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Effect of long term administration of halofantrine hydrochloride (halfan) on the morphology and histology of the testes of adult wistar rat

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ABSTRACT: *The effect of halfan on the body weight, morphology and histology of the testes of adult wistar rats were studied. Twenty male adult wistar rats were divided into four groups with five rats in each group, and the drug at a dose of 0.05 mg/ 100 g, 0.1 mg/ 100 g, 0.15 mg / 100 g body weight were administered orally to group B, C and D respectively, three times at six hourly intervals for five days. Control group (group A) received 0.05 mg / 100g of normal saline. The data were analyzed using ANOVA for testicular variables and student t-test for the body weight and P <0.05 was considered as level of significance. The result showed no significant (p > 0.05) morphological differences in the testes of all groups and decrease in body weight was only significant (p < 0.001) in group D. The drug has no adverse effect on the histological structure of the testes using therapeutic dose, but causes some degenerative changes in seminiferous tubule at higher doses. Apparently, halfan used in the treatment of malaria has no adverse effects on body weight as well as on morphology and histology of testes of adult wistar rats*

KEYWORDS: *Halfan, histology, malaria, morphology, testes*

I. INTRODUCTION

Malaria is a vector borne infectious disease caused by protozoan parasite which is the most prevalent of all tropical diseases causing many deaths each year. There are approximately five hundred and fifteen million cases of malaria, killing about one to three million people, the majority of whom are young children in sub Saharan Africa [1]. Chemotherapy of malaria involves the use of specific antimalarial drugs, and the development of different types of antimalarial drugs was achieved after malarial parasite Plasmodium falciparum was successfully cultured [2]. In Nigeria probably in the other parts of West Africa, the predominant parasite is *Plasmodium falciparum* (75%), *Plasmodium malariae* (15%), and *Plasmodium ovale* (3%) and *Plasmodium vivax* which is not found in the West Africa [3] The most commonly used antimalarial drug is chloroquine but the appearance of the resistance strain of the malarial parasite in 1960s led to development of other antimalarial drug called halofantrine hydrochloride with trade name halfan in a research collaboration between Walter Reed Army Institute for research (WRAIR) and Smith Kline French [4]. Halfan is a phenantrine methanol antimalarial drug which is schizonticidal with a high degree of activity against the asexual erythrocytic stage of malaria parasites [5, 6] Each 5 mls of white suspension of halfan contains 100 mg of halofantrine hydrochloride [7].

Although the mechanism of action of halfan is still uncertain, it was suggested to have a similar action with chloroquine and quinine in some respect, and chloroquine has been shown to have teratogenic effect on leydig cells of mature male wistar rats [8]. The testes have been reported to be affected by several agents such as hormones [9, 10, 11, 12, 13], traumatic injury [13, 14], chemical agent such as drugs [13], genetic factor [15]. It was shown that halfan causes significant increase in the level of serum enzymes such as aspartate transferase (AST), alkaline transferase (ALT) and white blood cells [16], and chronic or prolonged administration of halofantrine and artesunate may adversely affected the process of spermatogenesis leading to infertility in males [17]. A recent study by Aprioku and Obianime, [18] reported that artemether caused no ($p<0.05$) significant effects on phosphatase enzymes whereas halofantrine and artemether-lumefantrine significantly ($p<0.05$) increased the serum levels of ACPT and ACPP, without significant effects on ALP.

The use of antimalarial drugs such as halfan is on the increase in Nigeria; probably due to increase in the incidence of chloroquine resistance noticed in the third world countries [19]. The present studies aims to reveal the effect of long term administration of halofantrine hydrochloride (halfan) on the morphology and histology of the testes in adult wistar rat.

II. MATERIALS AND METHODS

2.1 Animals Maintenance and grouping

Twenty male adult wistar rats were caged in a well ventilated room in the animal house for a period of two weeks for proper acclimatization. Their weight ranged from 142-214 g after the period of acclimatization. The animals were grouped into four, A, B, C and D, with five rats in each group. Group A serves as control while groups B C and D serve as treatment groups.

