GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL 14, NO. 2, 2008: 175 - 177

 ISSN 1118-0579

EFFECT OF CHLOROQUINE TREATMENTS ON SPERM COUNT AND WEIGHT OF TESTES IN MALE RATS

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(Received 15, March 2006; Revision Accepted 13, December 2007)

ABSTRACT

Male albino rats were administered with acute and chronic treatment doses of Chloroquine Phosphate intraperitoneally to evaluate its effects on sperm count, weight of testes and epididymes at these recommended doses. The acute and chronic treatments caused a decrease in mean weight of testes of 1.05 ± 0.055g and 0.83 ± 0.082g respectively compared to the mean for the control of 1.08 ± 0.075g. The chronic treatment gave significant ($p < 0.01$) decrease in weight of testes. There were also decrease in the mean weights of the epididymes of both acute and chronic treatments of 0.45 ± 0.055g and 0.27 ± 0.082g respectively compared to mean for the control of 0.47 ± 0.52g. The chronic treatment also gave significant ($p < 0.01$) decrease in weight of the epididymes. Concomitantly, a reduction in mean sperm count of the acute and chronic-treated rats were recorded as 45.80 and 45.50 $x10^6$ sperm/ml compared to 45.85 $x10^6$ sperm/ml for the control. The chronic-treated rats had significant ($p < 0.01$) decrease in mean sperm count. A direct relationship between the mean sperm count and weight of testes and epididymes were observed. These effects on sperm count, weight of testes and epididymes is suggestive that Chloroquine phosphate is toxic in male albino rats as a model, particularly for the chronic (or suppressive) treatment dose.

KEYWORDS: Sperm Count, Gonads, Testes, Epididymes, Toxicity, Chloroquine, Rats.

INTRODUCTION

Chloroquine is an anti-malarial drug used in the prevention or treatment of malaria (Sewester, 1994). It is active against the erythrocytic forms of plasmodium (Sewester, 1994; Robert, 1998). Periodically; the blood merozites burst from the ruptured cells and invade a new group of erythrocytes, beginning the process anew. This periodic (every 3-4 days) rupturing of infected erythrocytes is responsible for the characteristic fever and chills that accompany acute attacks of malaria (Hall, 1973).

Chloroquine has also been used to suppress rheumatoid arthritis and in the treatment of scleroderma, polymyositis and sarcoidosis (Spideman *et al.*, 1994). It may also act by blocking the enzymatic synthesis of DNA and RNA in both mammalian and protozoan cells forming a complex with DNA that prevents replication or transcription to RNA (Robert, 1998).

Malaria is a parasitic disease that is still prevalent in many areas of the world, especially Africa, South East Asia, Central and South America (Nampoorly *et al.*, 1992). Malaria affects hundreds of millions of people worldwide and kills over two million people every year. Due to the fact that synthetic drugs, many a times are not able to subdue malaria parasite strains because of resistance (Mackinnon *et al.*, 1997). This leads to abuses and over-use of such drugs.

Irreversible retinal damage has been observed in some patients who had received long term or high dosage chloroquine therapy (Ette *et al.*, 1987) and may progress even after cessation of Chloroquine therapy (Masimirembusa *et al.*, 1994). Patients on long term therapy with this preparation have been reported to have shown evidence of muscular weakness (Ulberg *et al.*, 1970).

Ihejirika (1992), reported a reduction in the number of leydig cells following chronic administration of Chloroquine on rats, which led to reduction in sperm count. Similar reductions in sperm count had also been reported by Hole (1993) in humans and Ekaluo *et al.* (2005) in rats following administration of analgesics at recommended doses. Okanlawon *et al.* (1992), reported the disruption of the process of spermatogenesis following chronic toxic administration of Chloroquine on rats. Ebong *et al.* (1999), showed a correlation between leydig cells reduction and levels of plasma testosterone following chronic administration of Chloroquine to male albino rats and also reported alterations of testicular morphology and a concomitant decline in plasma testosterone levels which may result in the inhibition of the development and maintenance of the secondary sex characteristics, leading to reduced virility and consequent infertility.

In view of above finding, this study set out to show effects of acute and chronic treatments of chloroquine on sperm count, weights of testes and epididymes in male albino rats as a model; using short-term *in vivo* assays.

MATERIALS AND METHODS

2.1 Experimental Design and Procedure

Eighteen (18) healthy and sexually mature male albino rats of 200-210 g body weight were obtained from the animal house of Department of Zoology and Environmental Biology, University of Calabar, Calabar for this study. The initial weights of all the rats were taken before treatment and weekly during treatment period. The animals were grouped into three groups with six rats per group in a Completely Randomized Design (CRD) and kept in conventional cages under standard laboratory conditions. Food and water was given *ad libitum*.

2.2 Drug Administration

Group A received the acute treatment (10mg of Chloroquine/kg body weight initially, 6 hours later they received 5mg/kg body weight and another 5mg/kg body weight intraperitoneally daily for 2 days). Group B received the chronic treatment (5mg of Chloroquine/kg body weight intraperitoneally weekly for 8 weeks). Group C served as the control and did not receive any Chloroquine treatment. Twenty four hours after treatment the rats were sacrificed by cervical dislocation and dissected to obtain the testes and epididymes for assessment of mean sperm count, weight of testes and epididymes.

2.3 Weight of Testes and Epididymes

The testes and epididymes were dissected out from

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the male rats, rinse in physiological saline (0.9% Sodium chloride) and blotted on filter paper. They were weighed using Scout Pro SPU 601 electronic weighing balance.

