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Comparative Activities of Chloroquine, Mefloquine and Sulphadoxine-pyrimethamine on *in vivo* and *ex vivo* Male Reproductive Functions in Mammalian Models

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Comparative reproductive activities of chloroquine, mefloquine and sulphadoxine-pyrimethamine were explored in albino Wistar rats and semen from West African Dwarf Buck (WADB) with a view to elucidating the mechanism of action of these drugs on malereproduction. Five adult male rats were administered 0.5 mL distilled water and served as the control. Five rats each were administered orally chloroquine (10 mg kg^{-1} b.w.), mefloquine (10 mg kg^{-1} b.w.) and sulphadoxine-pyrimethamine (5 mg kg^{-1} b.w.) orally, for four weeks. Each group had it's own recovery group. Sperm counts, motility and morphology were reduced in rats treated with these drugs in the order mefloquine ($p<0.05$)> chloroquine > sulphadoxine-pyrimethamine. There was an appreciable recovery in the motility of sperms in all recovery groups. Semen samples from WADB were extended separately with chloroquine, mefloquine and sulphadoxine-pyrimethamine. Extender 1 (first control) had no PENSTRIP (Penicillin and Streptomycin combination) while extender 2 (standard extender; second control) had PENSTRIP. Semen in extenders 3, 4 and 5 were treated with chloroquine, mefloquine and sulphadoxine-pyrimethamine, respectively. Spermatozoa progressive motility in these extenders examined under the microscope at 24 h for 5 days significantly reduced in mefloquine ($p<0.01$), slightly with chloroquine and unchanged with sulphadoxine-pyrimethamine. The pH of the extenders was significantly reduced in duration dependent manner in mefloquine while it remained unchanged with chloroquine and sulphadoxine-pyrimethamine. The results suggest the safety of sulphadoxine-pyrimethamine and chloroquine in preservation of semen *ex vivo* while the negative impact of mefloquine could reside within the testis or epididymis.

Key words: Chloroquine, mefloquine, sulphadoxine-pyrimethamine, male reproduction, *in vivo*, *ex vivo*

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INTRODUCTION

Malaria remains an endemic disease in up to 102 countries, a scourge to which about 40% of the world population is exposed. Consequently many antimalarial drugs are used in the treatment of all forms of malaria. Among the commonly used antimalarial drugs are chloroquine, mefloquine and sulphadoxine-pyrimethamine combination (fansidar). Chloroquine a -4 aminoquinoline is the most commonly used drug in the treatment of malaria worldwide and for rheumatoid arthritis therapy (Adelusi and Salako, 1982). Mefloquine hydrochloride is a synthetic 4-quinoline methanol derivative chemically related to quinine. Mefloquine is used in prophylaxis and treatment of chloroquine-resistant and multi-drug resistant falciparum malaria (Goldsmith, 1995). Sulphadoxine-pyrimethamine has been provided to be an effective and a safe antimalarial drug (Salako *et al*., 1990). Sulphadoxine-pyrimethamine has also been reported as a curative agent for treating individual malaria patients and for reducing the reservoir of *Plasmodium falciparum*, where resistance to 4-aminoquinolines has been reported (Strickland *et al*., 1986).

However, it is important to note that many antimalarial drugs have been implicated in male infertility. Consentino *et al*. (1990) showed the ability of pyrimethamine to cause spermatogenic arrest and male infertility in mice in a dose dependent manner. Chloroquine has been found to have a dose-related reduction in fertility of male rats (Adeeko and Dada, 1994; 1998). Sulphasalazine has also been shown to cause reduced sperm motility (O'Morain *et al*., 1984). Recently, we reported the reversible gonadotoxic activity of a newly introduced antimalarial drug, artemether in rats (Raji *et al*., 2005c). Moreover quinine, quinacrine and chloroquine had earlier been reported to inhibit Leydig cell steroidogenesis, which suggest an antifertility action (Sairam, 1978). Interestingly, medicinal plant extracts with potent antimalarial activities have also been reported to have male antifertility actions. For example, *Quassia amara* which was reported to be highly potent against chloroquine resistant *Plasmodium falciparum* (Trager and Polonsky, 1981) produced significant reduction in epididymal sperm counts, serum levels of testosterone, luteinizing hormone and follicle stimulating hormone in male rats (Raji and Bolarinwa, 1997; Parveen *et al*., 2003). Joshi *et al*. (1996) 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*. (2003; 2005a) 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 (Gbile, 1986).

