

Full Length Research Paper

Haemolytic effects and changes in serum enzymes in normal rats exposed to halofantrine hydrochloride overdose

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The levels of serum enzymes and haemolytic effects of overdose of halofantrine hydrochloride were determined in adult male rats. The animals were grouped into four groups and were orally administered halofantrine hydrochloride in normal saline: 0 mg/kg (control), 4 mg/kg (under-dose), 8 mg/kg (normal dose) and 16 mg/kg (overdose) in three repeated doses at 6 h interval. The changes in serum enzyme levels were determined by monitoring the levels alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total serum albumin. The haemolytic effect of the drug was monitored by the changes in Packed Cell Volume (PCV), total bilirubin and direct bilirubin. There were significant increases in the ALT, AST and ALP levels in both the normal dose and overdose when compared with the control. The reduction in total serum albumin in normal dose and overdose was also significant ($p < 0.05$). The result also revealed a significant decrease in PCV and increase in total and direct bilirubin ($p < 0.05$) in the overdose groups. The result is indicative of the hepatotoxicity and haemotoxicity of halofantrine hydrochloride in normal dose and overdose conditions.

Key words: Halofantrine hydrochloride, haemotoxicity, hepatotoxicity, serum enzymes.

INTRODUCTION

The ravaging effect of malaria parasite in the world today can not be over emphasized. About 300 to 500 million people being infected with malaria with about 1 to 2 million mortality each year. Tropical Africa contributes mostly to this number as control of malaria has been particularly problematic because of the high transmission rates and the overall low socio-economic level. This notwithstanding, the advent of mutant strains of malaria parasite which defy existing chemotherapy has led to the introduction of more effective chemotherapeutic agents to combat the scourge. In Nigeria today Halofantrine hydrochloride is a household name for the treatment of malaria.

It is a phenanthrene methanol compound with schizonticide activity against all four malaria species. Halofantrine hydrochloride is a highly lipophilic anti-malaria drug which is effective against multidrug resistant strains of *Plasmodium falciparum* (Boudreau, 1988).

The prescribed effective dose of Halofantrine hydrochloride for treatment of malaria in human is 24 mg/kg administered as three divided doses of 8 mg/kg at six hourly intervals with an observation period of 1 h after each 8 mg/kg dose. Though the mechanism of action of Halofantrine hydrochloride has not been clearly elucidated, it is mainly metabolized in human by CYP3A4 (Halliday et al., 1995), with the eventual production of its principal metabolite N-desbutylhalofantrine (BHAL) excreted in faeces (Brocks, 2000). The median parasite clearance and fever clearance times, from the first dose, were 26 h and 30 h, respectively (Khan et al., 2006; Rao

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and Kamalakar, 1993). The pharmacokinetic properties of halofantrine hydrochloride in rats are very much related to those observed in humans (Brocks and Toni, 1999). This has led to the increasing use of animal models in the study of the characteristics of Halofantrine hydrochloride (Porter et al., 1996; Humberstone et al., 1996; Brocks, 2002). Halofantrine hydrochloride is highly lipophilic, a factor responsible for its potential hepatotoxicity and haemolytic influences on the liver and red cells respectively. In this study, the toxicological effect of different doses of Halofantrine hydrochloride on some biochemical and haematological parameters were evaluated in normal rats.

MATERIALS AND METHODS

Chemicals and assay kits

Halofantrine hydrochloride, copper sulphate pentahydrate, disodium potassium phosphate anhydrous and sodium dihydrogen phosphate (anhydrous) were products of British Drug House (Poole, England). Malondialdehyde (MDA), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), and ferrous sulphate hexahydrate were products of Sigma Chemical Company (St Louis, Mo. USA). Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) assay kits (Randox, England). Quantichrom™ BCG Albumin assay kit, Follin-cicaltaeu, sodium potassium tartrate were products of Patel industries (Mumbai, India). Other chemicals used for the study were of analytical grade.

Experimental animal

The experimental animals (*Rattus norvegicus*) all male, which weighed 85 – 120 g used for the research work was obtained from the animal house of the College of Health Sciences, Igbinedion University, Okada. They were acclimatized and housed in plastic cages. The rats were fed and given water *ad libitum*. The temperature of the room was maintained at $25 \pm 2^\circ\text{C}$ throughout the duration of the study.

