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Halofantrine (Anti-Malaria) Toxicity in Wister Rats: Biochemical Evaluation of Hepatic Dysfunction

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Abstract: Halofantrine is a phenanthrene methanol, belonging to the aryl-amino alcohol family, which is widely prescribed for the treatment of infections with chloroquine-resistant strains of *Plasmodium falciparium*. This study examined biochemical evaluation of hepatic dysfunction as a result of halofantrine toxicity in Wistar rats. Various concentrations of halofantrine (30, 60 and 90 mg kg^{-1} were administered to the three groups of Wistar rats. The fourth group of animals received distilled water (control). The body weight changes and the relative weight of the liver were measured. The serum hepatospecific markers such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP) activities and serum total bilirubin level were also estimated. The halofantrine treated groups had a significant increase in the relative weight of their livers ($p<0.05$) when compared with control. There was also significant increase in all the enzymes and total bilirubin ($p<0.05$) when compared with control. The results indicated that halofantrine might have hepatotoxic effect in Wistar rats.

Key words: Halofantrine, hepatic dysfunction, toxicity, AST, ALP

INTRODUCTION

Malaria is an infectious diseased that continues to be associated with considerable morbidity and mortality and significant social and economic impact in developing countries. According to the World Health Organisation (WHO) Malaria is endemic in 91 countries, predominantly in Africa, Asia and Latin America with about 40% of the world's population at risk (WHO, 1996). It is caused by parasites that belong to the genius plasmodium with four different species namely *Plasmodium falciparium, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae*.

The resistance of these parasites especially *Plasmodium falciparium* to most antimalarial drugs is rampant and on the increase too. This is due to remarkable adaptability of the parasites to drugs and man's abuse of malaria drugs for prophylaxis and treatment of undiagnosed fever in endemic areas.

Halofantrine, a lipophilic phenanthrenemethanol belonging to the aryl amino alcohol is used for the treatment of acute uncomplicated multi-drug resistant malaria (Philips-Howard and Wood, 1996). It is schizonticidal with high degrees of activity against the asexual erythrocytic stage of malarial infections caused by single or mixed infections of *Plasmodium falciparium* or *Plasmodium vivax*. It has limited effect against the exoerythrocytic or gametocyte stages of malaria parasites, (Smith, 1998).

Clinical treatment with halofantrine is often accompanied by serious side effects such as abdominal pain, diarrhoea, prolongation of QTc interval and arrythmias that could be fatal. However, an increasing number of reports describing serious complications in the last few years have raised some doubt about the safety of halofantrine (Touze and Fourcarde, 1997) Halofantrine has been reported to be cardiotoxic (Nosten *et al*., 1993; Touze and Fourcarde, 1997). This may be similar to that of chloroquine, quinine and mefloquine by forming toxic complexes with ferritoporphyrin IX that damage the membrane of the parasite or organs. Also several studies have shown that other antimalarials such as chloroquine and quinine are hepatoxic (Okonkwo *et al*., 1997; Debra and Megan, 1999).

Self-medication is especially common in developing countries like Nigeria and sometimes at dosages above the therapeutic dose. It has been shown that drugs that are effective in malaria treatment may cause damage to certain organs of the body, it is therefore important for drugs to be avoided and only to be safely administered when necessary. This study was therefore, carried out on the biochemical parameters as indices for assessing halofantrine-induced hepatotoxicity.

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MATERIALS AND METHODS

Animals: Twenty four Wistar rats bred in the Central Animal House of College of Medicine and Health Sciences, Imo State University, Owerri, Nigeria were used in the present study. They were maintained at a temperature range of 25 to 30°C and a 12 h light 12 h dark cycle. They were fed with commercial growers mash, product of Tops Feeds Ltd, Sapele, Nigeria. Water and feed were provided *ad libitum* and this continued until the rats weighed between 200-300 g.

Drugs: Halofantrine (HAL FAN) (20 mg mL^{-1} suspenion) used in this study was the product of Smith Kline and French Laboratories, Nanterre, Cedar, France and was purchased from a standard pharmacy shop in Owerri, Nigeria. The drugs suspension was administered to the animals on the basis of their body weight. The suspension of halofantrine was administered orally using cannula.

Experimental designs: Animals were randomly assigned to 4 experimental groups (n = 6×4 groups) each having similar body weights.

Group I: (Control): Animals received distilled water.

Group II: Animals in this group were given 30 mg kg^{-1} halofantrine suspension in three divided doses.

Group III: Animals in this group were given 60 mg kg^{-1} halofantrine suspension in three divided doses.

Group IV: Animals in this group were given 90 mg kg^{-1} halofantrine suspension in three divided doses.

The drugs were administered orally for a period of 14 days. All the animals were allowed free access to food and water till the end of the experiment.

Blood sample collection: Twenty four hours after the last doses were administered, the animals were weighed and then anaesthetized with chloroform vapour, quickly brought out of the jar and sacrificed. Whole blood was collected by cardiac puncture from each animal into clean dry centrifuge tubes. The blood were allowed to stand for about 30 min to clot and further centrifuged at 10,000 rpm for 5 min using Wisperfuge model 1384 centrifuge (Samson, Holland). Serum was separated from clot with Pasteur pipette into sterile serum sample tubes for the measurement of biochemical parameters. The liver from both control and test animals were removed and immediately washed with physiological saline and weighed.