The mechanisms by which most antimalarial agents induce infertility and the site of action have not been fully elucidated. It is thought that *in vivo* studies supported by *ex vivo* analyses of the actions of these drugs in male reproduction will throw more light on these. The present study was therefore designed to investigate the impact of chloroquine, mefloquine and sulphadoxine-pyrimethamine on male reproductive functions *in vivo* and on sperm storage *ex vivo*.

MATERIALS AND METHODS

Animals and drugs: The study was conducted between July 2003 and April 2004 in the department of Physiology, university of Ibadan, Nigeria. Wistar strain albino rats (200-220 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 a photoperiod-controlled environment (12 h dark: 12 h light cycles; 24-25°C) and fed with rat cubes (Ladokun feeds, Nigeria, Ibadan) and water *ad libitum*. Chloroquine (Glaxo), mefloquine (Smithkline-Beecham) and sulphadoxine-pyrimethamine (Smithkline-Beecham) were obtained from the University of Ibadan Health Centre and dissolved individually in distilled water. Water was chosen as the vehicle because the drugs were readily soluble in it.

Experiment I: *In vivo* study: A total of thirty-five male rats divided into seven groups were treated as follows: Group I rats were administered 0.5 mL distilled water (orally) and served as the control. Group II rats received 10 mg kg^{-1} b.w. chloroquine, Group III rats received 10 mg kg^{-1} b.w. mefloquine and Group IV rats received 5 mg kg^{-1} b.w. sulphadoxine-pyrimethamine for four weeks. Each drug treated group had it's own recovery from which drug treatment was discontinued for two weeks before the rats were sacrificed by exsanguinations under urethane anaesthesia 24 h after the last treatment. The liver, testes, and epididymis were removed and weighed. Epididymal sperm counts, motility and morphology were evaluated as earlier described (Raji *et al*., 2005b). Sperm counts were expressed in million mL^{-1}.

***Ex vivo* study: Animals:** One healthy adult (3 years old) male West African Dwarf Buck (WADB) (*Carpora hircus* L.) weighing 20 kg was used for the *ex vivo* study for ease of semen collection which was done using electro ejaculation method (Moss *et al*., 1999). The animal was certified healthy and fertile by a veterinary Doctor and was kept away from mating for 2 days prior to the study.

Collection of semen samples: Semen was collected from the buck into a pre-warmed (27°C) glass tube using electro-ejaculation stimulating machine with a rectal probe. The semen quality (sperm count, motility, pH, volume, colour and percentage life spermatozoa) was quickly assessed (Raji *et al*., 2005b). It was thereafter extended in individual concentration of chloroquine, mefloquine, and sulphadoxine-pyrimethamine previously prepared and stored at 5°C.

