First International Journal of Andrology
ANDROLOGIA

ORIGINAL ARTICLE

Sperm characteristics, antioxidant status and hormonal profile in rats treated with artemisinin

E. O. Farombi, I. A. Adedara, A. O. Abolaji, J. P. Anamelechi & J. O. Sangodele

Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria

Keywords
Artemisinin—endocrine—oxidative stress—rats—reproductive toxicity

Correspondence
Prof. Ebenezer Olatunde Farombi, Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.
Tel.: +234 8023470333;
Fax: +234 2 8103043;
E-mails: olatunde_farombi@yahoo.com; eo.farombi@mail.ui.edu.ng

Accepted: August 6, 2013

doi: 10.1111/and.12170

Summary

The indiscriminate use, abuse and patients' noncompliance to normal prescription of artemisinin and its derivatives are a common practice during the treatment for drug-resistant malaria parasites in most developing countries. This study investigated the influence of artemisinin on the testicular and epididymal sperm antioxidant systems as well as on the plasma levels of hormones from the pituitary and thyroid components of the brain–pituitary–testicular axis. Oral exposure of rats to 0, 7 and 35 mg kg^{-1} artemisinin for 7 days showed that the testicular antioxidant status at both therapeutic dose (7 mg kg^{-1}) and overdose (35 mg kg^{-1}), and the sperm antioxidant status at therapeutic dose of artemisinin remained unaffected compared with control. However, increased hydrogen peroxide and lipid peroxidation levels were accompanied by a concomitant decrease in glutathione peroxidase and glutathione-S-transferase activities as well as glutathione level in spermatozoon of rats administered with overdose of artemisinin. While plasma levels of all the hormones investigated remained unaffected, severe epididymal degeneration with concomitant decrease in sperm quantity and quality was observed in rats treated with overdose of artemisinin compared with control. Overall, induction of oxidative stress in the epididymis, but not in the testes, could cause reproductive deficits in individuals unduly undergoing artemisinin therapy.

Introduction

Malaria is a serious mosquito-borne infectious disease of humans and remains a major public health challenge in endemic regions including countries from South and Central America, Africa and Asia. Malaria has been reported to be the major cause of mortality in children below the age of five (Aultman *et al.*, 2002). A variety of antimalarial medications are available, but their effectiveness has faced several challenges such as the development of resistance of the parasite and adverse effects of antimalarial agents (Wellems & Plowe, 2001; Yeung *et al.*, 2004), thus creating a need for new drugs that are well tolerated and simple to use. Artemisinin (Fig. 1) is an endoperoxide sesquiterpene lactone which occurs naturally in leaves and flowers of *Artemisia annua* L (Boareto *et al.*, 2008). The unusual peroxide bridge in artemisinin is believed to be responsible for the drug's mechanism of action. Artemisinin and its derivatives including artesunate, dihydroartemisinin (DHA), artemether and arteether are the current drugs of choice for malaria treatment and have been reported to be highly efficacious with minimal toxicity (Mulenga, 1998; Haynes, 2001).

The effectiveness of artemisinin-based treatments has been reported to be due to the high schizonticidal potentials and has made them to be the main chemotherapeutic agents for malaria treatment (WHO, 1995; Nosten & White, 2007). However, owing to the effectiveness of artemisinin and its derivatives in the treatment for malaria, the indiscriminate use, abuse and patients noncompliance to therapeutic regimens are common among the population where malaria is prevalent. Nowadays, a major percentage of infertility problems affect the males (Agarwal *et al.*, 2008). Data on adult couples in African countries showed that 10–25% are subfertile, and male-factor infertility accounts for 30–40% of these cases with Nigeria having approximately 12 million infertile persons (Giwa-Osagie, 2003). The deterioration of male reproductive health of humans and animals has been attributed to several causative factors including environmental

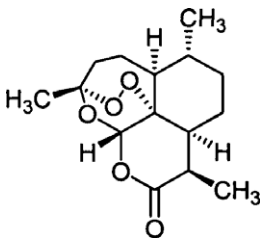

Fig. 1 Chemical structure of artemisinin.

contaminants, therapeutics and lifestyle factors. The adverse effects of these xenobiotics may occur via the hypothalamic–pituitary–gonadal axis or a direct toxic effect on the gonads. There is a need to avoid the risk of infertility that could result from malaria chemotherapy. The evaluation of these antimalarial drugs for possible reproductive toxicity especially at doses higher than recommended doses becomes very important as both malaria and infertility are of global concern.