Experimental design

The animals were randomly selected and grouped into four, with each group containing ten rats each. Group A: contained the control animals, which were placed on placebo with normal saline. Group B: contained animals that were administered oral dose of 4 mg/kg in normal saline representing under-dose. Group C: contained animals that were administered oral dose of 8 mg/kg in normal saline representing the normal dose. Group D: contained animals that were administered oral dose of 16 mg/kg in normal saline representing over dose. The administration was repeated three times at 6 hours interval. The animals were sacrificed after three days by cervical dislocation; blood samples were collected by cardiac puncture. The liver was decapsulated and perfused in physiological saline, the organ was homogenized in 0.1M phosphate buffer (pH 7.2).

Haematological assay

Packed cell volume (PCV)

Non-heparinized capillary tubes (75 mm long with bore of 1.155 ±

0.085 mm) heat sealed at one end was filled up to 2/3 length with whole blood, was spun at 12,000 g (10,000 rpm) for five minutes in a micro-hematocrit centrifuge. The packed cell volume was determined using the graduation in the standard hematocrit chart and recorded as percentage of whole blood.

Biochemical parameters

Alanine transaminase (ALT)

This enzyme catalyses the transamination of alanine in the presence of alpha-keto glutarate with the concomitant generation of glutamate and pyruvate, which then react with 2,4-dinitro-phenylhydrazine forming a coloured complex of hydrazone derivative of pyruvate, which can be measured at 546 nm. The activity in (U/L) was determined from the standard calibration curve.

Aspartate transaminase (AST)

This enzyme catalyses the transamination of aspartate in the presence of alpha-keto glutarate with the concomitant generation of glutamate and oxaloacetate, which then react with 2,4-dinitro-phenylhydrazine forming a coloured complex of hydrazone derivative of pyruvate, which can be measured at 546 nm. The activity in (U/L) was determined from the standard calibration curve.

Alkaline phosphatase (ALP)

This enzyme catalyses the hydrolysis of esters of phosphoric acid with the subsequent release of a phosphate group. P-nitrophenol phosphate was used as the substrate, a colourless solution which turns yellow upon hydrolysis. The coloured complex can be measured at 405 nm. Enzyme activity (U/L) = $3300 \times$ change in absorbance at 405 nm/change in time (min).

Total serum albumin (BCG)

Bromocresol green forms a coloured complex specifically with albumin. The intensity for the colour measured at 620 nm, is directly proportional to the albumin concentration in the sample. The concentration of total serum protein (g/dl) is determined from the standard calibration curve.

Malondialdehyde (MDA) assay

Principle of assay

Malondialdehyde are released from the cleavage of peroxide intermediates generated from lipid peroxidation. Thiobarbituric acid reacts with malondialdehyde to form a coloured complex which absorbs light at 532 nm. The MDA level was determined from the standard calibration curve.

Procedure of assay

One millilitre aliquot of each homogenate supernatant was incubated for two hours at 37°C with three millilitres of ferrous sulphate/phosphate buffer solution. One millilitre of each mixture was added to a tube containing two millilitres trichloroacetic acid (TCA). After centrifugation at 700 g for ten minute, two millilitres of each supernatant was added to one millilitre of 2-thiobarbituric acid

Table 1. The effect of Halofantrine hydrochloride administration on the total serum albumin and serum enzyme activities.

Study groups	Alanine transaminase (U/L)	Aspartate transaminase (U/L)	Alkaline phosphatase (U/L)	Total serum albumin (g/dl)
Group A	1.41 ± 0.063	1.30 ± 0.070	4.833 ± 0.1467	1.68 ± 0.479
Group B	1.51 ± 0.045	1.90 ± 0.119	5.04 ± 0.094	1.62 ± 0.203
Group C	2.56 ± 0.173	3.93 ± 0.714	16.13 ± 2.017	1.20 ± 0.164
Group D	4.59 ± 0.186	4.95 ± 0.420	29.78 ± 7.303	0.96 ± 0.129

Values * given as mean ± standard deviation had significant differences, when compared with the control (p < 0.05).

