studies on the safety of this regimen with focus on the effects of the drugs on certain cardiovascular disease indices have been carried out in our laboratory (Adebayo *et al.*, 2011). This study is therefore aimed at comparing the effects of the therapeutic doses of artesunate, amodiaquine and their combination on some liver enzymes, malondialdehyde concentration and hepatic drug biotransformation.

MATERIALS AND METHODS

A total of 20 male albino rats (*Rattus norvegicus*) (121g-124g) were obtained from the Animal Breeding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Artesunate and amodiaquine were products of Gunadong Luck-Sun, China. The alkaline phosphatase, alanine and aspartate aminotransferase assay kits were obtained from Quinica Clinica Aplicada S.A, Spain. All other reagents were of analytical grade and were prepared in all glass-distilled water. The reagents were stored in clean, airtight reagent bottles.

Animal Handling/ Grouping

The rats were maintained on normal rat chow and water *ad libitum* and were housed in wooden cages. Animal husbandry and experimentation were consistent with the *Guiding Principles in the Use of Animals in Toxicology* (Derenlanko, 2000). The experimental animals were randomly divided into four groups (of five rats each). Group I (control) received an appropriate volume of distilled water for 3 hours before the administration of 120 mg/kg b.w. hexobarbital sodium intraperitoneally. Group II (arte) received 2mg/kg b.w. artesunate for 3 hours before the administration of 120 mg/kg b.w. of sodium phenobarbitone and Group III (amod) received 6.12 mg/kg b.w. amodiaquine for 3 hours before the administration of 120 mg/kg b.w. of sodium phenobarbitone while Group IV (art+amo) received same dose of artesunate and amodiaquine with sodium phenobarbitone for 3 hours before the administration of 120 mg/kg b.w. of sodium phenobarbitone. The administration of artesunate and amodiaquine was done orally and twice daily for 3 days to evaluate the safety of the combination therapy. The animals were sacrificed 24 hours after the last dose on the third day of administration.

Hexobarbital Sleeping Time

Hexobarbital sleeping time was determined according to Shapiro *et al.* (1989). The principle is based on the duration of time from loss of righting reflex to the time of spontaneous righting following sodium phenobarbitone administration. After loss of righting reflexes, the rats were placed on their back and the length of time between the loss of righting reflexes and the time of gain of consciousness was determined.

Preparation of Liver Homogenate

The albino rats were starved overnight and sacrificed by cervical dislocation and the liver was rapidly excised and homogenised with 0.25M of ice cold sucrose solution. The liver homogenate was then centrifuged at 5000 rev/min for 20 minutes after which the residue/debris was discarded.

Assay of Biochemical Parameter

Alkaline phosphatase (ALP) activity was determined by the method described by Wright *et al.* (1972) while alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were determined by the method of Reitman and Frankel (1957).

Determination of Malondialdehyde Concentration

Malondialdehyde (MDA) concentration was determined by measuring the formation of thiobarbituric acid reacting substances described by Varshney and Kale (1990).

Statistical Analysis

All data are presented as mean $\pm$ standard deviation. Statistical analyses were carried out using Duncan Multiple Range test. In all cases, probability level of 95% was taken as significant (Montgomery, 1976).

RESULTS

The effects of administration of artesunate and/or amodiaquine on rat hepatic enzyme activities are represented in Figures 1-3. The activities of rat liver alkaline phosphatase and aspartate aminotransferase were significantly reduced ($p < 0.05$) following the single and combined administration of artesunate and amodiaquine as shown in Figures 1 and 2 respectively. Figure 3 revealed that there was no significant change ($p > 0.05$) in the activity of rat liver alanine aminotransferase (ALT) following the single and combined administration of artesunate and amodiaquine. In Figure 4, there was no significant change ($p > 0.05$) in the MDA content of rat liver following the single and combined administration of artesunate and amodiaquine. The effect of administration of artesunate and/or amodiaquine on rat hexobarbital sleeping time is shown in Figure 5. The co-administration of artesunate with amodiaquine significantly shortened ($p < 0.05$) rat hexobarbital sleeping time while it is significantly prolonged ($p < 0.05$) upon separate administration of artesunate and amodiaquine.