Preparation of semen extenders: Extenders are liquid substances used when preserving semen samples for long period to allow the spermatozoa retain their functions and remain alive. Extenders also provide nutrients for the spermatozoa and prevent growth of harmful bacteria in the semen. In this study extenders were prepared prior to semen collection. Extender 1 was the control extender and contained 90 mL of citrate buffer (2.9% w/v), 10 mL of egg yolk (Raji *et al*., 2005b; Berndtson and Foote, 1976). The pH of the extenders was maintained at 7.43 by means of buffers and kept in water bath at 37°C. Extender 2 (PENSTRIP) prepared by mixing 0.5 mL Penicillin with 0.5 mL Streptomycin, was the standard extender in which sperms survive. Extender 3 (test extender) was prepared by diluting a stock solution of chloroquine (50 mg mL^{-1}) to give graded concentrations of 2.5, 5.0, 7.5 and 10.0 mg mL^{-1}. One mL each from the doses was added to 10 mL of extender 1 and extender of 2 separately. Extender 4 (test extender) was prepared by diluting a stock solution of mefloquine (50 mg mL^{-1}) to give graded concentrations of 2.5, 5.0, 7.5 and 10.0 mg mL^{-1}. One mL each from the doses was added to 10 mL of extenders 1 and 2 separately. Extender 5 (test extender) was prepared by diluting a stock solution of sulphadoxine-pyrimethamine (50 mg mL^{-1}) to give graded concentrations 2.5, 5.0, 7.5 and 10.0 mg mL^{-1}. One mL each from the doses was added to 10 mL of extenders 1 and 2 separately. Each test dose was then added to 5 mL of extended semen in a sterilized universal bottle. The bottle was gently turned for proper mixing and the progressive motility was determined immediately. The remaining samples were stored at 5°C in the refrigerator and the progressive motility was determined 24 hourly for 5 days.

Progressive sperm motility study: The progressive motility of the extended semen sample of graded concentrations of chloroquine, mefloquine and sulphadoxine-pyrimethamine was done at an interval of 24 h for 5 days as previously described (Raji *et al*., 2005b).

Statistical analysis: Data are expressed as mean± standard error of the mean. The test of significance between two groups was estimated using the Students' t-test and ANOVA for more than two groups. $p<0.05$ was accepted to be significant.

RESULTS

Individual effects of chloroquine, mefloquine and sulphadoxine-pyrimethamine on body and organ weights of male albino rats *in vivo*: The control and drug treated rats showed a slight increase in body weight between the beginning of the study and autopsy (Table 1). There was no significant change in the mean testicular and epididymal weight of both drug treated and recovery groups when compared with the respective control groups.

Effects of chloroquine, mefloquine and sulphadoxine-pyrimethamine on sperm characteristics of rats *in vivo*: The total sperm count of drug treated rats showed a significant change ($p<0.01$) when compared with the control. There was also a significant decrease in percentage sperm motility ($p<0.01$) of rats in all groups in the order mefloquine > chloroquine > sulphadoxine-pyrimethamine when compared to that of the control. There was also an increase in the percentage of non-motile sperms in these rats when compared with the control. The recovery groups showed normal sperm counts, motility and morphology (Table 2).

Effects of chloroquine, mefloquine and sulphadoxine-pyrimethamine on sperm storage *ex vivo*: Table 3 shows the effect of chloroquine on semen storage using extenders 1 and 2 as controls. Only a slight insignificant change in percentage motility of spermatozoa was recorded in the drug treated extenders when compared with the control. Table 4 shows the effect of mefloquine on semen storage, using extenders 1 and 2 as controls. It was observed that mefloquine did not favour semen storage: the sperm percentage motility was significantly reduced ($p<0.01$) at all concentrations of the drug. The pH of the extender also reduced in a duration dependent manner. Table 5 shows the effect of sulphadoxine-

Table 1: Mean body weights of male rats treated with chloroquine, mefloquine and sulphadoxine-pyrimethamine

Group	Body weight (g)	Liver (g)	Testis (g)	Epididymis (g)
Control	220.0±7.09	4.67±0.13	0.53±0.04	0.92±0.05
Chloroquine, 10 mg kg^{-1} b.w.	221.0±9.50	6.30±1.10	1.15±0.03	0.90±0.04
(Chloroquine recovery)	222.0±9.10	5.46±0.52	1.15±0.03	0.91±0.05
Mefloquine, 10 mg kg^{-1} b.w.	236.0±10.10	6.25±0.02	0.97±0.01	0.88±0.06
(Mefloquine recovery)	219.5±10.50	9.09±0.03	1.32±0.01	0.90±0.03
Sulphadoxine-pyrimethamine 5 mg kg^{-1} b.w.	223.5±10.41	7.43±0.03	1.28±0.03	0.91±0.05
(Sulphadoxine-pyrimethamine recovery)	228.5±7.50	7.46±0.04	1.59±0.03	0.90±0.04

