

Full Length Research Paper

Role of oxidative stress in therapeutic administration of artesunate on sperm quality and testosterone level in male albino rats

Shittu, S. T.^{1*}, Oyeyemi, W. A.², Okewumi, T. A.² and Salman, T. M.³

¹Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

²Department of Physiology, Faculty of Basic Medical Science, Madonna University, Elele campus, Nigeria.

³Department of Physiology, Faculty of Basic Medical Science, University of Ilorin, Ilorin, Nigeria.

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The effects of antioxidants, vitamins C and E, on sperm quality, testosterone levels, superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration were studied in artesunate treated rats. 25 male rats (160 to 250 g) divided into five groups were used for the study. Group 1 animals received normal saline and served as control while groups 2 to 5 received artesunate in therapeutic doses of 2.9 mg/Kg body weight on day 1 and 1.45 mg/Kg body weight on days 2 to 5 orally. Following artesunate pretreatment, groups 3, 4 and 5 rats received vitamin C (100 mg/kg), vitamin E (100 mg/kg) and a combination of both respectively orally for the 5 days. There was no significant difference in sperm viability and motility in all groups while count significantly ($p < 0.05$) increased in group 3 animals treated with vitamin C. Serum testosterone level was significantly increased ($p < 0.05$) in groups 4 and 5. The MDA concentrations were significantly increased ($p < 0.05$) while SOD activity concurrently decreased significantly ($p < 0.05$) in groups 2, 3, and 5 indicating an oxidative-counter oxidative relationship. It was thus concluded that artesunate at therapeutic doses and duration had no significant effect on sperm quality and serum testosterone level in male rats while vitamin C, and combination of vitamins C and E tend to promote reproductive functions in artesunate-treated male rats.

Key words: Artesunate, vitamin C, vitamin E, sperm quality, superoxide dismutase activity, malondialdehyde concentration.

INTRODUCTION

Artesunate, a synthetic derivative of artemisinin which are major advance in anti-malarial treatment, is the most widely available of the artemisinin-related compounds (Woodrow et al., 2005). Oral artesunate either alone or in combination usually with other anti-malarial drug is increasingly used throughout the tropical World (WHO, 2003, 2008).

Most anti-malarial agents have been reported to possess various degrees of anti-fertility activities (Adeeko and Dada, 1998; Raji et al., 2005; Jewo et al., 2008; Obianime and Aprioku, 2009, 2011). Reactive oxygen

present in the artemisinin molecule due to its internal peroxide group established its linkage with oxidative stress (Ames et al., 1985). Oxidative stress has been reported as one of the means through which spermatozoa lose its functions (Aitken and Charkson, 1987).

Artesunate is considered to have high safety margins (Nosten and White, 2007), but its mechanism of action have been reported to involve the release of reactive oxygen species (ROS) that may lead to oxidative stress (Cumming et al., 1997; Paul et al., 2010). Functionally, low levels of ROS are beneficial and have been shown to stimulate sperm capacitation, enhance zona pellucida binding and promote acrosome reaction. In contrast, high levels of ROS are harmful and lead to lipid peroxidation of sperm plasma membrane and DNA fragmentation. Increased lipid peroxidation is associated with impaired

*Corresponding author. E-mail: drshittu@gmail.com. Tel: +2348052644680.

sperm motility and diminished capacity for sperm-oocyte fusion (Agarwal et al., 2003). There are natural anti-oxidants such as superoxide dismutase present in the body to prevent oxidative stress. Because spermatozoa are such specialized cells, they lack the common cytoplasmic antioxidants found in other cell types and are therefore particularly sensitive to oxidative damage (Wen, 2006). Vitamins C and E are potent antioxidants that are useful in the body to maintain redox homeostasis (Padayatty et al., 2003). These vitamins have been reported to have protective effect on spermatozoa against endogenous oxidative DNA damage and membrane damage (Sikka, 2004). Vitamin C may benefit fertility in its ability to promote collagen synthesis, its role in hormone production and its ability to protect cells from free radicals (Luck et al., 1995). Vitamin E inhibits peroxidation of polyunsaturated fatty acids (PUFA), which is especially important in spermatozoa due to their high PUFA content (Bolle et al., 2002).

