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Evaluation of the Toxic Effects of Dihydroartemisinin on the Vital Organs of Wistar Albino Rats

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Abstract: Problem statement: The current surge in the use of Artemisinin based Combination Therapy (ACT) in the treatment of falciparium resistant malaria in the tropics and the potentials of Dihydroartemisinin (DHA), a member of the artemisinin family, to produce toxic effect in the vital organs (such as the liver, heart, lungs, intestine, spleen kidney and blood cells) necessitated this research. **Approach:** Rats were treated once orally with DHA (2 mg kg^{-1}) on day 1 and then (1 mg kg^{-1}) on day 2-5 and the treatment was repeated 7 days after the first treatment. The animals were sacrificed 24 h after the second treatment for toxicity studies on the vital organs and liver enzymes activity tests: Serum Alanine amino Transferase (ALT), Serum Aspartate amino Transferase (AST) and serum Alkaline Phosphatase (ALP) tests. **Results:** Results indicated that dihydroartemisinin significantly ($p<0.05$) elevated the Packed Cell Volume (PCV), the total White Cell Count (WBC), the percentage neutrophil count (NC) and the percentage Lymphocyte Count (LC). DHA did not affect the serum levels of ALT, AST and ALP, as all the values fell within the normal laboratory range. Histopathological studies revealed no evidence of toxicities in the heart, liver, lungs, intestine, spleen and kidney. The DHA treated and control rats exhibited 75.87 and 29.76% increase in the mean body weight respectively at the end of the second treatment. **Conclusion/Recommendations:** Oral DHA had no deleterious effects on the hematological parameters, did not alter the values of serum liver enzymes and is devoid of obvious toxic effects on vital organs at the doses tested, while the effects on the WBC also suggested potentials of immunomodulatory effects.

Key words: Dihydroartemisinin, toxic effects, antimalarial, rats

INTRODUCTION

Dihydroartemisinin is a member of the artemisinin group of antimalarial drugs which are currently employed in the treatment of both complicated and uncomplicated malaria. Artemisinin antimalarial drugs derived from the extract of a Chinese herb quinhaosu used for the treatment of fevers have in the past three decades been found to be efficacious in clinical management of chloroquine-resistant malaria[1-3]. The use of artemisinin and its derivatives for the management of falciparum resistant and uncomplicated malaria is now the gold standard in malaria endemic areas of Asia and Africa South of Sahara including Nigeria[4]. The use of artemisinin and its derivatives has been on the increase in the tropics since the advent of chloroquine-resistant falciparum strains[4,5]. The emergence of parasites resistance to chloroquine and Sulfadoxine-Pyrimethamine (SP) led to the introduction of artemisinin containing combination drugs[6], known as Artemisinin-based Combination Therapies (ACT's). A typical example of ACT's is the combination of artemisinin with mefloquine which provided much improved cure rates in South East Asia[6]. Like other therapeutic agents, artemisinin may not be devoid of side effects or toxicities in both human and animal studies. The adverse effects reported in patients included gastrointestinal (nausea, vomiting, abdominal pain) and neurological (convulsions, dizziness, impairment of consciousness and vertigo) effects[7]. Also reported were allergic reactions[8], haemolysis[9] and mild hearing loss[10]. In animal studies side effects documented include neurotoxicity[11,12] and contra-gestational effects in animals[4,13]. The fact that artemisinin anti-malarials originated from a Chinese herb and did not undergo extensive rigors of orthodox drug development, calls for continuous animal toxicity

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studies on these drugs[14]. Much has not been reported on the organic and systemic toxicity of artemisinin in laboratory animals. This study was therefore designed to evaluate the toxicity of DHA on vital organs and hematological parameters in rats.

MATERIALS AND METHODS

Animals: Adult Wistar rats of both sexes (106-140 g) were obtained from the small animal breeding section of the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. Animals were allowed to acclimatize for two weeks in the Laboratory before drug treatments and allowed free access to food and water.

Reagents: Dihydroartemisinin (Beijin Cotec Co., China); ethanol (Aldrich); Xylene; paraffin wax; Haematoxylin and Eosin dye; Phosphate buffer (pH 7.4); aspartate amino transferase (AST) substrate; aspartic acid; α-ketoglutarate; Alanine Amino Transferase (ALT) substrate; L-alanin; L-ketoglutarate; pyruvate standard; carbohydrate Buffer (pH 10); phenyl-substrate; phenol; Sodium hydroxide (0.5 N); Sodium bicarbonate (0.5 N); 4-amino anti-pyrine and potassium ferricyanide.

