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HAEMATOLOGICAL AND BIOCHEMICAL EFFECTS OF HALOFANTRINE HYDROCHLORIDE (HALFAN) ON FEMALE ALBINO RATS

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ABSTRACT

Malaria has remained a major killer disease in the tropics and its treatment has been greatly hindered by increased drug resistance. This study evaluated hematological and biochemical effect of doses of Halofantrine hydrochloride (HALFAN) a phenanthrene methanol drug used for the therapeutic treatment of malaria on female albino rats. Twenty (20) female albino rats divided into four (4) groups (A-D) of five (5) rats each were used. Group A (control) received 0.1 mg/kg body weight of normal saline, while groups B, C and D received 0.1mg/kg body weight of Halofantrine hydrochloride administered orally three times at six hourly interval for two, four and six weeks duration respectively. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol (TC), triacylglycerol (TG), high density lipoprotein (HDL), and low-density lipoprotein (LDL) increased significantly ($P < 0.05$). The full blood count increased in a dose dependent manner. There was significant increase ($P < 0.05$) in the body weight of the rats. The results have shown that the drug could induce toxicological effect as the activities of ALT, AST and ALP increased significantly ($p<0.05$). The white blood cell count also increased in a dose dependent manner. These findings suggest that the drug might have some hepatotoxic effects.

KEYWORDS: *Biochemical parameters, albino rats, Halofantrine Hydrochloride, Malaria, Toxicology*

INTRODUCTION

Malaria has remained the most prominent and ancient disease that has bedevilled mankind globally. In 2017, global malaria incidence and its associated mortality were reported to have decreased by over 40% and 60% respectively. Notwithstanding, more than 200 million new cases and about 430, 000 deaths was recorded in the same year (WHO, 2018). Malaria is caused by parasitic protozoan transmitted by female *Anopheles* mosquito and kills millions of people in

the tropics (WHO, 2017). In man, six species of plasmodium namely: *P. falciparum, P. vivax, P. ovalecurtisi, P. ovalewallikeri, P. malariae and P. knowlesi* are known to cause infections. More than 90% of malaria cases in sub-saharan Africa are caused by *Plasmodium falciparum* (Alaebo *et al.,* 2018b) Out of this number, 70% affects mostly children below age of 5 years (WHO, 2018). Number of deaths recorded for malaria yearly has been reported to equal number of deaths from AIDS in fifteen years (Arnott *et al.*, 2012). Despite the advances in medical research, malaria has remained a monster difficult to summount due to plasmodium parasite resistance to chemo-prophylactic and chemotherapeutic agents; resistance of mosquito vector to pyrethroids impregnated in bed nets and other insecticides (Arnott *et al.*, 2012). There is also the challenge of inability to coordinate as well as maintain malaria control resources by health and public workers. Globally, malaria control measures is targeted at preventing human contact with the vector by using insecticide-treated nets, early diagnosis and treatment while chemo preventive measures is used for pregnant women and children (Alaebo *et al.,* 2018a).

The resistance of *P. falciparum* to most of the anti- malarial drugs is spreading. This is due not only for the remarkable adaptability of the parasite, but also to man's misuse and overuse of drugs for prophylaxis and inadequate routine treatment of undiagnosed fever in endemic areas. The spread of drug-resistance *Plasmodium falciparum* has encouraged the need to develop new anti-malarial compounds. Amongst the numerous antimalarials used for malaria treatment is Halfan (pharmacologically known as Halofrantine) developed by Walter Reed Army Institute of Research between 1960s-1970s (Cosgriff *et al.,* 1982).

It is an anti-malarial drug that is schizonticidal with a high degree of activity against the erythrocytic stage of malaria parasite. It is indicated for the treatment of acute malaria in patients with single or mixed infections of *P. falciparum* and *P. vivax*. (Alaebo *et al.,* 2018b). Halofantrine has not been shown to be mutagenic either in animals or in tests utilizing carbohydrate (CHO) chromosome aberration test and dominant lethal assay in rats. However, overdose of halfan has been shown to be embryocidal in rats (Baird, 2013). In developing countries like Nigeria, with widespread practice of self-medication and drug abuse especially amongst pregnant women and children, there is need to investigate the effect of halfan- a common anti-malarial using some biochemical parameters and hence this study. It has been shown that the gonads are affected by series of factors like exposure to certain types of drugs and physical agents, irradiation and hypoxia (Heywood and Wardsworth, 1980). Some anti-malaria drugs are much more abused and some have been found to be teratogenic. For example, pyrimethamine has been implicated in several forms of skeletal anomalies when administered to pregnant rats in doses higher than the therapeutic dose (Akpaffiong *et al.,* 1986).Studies on reproductive and developmental toxicity carried out on male using Halfan showed that there was no drug related effects on male fertility or reproductive

performance in low or mild groups (Reno, 1982). The epididymis and seminal vesicles were organs of toxicity in the high dose group. Oral administration of excess doses of 80mg/kg Halfan produced scarring and necrosis of the skin (Reno, 1982).

