

Comparative Effects of Halofantrine Hydrochloride and Artesunate on the Testis of Guinea Pigs

S.M. Okey and K.V. Olorunshola

Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria

Abstract: Halofantrine Hydrochloride and Artesunate are both recent antimalarial drugs from the class of phenanthrene methanol and Artemisinin respectively used in the effective combat of malaria parasite in Africa. The aim of this research was to study the effects of these antimalarial drugs on the testicular weight and sperm concentration of guinea pigs. Fifteen male adult guinea pigs weighing between 230-700 g were divided into three groups of five guinea pigs each. First group, control was given 0.9% normal saline once daily for five days. The second group was given Halofantrine Hydrochloride 7 mg/kg (in three doses) in one day orally. The third group was given Artesunate with starting dose of 1.4 mg/kg twice the first day and a sustenance dose of 0.7 mg/kg twice daily for four days orally. The guinea pigs were all anesthetized the one day after the last dose. There was no significant difference in the testicular weight and sperm concentration between the control and experimental groups ($p > 0.05$). We concluded that Halofantrine Hydrochloride and Artesunate given at normal therapeutic doses, have no adverse effect on sperm concentration and testicular weight, but have the potential to cause oligospermia following prolong use.

Key words: Artesunate, guinea pigs, halofantrine hydrochloride, malaria, sperm concentration, testis

INTRODUCTION

The burden of malaria, its complications such as anaemia, cerebral malaria, black water fever, Chloroquine resistance, insecticide resistance and poverty in Sub-Saharan Africa has resulted in reliance upon case management as the primary approach to malaria control (Greenberg *et al.*, 1989; Trape *et al.*, 1998).

In order to combat further resistance of malaria parasite to combined therapy, more recent and effective anti-malaria drug such as Artesunate and Halofantrine Hydrochloride has been developed (Rosenthal and Goldsmith, 2001).

Artesunate like its parent artemisinin is a rapidly acting well tolerated endoperoxide compound (Zhang *et al.*, 2001) It is commonly used in chemotherapy of malaria fever and has adverse effects such as nausea, vomiting and diarrhea. Irreversible neurotoxicity has been reported in animals, but only after doses much higher than those used to treat malaria (Rosenthal and Goldsmith, 2001).

Halofantrine Hydrochloride is a phenanthrene methanol antimalaria drug that is also effective against asexual forms of multidrug resistant plasmodium falciparum malaria (Karbwan and Bangchang, 1994). It has adverse effects such as pulmonary edema, asthenia, chest pain, cardiac toxicity, abdominal pain, diarrhea,

nausea, vomiting, orthostatic hypotension, convulsion, pruritis, headache, cough, dyspepsia (Beecham, 1998; Goa and Bryson, 1992; Karbwan and Bangchang, 1994).

Preliminary reports by Dapper *et al.* (2002) on Halofantrine Hydrochloride administration on the male reproductive system indicated increase spermatogenesis and possible degenerative changes at higher doses. To the best of our knowledge, there is no report on the possible side effects of Artesunate on the male reproductive system at the time of this research.

Hence the aim of this study is to compare the effects of Halofantrine hydrochloride and Artesunate acute administration on the testes of guinea pigs following short time administration.

MATERIALS AND METHODS

Experimental animals: 15 guinea pigs weighing between 230 and 700 g were used for the study. They were housed in the animal house of the department of Human Physiology, Ahmadu Bello University, Zaria. They were allowed free access to feed and water *ad libitum*.

Drug and chemicals: Halofantrine hydrochloride (Smithkline Beecham Pharmaceuticals) and Artesunate (CIPLA Limited Patalganga, India) was obtained from

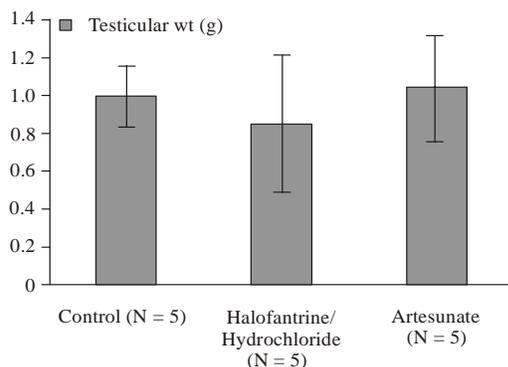


Fig. 1: Mean testicular weights of control, artesunate, halofantrine group of guinea pigs

