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Gonadotoxicity Evaluation of Oral Artemisinin Derivative in Male Rats

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Male Wister albino rats were exposed to artemether by gavage at dosages of 25, 50 and 75 mg kg^{-1} day^{-1} for 1, 2 and 3 days. The control groups received sterile water (control 1) and 5% ethanol (vehicle for artemether, control 2). The maximum volume injected in all groups was 0.5 mL. Rats administered the highest dose for three days were mated with female rats to determine the fertilizing capacity of their epididymal sperms and fertility status. Artemether significantly reduced ($p<0.05$) the progressive sperm motility, viability, sperm count and serum testosterone levels in dose and duration dependent manners, factors that may impair fertility. None of the untreated cohabited female rats got pregnant throughout the period of the study. These changes were restored in recovery experiments. The results suggest that artemether could induce reversible infertility in rats.

Key words: Artemether, antimalarial, testosterone, sperm motility, gonad, rat

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INTRODUCTION

Artemether is an antimalarial drug used in the treatment of all forms of malaria due to *Plasmodium falciparum, Plasmodium ovale* and *Plasmodium malariae* and it is the most active derivative of a new class of antimalarial drugs that are chemically unrelated to existing drugs[1]. Its efficacy against multi-drug resistant falciparum malaria and its potential to delay antimalarial drug resistance have led to its increasing use[2] in recent years.

Many antimalarial drugs have been implicated in male infertility. For instance, chloroquine, quinine and quinacrine have been reported to inhibit Leydig cell steroidogenesis and fertility in male[3]. Chloroquine has also been reported to reduce sperm motility and hence fertility by a reduction in the average number of fetuses of cohabited female rats[4]. Pyrimethamine, an anaphylactic antimalarial drug has been shown to cause spermatogenic arrest and male infertility in mice[5]. Interestingly antimalarial medicinal plant extracts have also been reported in experimental male infertility. For example, *Quassia amara* which was reported to be highly potent against chloroquine resistant *Plasmodium falciparum*[6] produced significant reduction in epididymal sperm counts, serum levels of testosterone, luteinizing hormone and follicle stimulating hormone in male rats[7,8]. Joshi *et al.*[9] also reported a mass atrophy of the spermatogenic elements and Leydig cells when *Azadirachta indica* extract was administered to male rats. More recently, Raji *et al.*[10,11] reported a dose dependent decrease in serum testosterone and luteinizing hormone when *Azadirachta indica* and *Morinda lucida* extracts were individually administered to male rats. These medicinal plants are commonly used in folkloric medicine to treat malaria and have been reported to possess antiplasmodium activities in mice[12].

The strong efficacy of artemether to various forms of malarial parasites made its introduction and use in malarial chemotherapy globally acceptable. It is therefore imperative to evaluate the reproductive activities of the drug in experimental model, since none is currently available. This study was therefore designed to evaluate the effects of artemether on male reproductive functions in albino rats.

MATERIALS AND METHODS

Animals: Wistar strain albino rats (190-230 g) obtained from the Central Animal house, College of Medicine, University of Ibadan were used for the study. The animals were fed with standard rat cubes (Ladokun feeds Nig. Ltd.) and water *ad libitum*. They were housed at room temperature under photoperiod-controlled environment (12 h light: 12 h dark light cycles).

Drug and dose regimens: Artemether (Rhone-Poulene Rorer International, France) was obtained from the University of Ibadan Health center and administered in 5% ethanol (vehicle for artemether) orally at doses of 25, 50 and 75 mg kg^{-1} day^{-1} to male rats for 1, 2 and 3 days. Each group has its corresponding positive and negative controls whose rats received sterile water and 5% ethanol, respectively.

Study protocol: The study was divided into three experimental sections as follows:

Experiment 1: Five male rats each were treated with 25, 50 and 75 mg kg^{-1} artemether. Each group was run for 1, 2 and 3 days.

Experiment 2- Recovery studies: Twenty-five male rats were divided into five equal groups and each group was administered sterile water only, 5% ethanol only, 25, 50 and 75 mg $kg^{-1}day^{-1}$ artemether, for three days and allowed to recover from the drug for another three days.

