

*Full Length Research Paper*

# **Serum testosterone concentration in chloroquine-treated rats: effects of ascorbic acid and alpha-tocopherol**

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The effects of ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) were studied on serum testosterone concentration in chloroquine-treated rats. Thirty five (35) adult male rats weighing 160 - 200 g were divided into seven groups of five (5) rats each. Group I rats served as the control and received 2 ml/kg of normal saline while Group II rats were treated with chloroquine (20 mg/kg). Groups III, IV, V, VI and VII rats were treated with chloroquine (20 mg/kg) and either vitamin C (14.3 or 100 mg/kg) or vitamin E (9.3 mg/kg or 100 mg/kg) or the combination of both. The drugs were administered orally for thirty five (35) days and at the end of the treatment, serum testosterone concentrations were determined. The results showed that chloroquine did not cause a significant change in serum testosterone concentration. In addition, the administration of the vitamins with chloroquine also did not cause any significant change in serum testosterone concentration when compared with the control. The results suggest that long-term administration of chloroquine could have no effect on testosterone concentration and the vitamins also could not cause any significant change in testosterone concentration in the presence of chloroquine.

**Key words:** Chloroquine, alpha-tocopherol, ascorbic acid, testosterone.

## **INTRODUCTION**

Several studies have shown that chloroquine, an antimalarial drug possesses reversible antifertility activities including reductions in sperm motility and viability (Adeeko and Dada, 1998; Salman and Ajayi, 2007), increase in the number of abnormal spermatozoa and a reduction in the weight of the testes and accessory sexual organs (Nichola et al., 1997). It also disrupts the seminiferous tubules and reduces Leydig's cells population and testosterone concentration in rats after six days of treatment (Ebong, 1999). Moreover, it had been reported to inhibit testosterone secretion in rat testes (Nduka, 1986).

Several authors have also reported the positive influence of antioxidant vitamins (vitamins A and E) on male reproductive functions. For instance, ascorbic acid

had been observed to protect human epididymis and spermatozoa against deoxyribonucleic acid damage (Fraga et al., 1991). It also improves semen quality and sperm motility in smokers (Dawson et al., 1992). Moreover, alpha-tocopherol, a lipid-soluble vitamin had also been reported to improve spermatogenesis, prevent loss of spermatogenesis (Mason, 1954; Marin-Guzman et al., 2000; Regina et al., 1999) and improve semen quality and cell viability in chicken (Franchini et al., 2001). This vitamin also increases the percentage of normal sperm in men (Therond et al., 1996) and protects sperm cells from morphological damage (Audet et al., 2004). Recently, Salman and Ajayi (2007) reported that administration of vitamins C and E ameliorated the reduction in sperm motility and viability induced by long-term chloroquine treatment in rats; suggesting that the antifertility effects of chloroquine are probably mediated via the generation of free radicals. However, the study did not investigate whether or not the antioxidant vitamins could have any effects on serum testosterone concentration in

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**Table 1.** Effects of ascorbic acid and alpha-tocopherol on serum testosterone concentration in chloroquine-treated rats.

Groups	Treatment	Testosterone concentration (ng/ml)
I	Normal saline	0.08 ± 0.04
II	CQ (20 mg/kg)	0.13 ± 0.04
III	CQ (20 mg/kg) + AA (14.3 mg/kg)	0.06 ± 0.02
IV	CQ (20 mg/kg) + AA (100 mg/kg)	0.08 ± 0.02
V	CQ (20 mg/kg) + AT (9.3 mg/kg)	0.09 ± 0.01
VI	CQ(20 mg/kg) + AT (100 mg/kg)	0.07 ± 0.02
VII	CQ (20 mg/kg) + AA (100 mg/kg) + AT (100 mg/kg)	0.13 ± 0.01

CQ = Chloroquine, AA = ascorbic acid, AT = alpha-tocopherol.

chloroquine- treated rats. The observations that chloroquine reduced testosterone concentration were based on short-term treatment. Available data however suggest that the effects of a number of antimalarial agents on testosterone concentration are duration-dependent (Raji et al., 2005a; Raji et al., 2005b). Information on the effects of chronic administration of chloroquine on testosterone concentration is still very scanty.

The present study was therefore designed to investigate the effects of long-term administration of chloroquine on testosterone concentration in rats. The effects of vitamins C and E on serum testosterone concentration in chloroquine-treated rats will also be investigated.

## MATERIALS AND METHODS

### Animal model

Wister strain albino rats (160 – 200 g) obtained from the Central Animal House, College of Medicine, University of Ibadan, were used for the study. The rats were housed in wire mesh cages under standard conditions (Temperature, 25 – 29°C, 12 h light and 12 h darkness cycles) and fed with standard rat pelleted diet and water. The study was generally conducted in accordance with recommendations from the declaration of Helsinki on guiding principles in the care and use of animals.

### Drugs

Tablets of chloroquine and ascorbic acid (Tuyil Pharmaceutical Industry, Nigeria) alpha-tocopherol (G.A. Pharmaceuticals, Athens, Greece) and Testosterone kit (Immunometrics, London, UK) were used for the study.

