EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 2
137-141
Year: 2018

THE TIME AND DOSE DEPENDENT EFFECTS OF HALOFANTRINE HYDROCHLORIDE ON THE BIOCHEMICAL LIVER INDICES OF GUINEA PIG

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Article Received on 10/12/2017 **Article Revised on 31/12/2017** **Article Accepted on 22/01/2018**

ABSTRACT
Halofantrine hydrochloride is an antimalarial drug, usually prescribed as a standby treatment of malaria in travellers and pregnant mothers. In this study the time and dose dependent effects of halofantrine hydrochloride on liver biochemical indices during 24 hours treatment period of malaria. 48 female guinea pigs were used in this study and were equally divided into 3 groups of 16 each. The treatment group B and C received 2.6mg and 1.3mg of halofantrine hydrochloride as clinical and subclinical doses respectively at 6 hours interval for 3 doses while group A the control received distilled water alone. Signs of toxicity were observed throughout the study. No death was recorded in any of the treatment groups and also the guinea pigs did not show any abnormalities in their physiological characteristics. The liver biochemical indices such aspartate aminotransferase, alanine aminotransferase, total protein and albumin were significantly elevated at $p<0.05$ however there was a significant reduction alkaline phosphatase enzyme at $p<0.05$. It was also observed that the effects of halofantrine on the liver enzymes follow a biphasic activity. Based on this result, it can be concluded that halofantrine hydrochloride has a biphasic activity on liver enzymes and hepatotoxic in clinical and subclinical doses during treatment period. It is therefore contraindicated in patient with livers diseases or those at risk of liver disease.

KEYWORDS: Halofantrine hydrochloride, biochemical indices, guinea pig, short-term treatment period.

INTRODUCTION

Malaria infestation is an endemic disease in the Sub-Saharan African. This is the most common cause of death in this region especially in Children. Malaria is caused by plasmodium parasites which is carried and transmitted to human through the bite of infected female anopheles mosquitoes. Symptoms present as an acute febrile illness and if not treated at the early stage of the disease process, it may progress to more complicated syndrome involving multiple organ dysfunctions.[1]

Human beings are infected by four species of the malaria parasite know as plasmodium species which include Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium falciparium. Of these four species, the most pathogenic and prevalent of the human malarias is the plasmodium falciparium.[1]

Malaria disease has remains a public health burden with increasing morbidity and mortality that poses major economic and developmental challenges in sub-Saharan Africa.[2] The spectrum of malaria illness may take various clinical forms, differing in pattern and severity, from uncomplicated to complicated and severe forms of malaria. The different effort has been put in place to control or eradicate the illness has been proven difficult. In 2007 a study done by Orimadegun shows that out of ten children admitted to children emergency units two of them suffer from severe forms of malaria and the complications associated with it.[3] Worldwide, there is a reported 90% of cases and 85% of the deaths have been attributed to malarial illness in sub-Saharan Africa where Plasmodium falciparum infection was found to be responsible for a number of all the morbidity and mortality.[4]

In 2015, the African region consider that about 90% of cases was attributed to malaria and 92% malaria related deaths and about 13 of these countries are with the Sub-Saharan Africa region which accounted for 76% of malaria cases and 75% of malaria related- deaths globally.[1]

Antimalarial medicines are drugs which can be used as treatment and preventions of malaria.[5] Malaria can be prevented in travellers to endemic region and pregnant mothers through chemoprophylaxis which suppresses the blood stage of the malaria infection. Some of medication

use includes sulphadoxine- pyrimethamine, halofantrine.[1]

Halofantrine hydrochloride is a 1, 3- dichloro-2-2-dibutylaminoethyl-6-trifluoromethyl-9-phenanthrenemethanol, a highly lipophilic drug, belonging to the aryl-amino-alcohol family and it is usually prescribed as a standby treatment in travellers to the tropics who develop febrile illness and has also been proposed as a radical cure to avoid the constraints of prolonged chemoprophylaxis.[6] It is effective against erythrocytic stage of all four plasmodium spices but not active against the hepatic stage or gametocytes.[5]