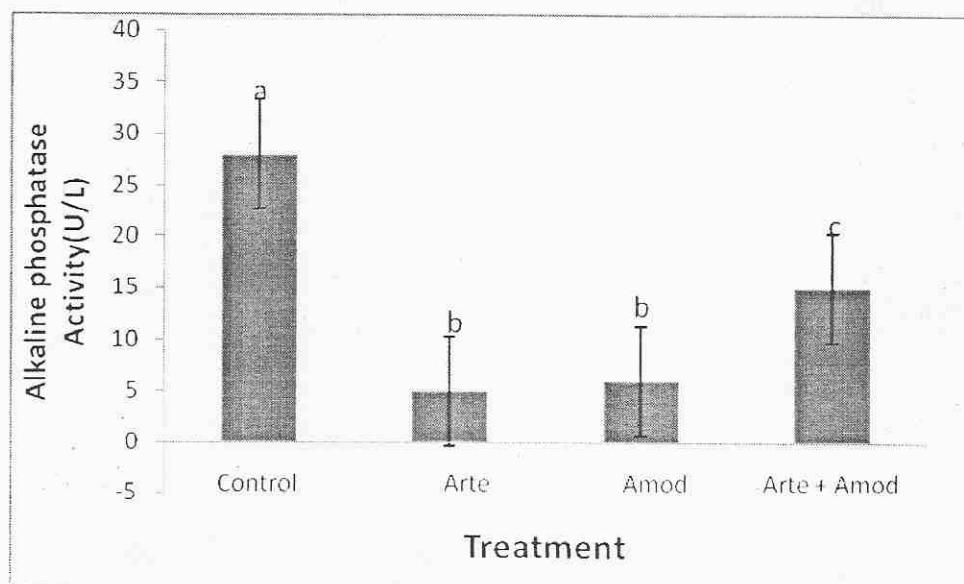


Figure 1: Effect of administration of artesunate and/or amodiaquine on alkaline phosphatase activity of rat liver.

Each value is a mean of 5 determinations $\pm$ S.D. Bars with different superscripts are significantly different at $P < 0.05$. Arte means artesunate; Amod means amodiaquine; Arte + Amod means artesunate plus amodiaquine.

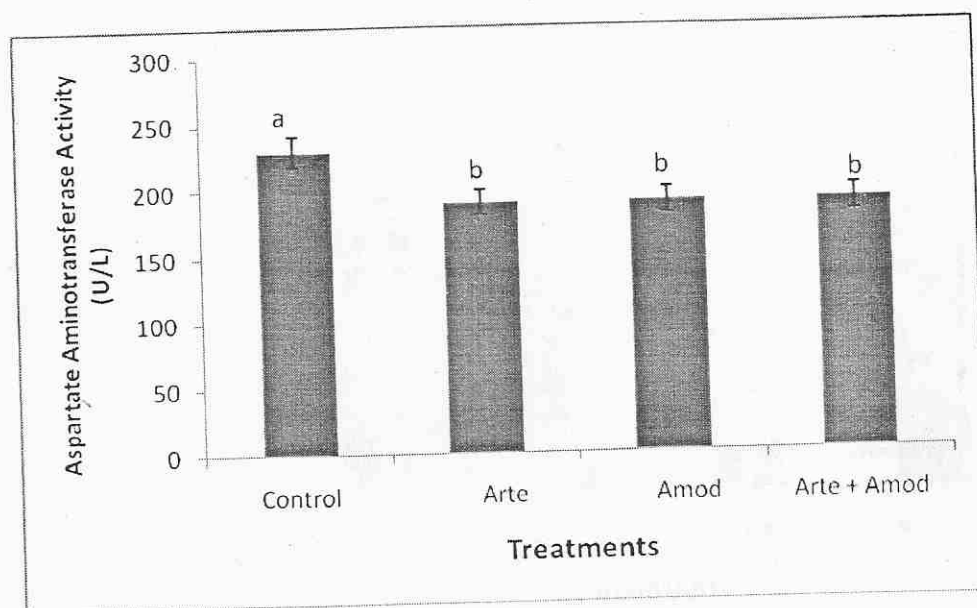


Figure 2: Effect of administration of artesunate and/or amodiaquine on aspartate aminotransferase activity of rat liver.

Each value is a mean of 5 determinations $\pm$ S.D. Bars with different superscripts are significantly different at $P < 0.05$. Arte means artesunate; Amod means amodiaquine; Arte + Amod means artesunate plus amodiaquine.