The semen is used for reproductive toxicity studies because it is a more accessible reproductive endpoint, which can be easily analysed by well-established procedures (WHO, 1999). The routine semen analysis involves the determination of sperm concentration, motility, viability and morphology. The present investigation was therefore undertaken to determine the effect of different concentrations of pure artemisinin on certain sperm characteristics, reproductive hormones, oxidative stress indices and histology of the testes and epididymis in Wistar rats.

Materials and methods

Chemicals

Artemisinin (Sigma Chemical Co., St Louis, USA, 98%) was a gift from Professor Andi Brisibe of the Molecular Bioscience Limited, Calabar, Nigeria. Epinephrine, 5,5′-dithio-bis-2-nitobenzoic acid (DTNB), hydrogen peroxide, thiobarbituric acid (TBA), 1-chloro-2,4-dinitrobenzene (CDNB), trichloroacetic acid (TCA), reduced glutathione (GSH), bovine serum albumin (BSA), Tris base and adenosine monophosphate (AMP) were purchased from Sigma Chemical Co. All other reagents were of analytic grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Experimental design

Thirty healthy adult male Wistar rats weighing approximately 155 ± 3 g, obtained from the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria, were randomly assigned to three groups of ten animals each. They were housed in plastic cages placed in a well-ventilated rat house, provided with rat pellets and water *ad libitum* and subjected to photoperiod of 12-h light/12-h dark. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. The experiment was performed according to the guidelines and approval of institutional animal ethics committee.

Group I rats were administered corn oil alone and served as control. Artemisinin was dissolved in corn oil and administered orally at therapeutic dose of 7 mg kg^{-1} body weight to a group II rats, while group III rats received an overdose of 35 mg kg^{-1} body weight daily for 7 days. The doses of artemisinin and the duration of experiment were chosen from previously published studies and the recommendation for artemisinin monotherapy (Adjuik *et al.*, 2004; Boareto *et al.*, 2008). Twenty-four hours after the last treatment, the blood from each group was drawn from retro-orbital venous plexus for hormonal assay before they were sacrificed by cervical dislocation. The testes, epididymides, seminal vesicles and prostate glands were quickly removed, weighed and kept frozen. The body weights of rats were recorded before exposure to various treatments and again before they were killed.

Sperm motility assay

The motility of the spermatozoon from the control and artemisinin-treated rats was evaluated according to the method of Zemjanis (1970). Briefly, epididymal spermatozoon was obtained by cutting the cauda epididymidis with surgical blades and released onto a sterile clean glass slide. The spermatozoon was then diluted with 2.9% sodium citrate dehydrate solution and thoroughly mixed to assess the sperm progressive motility with the aid of a microscope within 2–4 min of their isolation and data expressed as percentages.

Sperm count

Epididymal sperm count was obtained by mincing the caudal epididymis in four volume of distilled water and filtering through a nylon mesh. The spermatozoa were counted by haemocytometer using the improved Neubauer (Deep 1/10 m; LABART, Munich, Germany) chamber according to Pant & Srivastava (2003).

Determination of daily sperm production and testicular sperm number

Daily sperm production was determined using seven frozen left testes from control and artemisinin-treated rats

according to Joyce *et al.* (1993). Briefly, after the testes were removed and weighed, they were homogenised for three minutes in 25 ml physiological saline containing 0.05% (v/v) Triton X-100. Sample aliquots of the 5.5 µl were then placed on the haemocytometer and counted twice at 100× magnification under a microscope to determine the average number of spermatids per sample. These values were used to obtain the total number of spermatids per testis, and this number was then divided by the testes weight to yield spermatids per gram of testes. Developing spermatids spend 4.61 days in rats. Therefore, the values for the number of spermatids per testis were divided by 4.61 to obtain daily sperm production.

Morphologic abnormalities and percentage viability assay

A portion of the sperm suspension placed on a glass slide was smeared out with another slide and stained with Wells and Awa's stain (0.2 g eosin and 0.6 g fast green dissolved in distilled water and ethanol in a 2 : 1 ratio) for morphologic examination and with 1% eosin and 5% nigrosine in 3% sodium citrate dehydrate solution for viability according to Wells & Awa (1970). A total of 400 spermatozoa from each rat were used for morphologic examination.