Numerous studies indicate the cardiotoxicity of halofantrine, which is due to its quinidine- like effects.[7] The effects of antimalarial drugs on the biochemical liver function and liver tissue has been studied. It was reported to have increased ALT and AST in quinine induced hepatotoxicity, however the studies of the biochemical effects of halofantrine has received little attention. As a result, this study aims to investigate the liver biochemical indices of cytotoxicity during the treatment of malaria with halofantrine hydrochloride.[8]

MATERIALS AND METHODS

Experimental drug

Halofantrine hydrochloride 8mg/kg dose given six (6) hourly intervals for 24 hour was used in the experiment. The drug was gotten from a pharmaceutical store in Port Harcourt, Rivers state, Nigeria to the Physiology laboratory in the University of Port Harcourt, Rivers Sate.

Experimental Animals

The experimental animals used in this study were male guinea pigs (*Cavia porcellus*). Forty-eight male guinea pigs with and average weight of 326 grams (0.326 Kilogram) were obtained from the animal house unit of the Department of Human Physiology of the University of Port Harcourt. They were divided into three groups – two experimental (clinical and sub-clinical) groups and control group with each containing twelve guinea pigs. The guinea pigs were fed with standard diet and water before and during the experiment period ad libitum. They were also acclimatized for a period of 14 days under standard environmental conditions of temperature, relative humidity and dark-light cycle prior to the commencement of the experiment.

Preparation of the Drug

125 mg Halofantrine hydrochloride dissolved in 42mls of distilled water was prepared at each dose using a cylinder. Each guinea pig received 1mls of the mixture as clinical dose which is equivalent to 2.6mg halofantrine hydrochloride and 0.5mls of the mixture as subclinical dose (1.3mg Halofantrine HCL) in the experimental groups.

Toxicological study

The prepared drug mixture was orally administered to the animals thrice at 6 hours interval through oropharyngeal cannula. Group A, the control group received distilled water, Group B, the clinical dose group received 1mls = 2.6mg Halofantrine HCL and group C, the sub-clinical group received 0.5ml = 1.3mg Halofantrine HCL. The guinea pigs were observed 4 hourly for clinical signs of lethargy, feeding habit, water intake, physiological characteristics and their weight been measured on 0 hour and 48 hours. During the experimental period the rats were allowed access to feeds and water ad libitum.

Sample Collection

At the end of drug administration, blood samples were collected through cardiac puncture of the animals using 10mls hypodermal syringes into lithium heparin bottles and were used for the estimation of biochemical liver indices. The samples were collected in the following order; 4 animals from each group at 6, 12, 18, and 24 hours after complete drug administration.

Estimation of Biochemical indices

The biochemical indices of the guinea pig were estimated using automated biochemical auto analyzer, which determines liver biochemical such as total protein, Albumin, Aspartate aminotransferase, Alanine aminotransferase and alkaline phosphatase.

Data Analysis

All the date was presented as mean ± standard error of mean (SE). The significance of difference among groups was assessed using one way and multiple way analyses of variance (ANOVA) and proceeds with Post Hoc test either Bonferroni with p-value < 0.05 was considered as significant.

RESULTS

Determination of Death rate

In the 48 hours of treatment period no death was recorded in both the experimental and control groups.

Clinical observation on Physiological Characteristics

During the course of the treatment period of 48 hours, daily observations of the animals were done on their physiological characteristics. It was initiated at 6 hours after the administration of Halofantrine hydrochloride first dose to the animals. Changes in the animal's physiological characteristics were recorded and presented in table 1 below.

Table 1: The Time and Dose Effects of Halofantrine HCL on Physiological Characteristics of the Guinea pigs.