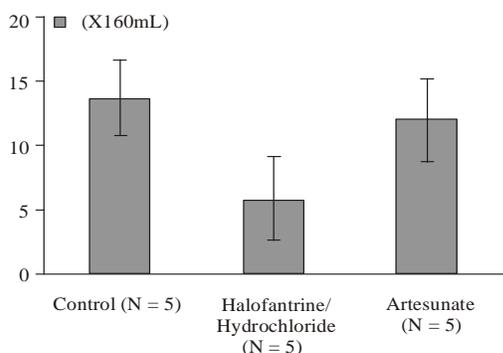


Fig. 2: Effect of treatments on sperm concentration of control, halofantrine, artesunate of guinea pigs

drug stores in Kaduna and Zaria respectively and reconstituted into a suspension using distilled water. All other chemicals used were of analytical grade.

Treatment protocol: The Guinea pigs were divided into three groups of five animals each:

- Group A:** served as control and was given 0.9% of normal saline once daily for five days.
- Group B:** was given 7 mg/kg of Halofantrine Hydrochloride, thrice at about six hours interval in one day.
- Group C:** was given 1.4 mg/kg of Artesunate twice the 1st day and a sustenance dose of 0.7 mg/kg twice for the next four days.

All regimens were administered orally by means of gavage. At the end of the experiment, the guinea pigs were anesthetized with chloroform.

Sperm collection and determination: The Guinea pigs were anesthetized using Chloroform and their reproductive organs were exposed, the testes were then

removed carefully, washed and weighed. The semen samples were obtained from the epididymis and mixed with 1ml of normal saline; sperm count determination involved making a 1ml dilution of the semen at dilution factor of 200. Using a red blood cell pipette, the diluted mixture was loaded on the heamocytometer by capillary action for counting of the sperm numbers. Using a light microscope at a magnification of X40, all sperm cell within the five large squares were counted, including sperm cell lying across the outermost lines at the top and right sides, but not the bottom or left sides. The sum of the number of sperm cells counted in the large squares were multiplied by million per ml.

Statistical analysis: Values of testicular weight in grams and sperm concentration were recorded as mean±SD, and statistically analysed by one- way analysis of variance (ANOVA). $p \leq 0.05$ was considered to be statistically significant.

RESULTS

The testicular weights of 0.84 ± 0.37 and 1.04 ± 0.29 (g) for Halofantrine hydrochloride and Artesunate treated group respectively were not significantly different ($p > 0.05$) from the testicular weight of 1.0 ± 0.16 (g) for control group, as shown in Fig. 1.

The mean sperm concentration of 5.80 ± 3.26 and 12.20 ± 3.07 (10^6 cells/mL) for Halofantrine hydrochloride and Artesunate treated group respectively revealed a reduction in sperm concentration when compared with the control group; 13.80 ± 2.94 (10^6 cells/mL) (Fig. 2). Although this is not significantly different ($p > 0.05$), when the treated groups were compared with the control group.

DISCUSSION

The results of this present study of the effect of Halofantrine Hydrochloride and Artesunate on sperm concentrations indicate highest mean of sperm concentration in control, followed by Artesunate group and lowest in the halofantrine group.

Although statistically, there is no significant difference ($p > 0.05$). This likely due to the normal manufacturer's therapeutic dose used for the administration. This is in contrast to the work of Dapper *et al.* (2002) where Halofantrine apparently increase spermatogenesis.

However, since acute administration of Halofantrine and Artesunate in the present experiment was able to cause a reduction in the number of spermatozoa in the

experimental animals as compared with that of the control, it may be implied that chronic or prolonged administration of Halofantrine and Artesunate may adversely affected the process of spermatogenesis leading to infertility in males, by causing degenerative changes in the seminiferous tubules as shown in the works of (Nwanjo *et al.*, 2007) were Dihydroartemisinin (an Artesunate derivative) treatment for 7days and 21days in rats resulted in a decrease in the sperm count.

Also in the works of Obianime and Aprioku (2009) Oxidative stress induced by Artesunate and its combinants in seminal fluid, is likely the mechanism by which these antimalarial drugs impair spermatogenesis hence causing reproduction dysfunction.

The difference of mean testicular weight which is highest in Artesunate group and lowest in Halofantrine group could be partially due to the effect of the drugs. Although this also is not statistically significant ($p>0.05$), but could be if a larger size of animals are used in such experiment.

In conclusion, Halofantrine Hydrochloride and Artesunate used for the treatment of malaria at normal therapeutic dose during acute administration have no adverse effect on sperm count and therefore may not cause infertility in males, but have the potential to cause oligospermia following prolong usage.

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