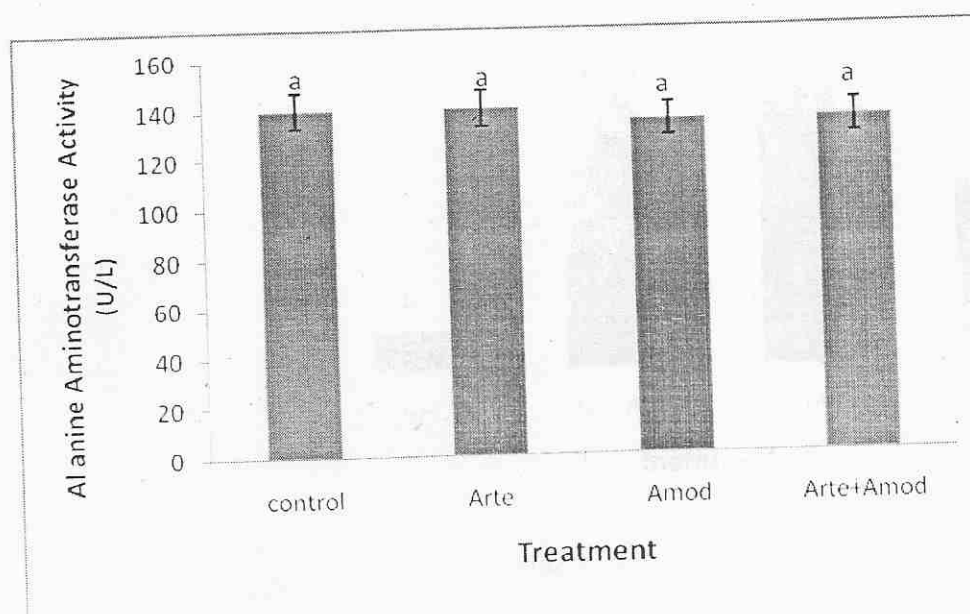


Figure 3: Effect of administration of artesunate and/or amodiaquine on alanine aminotransferase activity of rat liver.

Each value is a mean of 5 determinations $\pm$ S.D. Bars with different superscripts are significantly different at $P < 0.05$. Arte means artesunate; Amod means amodiaquine; Arte + Amod means artesunate plus amodiaquine.

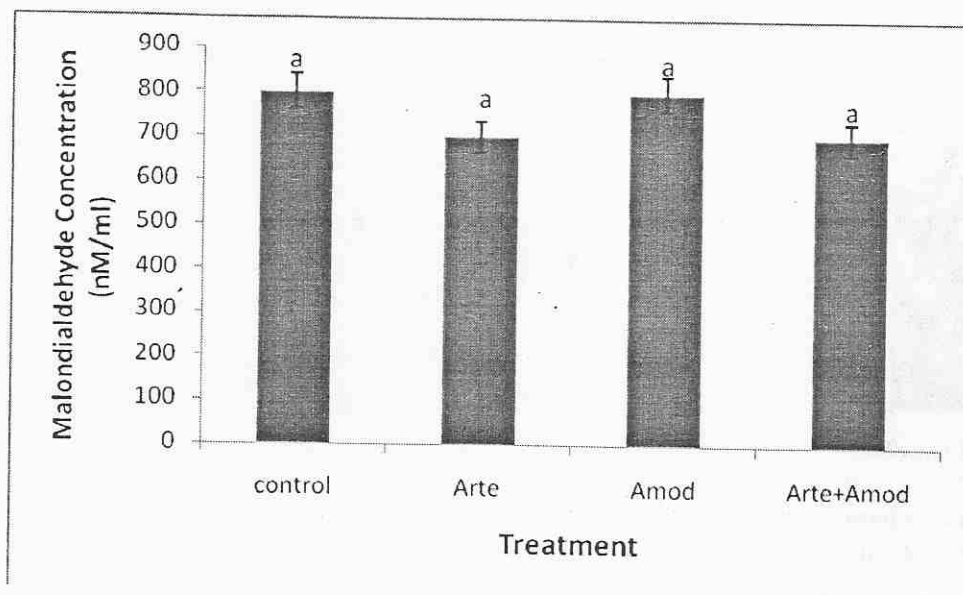


Figure 4: Effect of administration of artesunate and/or amodiaquine on malondialdehyde concentration of rat liver.

Each value is a mean of 5 determinations $\pm$ S.D. Bars with different superscripts are significantly different at $P < 0.05$. Arte means artesunate; Amod means amodiaquine; Arte + Amod means artesunate plus amodiaquine.