	Groups		
Physiological Characteristics	**A**	**B**	**C**
Motility	Active	Active	Active
Skin and fur colour	No	No	No
Mucosa of the eyes	No	No	No
Nose mucosa	No	No	No
Bleeding	No	No	No
Salivation convulsions	No	No	No
Tremors	No	No	No
Diarrhoea	No	No	No
Coma	No	No	No

Key:
No = No abnormality detected throughout the experimental period.
Active = still active in the case throughout the experimental period.
Group A (control) = administered with distilled water.

The Effects of Halofantrine hydrochloride on Liver Biochemical Indices

Blood samples were collected on the 6, 12, 18, and 24 hours after drug administration for the estimation of the serum level of Total protein, Albumin, Aspartate aminotransferase, Alanine aminotransferase and alkaline phosphatase. The result obtained from the estimation are show in table 2, 3, 4, 5 and 6 respectively and also illustrated graphically in figure 1, 2, 3, 4 and 5 respectively.

Table 2: The Time and Dose Dependent Effects of Halofantrine HCL on Total Protein of the Guinea pigs.

Groups (n=6)	**A**	**B**	**C**	**ANOVA**
Time (hours)	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**P-value**
6	56.5±3.8	54.0±2.7	53.5±0.3*	P<0.05
12	55.9±3.8	61.8±3.9	59.0±3.4	P>0.05
18	56.8±3.8	55.0±1.3	53.5±0.3	P>0.05
24	56.4±3.8	64.0±0.7*	59.5±2.3	P<0.05
Key:				
Group A: administered with distill water			**Unit:** g/dl	
Group 2: administered with 2.6mg/kg body weight				
Group 3: administered with 1.3mg/Kg body weight				

Analyzed using ANOVA one-way (significance level at p<0.05).

Table 3: The Time and Dose Dependent Effects of Halofantrine HCL on Albumin of the Guinea pigs.

Groups (n=6)	**A**	**B**	**C**	**ANOVA**
Time (hours)	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**P-value**
6	29.4±2.2	35.0±1.3*	34.0±0.6*	P<0.05
12	29.3±2.0	29.5±2.0	31.0±3.0*	P<0.05
18	28.8±2.2	35.5±0.3*	34.0±0.7*	P<0.05
24	29.0±2.1	42.0±1.3**	37.5±0.3**	P<0.05
Key:				
Group A: administered with distill water			**Unit:** g/dl	
Group 2: administered with 2.6mg/kg body weight				
Group 3: administered with 1.3mg/Kg body weight				

Analyzed using ANOVA one-way (significance level at p<0.05).

Table 4: The Time and Dose Dependent Effects of Halofantrine HCL on Aspartate aminotransferase (AST) of the Guinea pigs.

Groups (n=6)	A	B	C	ANOVA
Time (hours)	Mean ± SE	Mean ± SE	Mean ± SE	P-value
6	37.8±2.8	43.0±1.3	35.0±2.0	P<0.05
12	37.4±2.6	41.0±0.8	42.3±3.0	P<0.05
18	37.8±2.0	28.5±1.0	30.5±1.7	P<0.05
24	37.6±2.1	32.5±1.2*	25.5±0.3*	P<0.05
Key:				
Group A: administered with distill water			**Unit:** IU/L	
Group 2: administered with 2.6mg/kg body weight				
Group 3: administered with 1.3mg/Kg body weight				

Analyzed using ANOVA one-way (significance level at p<0.05).

Table 5: The Time and Dose Dependent Effects of Halofantrine HCL on Alanine aminotransferase (ALT) of the Guinea pigs.

Groups (n=6)	A	B	C	ANOVA
Time (hours)	Mean ± SE	Mean ± SE	Mean ± SE	P-value
6	27.0±2.8	35.0±3.3*	27.0±2.0	P<0.05
12	27.0±2.6	29.0±1.6	31.3±2.2	P<0.05
18	27.0±2.0	22.0±1.3*	25.5±1.7	P<0.05
24	27.0±2.1	25.5±1.7*	20.5±1.3*	P<0.05
Key:				
Group A: administered with distill water			**Unit:** IU/L	
Group 2: administered with 2.6mg/kg body weight				
Group 3: administered with 1.3mg/Kg body weight				

Analyzed using ANOVA one-way (significance level at p<0.05).