Preparation of plasma

Five millilitres of the whole blood collected from each rat in centrifuge tubes containing anticoagulant (EDTA) was immediately centrifuged at 3000 ***g*** for 10 min at 4 °C and stored at −20 °C prior to hormone assaying.

Evaluation of plasma testosterone, luteinising hormone (LH) and follicle-stimulating hormone (FSH) concentrations

The plasma concentrations of testosterone, LH and FSH were assayed using the commercial enzyme immunoassay kits specific for rats according to the manufacturer's instructions [testosterone (EIA-5179) from DRG Diagnostics, whereas FSH (RPN 2560) and LH (RPN 2562) from Amersham, UK]. The sensitivity of the testosterone assay was 0.06 ng ml^{-1} and with negligible cross-reactivity with other androgen derivatives, such as androstenedione, 5α–dihydrotestosterone and methyl testosterone. The intra-assay coefficients of variation for the testosterone assay were 5.2%. The sensitivity of LH was 0.07 ng at 80%, whereas FSH sensitivity was 0.03 ng at 98%. The intra-assay coefficients of variation were 3.5% for LH and 3.4% for FSH.

Evaluation of plasma triiodothyronine and thyroxine concentrations

The total plasma triiodothyronine and thyroxine concentrations were determined using the commercial enzyme immunoassay kits (DiaSorin, Sauggia, Italy) according to the manufacturer's instructions. Intra-assays coefficients of variation for total thyroxine were 2.5–3.2%, whereas for total triiodothyronine, 3.3–6.3%. Sensitivity of the assays was 80 pg ml^{-1} for total thyroxine and 310 pg ml^{-1} for total triiodothyronine. All the samples were assayed on the same day to avoid the inter-assay variation. The total plasma triiodothyronine and thyroxine concentrations were expressed as ng/dl and ug/dl respectively.

Collection of epididymal spermatozoon

The spermatozoa from the control and artemisinin-treated rats were collected according to Adedara & Farombi (2012). Briefly, spermatozoa were collected as quickly as possible after a rat was killed. Each right cauda epididymis was placed in eight volume of cold phosphate buffer saline solution and cut with surgical blades into pieces. The solution was pipetted several times to obtain the sperm suspension and then filtered through a nylon mesh. The sperm suspensions were subsequently homogenised at 4 °C with a glass Teflon homogeniser for 10 s and centrifuged at 2000 ***g*** for 10 min to obtain the supernatant, which was used for biochemical assays.

Biochemical assays

The right testes were homogenised in four volume of 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride. The resulting homogenate was centrifuged at 10 000 ***g*** for 15 min at 4 °C, and the supernatant was thereafter collected for the estimation of superoxide dismutase (SOD) activity by the method described by Misra & Fridovich (1972). Catalase (CAT) activity was determined using hydrogen peroxide as a substrate according to the method of Clairborne (1995). Glutathione-S-transferase (GST) was assayed by the method of Habig *et al.* (1974). Reduced GSH was determined at 412 nm using the method described by Jollow *et al.* (1974). Glutathione peroxidase (GPX) activity was determined according to the method of Rotruck *et al.* (1973). Testicular 5′ nucleotidase (5′-NTD) was determined following the method described by Goodlad & Clark (1982). Protein concentration was determined by the method of Lowry *et al.* (1951). Hydrogen peroxide generation was assessed by the method of Wolff (1994). Lipid peroxidation was quantified as MDA according to the method

described by Farombi *et al.* (2000) and expressed as Unit MDA/mg protein.

Histopathology

Testes and epididymis biopsies were carefully removed and fixed with Bouin's solution. After dehydration procedures, the samples were blocked in paraffin. Sections of 4–5 μm were prepared by a microtome and stained with haematoxylin and eosin (H & E). All slides were coded before examination with light microscope and photographed using a digital camera by investigators who were blinded to control and artemisinin-treated groups.

Statistical analysis

Statistical analyses were carried out using one-way analysis of variance, followed by Student's *t*-test to compare experimental groups. $P < 0.05$ was considered statistically significant.

Results

Artemisinin treatment did not affect the body weight gain and organ weights in rats

Effect of therapeutic dose (7 mg kg^{-1}) and overdose (35 mg kg^{-1}) of artemisinin on the absolute and the relative weights (organ weight/100 g body weight) of testes, epididymis, seminal vesicle and prostate gland was determined following treatment period. The data presented on body weight gain and organs weights in Table 1 showed there was no significant differences observed in the artemisinin-treated rats and those of the control group.