Experiment 3- Fertility studies: Twenty-five male rats divided into three equal groups were treated, respectively with sterile water only, 5% ethanol only, 25, 50 and 75 mg $kg^{-1}day^{-1}$ artemether, for three days. They were then introduced to proestrous female rats (200 g) of proven fertility at a ratio of 2 males to 3 females. A single time point fertility test for each rat was carried out using the following formula:% Fertility success = number of pregnant female rats divided by number of mated female rats multiplied by 100 as earlier described[11].

Rats were weighed and sacrificed by exsanguinations under 25% urethane anesthesia (0.6 mL/100 g body weight) about 24 h after the last artemether administration. Blood was collected from each rat via cardiac puncture from which the serum was separated. Testosterone concentration in the serum was measured using the enzyme immunoassay (EIA) technique as previously described[11].

Sperm characteristic analysis: Semen collected from the cauda epididymis was used in the determination of the progressive sperm motility, viability, count and morphology[11,13].

Statistical analysis: Data were presented as Mean±SEM and statistical analyses were carried out using the Student's t-test and ANOVA. Significant difference was accepted at $p<0.05$.

RESULTS

Effect of artemether on body weight: There was no significant change in body and reproductive organ

Table 1: Effects of artemether on sperm parameters after 1, 2 and 3 days of treatments and recovery

Groups	Sperm motility (%)	Sperm count (10^6/mL)	Sperm viability (%)
1 day treatment			
Control 1(Sterile water)	95.00±0.45	8.30±0.04	98.00±0.35
Control 2 (Ethanol)	70.80±2.67*	7.67±0.03*	90.00±0.95*
25 mg kg^{-1} b.w.	66.60±1.86*	7.43±0.05*	90.40±0.60*
50 mg kg^{-1} b.w.	53.30±1.76*	7.27±0.05*	90.00±1.61*
75 mg kg^{-1} b.w.	50.00±5.24*	6.50±0.05*	85.00±0.63*
2 days treatment			
Control 1(Sterile water)	95.00±0.45	8.30±0.04	98.00±0.35
Control 2 (Ethanol)	67.00±2.00*	7.20±0.07*	85.00±2.60*
25 mg kg^{-1} b.w.	46.60±1.83*	5.83±0.98*	80.00±3.54*
50 mg kg^{-1} b.w.	36.60±1.83*	5.11±0.05*	76.60±1.85*
75 mg kg^{-1} b.w.	33.30±1.91*	4.90±0.03*	75.00±1.58*
3 days treatment			
Control 1(Sterile water)	95.00±0.45	8.30±0.04	98.00±0.50
Control 2 (Ethanol)	56.70±1.74*	7.03±0.05*	80.00±0.65*
25 mg kg^{-1} b.w.	33.30±1.91*	5.23±0.04*	78.10±0.64*
50 mg kg^{-1} b.w.	31.60±0.93*	4.80±0.09*	75.90±0.64*
75 mg kg^{-1} b.w.	23.50±0.74*	3.20±0.07*	73.00±0.27*
Recovery			
Control 1 (Sterile water)	95.00±0.45	8.30±0.04	98.00±0.35
Control 2 (Ethanol)	90.00±0.71*	7.56±0.19*	96.00±0.71*
25 mg kg^{-1} b.w.	76.00±0.71*	7.37±0.10*	93.32±0.99*
50 mg kg^{-1} b.w.	70.00±1.10*	6.73±0.05*	90.00±0.95*
75 mg kg^{-1} b.w.	63.32±0.87*	6.10±0.11*	90.00±1.61*

Table 2: Effects of artemether on sperm morphological parameters

Groups	Control (Sterile water)	Control 2 (Ethanol)	25 mg kg^{-1} b.w.	50 mg kg^{-1} b.w.	75 mg kg^{-1} b.w.
Day 1					
% Abnormal sperms	1.18±0.42	6.45±1.94	6.27±1.66	7.45±2.01	7.91±2.34
Day 2					
% Abnormal sperms	1.18±0.42	9.27±2.83	7.64±2.43	8.18±2.89	9.36±2.82
Day 3					
%Abnormal sperms	1.18±0.42	10.18±3.01	9.55±2.39	10.91±3.17	11.09±2.46
Recovery					
%Abnormal sperms	1.18±0.42	7.18±2.06	7.18±2.33	7.09±2.10	7.27±2.36