With increasing demand for artesunate in the treatment of malaria due to resistance to some anti-malarial drugs like chloroquine which had also been reported to have adverse effect on sperm quality with its amelioration by vitamin C and vitamin E (Salman and Ajayi, 2006); and in the light of spermatotoxicity effect of artesunate reported in male rats due to over dose usage (Jewo et al., 2008; Izunya et al., 2010) and decreased sperm quality and serum testosterone level of male guinea pigs treated with artesunate (Obianime and Aprioku, 2009; 2011). Although, the studies of Obianime and Aprioku (2011) implicated oxidative stress following therapeutic administration of artesunate but it was not evaluated, it is then desirable to evaluate the effect of artesunate at therapeutic dose and duration as well as probable role of antioxidants vitamins C and E on sperm quality, testosterone level, and indices of oxidative stress such as superoxide dismutase activity and malondialdehyde concentration in male albino rats.

MATERIALS AND METHODS

Animals and drugs

Twenty five (25) male albino rats of wistar strain weighing between 160 to 250 g were used for the study. The animals were housed and acclimatized for two weeks in the Central Animal house of the Faculty of Basic Medical Science, College of Health Sciences, University of Ilorin, Ilorin, Nigeria. They were fed on standard rat pellet diet (Ladoko Feeds, Nigeria) and were allowed access to tap water *ad libitum*. The animals were maintained under standard laboratory conditions and were subjected to natural photoperiod of 12 h light: dark cycle. All experimental protocols and handling were in compliance with the NIH publication No 85-23 guidelines (NIH publication revised, 1985). Artesunate, vitamins C and E were of analytical grade and products of Tuyil Pharmaceutical Ltd. Ilorin, Kwara state, Nigeria.

Treatments

The animals were randomly divided into five groups of five rats.

Group 1 animals served as control and were given normal saline in same volume as the treated groups received for 5 days by oral gavage. Animals in group 2 were given 2.9 mg/Kg body weight of artesunate in the first day orally, and 1.45 mg/Kg body weight of artesunate for the remaining four days. Group 3, 4 and 5 rats were treated with artesunate as in group 2 but followed with 100 mg/kg of vitamin C, 100 mg/kg of vitamin E or a combination of both respectively for the entire duration of the study. At the end of the 5 days treatment, all rats were anaesthetized by 0.6 ml/100 g body weight of 25% urethane and the testis and epididymis were excised. Blood was taken by intra-cardiac puncture for serum estimation of testosterone, superoxide dismutase activity and malondialdehyde concentration. The animals were thereafter euthanized by cervical dislocation.

Sperm characteristic analysis

The testes from each rat were carefully exposed and one of them was removed together with its epididymis. The epididymis was separated and the epididymal fluid was collected from the caudal part. The progressive sperm motility and sperm count were determined as recommended by WHO (1987). The numbers of motile spermatozoa were calculated per unit area and expressed as percentage motility. Sperm counts were done using a haemocytometer and the results were expressed as million/ml of sperm suspension (Baker, 2007), briefly the epididymis was homogenized in 5 ml normal saline and the homogenate were further diluted to 1/200 and 10 μ l of the aliquot was loaded on a side of the Neubauer counting chamber and placed in a moist chamber for 10 to 15 min to allow settling of the spermatozoa. Thereafter, it was viewed at 400x magnification, the central grid was located and sperm cells were counted in five squares within the central grid.

Sperm viability was carried out by preparing a uniform smear of the spermatozoa on slides with eosin/nigrosin stains and allowed to air dry. Live sperm appear white and the dead sperm were stained red against a dark background when viewed under light microscope. Hundred sperm cells were counted per slide in order to obtain the percentage of live/death ratio (Baker, 2007).