Table 6: The Time and Dose Dependent Effects of Halofantrine HCL on Alkaline Phosphatase of the Guinea pigs.

Groups (n=6)	A	B	C	ANOVA
Time (hours)	Mean ± SE	Mean ± SE	Mean ± SE	P-value
6	130.3±2.4	25.0±2.8	67.0±1.2	P<0.05
12	130.4±3.6	30.0±0.8	96.0±2.3	P<0.05
18	130.8±2.8	29.5±3.8	53.5±2.7	P<0.05
24	130.6±3.4	40.0±1.2	26.5±0.3	P<0.05
Key:				
Group A: administered with distill water			**Unit:** IU/L	
Group B: administered with 2.6mg/kg body weight				
Group C: administered with 1.3mg/Kg body weight				

Analyzed using ANOVA one-way (significance level at p<0.05).

DISCUSSION

42 hours toxicity study of the clinical and subclinical doses of halofantrine hydrochloride do not have any adverse effects on the physiological characteristic of the guinea pigs as shown in table

In 1998 it was established by Guillouzo that many xenobiotic substances are potentially hepatotoxic.[9] Their ability to produces liver damage in vivo often results from the interactions of the uptake of a series of complex cellular processes involved in the uptake, biotransformation and elimination of these potentially toxics compounds.

The time and dose dependent effects of halofantrine hydrochloride on liver biochemical indices of guinea pigs were investigated and all the measured indices such aspartate aminotransferase, alanine aminotransferase, total protein, and albumin in this study were significantly increased at $p < 0.05$ in both clinical and subclinical doses except alkaline phosphatase that was significantly decreased at p<0.05 as compared to the control group as shown in table 1, 2, 3, 4, 5, and 6 above.

In acute hepatotoxicity, liver enzymes are mobilized and are usually elevated however they tend to decreased with prolong intoxication due to the damage of the liver parenchymal.[10] Increase in liver enzymes such as alanine aminotransferase and aspartate aminotransferase are common findings in acute liver toxicity, while increased activity of these enzymes may be found in damage tissue.[11,12] This study shows an increase in these enzymes indicating liver parenchymal damage at the clinical and subclinical doses of halofantrine

hydrochloride on guinea pigs as compared to the report of Smith e. al., in 1998 where an increase in aspartate aminotransferase was recorded in oral acetaminophen administration to rats inducing hepatotoxicity.[13]

Also Mizutani et. al., in 1999, reported an increase in serum alanine aminotransferase activity in metronidazole induced hepatotoxicity in mice.[14] Exposure halofantrine causes pathologic changes in the guinea pigs which include moderate portal triaditis to severe hepatic degeneration and these changes are dose dependent.[15]

This study shows that halofantrine hydrochloride as an oxidant causes hepatotoxicity at clinical and subclinical doses and produces mild to moderate degrees of hepatocellular damage with the treatment period of twenty-four to forty-eight hours and these effects are dose and time dependent as shown in the elevation of liver enzymes in the clinical and subclinical doses.

CONCLUSION

At the end of study it has been established that halofantrine hydrochloride, a drug use in there biochemical of the patient should be known. Further studies are recommended to ascertain the treatment of malaria was found to cause significant hepatotoxicity at clinical and subclinical doses that is time dependent. It has the potential of causing hepatocellular damage despite its antimalarial effects which may not be helpful in patient with any form of liver disease or at risk of liver disease as this drug may be harmful to them. It is therefore recommended that before halofantrine hydrochloride is administered, the baseline laboratory value liver function test should be available.

ACKNOWLEDGEMENT

I will like to acknowledge people who directly or indirectly involved and helped me to complete this study; Mr. Elechi-Amadi Kamzi Nnosike, Miss Oron Martha Dickson, Miss Abiakam Ijeoma G.

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