Table 2: Sperm counts, motility and abnormal morphology of male rats treated individually with chloroquine, mefloquine and sulphadoxine-pyrimethamine

Group	Sperm count million mL^{-1}	% Sperm motility	% Abnormal sperm morphology
Control	7.81±0.08	89.00±0.56	1.05±0.40
Chloroquine, 10 mg kg^{-1} b.w.	6.62±0.05	67.50±0.40	36.80±0.50
(Chloroquine recovery)	6.87±0.07	70.00±0.45	47.75±0.45
Mefloquine, 10 mg kg^{-1} b.w.	3.89±0.04	35.60±0.70	78.60±0.80
(Mefloquine recovery)	5.78±0.07	40.50±0.80	70.00±0.65
Sulphadoxine-pyrimethamine 5 mg kg 1 b.w.	7.52±0.09	80.00±0.95	23.40±0.50
(Sulphadoxine-pyrimethamine recovery)	7.56±0.05	85.50±0.60	12.70±0.70

Table 3: Effects of chloroquine on progressive sperm motility in extended buck semen stored at 5°C

Chloroquine	Extender 1 (EYC)* as Control	Extender 2	Extender 3				pH
% Concentration of chloroquine mg mL^{-1}	Nil	Nil	2.5	5.0	7.5	10.0	
% Motility immediately after the addition	80.00±0.00	80.00±0.00	80.00±0.00	80.00±0.00	80.00±0.00	80.00±0.00	7.43
% Motility after							
24 h	70.00±0.41	80.00±0.50	60.00±0.00	60.00±0.51	50.00±0.00	40.00±0.32	7.43
48 h	50.00±0.45	60.00±0.00	50.00±0.40	40.00±0.40	40.00±0.41	40.00±0.50	7.42
72 h	30.00±0.00	50.00±0.40	30.00±0.00	20.00±0.00	10.00±0.31	5.00±0.71	7.42
96 h	20.00±0.00	40.00±0.00	20.00±0.20	10.00±0.21	5.00±0.71	Nil	7.40
120 h	Nil	Nil	Nil	Nil	Nil	Nil	7.40

*EYC: Egg Yolk Sodium Citrate. pH at chloroquine concentration 10.0 mg mL^{-1} was measured

Table 4: Effects of mefloquine on progressive sperm motility in extended buck semen stored at 5°C

Mefloquine	Extender 1 (EYC)* as Control	Extender 2	Extender 4				pH
% Concentration of mefloquine mg mL^{-1}	Nil	Nil	2.5	5.0	7.5	10.0	
% Motility immediately after the addition	70.00±0.00	70.00±0.00	70.00±0.00	70.00±0.00	70.00±0.00	70.00±0.00	7.43
% Motility after							
24 h	60.00±1.41	60.00±0.71	5.00±0.00	5.00±0.71	Nil	Nil	7.10
48 h	50.00±1.14	50.00±0.00	Nil	Nil	Nil	Nil	7.01
72 h	40.00±0.00	40.00±1.41	Nil	Nil	Nil	Nil	6.61
96 h	20.00±0.00	20.00±0.00	Nil	Nil	Nil	Nil	6.59
120 h	Nil	Nil	Nil	Nil	Nil	Nil	6.54

*EYC: Egg Yolk Sodium Citrate. pH at mefloquine concentration 10.0 mg mL^{-1} was measured

Table 5: Effects of sulphadoxine-pyrimethamine on progressive sperm motility in extended buck semen stored at 5°C