Analysis of serum testosterone levels

Serum testosterone level was determined with the tube-based enzyme immune assay (EIA) method. The EIA testosterone kit obtained from C.C Obi (Lagos, Nigeria) was a product of immunometrics (London, UK). The procedures for the assay as contained in the manufacturer's manual were strictly followed; the within assay variation was 8.1% and sensitivity was 0.3 ng/ml. The optical density was read by using spectrophotometer that was sensitive at wavelength of 450.

Determination of MDA concentration

The assay method of Hunter et al. (1963) modified by Gutteridge and Wilkins (1982) was adopted. Malondialdehyde, a product of lipid peroxidation, when heated with 2-thiobarbituric acid (TBA) under acid conditions forms a pink colored product which has a maximum absorbance of 532 nm. 2 ml of 0.7% TBA and 1 ml of glacial acetic acid were added to 2 ml of serum. The mixture was thoroughly mixed and incubated in water bath at 80°C for 20 min. It was then allowed to cool and centrifuged at 400 rev/min for 10 min. Absorbance of the supernatant was read at 532 nm against a blank wherein serum was substituted with distilled water. The results were expressed as nanomoles MDA/ml.

Table 1. Effects of vitamins C and E on sperm motility, viability, count, testosterone level, MDA concentration and SOD activity in Artesunate treated rats.

Parameter	Control	Art	Art+C	Art+E	Art+C+E
Sperm motility (%)	61.00 ± 6.00	57.17 ± 8.60	75.00 ± 5.00*	54.00±3.54	59.00±7.14
Sperm viability (%)	67.00 ± 7.35	56.67 ± 7.60	69.00 ± 4.30	56.00±5.10	76.00±4.30
Sperm count (10 ⁶ /ml)	27.60 ± 1.66	27.20 ± 1.39	32.80±1.02*	27.20±0.73	29.20±1.02
Testosterone (ng/ml)	0.12 ± 0.02	0.14 ± 0.04	0.10 ± 0.00	0.22±0.07*	0.22±0.05*
MDA Conc. (nM/ml)	0.88 ± 0.15	5.71 ± 0.87*	4.98±0.23*	0.77±0.12	2.80±0.13*
SOD activity (Specific activity/min)	16.17 ± 2.72	10.43 ± 1.33*	13.20±2.94*	17.10±1.99	12.8±0.58*

Values are mean ± SEM (n = 5 rats per group). Art, Artesunate only treated; Art+C, artesunate and vitamin C; Art+E, artesunate and vitamin E; Art+C+E, artesunate and combination of vitamins C and E. *Significant difference (p < 0.05) when compared with control.

Determination of superoxide dismutase (SOD) activity

A method originally described by Misra and Fridovich (1972) was employed. This method involves inhibition of epinephrine auto-oxidation, in an alkaline medium at 480 nm in an ultraviolet spectrum. For the determination of specific activity of SOD in 1 ml of blood serum, the rate of autoxidation of epinephrine was noted at 30 s intervals in all groups. Briefly, the serum was diluted to make a 1/10 dilution. 0.2 ml of the aliquot was supplemented with 2.5 ml of 0.05 M phosphate buffer (pH 10.2) and equilibrated at room temperature. 0.3 ml of 0.3 nM adrenaline solution was then added to the reference and the test solution, followed by mixing by inversion and absorbance at 480 nm was monitored every 30 s over 150 s. Increase in absorbance per minute was obtained and used to calculate percentage inhibition. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation as 1 unit of SOD specific activity which is the amount of SOD to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 min.

Statistical analysis

The data were expressed as mean and standard error of mean (mean ± SEM). Statistical significance between the groups was assessed by one way analysis of variance (ANOVA) and students't-test was used to compare difference between two means. Differences in means were considered significant at P < 0.05. All analyses were performed using SPSS 15 package.

