# Antifertility Activity Of Dihydroartemisinin In Male Albino Rats

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**Citation**

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**Abstract**

The effects of dihydroartemisinin on epididymal sperm characteristics and testosterone levels in male albino rats were studied. Dihydoartemisinin treatment for 7 days and 21 days in the rats resulted in a decrease in the sperm count, sperm motility and sperm viability. The abnormalities noticed in sperms were “curved mind piece” and small and pyriform heads observed only in some groups. The sperm motility, disability and counts were significantly reduced in all the treated groups when compared with the control and the reductions were both dose and duration dependent. There was a significant decrease in serum testosterone levels in rats treated with an initial dose of 4.4mg/kg body weight of dihydroartemisinin ($P< 0.05$) when compared with control. These observations suggest that the effects are probably due to an androgen deficiency caused by the anti-androgens property of the dihydroartemisinin.

## INTRODUCTION

Artemisinin and their derivatives have been recommended for the treatment of severe and complicated malaria. They are very active anti-malaria drugs producing up to ten thousand fold reductions in parasite biomass per a sexual cycle and they reduce malaria transmissibility (white, 1999).

Many anti-malaria and antibiotic agents have been reported to have anti-fertility actions. For instance the antisteroidogenic and anti-fertility actions of quinine and chloroquine have been well documented (Meisel et. al., 1993; Adeeko and Dada, 1998). The effects of anti-malaria chloroquine on peripheral level of testosterone, gonadotrophin and sperm motility have been studied (Sehuster and Canfield, 1989). With the increased efforts in the development of more portent anti-malaria agents as a result of the challenge posed by the resistant strains of the malaria parasite, the evaluation of these anti-malaria agents for possible anti-fertility actions becomes important, this is necessary since both malaria and infertility are worldwide phenomena and the need to avoid the risk of infertility resulting from malaria chemotherapy.

In the absence of information on the reproductive toxicity of artemisinin and its derivatives the present investigation was therefore undertaken to determine in male albino rats the effect of artemisinin on certain parameters namely total sperm count, sperm motility, sperm viability and serum testosterone levels.

## MATERIALS AND METHODS

### ANIMALS

Twenty-four Albino rats (150-200g) obtained from the Animal House of College of Medicine and Health Science, Imo State University, Owerri were used for this study. The rats were housed in wire mesh cages under standard conditions (temperature 25-300C, 12hr light and 12hr darkness cycle). They were allowed free access to water and feed (product of Pfizer, Nigeria Limited, Benin, Edo State) throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals.

### EXPERIMENTAL DESIGN

Four experimental groups of six Albino rats each with similar body weights were used. Rats in group A served the control and received only distilled water.

Group B rats had 2.2 mg/kg body weight of dihydrartermisinin the first day, and then 1.1 mg/kg body weight for subsequent six days being the normal duration for the treatment of malaria (Churchbells, 2000).

Group C rats: the drug was administered to animals in group C at the recommended doses of 4.4 mg/kg body weight the

first day, then 2.2 mg/kg body weight for the subsequent six days.

In group D rats, dihydroartemisnin were administered orally at the recommended doses (2.2 mg/kg body weight the first day, 1.1 mg/kg body weight) or subsequent twenty days (chronic administration)

## BODY AND ORGAN WEIGHTS

Initial and final body weights of the animals were recorded. At the end of the treatment period the animals were sacrificed under chloroform anesthesia 24 hours after the last dosing of the respective treatment duration. The testis was removed and weighed.

## HORMONAL ASSAY

Blood was collected by cardiac puncture from the rats in each study group after anaesthetizing them with chloroform. The blood sample was spun at 2500 rpm for 10min using Wisperfuge model 1384 centrifuge (Tamson, Holland) at 10-250C. Serum samples were assayed for testosterone using enzyme linked immunoassay (EIA) technique.

## SPERM MOTILITY, VIABILITY, COUNTS AND MORPHOLOGY

The caudal epidermis was dissected out, an incision (about 1mm) was made in the caudal epididymis. Sperm fluid was then squeezed onto the microscope slide. Epididymal sperm was assessed by calculating motile spermatozoa per unit area and was expressed as percent motility.

Epididymal sperm counts were made using the haemocytometer and were expressed as million/ml of suspension. The sperm viability was determined using Eosin/Nigrosin stain (Raji et al, 2003).

## RESULTS

## BODY AND ORGAN WEIGHT CHANGES

Table 1 shows the effect of dihydroartemisinin on the body weight of rats. There was significant increase in the relative weight of testis in all the treated groups when compared with the control. All the groups showed no significant body weight again or loss throughout the experimental period.

**Figure 1**

Table 1: Body weight and organ weight of male rats treated with dihydroartemusinin.

| | Mean Initial Body weight (g) | Mean Final Body Weight (g) | Mean Body Weight Change (g) | Mean Weight of testis (g) |
|---|---|---|---|---|
| GroupA(Control) | 158.24 ± 6.8 | 162.12 ± 8.2 | 3.88 ± 0.48 | 0.72 ± 0.08 |
| Group B | 161.66 ± 9.6 | 164.46 ± 6.4 | 2.80 ± 0.36 | 1.28 1.02* |
| Group C | 155.22 ± 10.2 | 158.46 ± 7.4 | 3.24 ± 0.32 | 1.32 ± 0.90* |
| Group D | 164.45 ± 6.5 | 167.12 ± 5.8 | 2.67 ± 0.58 | 1.34 ± 01.0* |

*Significantly different from control group (p<0.05).

## SPERM MOBILITY

The sperm mobility was significantly reduced (p<0.01) in rats treated 7 days at both low and high doses when compared with control, whereas there no significant change in the mobility of those treated for 21 days at the low dose.