Table 1 Effects of artemisinin on body weight gain, absolute and relative organ weights in male rats

End point	Control	7 mg kg^{-1}	35 mg kg^{-1}
Body weight gain	23.71 ± 9.03	22.14 ± 9.21	25.71 ± 8.43
Testes	1.27 ± 0.48	1.20 ± 0.53	1.19 ± 0.37
Testes (g/100 g bw)	0.73 ± 0.17	0.68 ± 0.13	0.69 ± 0.18
Epididymis	0.53 ± 0.01	0.54 ± 0.02	0.61 ± 0.61
Epididymis (g/100 g bw)	0.31 ± 0.02	0.32 ± 0.03	0.34 ± 0.01
Seminal vesicle	0.25 ± 0.03	0.24 ± 0.11	0.26 ± 0.22
Seminal vesicle (g/100 g bw)	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.02
Prostate gland	0.12 ± 0.04	0.13 ± 0.03	0.11 ± 0.04
Prostate gland (g/100 g bw)	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01

The data are expressed as mean ± SD for ten rats per group.

Alteration of antioxidant enzymes activities in testes and spermatozoon of artemisinin-treated rats

Table 2 reveals the activities of antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) in the testes and spermatozoon following administration of artemisinin in rats after 7 days. Acute administration of therapeutic dose and overdose of artemisinin caused a significant increase in the SOD and CAT activity, whereas activities of GST and GPx were unaffected in testes of the treated rats when compared with control group. Moreover, the activities of these antioxidant enzymes remain unaffected in spermatozoon of rats administered with therapeutic dose of artemisinin. Conversely, the significant increase in the SOD activity was accompanied by a marked decrease in GST and GPx activities without affecting CAT in spermatozoon of the rats treated with overdose of artemisinin when compared with the control.

Overdose of artemisinin induced oxidative stress in spermatozoon, but not in testes of rats

The results presented in Fig. 2 showed that both therapeutic dose and overdose of artemisinin treatment did not significantly alter the levels of GSH, H_2O_2 and MDA in testes of the treated rats. However, overdose of artemisinin markedly decreased GSH level and significantly increased the levels of H_2O_2 and MDA in the spermatozoon of the treated rats above the control.

Overdose of artemisinin did not affect hormonal homeostasis in rats

The influence of artemisinin administration on plasma concentrations of LH, FSH, testosterone, T_3 and T_4 in rats is presented in Table 3. Hormonal homeostasis was unaffected in rats exposed to both therapeutic dose and the overdose of artemisinin. Circulatory concentrations of LH, FSH, testosterone, T_3 and T_4 testosterone levels in artemisinin-treated rats were not statistically different from control rats.

Overdose of artemisinin decreased epididymal sperm quantity and quality in rats

Effects of therapeutic dose and overdose of artemisinin on sperm characteristics after the treatment period are presented in Fig. 3. The rats administered with the therapeutic dose of artemisinin demonstrated significant decrease in sperm motility, while the epididymal sperm

Table 2 Testicular and sperm antioxidant enzymes activities following artemisinin treatment in male rats

	Testes			Spermatozoon		
End point	Control	7 mg kg^{-1}	35 mg kg^{-1}	Control	7 mg kg^{-1}	35 mg kg^{-1}
SOD	1.82 ± 0.53	2.51 ± 0.11*	3.35 ± 0.35*	0.66 ± 0.27	0.84 ± 0.18	0.98 ± 0.23*
CAT	62.62 ± 3.73	64.53 ± 3.62*	72.09 ± 2.01*	22.74 ± 5.87	24.49 ± 4.71	24.76 ± 3.23
GPx	19.32 ± 0.41	17.67 ± 0.43	16.34 ± 0.55	35.04 ± 2.28	34.71 ± 2.11	25.04 ± 1.17*
GST	6.41 ± 0.35	7.92 ± 0.24	8.85 ± 0.17	1.05 ± 0.14	0.84 ± 0.11	0.75 ± 0.07*

SOD activity (Units per milligram protein), CAT activity (μmole H_2O_2 consumed per minute per milligram protein), GPx activity (Units per milligram protein), GST activity (μmole CDNB–GSH complex formed per minute per milligram protein). The data are expressed as mean ± SD for ten rats per group.
*$P < 0.05$ against control.