Methods:

Drug treatment: The study was done in two phases. In the first phase, 10 rats were treated with DHA (2 mg kg^{-1}) on the first day (day 1) and subsequently (1 mg kg^{-1}) daily for four days (day 2-5), while the other set of 10 rats were given normal saline (control). The animals were then allowed to rest for one week with free access to water and feed. In the second phase, another five-day treatment of DHA (2 mg kg^{-1}) on day 13 and (1 mg kg^{-1}) on day 14-17, was repeated while the control group still received normal saline. After 24 h of the repeated drug treatment, blood samples were collected from both the DHA-treated and control rats for haematological investigations and serum enzymes assay. The blood samples were collected through the subclavian artery which is very close to the pulmonary blood supply. After blood collection, the animals (both the DHA-treated and control groups) were sacrificed to distinctly display the lungs, hearts, liver, spleen, intestine and kidney for gross anatomical examination. Slides of micro section of these organs were prepared using conventional methods[15] and the photomicrographs were taken for histopathological investigations. Treated animals were compared with the control ones.

Statistical analysis: The results were presented as mean ± SEM and subjected to a two-tailed T-test. Values at $p<0.05$ were taken as significant.

RESULTS

Effects of DHA on body weight: A comparison of the Mean Body Weight (MBW) of the rats before and after treatment showed that both the DHA-treated and control rats manifested increase in body weight however the DHA-treated rats exhibited a significant ($p<0.05$) increase in mean body weight compared with the control. The percentage increase in weight of DHA-treated rats was 30.16 and 75.87% after first and second treatments respectively (Table 1).

Effects of DHA on serum ALT, AST and ALP enzyme activities: The results showed that the serum Alanine Amino Transferase (ALT), Asparatate amino Transferase (AST) and Alkaline Phosphatase (ALP) enzymes activities of DHA-treated rats were not significantly altered Table 2. However there was an elevation in AST value after first treatment and in all ALT values after first and second treatments which was significant compared to the control but fell within the normal range of enzyme activity (Table 2).

Table 1: Effects of DHA on body weight

	MWG_{1-5}		MWG_{13-17}	
	(gm)	Percent	(gm)	Percent
DHA	38.10±0.23*	30.16	96.62±1.42*	75.87
Control	3.40±0.29	2.68	37.80±1.07	29.76

MWG_{1-5} = Mean Weight Gain of rats after first treatment, MWG_{13-17} = Mean Weight Gain of rats after second treatment, *: Significant at $P<0.01$, n = 10

Table 2: Effect of DHA on liver enzyme activity

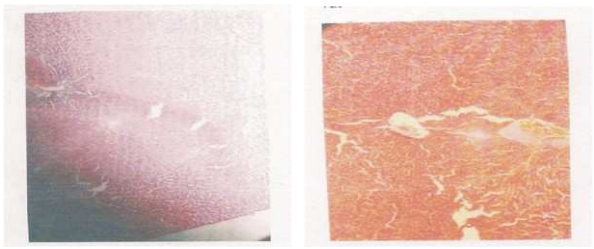
	ALT		AST		ALP	
Treatment	Day_{1-5}	Day_{13-17}	Day_{1-5}	Day_{13-17}	Day_{1-5}	Day_{13-17}
DHA	9.00±1.33*	7.00±1.21*	9.50±0.64*	6.50±0.70	4.00±0.40	3.75±0.38
Control	5.00±0.58	6.06±1.04	6.50±0.23	6.50±0.23	3.75±0.40	4.13±0.39

Values are mean ± SEM. n = 10, *: $p<0.05$ compared to the control, ALT: Serum alanine amino transferase, AST: Serum aspartate amino transferase, ALP: Serum alkaline phosphatase, Day_{1-5}: First treatment, Day_{13-17}: Second treatment

Table 3: Haematological effects of DHA

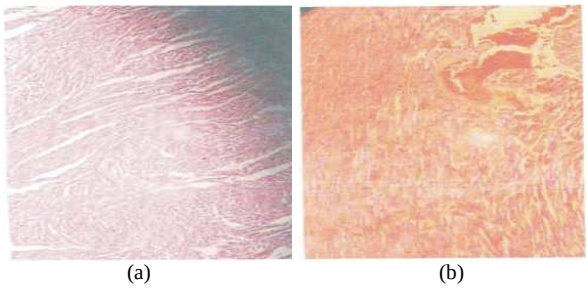
	PCV		WBC		NC		LC	
Treatment	Day_{1-5}	Day_{13-17}	Day_{1-5}	Day_{13-17}	Day_{1-5}	Day_{13-17}	Day_{1-5}	Day_{13-17}
DHA	45±0.58*	46±0.58*	9050±577.35*	12025±831.23**	24.67±**76.0	26±1.15**	76±1.15*	82±1.00**
Control	40±1.15	40±1.15	4700±152.75	5050±172.24	10.00±0.58	11±0.58	70±1.15	70±1.15

p< 0.05; **: p<0.01; Values are Mean ± Standard error mean; n: 10; PCV: Packed Cell Volume; WBC: White Blood Cell; NC: Percentage Neutrophil Count, LC: Percentage Lymphocyte Count, Day_{1-5}: First treatment, Day_{13-17}: Second treatment