The decrease in mobility was dose-dependent in rats that were treated 7 days where 55% and 45% were recorded for rats treated with 2.2 mg/kg body weight the first day then 1.1 mg/kg body weight for subsequent six days and 4.4 mg/kg body weight the first day then 2.2 mg/kg body weight for subsequent six days respectively (Table 2).

## SPERM VIABILITY

The sperm viability reduced significantly (p<0.05) in all the treated groups when compared with the control. The changes in sperm viability were both duration-and dose dependent. Sperm viability was significantly higher (p<0.05) in rats treated with initial 2.2 mg/kg of the drug than in those treated with initial 4.4 mg/kg of the drug for 7days (Table2).

## SPERM COUNTS

The mean epididymal sperm number was significantly reduced in all the treated groups when compared with the control. There was no significant difference in sperm counts of rats that were treated with the same does for 7 days and 21 days. However, in rats treated for 7 days, the sperm counts were significantly higher (p<0.05) in those treated with initial 2.2 mg/kg than those treated with initial 4.4 mg/kg weight (Table 2).

**Figure 2**

Table 2: Effect of Dihydroartenusinin on sperm Characteristics and Testosterone in Male albino Rats.

| Groups | Sperm Motility (%) | Sperm Viability (%) | Sperm counts (million) | Testosterone Conc. (ng/ml) |
|---|---|---|---|---|
| A | 82.00 ± 1.05 | 90.00 ± 3.20 | 67.20 ± 550 | 1.60 ± 0.16 |
| B | *55.00 ± 10.5 | 65.00 ± 5.80 | *68.00 ± 8.20 | 1.52 ± 0.30 |
| C | *45.00 ± 6.20 | **40.00 ± 4.0 | **50.00 ± 6.40 | 1.16 ± 0.25* |
| D | 73.00 ± 5.60 | *54.00 ± 6.50 | *62.00 ± 3.60 | 1.48 ± 0.20 |

* Significantly different from control (A) group ($p<0.05$).

** Significantly different from both Group A and B ($p<0.05$).

## SPERM MORPHOLOGY

The most common abnormality of the epididymal sperm was ‘curved mid piece' found in all the treated groups which were mainly secondary and tertiary. However, primary abnormalities characterized by small and pyriform heads were observed only in rats that were treated with 4.4 mg/kg body weight of artemisinin the first day and 2.2 mg/kg body weight for the subsequent six days.

## SERUM TESTOSTERONE LEVEL

The serum testosterone levels in group A.B.C. and D were 1.60 ± 0.16, 1.52 ± 0.3, 1.16 ± 0.25, 1.48 ± 0.2 ng/ml respectively. There was no significant difference in the serum levels of testosterone in treated rats when compared with their control counterparts, however, it was found out that there was a significant decrease in serum testosterone levels in rats treated with initial dose of 4.4 mg/kg body weight of dihydroartemisinin ($p<0.05$) when compared with the control.

## DISCUSSION

Reduction in fertility and sterile mating recorded after treatment of male rats with Chloroquine and halofantrine have been reported to be due to impairment in sperm motility (Adeeko and Dada, 1998. Orisakwe et. al., 2003). Treatment with such antimalarial drugs result in reducing the sperm count, motility, fertility and viability, as well as increasing the amount of abnormal sperms. It has been suggested that these drugs cause androgen depletion at the targets levels. Particularly in the caudal epididymis, thereby affecting the physiological maturation of sperm (Adeeko and Dada, 1998).

The result of this present study revealed that dihydroartemisinin could cause reproductive impairment in male rats. The lack of effects on the body weight gain as well as the absence of chemical and behavioral alterations on treated animals suggest that dihydroartemisinin did not include systemic toxicity at the doses treated. However, the observed increase in relative weight of testis in this study indicates that the drug may have toxic effect on this organ. It has been reported that increase or decrease in either absolute or relative weight of an organ after administering a chemical or drug is an indicator of the toxic effect of that chemical (Simons et al, 1995).

The significant reduction in the sperm motility of rat that were treated initially with 2.2 mg/kg body weight of drugs and those treated initially with 1.1mg/kg body weight suggest that the drug was able to permeate the blood-testis barriers. The decrease in sperm motility caused by chemical agents has earlier been reported to be due to their ability to permeates the blood-testis bearer (Baldessarini, 1980) and thus, creating a different microenvironment in the inner part of the wall of the seminiferous tubules from that in its outer part (Bloom and Faweett, 1975).

Drugs that affect the testicular functions will affect the quality and quantity of spermatozoa. The mean epididymal sperm number was significantly reduced ($P<0.05$) in all the treated groups. There was no significant decline in serum levels of testosterone in all the treated rats when compared with the control. The significant difference in the sperm motility, viability and counts of those rats provides evidence for the significant reductions in the circulating androgen levels. Testosterone is required for the growth (Meridian et. al., 1987) and in association with follicle stimulating hormone, acts on the semniferous tubules to initiate and maintain spermatogenesis (Christensen, 1975).

In this study, the increased percentage of abnormal sperm, reduced sperm count, motility and speed, may have resulted from the alteration in the epididymal milieu, probably due to androgen deficiency consequent to the anti-androgenic property of dihydroartemisinin.

Thus, it is likely that reproductive toxicological risk would occur with the doses of dihydroartemisinin commonly consumed as anti-malaria drug by humans. However, this extrapolation should be made with caution, since the real human risk cannot be assessed on the basis of the present study. So further studies are necessary with human models to find out the effects of this drug to the testis of men.

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