The anti-malaria activity of the phenanthrene methanols was first recognized during the World War II (Wiselogle *et al.,* 1946).The problem of drugs resistance experienced in the 1960's and the failure of the measures taken to control malaria infection prompted the Walter Reed Army Institute of Research (WRAIR) in Washington DC to discover this drug. Collaboration between WRAIR, Smith Kline and French began in 1983 and World Health Organization (WHO) started conducting clinical trials in Zambia and Columbia in 1987 (WHO, 1998).This study aimed to assess the effect of halfan on some biochemical parameters of female albino rats. The study seeks to test the hypothesis that prolonged administration of halfan is toxic.

MATERIALS AND METHODS

Animal Maintenance and Grouping

Twenty (20) female Wistar rats (160-215g) purchased from Chris Animal Farm, MgbakwuAwka, Anambra state, Nigeria, were acclimatized under standard housing conditions. The rats were randomly divided into four (4) groups of 5 rats each. Group A served as control while B, C and D were the treatment groups administered oral doses of 0.1 mg/kg body weight halfan for 2, 4 and 6 weeks respectively. Ethical guidelines approved by the Ethical Committee of National Institute of Health, USA as approved by the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

Blood Samples Collection

At the end of the treatment period, the rats were sacrificed by exposing them to an overdose of chloroform soaked in cotton wool placed in an anaesthetic box covered with a lid. Blood samples were drawn from the heart of each sacrificed rat from all groups by puncture, and blood samples were collected in EDTA specimen bottles for haematological analysis. The clear serum was obtained by centrifuging the whole blood and estimating AST, ALT and ALP.

Drug Administration

Halofantrine hydrochloride used in this research was produced by SmithKline and French Laboratories. The drug suspension was administered to the animals on the basis of their body weight. Usually, 5ml of the drug suspension contain 100 mg of Halofantrine hydrochloride (Halfan). The therapeutic dose for the experimental animals was thus 0.1mg/kg body weight as against the therapeutic dose of humans, which is 10 mg/kg at six hourly doses. The suspension of halofantrine was administered orally with the aid of an orogastric tube attached to needle and syringe.

Haematological Analysis

Haematological parameters were determined using automated auto haematology analyzer (Mindray-BC-28000). The haematology parameter that were analyzed include white blood cell (WBC), red blood cell (RBC), Heamoglobin (HB), packed cell volume (PVC), platelets (PLT).

Biochemical Analysis

Determination of Liver Function Markers

Determination of Aspartate Aminotransferase Activity: The method of Reitman and Frankel (1957) described by Randox laboratories, the United Kingdom using Randox kits, was used for this study. Principle: The activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Determination of Alanine Aminotransferase Activity: This was also done using the method of Reitman and Frankel (1957). Principle: The activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4, -dinitrophenylhydrazine.

Determination of Alkaline Phosphatase Activity: The activities of alkaline phosphatase were evaluated using the methods of Kind and King (1972). Principle: Serum alkaline phosphatase hydrolysis yields a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein, which at alkaline pH, turns pink and can be determined photometrically.

Determination of Lipid Profile Biomarkers

Determination of total cholesterol concentration

Total cholesterol was determined using the enzymatic colourimetricchod-pad test method described by Allain et al. (1974), with Randox laboratory test kits. Principle: The sample's free and esterified cholesterol originates utilizing a coupled reaction where serum cholesterol reacts with enzymes to produce a coloured complex whose intensity is proportional to the serum cholesterol concentration and is measured spectrophotometrically.

Determination of Triglycerides Concentration

This was also determined spectrophotometrically using the method of Tietz *et al*. (1990) Principle: The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinonemine formed from hydrogen peroxidases, 4-amino phenazone and 4- chlorophenol under the catalytic influence of peroxidase.

Determination of High-density Lipoprotein Concentration

This was evaluated by Grove (1979) methods, as described in the Randox Laboratory test kit. Principle: It involves a precipitation reaction with phosphotungstate and magnesium ion where the supernatant contains HDL, which is measured spectrophotometrically.