Table 3: Effects of artemether on serum testosterone levels

Groups	Control (Sterile water)	Control 2 (Ethanol)	25 mg kg^{-1} b.w.	50 mg kg^{-1} b.w.	75 mg kg^{-1} b.w.
1 day treatment	2.00±0.05*	1.04±0.04*	0.09±0.04	0.08±0.04	0.10±0.03
2 days treatment	2.01±0.04*	1.06±0.03*	0.09±0.03	0.08±0.03	0.09±0.04
3 days treatment	2.00±0.05*	1.05±0.04*	0.08±0.04	0.07±0.03	0.07±0.04
Recovery	2.02±0.03*	1.04±0.03*	1. 09±0.05	1.10±0.04	1.50±0.05

*$p<0.05$

weights of artemether treated rats when compared with the controls (Data not shown). This trend was also observed in the recovery group.

Effect of artemether on sperm parameters (sperm counts, viability and motility and morphology): Administration of artemether at 25, 50 and 75 mg kg^{-1} day^{-1} significantly reduced ($p<0.05$) the progressive sperm motility, sperm count and sperm viability in dose and duration dependent manners when compared with the controls (Table 1). Drug withdrawal resulted in gradual restoration of sperm parameters (Table 1). In almost all the test groups, the most common abnormality encountered was the "simple bent tail". Administration of artemether caused dose and duration dependent increase in the number of abnormal sperms when compared with the controls. However there was a significant decrease ($p<0.05$) in the number of abnormal sperms encountered in the recovery group (Table 2).

Effect of artemether on serum testosterone level: Serum testosterone levels of artemether treated rats significantly reduced in dose and duration dependent manners when compared with the controls. However there was an appreciable increase in serum testosterone levels of rats in the recovery group (Table 3).

Fertility studies: All rats that received the various doses of the drug did not produce any litter. The control groups sired 8.00±2.00 and 7.10±1.80 physically normal litters, respectively.

DISCUSSION

The results suggest that artemether could cause reversible impairment to reproductive activity in male albino rats. Gonadotoxic effects of many antimalarial agents have been demonstrated in many animal species including human. Antimalarial drugs such as chloroquine and quinines[3,4,14] have been reported to be toxic to germ cells and cause spermatogenic arrest. Chloroquine has also been demonstrated having varied effects on male reproductive functions including fertility reduction in the male rats[15] and complete obliteration of Leydig cell response to leutropin and hormones having leutropin like activity *in vitro*[3]. Chloroquine was reported to have an activating influence on bovine sperm respiration and motility *in vitro* as well as stimulatory to aged porcine spermatozoa motility[16]. Adeeko and Dada[4] also reported that chloroquine caused a dose dependent reduction in fertility of male rats as evidenced by a reduction in average number of fetuses of cohabited females. Okanlawon and Ashiru[14] showed that in chloroquine treated rats; there was a disruption of spermatogenesis, which was accompanied by a decline in serum concentrations of testosterone in these rats. These effects of chloroquine were reported in all cases to be reversible.

In the present study artemether caused significant reduction in sperm motility in dose and duration dependent manners while there was an increase in the number of dead caudal epididymal sperm. Sperm motility

is usually acquired partly in the epididymis raising the suspicion that the deleterious effect of artemether is on the storage of sperms in the epididymis. These observed reductions in sperm functions might be related to the altered micro-environment in the epididymis[17] and/or the drug might be toxic to maturing or mature spermatozoa in the epididymis[18]. The decrease observed in sperm count could then be the result of the increase in number of dead spermatozoa. Artemether appears to be different from other antimalarial drugs in its having an endoperoxide bridge thereby generating single oxygen and free radicals[2]. Free radicals have been proposed as a major cause of defective sperm function in cases of male infertility[19]. The reduced serum testosterone concentration during artemether administration confirmed that the drug could suppress Leydig cell steroidogenesis leading to reduction in testosterone production and hence reduced sperm motility and fertility potentials. This probably led to the inability of the untreated female rats to conceive upon cohabitation with artemether treated male rats. The actions of artemether appear reversible within the therapeutic doses and durations employed in this study. It is obvious that spermatogenic cycle was not covered and whether the reproductive organs could concentrate or store artemether is also not known yet. Ongoing studies in our laboratory will address these issues and the detail probable mechanism of action of artemether on reproductive organs.

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