(a) (b)

Fig. 1: Photomicrographs of the liver; (a): DHA-treated rats; (b): Control rats



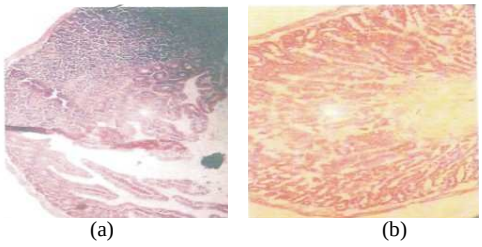
(a) (b)

Fig. 2: Photomicrographs of the heart; (a): DHA-treated rats; (b): Control rats

Haematological effects of DHA: DHA significantly increased the Packed Cell Volume (PCV), White Blood Cells (WBC), percentage Neutrophil Count (NC) and percentage Lymphocyte Count (LC) at varying degrees when compared with the control groups. DHA produced significant increase ($p<0.01$) in the PCV, WBC and NC counts and also indicated a significant increase ($p<0.05$) for the LC counts (Table 3).

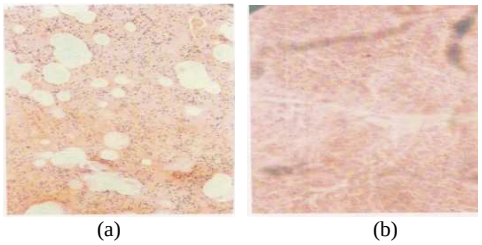
Gross anatomical observations: Gross anatomical observations of the heart, liver, spleen, intestine, lungs, kidneys and blood of the DHA-treated rats revealed a congested darker red appearance (especially with the liver, the spleen and the kidney) when compared with the control rats. This is may indicate that the blood cells of the DHA treated rats were denser than those of the control rats.

Histopathological Investigations: The photomicrographs of the vital organs of both the DHA-treated and control rats indicated no evidence of toxicity (Fig. 1-6).



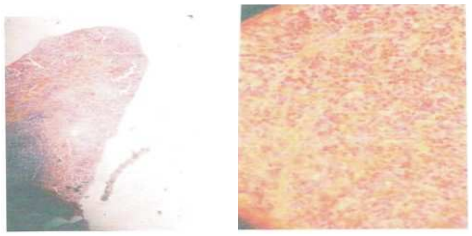
(a) (b)

Fig. 3: Photomicrographs of the intestine; (a): DHA-treated rats; (b): Control rats



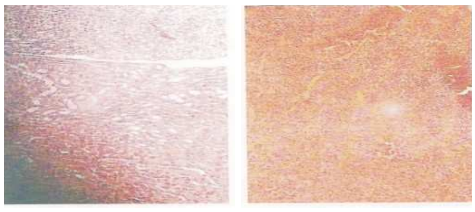
(a) (b)

Fig. 4: Photomicrographs of the lungs; (a): DHA-treated rats; (b): Control rats



(a) (b)

Fig. 5: Photomicrographs of the spleen; (a): DHA-treated rats; (b): Control rats



(a) (b)

Fig. 6: Photomicrographs of the kidney; (a) DHA-treated rats (b): Control rats.

DISCUSSION

The DHA-treated rats manifested a significant increase in Mean Body Weight (MBW) compared to the control. Increase in body weight after drug administration usually indicate absence of toxicity as decrease in body weight is an index of toxic effect of a compound[16]. The enzyme assay studies revealed no significant increase in the liver enzymes of DHA treated rats. This is an indication that DHA does not possess the potential of inducing both liver and cardiac malfunctions. High values of serum ALT have been found in liver necrosis, hepatitis and toxic liver diseases[17]. The AST is one of the plasma cardiac markers that increase sequentially after acute myocardial infarction[18]. The serum Alkaline Phosphatase (ALP) level of the DHA-treated rats was within the normal range indicating that DHA treatment did not adversely affect the hepatocytes. These results were confirmed by the intactness of the liver and the heart of the DHA-treated rats as shown by the photo-micrographs (Fig. 1 and 2). The significant ($p<0.05$) increase in the Packed Cell Volume (PCV) of the DHA-treated rats is an indication that the red blood cell volume was increased by DHA treatment. An increase in the PCV suggests an increase in the oxygen carrying capacity of the blood. The significant ($p<0.05$) increase in WBC, percentage neutrophil and percentage lymphocyte counts is an indication of the immune boosting capacity of the DHA in rats. Neutrophils and lymphocytes play a role in the antibody dependent cellular cytotoxicity to invading pathogens in the body[19]. WBC plays an important role in the body's defense mechanisms. The DHA-treated rats were found to have no basophils. Basophils are usually present only when there is severe infection.

CONCLUSION

The 5 day oral administration of dihydroartemisin whether given once or repeated after an interval of one week, produced no toxic effects on the vital organs and blood of rats while the effects on the WBC suggested potentials of immunomodulatory effects.

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