Sulphadoxine-pyrimethamine	Extender 1 (EYC)* as Control	Extender 2	Extender 5				pH
% Concentration of sulphadoxine-pyrimethamine mg mL^{-1}	Nil	Nil	2.5	5.0	7.5	10.0	
% Motility immediately after the addition	70.00±0.00	70.00±0.00	70.00±0.00	70.00±0.00	70.00±0.00	70.00±0.00	7.43
% Motility after							
24 h	30.00±1.41	60.00±0.70	40.00±0.00	50.00±0.71	60.00±0.00	60.00±1.41	7.43
48 h	20.00±1.14	40.00±0.00	40.00±1.21	50.00±1.40	40.00±1.31	40.00±1.40	7.43
72 h	10.00±0.00	30.00±1.40	30.00±0.00	40.00±0.00	30.00±0.70	30.00±0.71	7.42
96 h	5.0±0.00	20.00±0.00	10.00±0.71	20.00±0.71	20.00±0.71	20.00±0.67	7.41
120 h	Nil	Nil	Nil	Nil	Nil	Nil	7.40

*EYC: Egg Yolk Sodium Citrate. pH at sulphadoxine-pyrimethamine concentration 10.0 mg mL^{-1} was measured

pyrimethamine on semen storage, using extenders 1 and 2 as controls. The percentage sperm motility was slightly but insignificantly reduced with sulphadoxine-pyrimethamine (more than those of chloroquine) when compared with the control.

DISCUSSION

The results showed that mefloquine and not probably chloroquine or sulphadoxine-pyrimethamine might cause reproductive impairment in male albino rat. Previous studies have shown that many antimalarial drugs possess some degree of antifertility action in male. Gonadotoxic effects of many antimicrobial agents (antibiotics and antimalarials) have been demonstrated throughout the animal kingdom. Approximately 50% of known causes of primary infertility are attributed to male factor (Yates *et al*., 1989); however the aetiology of male factor infertility is poorly understood. While certain individuals may be genetically predisposed to being subfertile (Reijo *et al*., 1996), there are many other factors, which have been implicated as potential causes of male infertility. Environmental pollutants such as smoking, consumption of alcohol and drug abuse have all been associated with male infertility. Now antimalarias; chloroquine and quinines (Sairam, 1978; Adeeko and Dada, 1994; 1998; Okanlawon and Ashiru, 1998) 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 (Vawva and Saade, 1987) and complete obliteration of Leydig cell response to leutropin and hormones having leutropin like activity *in vitro* (Sairam, 1978). 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 (Egbunike, 1989). Adeeko and Dada (1998) 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. In chloroquine treated rats; there was a disruption of spermatogenesis, which was accompanied by a decline in serum concentrations of testosterone (Okanlawon and Ashiru, 1998). These effects of chloroquine were reported in all cases to be reversible. Consentino *et al*. (1990), showed the ability of pyrimethamine to cause spermatogenic arrest and male infertility in mice in a dose dependent manner, though all the animals returned to their normal fertility status upon cessation of drug administration. The results of the present *ex vivo* study showed that sulphadoxine-pyrimethamine and chloroquine favoured semen storage. There was no previous report on the effects of antimalarial agents on semen storage *ex vivo*. The results of the present *ex vivo* study also clearly indicate that the site of action of chloroquine and sulphadoxine-pyrimethamine is not likely to be epididymis alone as it is likely to be for mefloquine. Reproductive toxins usually exert their adverse effects on pre-testicular, testicular or post-testicular sites and they may impair spermatogenesis directly or indirectly. Mefloquine might be acting at both the testicular and epididymal levels going by the results of the present *in vivo* and *ex vivo* studies. This findings being reported for the first time could be related to increased acidity produced by the drug or its metabolites. Mefloquine is highly bound to plasma proteins, concentrated in red blood cells and extensively distributed to tissues, including the central nervous system. Mefloquine is cleared in the liver and it's acid metabolites are slowly excreted mainly in the faeces. It's elimination half-life, which varies from 13 to 33 days, tend to be shortened in patients with acute malaria. The increase in acidity caused by mefloquine metabolites could have resulted in low sperm count, low sperm motility and increase in the number of abnormal sperm morphology.

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