2.2 Drug administration

The drug was administered orally at six hourly interval three times daily for five days to each rats in the treatment group with aid of butterfly tube attached to needle and syringe. Each 5mls of halfan contains 100mg of halofantrine hydrochloride and the number of mls per dose is 25mls adult human. Hence, the equivalent dose in mls per 100 g considering average weight of 55kg (55000g) is 25 x 100 / 55000 which is equal to 0.05 ml / 100 g. Therefore, the doses given to respective groups are as follows; Group B with average weight of 158 g received 0.05 ml x 158 g / 100 g which was 0.1 ml of halfan. Group C with average weight of 167 g received 0.05 ml x 167 g / 100 g which was 0.1 ml of halfan and the double therapeutic dose was 0.2 ml. Group D with average weight of 201g received 0.05 ml x 201g / 100 g which was 0.1ml of halfan and the triple therapeutic dose was 0.3 ml. Group A with average weight of 151g received 0.05 ml x 151 g / 100 g which was 0.1 ml of normal saline.

2.3 Morphometery and histological techniques

The rats were sacrificed on the sixth day using a box containing chloroform and all the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education were observed. The testes were dissected out. The length and width of the testes were measured using divider and ruler whereas the weight was measured using weighing balance. The shape and color of the testes were observed using a hand lens. The tissues were fixed in bounce's fluid and processed using routine H and E histological techniques.

2.4 Statistical analysis

The data were expressed as mean ± standard error of mean. The student t-test was used to analyze the differences in the body weight before and after the experiment, and one way analyses of variance (ANOVA) was used determine the differences of testicular variables across the groups and $P < 0.05$ was considered as level of significance.

III. RESULTS

3.1 Physical Examination and Body Weight Analyses

Physical examination of the animal shows normal activities in the control group, whereas in the treatment groups (B, C and D) there was decrease in the activities in a dose dependent manner. One of the animals died in the control group in the course of the experiments.

The statistical analyses of the animals' body weight (Table 1) show no statistically significant differences ($p > 0.05$) before and after the experiment in group A, B and C, but a statistically significant differences ($p < 0.001$) was observed in the group D (a higher dose group).

Table 1: Statistical analysis of body weight (g) of the wistar rats

Groups	Before experiment	After experiment	P value
	Mean ±SEM	Mean ±SEM	
A (n=4)	150.6 ± 2.402	163.5 ± 2.901	0.061
B (n=5)	157.6 ± 8.394	150.8 ± 5.987	0.202
C (n=5)	166.6 ± 2.441	165.2 ± 4.641	0.611
D (n=5)	201.0 ± 4.775	159.8 ± 6.468	0.001

3.2 Morphometric Analyses

The gross morphological examination of the testes shows no clear differences in the shape and colors of the testes in all groups. Table 2 shows statistical analyses of the testicular variables (length, weight and weight). The result shows no statistically significant differences ($P > 0.05$) in the variable studies within the groups

Table 2: Statistical analysis of testicular weight, length and width of wistar rats

Variables	Group A (n=4)	Group B (n=5)	Group C (n=5)	Group D (n=5)	P value
			Mean ± SEM		
Length(cm)	1.850 ± 0.0289	1.860 ± 0.0600	1.960 ± 0.0510	1.880 ± 0.0374	0.377
Width (cm)	1.075 ± 0.0479	1.040 ± 0.0245	1.140 ± 0.0245	1.100 ± 0.000	0.094
Weight (g)	1.150 ± 0.0289	1.080 ± 0.0735	1.120 ± 0.0490	1.120 ± 0.0200	0.808

3.3 Histological Examinations

The photomicrograph (Fig. 1) of the testes in group A (control group; 0.1 ml of normal saline) shows section of the seminiferous tubules (ST) with cells at different stages of cell division. In between the seminiferous tubules are sparsely arranged interstitial cell of Leydig (IC) located within interstitial connective tissue (ICT). The lumen of seminiferous tubules also appeared clearly.