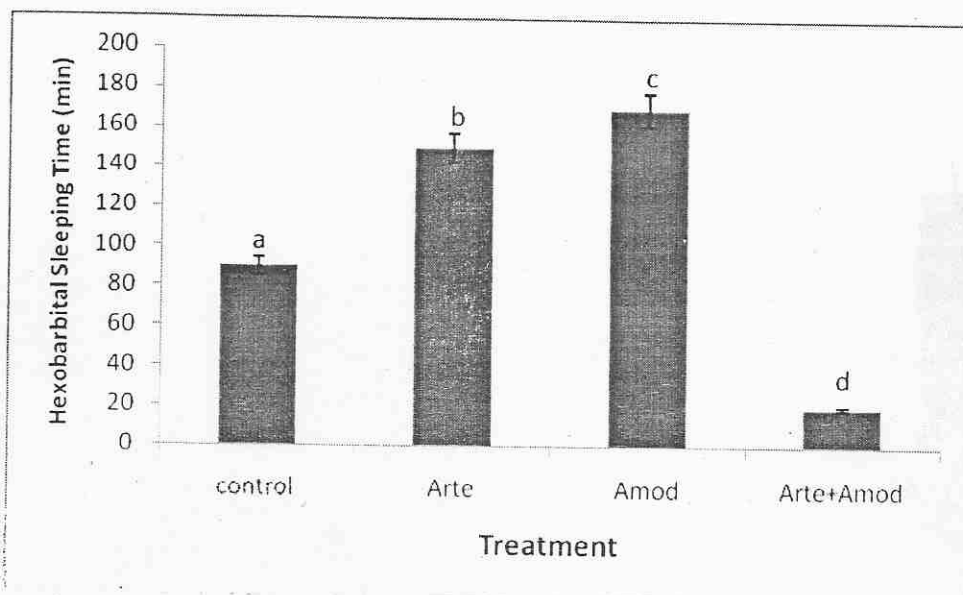


Figure 5: Effect of administration of artesunate and/or amodiaquine on Rat Hexobarbital Sleeping Time.

Each value is a mean of 5 determinations $\pm$ S.D. Bars with different superscripts are significantly different at $P < 0.05$. Arte means artesunate; Amod means amodiaquine; Arte + Amod means artesunate plus amodiaquine.

DISCUSSION

In general, artesunate and its derivatives are rapidly absorbed from the gastrointestinal tract and are primarily metabolized in the liver (Dhingra *et al.*, 2000). In the liver they are quickly hydrolyzed *in vivo* to dihydroartemisinin (the physiologically active form of all the derivatives) and eliminated from the plasma with a half-life ranging from 45 minutes to 11 hours (Titulaer *et al.*, 1990; Meshnick *et al.*, 1996). Thus the significant increase in MDA content in the liver of rats administered only artesunate may be due to their exposure to the drug plus the fact that the liver is the major organ of xenobiotic metabolism. This increase suggests that artesunate is capable of inducing oxidative stress which may overload the cell's endogenous detoxification mechanism, with the drug and its liver detoxification metabolite (possibly radicals) interacting with and exacerbating hepatic lipid peroxidation. Alkaline phosphatase has been employed to assess the integrity of plasma membrane and endoplasmic reticulum (Akanji *et al.*, 1993). The significant decrease in the activity of rat liver alkaline phosphatase observed following the administration of artesunate and amodiaquine suggests that the integrity of the various membrane systems has been compromised. Therefore significant reduction in the activity of aspartate aminotransferase activity of rat liver in the groups administered artesunate, amodiaquine and co-administration of both drugs may also be attributed to the leakage of the enzyme to the extracellular fluids (Akanji *et al.*, 1993). Hexobarbital sleeping time is the time taken for normal and healthy rats between losses of righting and regain of righting under the influence of a sleeping drug (Farombi *et al.*, 2000). Righting response is the ability of the animal when placed on its back on flat surface to snap over on its paw (Shapiro *et al.*, 1989). The extension in rat hexobarbital sleeping time is an indication of effect of drug on metabolizing enzymes, thus causing delay in the detoxification of phenobarbitone (Anderson, 1988). The co-administration of artesunate and amodiaquine that significantly shortened the hexobarbital sleeping time suggests the induction of drug metabolizing enzymes (European Medicines Agency, 2012) by the concurrent administration of artesunate and amodiaquine thus reflecting a reduction in the time of spontaneous righting (Shapiro *et al.*, 1989). This may be due to antagonistic effect of the two drugs.

The current use of combination of drugs to combat diseases/infections has posed serious concern about the possibility of drug-drug interaction that may potentiate toxicity. The results obtained from this study suggest that despite the acclaimed potency, artesunate and /or amodiaquine in the treatment of malaria may interfere with drug metabolism. The altered hexobarbital sleeping time, ALP and AST activities suggest altered and deranged membrane structure and functions. This may cause damage to hepatic cells as well as disrupt amino acid metabolism.

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