Determination of Low-Density Lipoprotein Cholesterol Concentration

Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation (Bhandari *et al*., 2013), LDL-C = [TC – {HDL-C+ (TG/5)}] where VLDL-C = (TG/5) (Bhandari *et al*., 2013).

Determination of Very Low-Density Lipoprotein Cholesterol Concentration

This was calculated according to the method of Wilson et al. (1981) as VLDL = 0.2 x TG (where TG is total glycerides)

Statistical Analysis

Statistical tests were performed using SPSS (version 11) package. The statistical significance between groups was analyzed using one-way analysis of variance (ANOVA). Significant

differences were determined by Fisher Least Significance Difference (LSD) post hoc test.

RESULTS

There were significant increases in the white blood cell count of the rats after 5 and 10 days of drug administration (Fig. 1). This shows that Halfan causes an increase in the white blood cell count of female wistar rats.

Table 1: Effect of Halofantrine hydrochloride on the haematology of female albino rats

PARAMETERS	CONTROL	WEEK 2	WEEK 4	WEEK 6
WBC (cmm)	3.8±0.1	4.0±0.1	4.4±0.6*	4.6±0.8*
RBC (cmm)	3.9±0.6	4.2±0.3	4.5±0.6*	4.9±1.0*
HB (g/dL)	7.2±1.2	9.9±1.0*	11.0±1.4*	11.5±2.1*
PVC (%)	21.7±3.5	29.4±2.8	32.2±4.2*	34.4±6.1*
PLT (µL)	169.0±13.7	184. 3±19.0	196.1±0.0*	220.6±30.4*

Values marked asterisks (*) are statistically significant (P< 0.05); WBC – White blood cell, RBC – Red blood cell, HB – Haemoglobin, PCV – Packed cell volume, PLT – Platelet

Result of the effect of Halofantrine hydrochloride on the haematology of female Wistar rats is as depicted in Table 1. The WBC, RBC and platelet counts of rats in groups C and D increased significantly ($p<0.05$) compared to their controls. Hemoglobin and PCV counts of all the experimental groups were all significantly ($p<0.05$) higher than their control.

Table 2: Effect of Halofantrine hydrochloride on some liver function parameters of female albino rats

PARAMETERS	CONTROL	WEEK 2	WEEK 4	WEEK 6
AST (IU/L)	6.9±5.1	10.7±3.8*	11.0±2.8*	13.7±3.4*
ALT (IU/L)	9.2±1.4	10.4±1.8	11.4±2.2*	12.7±2.7*
ALP 9IU/L)	67.5±5.9	75.0±7.6*	73.6±8.38	85.8±7.8*

Values marked asterisks (*) are statistically significant (P< 0.05); AST – Aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, TB – Total Bilirubin, CB – conjugated bilirubin

Result of the effect of Halofantrine hydrochloride on some liver function parameters of female Wistar rats is as presented in Table 2. All the treatment groups showed an increase in all the liver function parameters (AST, ALT, and ALP) studied. The activity of AST was significantly ($p <0.05$) increased in all the treatment groups compared to the control. Rats in groups C and D had significantly ($p<0.05$) increased ALT activity compared to the control. The activity of ALP was significantly ($p<0.05$) increased in groups B and D rats compared to the control.

Table 3: Effect of Halofantrine hydrochloride on some lipid function parameters of female albino rats

PARAMETERS	CONTROL	WEEK 2	WEEK 4	WEEK 6
TC (mmol/L)	3.2±0.4	3.7±0.2*	3.9±0.3*	4.6±0.6*
TG (mmol/l)	0.6±0.1	0.8±0.1*	1.0±0.2*	1.1±0.2*
HDL (mmol/l)	1.3±0.1	1.3±0.1	1.4±0.1*	1.5±0.1*
LDL (mmol/l)	2.6±0.2	2.7±0.1	3.1±1.1*	3.1±0.2*

Values marked asterisks (*) are statistically significant (P< 0.05); TC – Total cholesterol, TG- Triacylglycerol, HDL- High density lipoproteins, LDL- Low density lipoproteins

Table 3 presents the result of the effect of halofantrine hydrochloride on lipid function parameters. Total cholesterol and triacyglycerols concentrations increased significantly in all the groups compared to the control. Concentrations of HDL and LDL increased significantly ($p<0.05$) in rats of groups C and D compared to the control.