RESULTS

The results of this study are shown in Table 1. There was no significant difference in the sperm viability of all the groups. Sperm counts motility and significantly increased in rats treated with artesunate and vitamin C (P < 0.05). The result also showed significant increase in serum testosterone level of rats in the group supplemented with vitamin E and the group that received a combination of the two vitamins. Lipid peroxidation, as shown by MDA concentration, significantly increased in groups administered with artesunate only, artesunate with vitamin C and artesunate with a combination of vitamins C and E (P < 0.05) while the group that received artesunate and vitamin E was not significantly different from the control. Superoxide dismutase activity significantly reduced (p < 0.05) in all rats treated with artesunate except those that

further received vitamin E only which apparently increased when compared with the control group. However, when compared with artesunate treated rats, SOD activity increased in all the groups but significantly only in the rats treated with artesunate and vitamin E.

DISCUSSION

This study observed an apparent but not significant reduction in sperm motility, viability and no difference in sperm count in the artesunate treated rats which is consistent with the available information on its insignificant reduction in sperm count (WHO, 2003; 2008). The significant increase in sperm count observed in the rats treated with artesunate and vitamin C is also consistent with the positive influence of vitamin C on male reproductive function earlier reported by Jewo et al. (2008) and Obianime and Aprioku (2011). Vitamin C had also been reported to improve semen quality in smokers (Yousef et al., 2003; Akmal et al., 2006) and in co-administration with chloroquine (Salman and Ajayi, 2006).

The observed increase in sperm viability of rats treated with artesunate, vitamins C and E although insignificant (p > 0.05) supports earlier report by Salman and Ajayi (2006) that vitamins C and E may ameliorate the antifertility effect of chloroquine in male rats. However, sperm motility and counts of this group were not different when compared with the control. The ability of vitamin C to synergistically regenerate vitamin E (Groff et al., 1995; Cossins et al., 1998) might explain the increase in sperm count and motility observed in the group treated with artesunate and a combination of vitamin C and E when compared with rats treated with artesunate and vitamin E only. Vitamin E may directly quench the free radicals such as peroxy and alkoxy (ROO[•]) generated during ferrous ascorbate-induced lipid peroxidation, thus it is suggested as major chain breaking antioxidant (Bansal and Bilaspuri, 2009) which confers a better stability on the sperm cells (Bansal and Bilaspuri, 2011).

Also, the result of this study shows that therapeutic dose of artesunate did not cause decrease in serum testosterone level in male rats. This is contrary to the decrease in serum testosterone level earlier reported (Jewo et

al., 2008), a discrepancy that may be attributed to an overdose usage. Vitamin E caused a significant increase in the serum testosterone level in the artesunate treated rats and combined administration of vitamin C and vitamin E also significantly increased serum testosterone level when compared with rats treated with artesunate and artesunate with vitamin C only. These effects taken together could be explained by the protective effect exerted by vitamin E on Leydig cell steroidogenesis (Chen et al., 2005) bearing in mind the regeneration of vitamin E by vitamin C (Cossins et al., 1998; Groff et al., 1995).

The observed superoxide dismutase activity in this study is in accordance with the reported complementary actions of all the antioxidants (Sikka, 1996). The significant increase in MDA concentration observed in artesunate treated rats may be due to the oxidative property of artesunate (Cumming et al., 1997; Paul, et al., 2010). This increase was significantly decreased in the vitamin C and combination of vitamin C and E ($p < 0.05$) treated groups; an observation which is in line with the report of Sikka (2004) documenting the antioxidative potency of vitamin C and vitamin E in the prevention of lipid peroxidation in sperm cells. It is worthy to note from this study that the decrease in SOD activity is characterised with a concomitant increase in MDA concentration thereby showcasing an oxidative and counter oxidative relationship. This indicated that the mechanism of action of artesunate on the apparent differences in the sperm characteristic is via induction of oxidative stress.

It was thus concluded from this study that artesunate given at therapeutic doses and duration had no significant effect on the sperm quality and serum testosterone level in male rats and the anti-oxidative activities of vitamin C, and combination of vitamin C and vitamin E promoted reproductive functions of artesunate treated rats.

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