2.4 Mean Sperm Count

This was carried out according to the method of Abdel-Rahman *et al.* (1999) modified by Ekaluo *et al.* (2005). The epididymal content was obtained by macerating known weights of epididymes in watch glass containing physiological saline in the ratio of 1:10 weight by volume. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80µm stainless mesh. The sperm cells were counted by cytometry to obtain the sperm count per milliliter. Five different counts were done for each sample, and the mean of the five counts were taken as the count for each male rat.

2.5 Statistical Analysis

Differences between the mean weights of the control and treatment groups were compared using the Analysis of Variance (ANOVA) test.

RESULTS

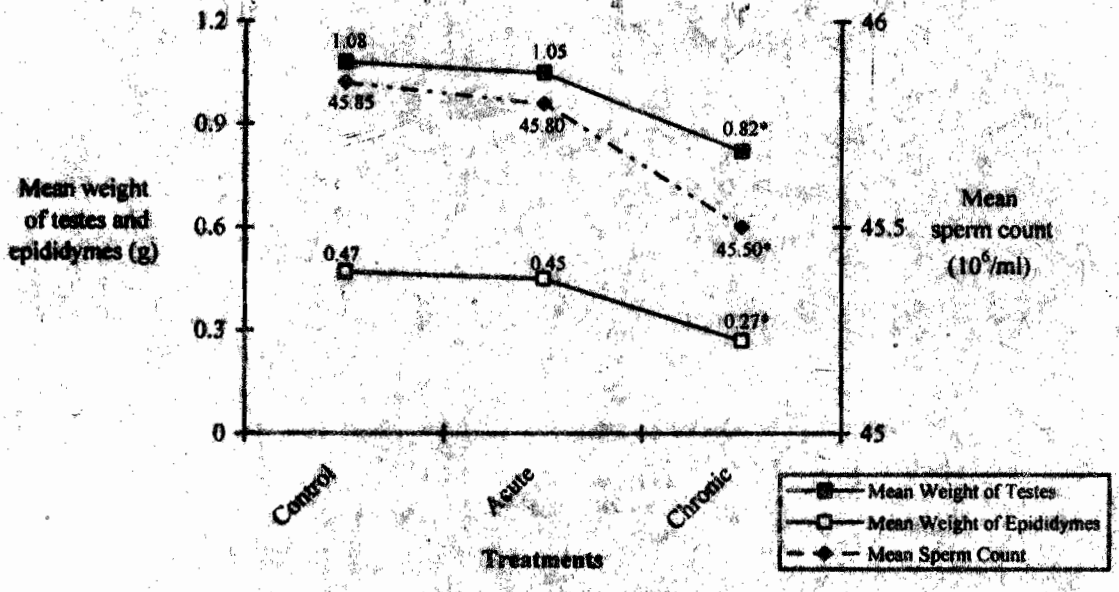
3.1 Mean Sperm count

The mean sperm counts of rats on chronic treatment were significantly ($p<0.01$) lower than those on control and acute treatment, while mean sperm counts of rats on control and acute treatment did not significantly ($p>0.05$) differ from each other. As shown on Table 1. There was also a positive relationship between mean of sperm count and mean of weights of testes and epididymes as shown on Figure 1.

Table 1: Mean Sperm Count, Weight of Testes and Epididymes

Treatment	Mean weight (g)		Mean Sperm Count ($\times 10^6$/ml)
	Testes	Epididymes	
Control	1.08 ± 0.750	0.47 ± 0.520	45.85
Acute	1.05 ± 0.055	0.45 ± 0.055	45.80
Chronic	0.83 ± 0.820*	0.27 ± 0.082*	45.50*

* Significantly less than control, $p < 0.01$, Analysis of Variance (ANOVA) test.



* Significantly less than control, $p < 0.01$, Analysis of Variance (ANOVA) test.

Figure 1: Relationship of Mean Sperm Count, Mean Weight of Testes and Epididymes

3.2 Weight of Testes

The weights of the testes of rats on chronic treatment were significantly ($p<0.01$) lower than those on control and acute treatment, while weights of testes of rats on control and acute treatment did not significantly ($p>0.05$) differ from each other. Table 1 shows the mean sperm count, weight of testes and epididymes for control and treatment groups.

3.3 Weight of Epididymes

The weights of the epididymes of rats on chronic treatment were significantly ($p<0.01$) lower than those on control and acute treatment, while weights of epididymes of rats on control and acute treatment did not significantly ($p>0.05$) differ from each other.

DISCUSSION

It was observed that there was a decrease in the weights of the testes and the epididymes of both treatment groups, that is, the acute and the chronic treatment groups but the decrease in the chronic group was significant ($p< 0.01$). The reduction in the weight of testes could be attributed to a

reduction in the number of leydig cells following the chronic administration of Chloroquine on rats, which agrees with the report of Ihejirika (1992).

The mean sperm count of the treatment groups showed a general reduction with the chronic treatment group being significantly ($p < 0.01$) reduced. The decrease in the mean sperm count may have been as a result of interference with spermatogenesis and testicular tissue degeneration leading to a reduction in the amount of germinal epithelium and the number of matured sperm cells (Hole, 1993; Ekaluo *et al.*, 2005). Ebong *et al.* (1999), also reported that chronic administration of Chloroquine leads to the alterations of testicular morphology and a concomitant decline in plasma testosterone levels.

In conclusion therefore, since Chloroquine is still one of the best known drug for the treatment of malaria; and it is usually sold over-the-counter and taken by people in the rural and some urban cities without prescription. Chloroquine should be used with caution, chronic (suppressive) treatment and abuses of the drug should be avoided as the arbitrary use may result in reproductive health dysfunctions. This may be evidenced in the effects such as reduction in sperm count, weight of testes and epididymes.

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