DISCUSSION

The liver is an important organ involved in drug metabolism and excretion of xenobiotics (Pandit *et al.,* 2012). Drug metabolism enzymes found in the liver strive to achieve homeostasis by detoxifying drugs (Upadhyay *et al.,* 2008). Any shift in this homeostasis favours generation of ROS that gives rise to oxidative stress hence impaired liver function (Upadhyay *et al.,* 2008). These ROS can bind to macromolecules like DNA, lipids and proteins to cause mutation, membrane injury and altered activity of proteins (Upadhyay *et al.,* 2010; Prince *et al.,* 2022). Hepatotoxic drugs induce ROS generation in the liver (Coleman *et al.,* 2007; Alaebo *et al.,* 2020).

In the present study, halofantrine hydrochloride significantly ($p<0.05$) increased the serum AST and ALT activities as compared with the control. It is a major organ that detoxifies and excretes both endogenous and exogenous compounds. Any alterations in its function lead to health impairment (Coleman *et al.,* 2007). This observed increase in AST and ALT could be due to membrane damage induced by the halofantrine hydrochloride (Joan *et al.,* 1987). The observed increase in ALP activity in groups B and D is also an indication of possible toxicity of the drug. ALP is not a specific and sensitive indicator of liver damage.

Lipid profile is a panel of blood tests that serve as an initial broad medical screening tool for the abnormality in lipids, such as total cholesterol, triacyglycerol, LDL and HDL. The result of this can be used to determine approximate risks for cardiovascular disease (Sniderman *et al.,* 2016). Body physiological changes usually alter blood total cholesterol level. The body stores energy in the form of triacylglycerol (TG). Low serum TG level favours good health while high TG level promotes heart diseases. Total cholesterol and total triacyglycerol levels in this study were significantly higher as days progressed for all the groups treated with halfan. In a similar manner also, the HDL and LDL levels also increased as time progressed but the groups treated with halfan for 4 and 6

weeks were significantly higher. The result has suggested that the use of halfan could predispose to cardiovascular diseases and toxicological effects (Doris *et al.,* 2022), especially under prolonged treatment.

High density lipoprotein (HDL) has been recognized as good cholesterol which at high concentration regulates the blood LDL cholesterol amount. It carries out its regulatory activity by transporting low density lipoprotein (LDL) from the arteries to the liver for elimination (Hunter *et al.,* 2010). Significant increase in HDL level observed in rats of 4 and 6 weeks groups suggest reduced risk of cardiovascular disorders while the increased LDL level suggests greater risk of atherosclerosis in the rats (Olukanni *et al.,* 2013). Hypercholesterolemia and low levels of high density lipoprotein cholesterol (HDL) are the major causes of increased atherogenic risk, (Bersot, 2011). The significant increase in blood TC and TG in the rats from 2 to 6 weeks relative to the control suggests that the drug halfan could have induced increased breakdown of lipid components in the rats. The result of the lipid profile indicates that halfan could contribute adversely to the cardiovascular disease.

Haematological parameters are used to check stress and disease in animals (Etim*et al.,* 2014). White blood cells (WBC) are part of the body's immune defense system that regulates immunological function (Ballarin *et al.,* 2004). Increased oxygen level leads to increase in RBC level (Ogunbajo*et al.,* 2009). Good transport of oxygen and absorbed nutrients is evidenced by increased PCV levels (Oguz *et al.,* 2000). Platelets regulates clotting and inflammation in the body (Golebiewska and Poole, 2015). Haemoglobin (Hb) is a metalloprotein that contains iron responsible for transporting oxygen to tissues. This transported oxygen is used to oxidize ingested food for the purpose of releasing energy needed for transport of oxygen out of the body (Omiyale *et al.,* 2012; Soetan *et al.,* 2013). The WBC, RBC, Hb, PCV and platelet levels obtained in this study increased with time in all the groups treated with halfan when compared to their controls. This could signify toxicity of the drug on the bone marrow of the female wistar rats, the increase in the whole blood count indicate a pathological condition (Osei-bimpong *et al.,* 2012). Ofem *et al.* (2013) reported that the administration of chloroquine and coartem on wistar rats for 3 days did not significantly alter the levels of RBC, Hb, PCV and differential WBC, and platelets indices but lead to significant reduction in MCV and MCHC in chloroquine recipients in comparison with control and coartem group respectively.

Our research finding was at avarice within the report of Agoreyo *et al.* (2012) who published that artemether drug caused a significant reduction in the hematological profiles of the animals in a dose dependent manner, discontinuous of the drug use, however showed gradual recovery of the depressed indices of the blood parameters.

Conclusion

Halofantrine hydrochloride has toxicological effects on the treated albino rats due to the increase in the white blood count which could signify toxicity of the drug on the bone marrow of the rats. The increased white blood

cell count indicates a pathological condition. The drug was discovered to cause increase on serum enzymes level in the experimental rats, suggesting hepatotoxicity of the drug.

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