Biochemical analysis: Serum total bilirubin level was estimated based on Van den Berg reaction (Malloy and Everlyn, 1937). Diazotised sulphonilic acid (0.5 mL) reacts with bilirubin in diluted serum (0.2 mL serum+1.8 mL distilled water) and forms purple coloured azobilirubin, which was measured at 540 nm. Activities of serum Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were assayed by the method of Reitman and Frankel (1957). 0.2 mL of serum with 1 mL of substrate (asparatate and α-ketoglutarate) for AST, alanine and α-ketoglutarate for ALT, in phosphate buffer pH 7.4) was incubated for an hour in case of AST and 30 min for ALT. 1 mL of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 mL of 0.4N NaOH was added and absorbance was read at 540 nm. Activities expressed as IU L^{-1}.

Based on the method of King and Armstrong (1934) alkaline phosphates activities was assayed using disodium phenylphosphate as substrate. The colour developed was read at 680nm after 10 min and activities of ALP expressed as IU L^{-1}.

Statistical analysis: Statistical evaluation of data was performed by using one-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1957).

RESULTS

Table 1 shows the effect of halofantrine on body weight changes of Wistar rats and mean relative liver weight. The control and 30 mg kg^{-1} halofantrine groups showed slight gain in body weight whereas the 60 and 90 mg kg^{-1} halofantrine groups had slight loss in body weight. There was significant increase in the relative weight of the liver in 60 and 90 mg kg^{-1} halofantrine groups ($p<0.05$) when compared with the control.

Table 1: Mean body weight changes and relative liver weight before and after treatment with halofantrine in Wistar rats

Groups	Mean initial weight (g)	Mean final weight (g)	Mean weight change (g)	Relative liver weight (g)
Control (I)	158.82±1.52	163.28±1.2	+4.46±0.56	5.08±0.26
II (30 mg kg^{-1})	157.2±1.43	160.15±1.38	+2.95±0.01	5.85±0.32
III (60 mg kg^{-1})	158.5±1.34	158.38±1.44	-0.12±0.01	7.46±0.41*
IV (90 mg kg^{-1})	159.6±1.86	158.01±1.6	-1.59±0.01	7.98±0.63*

*Significantly different from control ($p<0.05$)

Table 2: Mean values of activities of serum AST, ALT, ALP and levels of bilirubin in normal and experimental rats

	Control (I)	Group I	Group II	Group III
ALT IU L^{-1}	48.62±1.6	62. 41±2.0*	68.42±2.4*	94.50±3.66*
AST (IU L^{-1})	52.68±2.38	88.47±4.2*	102.1±3.6*	107.6± 4.2*
ALP (IU L^{-1})	81.5±5.8	100.2±5.6*	106.03±6.2*	105.8±5.8*
Bilirubin (μmol L^{-1})	4.88±1.6	6.58±2.0*	7.02±4.8*	7.25±7.0*

*Significantly different from control ($p<0.05$)

The changes in the mean value of serum bilirubin and serum hepatospecific markers in all the groups are shown in Table 2. There was a significant increase in the levels of total and conjugated bilirubin concentrations and AST, ALT and alkaline phosphatase activities in both 60 and 90 mg kg^{-1} halofantrine groups ($p<0.05$) when compared with control while 30 mg kg^{-1} halofantrine groups showed significant increase only in serum ALT and AST activities ($p<0.05$) when compared with the control. Two animals died in the 90 mg kg^{-1} dose group while one animal died in 60 mg kg^{-1}, no death was seen in 30 mg kg^{-1} halofantrine group.

DISCUSSION

The observed increase in the relative weight of the liver in this study indicates that the drug might have toxic effect on this organ. It has been reported that increase or decrease in either absolute or relative weight of an organ after administering a chemical or drugs is an indication of the toxic effect of that chemical (Simons *et al*., 1995).

The results of this study revealed that halofantrine might have deleterious effects on the liver of Wistar rats. Serum AST, ALT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage because they are cytoplasmic enzymes released into circulation after cellular damage (Sallie *et al*., 1991). The increased activities of AST, ALT, ALP and the level of bilirubin in serum indicate halofantrine-induced hepatocellular damage. Liver enzymes are usually raised in acute hepatoxicity but tend to decrease with prolonged intoxication due to damage to the liver cells (Cornellius, 1979). This was confirmed by an earlier study (Obi *et al*., 2004) in which the marked elevations of hepatic marker molucules were reported. Other antimalarials such as chloroquine (Pari and Murugavel, 2004) amodiaquine and quinine (Debra and Megan, 1999) are also reported to induce hepatic damage.

It has been suggested that halofantrine by virtue of its lyophilic character (Hurberstone *et al*., 1996) can be expected to permeate biomembraneous barriers (Bloom and Fawceth, 1973). Pretreatment with halofantrine has evidently been shown to inhibit Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase pumps. Also many of the cell proteases and phospholipase are activated in the presence of calcium ions. The activation of phospholipases has a damaging effect on the membrane, causing release of free fatty acids and phospolipids. These are toxic and can also initiate arachidonic acid production resulting in production of free radicals (Murphy *et al*., 1983). It has been reported that halofantrine pre-treatment enhances the levels of Nicotinamide Adenine Diphosphate (NADPH), cytochrome C reductase and cytochrome Gs, both enzymes being involved in metabolism of endogenous and exogenous compounds (Voznessensky and Schenkman, 1994). The enhancement of these 2 enzyme systems will obviously lead to accumulation of free radical oxygen species, since the enzymes have been shown to generate superoxide radical (Gibson and Sket, 1992). These may in part explain the level of bilirubin in serum manifested in the halofantrine induced hepatocellular damage in Wistar rats.

This study has established the hepatoxic potential of high doses of halofantrine in Wistar rats. The dose used in this research is high in comparison with the therapeutic dose levels in humans because small laboratory animals eliminate drugs faster than humans (Laumann *et al*., 1995). From this study, however, it is found that halofantrine induced hepatocellular damage at high concentrations causes increase in serum hepatospecific markers in Wistar rats.

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