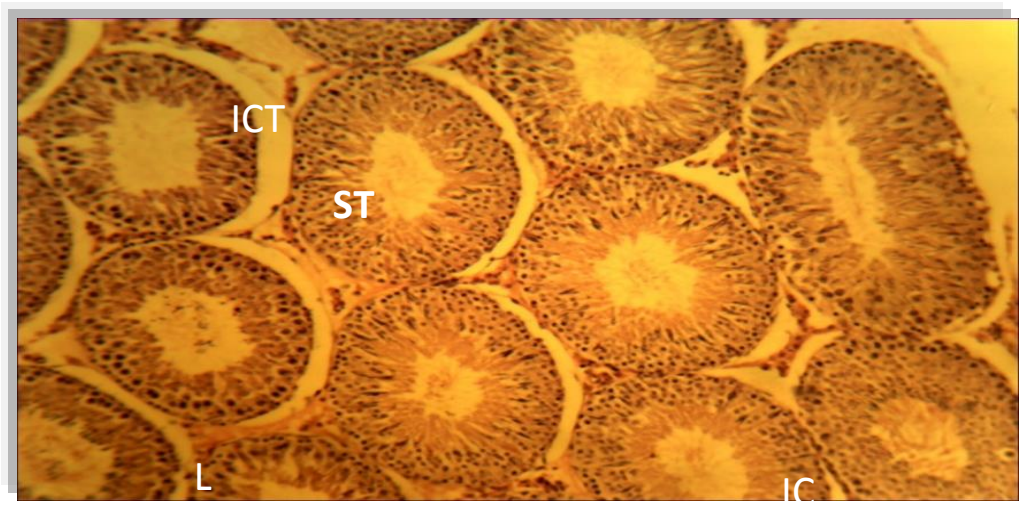

Figure 1: Control group (0.1ml of normal saline), H & E x 100, ST-seminiferous tubule, L -lumen, IC – interstitial cells, ICT- interstitial connective tissue

The photomicrograph (Fig. 2) of group B (0.1 ml of halfan) shows normal histology of the testes as seen in the control group. The photomicrograph (Fig. 3) of group C (0.2 ml of halfan) shows areas of degenerated seminiferous tubules (DST). The photomicrograph (Fig. 4) of group D (0.3 ml of Halfan) also shows areas of degenerated seminiferous tubules (DST) in more severe form compared to group C