Table 2. The effect of Halofantrine hydrochloride administration on the total serum bilirubin, serum conjugated bilirubin, packed cell volume and malondialdehyde.

Haematological parameters	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Packed cell volume (%)	MDA (nmol/l)
Group A	0.136 ± 0.010	0.025 ± 0.0003	43.28 ± 1.825	13.52 ± 5.032
Group B	0.198 ± 0.010	0.030 ± 0.0008	39.5 ± 0.548	16.12 ± 2.651
Group C	1.561 ± 0.039	0.200 ± 0.0095	34.73 ± 1.717	27.43 ± 3.222
Group D	1.538 ± 0.095	0.220 ± 0.0216	28.70 ± 1.061	34.76 ± 2.842

Values * given as mean ± standard deviation had significant differences, when compared with the control (p < 0.05).

(TBA) solution. The tubes were boiled for ten minute and allowed to cool and the absorbance read at 532 nm.

Calculation

Thiobarbituric acid reactive substance of the samples was calculated as malodialdehyde (MDA) equivalents, using serial dilutions of MDA standards (10 nmol/ml).

Total bilirubin assay

Principle

Conjugated bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin was determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Calculation

Total bilirubin (mg/dl) = 10.8 × Absorbance (578 nm)
 Conjugated bilirubin (mg/dl) = 14.4 × Absorbance (546 nm)

STATISTICAL ANALYSIS

The results obtained in the research work were expressed as mean ± standard deviation. The difference between mean values were assessed for significance by student t-test (SPC-XL, 2000) and considered significance at P < 0.05.

RESULTS

Table 1 shows the effect of halofantrine hydrochloride

administration on the total serum albumin and serum enzyme activities. Alanine transaminase, aspartate transaminase, alkaline phosphatases and malodialdehyde (MDA) showed a dose dependent increase in activity. Table 2 shows the effect of Halofantrine hydrochloride administration on the total serum bilirubin, serum direct bilirubin and packed cell volume (PCV). The PCV decrease was dose dependent and significant in both normal dose and over dose groups.

DISCUSSION

The liver is the site of synthesis of the most abundant plasma protein (albumin). Albumin accounts for 60% of total serum protein. The functions of albumin includes the maintenance of colloid osmotic pressure and binding of key substances such as drugs (Kim et al., 1998), which makes it a reliable marker for diagnosis of liver diseases (Benoit et al., 2000). Our study indicated a significant decrease in albumin both in rats in which the normal dose and over dose were administered. It is probable that the decreased serum albumin observed in this study was as a result of necrosis as previously suggested (Goldwasser and Feldman, 1997) or as a result of direct interference with albumin synthesizing machinery in hepatocytes.

In agreement with previous study by Obi et al. (2004), we observed a dose-dependent increase in the activities of alanine transaminase, aspartate transaminase and alkaline phosphatase. This observed increase was significant in both normal dose and over dose groups. This may be attributed to the supposed mechanism of action of

Halofantrine which may be similar to that of chloroquine, quinine and mefloquine, forming toxic complexes with ferritoporphyrin IX that damages the membrane of the parasite and possibly the host (Slater, 1993). Since Halofantrine hydrochloride is a schizonticide mainly metabolized in humans by CYP3A4 (Halliday et al., 1995), it therefore suggests accumulation and detoxification in the liver with subsequent imminent damages (Amin and Geoffrey, 2007).

The packed cell volume (PCV) decrease was dose-dependent and significant in both normal dose and over dose groups. This finding is in agreement with results obtained from most recently introduced antimalaria drugs (Omotuyi et al., 2008, Udosen and Akpan, 2003). We observed a significant increase in the levels of MDA in both normal dose and over dose groups. This increase may have resulted from the reactive complex formed by the drug or its metabolites (Slater, 1993).

The distribution of Halofantrine hydrochloride is highly dependent on the plasma protein concentrations (Daniyan et al., 2008). It has also been established that in *P. falciparum* infected individuals there is reduced plasma protein level (Ahmed et al., 2003). There is a possibility that the toxicity observed in normal animal model in this work was as a result of the levels of plasma protein that aided the drug distribution and concomitant toxicity.

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