### Experimental design

Thirty five (35) male rats were divided into seven groups of five (5) animals per group. Group 1 consists of rats which received 2 ml/kg of normal saline and served as the control while Group II rats received chloroquine (20 mg/kg). Groups III, IV, V, VI and VII were treated with chloroquine (20 mg/kg) and vitamin C (14.3 mg/kg), chloroquine (20 mg/kg) and vitamin C (100 mg/kg), chloroquine (20 mg/kg) and vitamin E (9.3 mg/kg), chloroquine (20 mg/kg) and vitamin E (100 mg/kg), and chloroquine (20 mg/kg) with vitamins C and E (100 mg/kg), respectively. The drugs were dissolved in normal saline and administered orally for thirty five (35) days.

### Hormone assay

Serum testosterone assay was carried out using the tube-based enzyme immunoassay (EIA) method (Raji et al., 2005). This is a standardized method used by WHO and part of its program for human reproductive research. The EIA testosterone kit was produced by Immunometrics (London, UK) and purchased from Nzemat (Lagos, Nigeria). The procedures for the assay as contained in the manufacturer's manual were strictly followed. The within assay variation was 8.1% and the sensitivity was 0.3 ng/ml. The optical density was read using a spectrophotometer (Jenway, 6300 spectrophotometer, UK) that was sensitive at wavelengths between 492 and 550 nm.

### Statistical analysis

Data were expressed as mean ± SEM and analyzed using student's t-test.  $P < 0.05$  was considered significant.

## RESULTS

The results showed that there was an apparent but insignificant increase ( $P > 0.05$ ) in serum testosterone concentration in chloroquine-treated rats. The estimated testosterone concentration of  $0.13 \pm 0.04$  observed in chloroquine-treated rats was similar to the value of  $0.08 \pm 0.04$  recorded for the control rats. The testosterone concentrations of the groups of rats that received either ascorbic acid or alpha-tocopherol with chloroquine were also similar with the control. However, there was an apparent but insignificant increase in the testosterone concentration of rats that received both vitamins with chloroquine (Table 1).

## DISCUSSION

The present study observed an insignificant increase in serum testosterone concentration following chronic administration of chloroquine. This finding is rather in contrast to the reduction in serum testosterone concentration induced by chloroquine after six days of treatment earlier reported. The reported decrease was said to be due to numerical reduction of the Leydig's cells (Ebong, 1999). The inability of chloroquine to cause a

significant change in serum testosterone as opposed to the reduction caused by short-term treatment may be due to the development of resistance against it induced by long-term treatment. Similar observation had been made with the extract of *Alstonia boonei* in which short-term treatment caused a significant decrease in serum testosterone concentration in rats while long-term treatment caused no significant change (Raji et al., 2005a). On the other hand, the apparent but insignificant increase in testosterone concentration could be construed as a result of stimulation of testosterone production in response to an earlier decrease that might have been caused by chloroquine at the earlier part of treatment since chloroquine had been reported to cause a reduction in testosterone concentration on short-term treatment. The observation is also similar to the report that chronic treatment with the extract of *Morinda lucida* caused a significant increase in serum testosterone concentration (Raji et al., 2005b). Both *A. boonei* and *M. lucida* are potent antimalarial agents.

The observation that vitamin C could not alter the serum testosterone concentration in the presence of chloroquine is of great interest. Previously, an increase in serum testosterone concentration in rats treated with vitamin C and a decrease in vitamin C-deprived rats had been reported. This was probably due to oxidative damages done to the Leydig's cells in Vitamin C-deprived rats and a stimulating effect on the Leydig's cells of rats treated with vitamin C (Steven, 2000). On the other hand, there are no effects of vitamin E deprivation on serum levels of testosterone, suggesting that the LH-testosterone feedback loop is not impaired. However, its deficiency has deleterious effect on germ cell proliferation and differentiation in rats and other animal species (Copper et al., 1987). Recently, the observation that vitamins C and E ameliorated chloroquine-induced impairment of sperm motility and viability suggests that the antifertility effects of chloroquine are probably mediated via the induction of free radical generation (Salman and Ajayi, 2007). It therefore appears that in the presence of chloroquine, the action of vitamin C was directed at preventing the damaging effects of chloroquine especially on the morphology of Leydig's cells which produce testosterone rather than stimulating the cells to increase testosterone production. Vitamins C and E are known to be potent free radical scavengers.

Even though vitamin E had been shown not to have a direct effect on testosterone concentration, the apparent but insignificant increase in testosterone concentration observed when both vitamins were administered with chloroquine suggests that it complements or potentiates the action of vitamin C. In other words, the two vitamins are synergistic in their actions. Salman and Ajayi (2007) had earlier made similar observation. This is also consistent with the reported ability of ascorbic acid to resuscitate alpha-tocopherol, preventing its damage during vigorous antioxidant activities (Vander et al., 1988) and reports that ascorbic acid protects cell membranes

and lipoprotein particles from oxidative damage by regenerating the antioxidant form of alpha-tocopherol (Gupta et al., 2005). It therefore seems likely that at higher doses, the combination of the two vitamins may be able to prevent the negative effects of chloroquine and at the same time stimulate testosterone production. Further studies will shed light into this.

The present findings therefore suggest that chronic administration of chloroquine could not have any significant effect on serum testosterone concentration and that in the presence of chloroquine, the vitamins would assume a protective rather than a stimulatory role on the Leydig's cells.

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