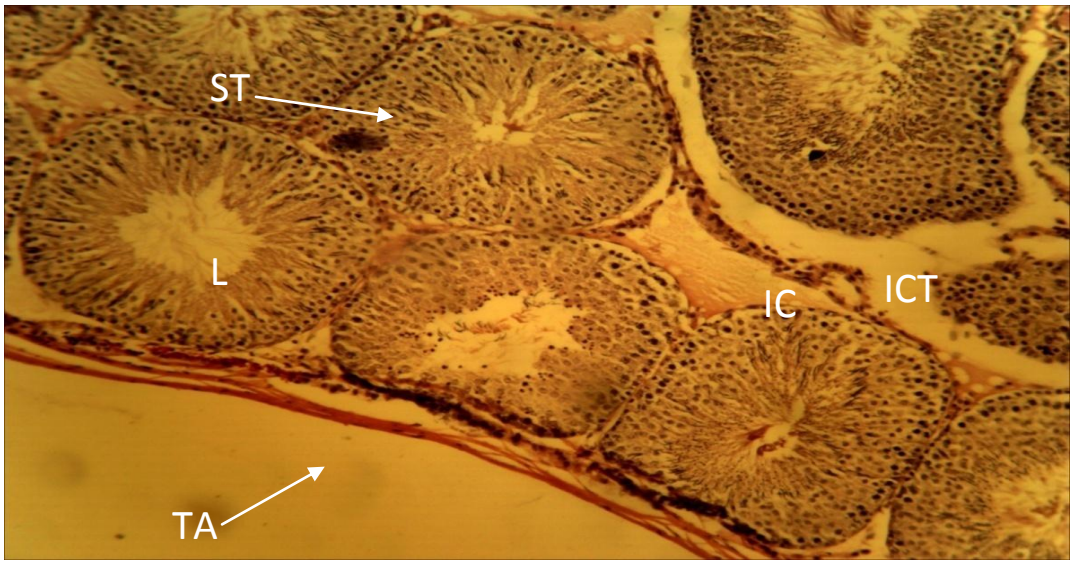

Figure 2: Group B (0.1ml of halfan) H & E x100, TA-tunica albuginea

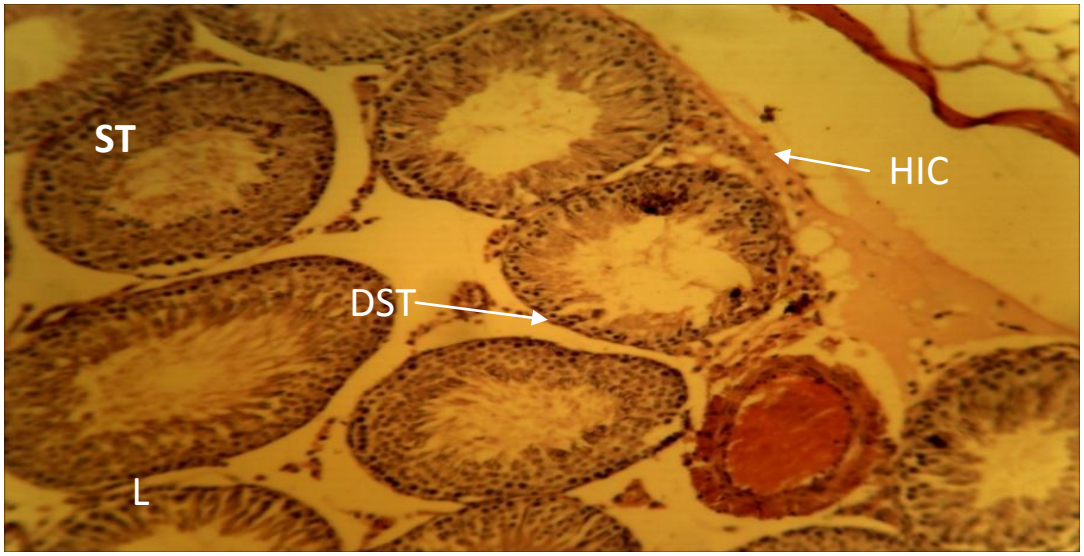

Figure 3: Group C (double therapeutic 0.2 ml of halfan) H & E x 100, HIC – hyperplastic interstitial cells, DST – degenerated seminiferous tubule,