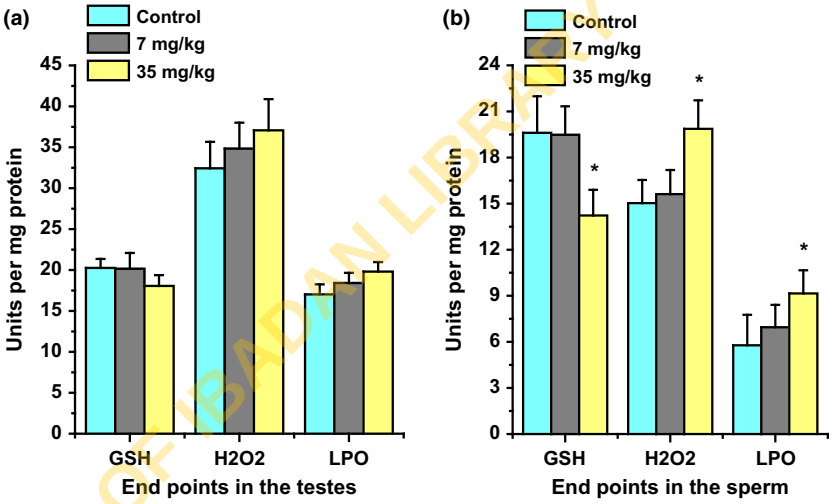

Fig. 2 Biomarkers of oxidative stress namely GSH, H2O2 and LPO levels were determined in testes (a) and sperm (b) of the control and artemisinin-treated rats after 7 day. Each bar represents mean ± SD of ten rats. Values with asterisks were significantly different from control (*$P < 0.05$).

Table 3 Hormonal profile in rats treated with artemisinin for 7 days

End points	Control	7 mg kg^{-1}	35 mg kg^{-1}
LH (ng ml^{-1})	7.28 ± 0.63	7.32 ± 0.81	7.36 ± 0.72
FSH (ng ml^{-1})	6.88 ± 0.15	7.03 ± 0.17	7.08 ± 0.11
Testosterone (ng ml^{-1})	4.03 ± 0.16	4.04 ± 0.14	4.08 ± 0.16
T_3 (ng dl^{-1})	0.23 ± 0.03	0.23 ± 0.02	0.22 ± 0.03
T_4 (μg dl^{-1})	1.13 ± 0.19	1.10 ± 0.17	1.11 ± 0.18

LH, luteinizing hormone; FSH, follicle-stimulating hormone; T_3, triiodothyronine; T_4, tetraiodothyronine.
The data are expressed as mean ± sd for ten rats per group.

number (ESN), viability and abnormality were unaffected. The administration of overdose of artemisinin resulted in a significant decrease in the ESN, sperm progressive motility, viability but elevated sperm abnormality significantly when compared with the control. The abnormalities observed in spermatozoon of artemisinin-treated rats were majorly tailless heads, curved mid-pieces and bent mid-pieces, whereas bent tails and rudimentary tails occurred less frequently.

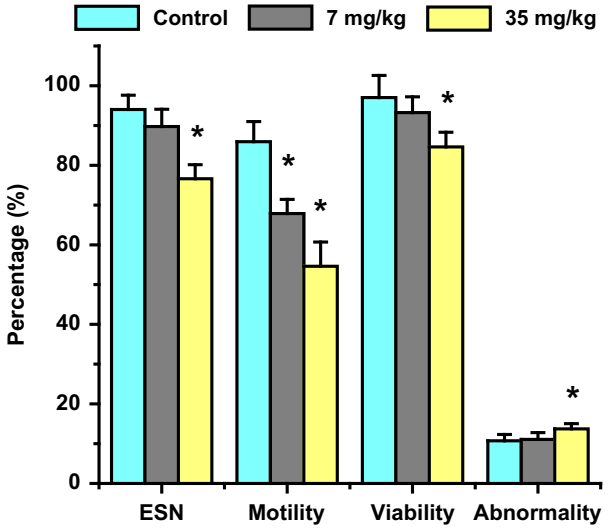

Fig. 3 Influence of artemisinin on sperm characteristics in experimental rats after 7 days. Epididymal sperm number (ESN). Each bar represents mean ± SD of ten rats. * Values differ significantly from control ($P < 0.05$).

Influence of artemisinin overdose on daily sperm production, testicular sperm number and testicular 5′-nucleotidase activity

Figure 4 shows the effects of artemisinin on the daily sperm production (DSP), testicular sperm number (TSN) and 5′-nucleotidase activity in the testes of the treated rats. There was no statistical significance observed in the daily sperm production and testicular sperm number in artemisinin-treated rats in comparison with control rats. However, there was a significant increase in the activity of 5′-nucleotidase in rats administered with overdose of artemisinin when compared with the control.