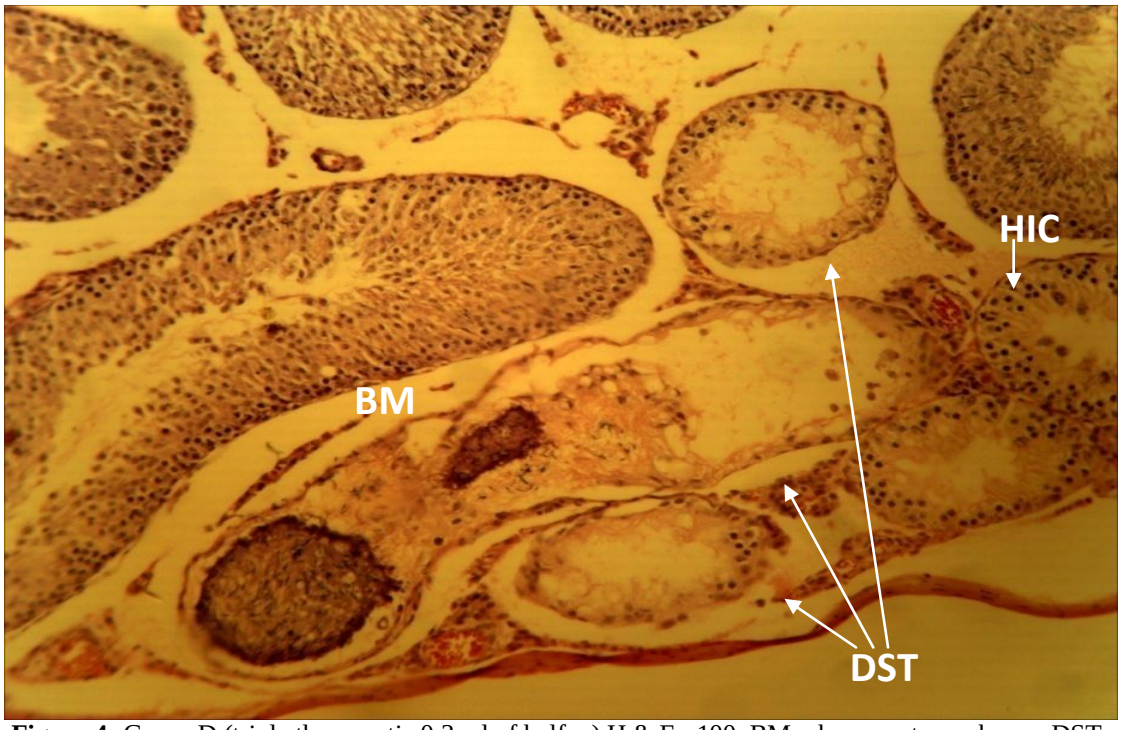

Figure 4: Group D (triple therapeutic 0.3 ml of halfan) H & E x100, BM – basement membrane, DST-Degenerated seminiferous tubule, HIC-hyperplastic interstitial cell

IV. DISCUSSION

The mechanism of action of halfan may still not be similar to chloroquine in some respect and the testes have shown to be sensitive to Halfan only at higher doses following long term administration of the drug. The decrease in the activities observed in the treatment groups can be linked to the diarrhoea associated side effect of halfan [7], with group D having higher rate of diarrhoea as well as decrease in the activities which led to significant ($p < 0.001$) decrease in the body weight in the group

The histological study of the testes indicates degeneration of the seminiferous tubules at higher doses which is not apparent morphologically. This is similar to recent studies on guinea pigs which show that difference of mean testicular weight was highest in artesunate treated group and lowest in Halofantrine treated group which is not statistically significant [20]. It was also reported that artemether-lumefantrine and halofantrine may be toxic to testicular tissues [17, 18, 21]. According to Ugochukwu et al. [16], halfan causes an increase in the white blood cell count as well as estradiol levels on adult female wistar rats. It was also observed

that synthetic estrogen not only caused a decrease in testicular size and activity but also a reduction of the sexual characteristics [9]. Testicular atrophy results from either local or generalized injury is generalized by degeneration of testicular tubules with either partial or complete arrest of spermatogenesis and relatively increased connective tissue volume resulting from the testicular atrophy containing islands of hyperplastic, eosinophilic Leydig cells. However, in the present study the effect produced by halfan on the histology of the testes occurred at the edge of the testes close to tunica albuginea with some of the seminiferous tubules intact

V. CONCLUSION

In conclusion, long term administration of halfan using normal therapeutic dose has no adverse effect on the body weight as well as on the morphology and histology of testes of adult wistar rats, but some degenerative changes in seminiferous tubules and decrease in the body weight were observed at higher doses

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