Overdose of artemisinin induced histopathological changes in testes and epididymis of rats

Histopathological changes observed with the light microscope in the testes and epididymis sections from control and artemisinin-treated rats are presented in Fig. 5. The testes of the control (A) and artemisinin (therapeutic)-treated (B) rats showed normal structurally and functionally active seminiferous tubules with many sperm and adequate numbers of the testicular epidermal cells. Testes of rats treated with overdose of artemisinin (C) showed mild oedema at the interstitium with reduced sperm numbers in the seminiferous tubules. The epididymis of the control (A) and artemisinin (therapeutic)-treated (B) rats showed normal architecture with adequate sperm cells, and the walls of the epididymis appear stratified and thickened. However, epididymis of rats treated with overdose of artemisinin (C) showed severe erosion of the epididymal lining, enlarged interstitium with fibrillar pink staining materials and few fibrocytes.

Discussion

In spite of the success in the design of drugs which are selectively toxic against microorganisms or parasites, while sparing the host cells, many chemotherapeutic agents have been reported to produce toxic adverse effects

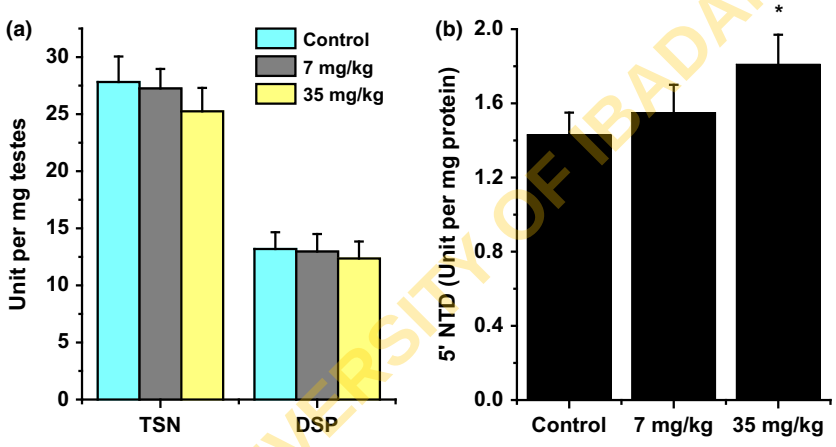

Fig. 4 Influence of artemisinin on testicular sperm number (TSN) and daily sperm production (DSP) (a) and testicular 5′ nucleotidase activity (b) in experimental rats after 7 days. Each bar represents mean ± SD of ten rats. *Values differ significantly from control ($P < 0.05$).

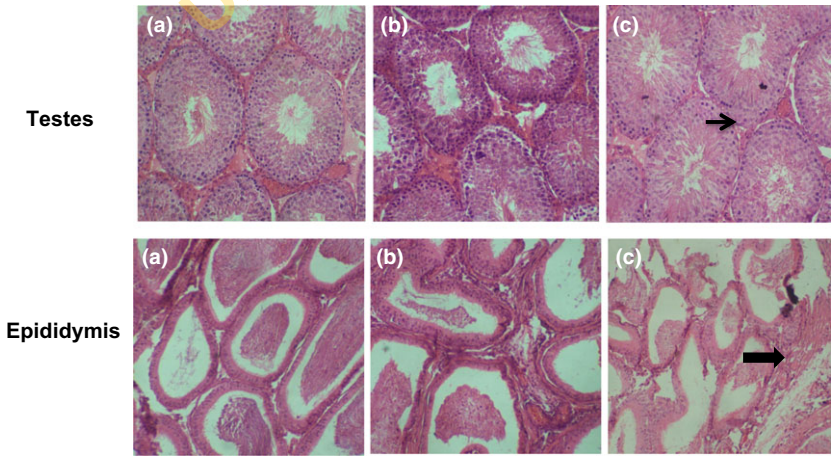

Fig. 5 Histopathology guide of organs from control (a), therapeutic dose (b) and overdose (c) of artemisinin-treated rats. The upper panel shows representative photomicrographs of testes from control and artemisinin-treated rats. Arrow head indicates oedema in testes of rats treated with the overdose of artemisinin. The lower panel reveals epididymis histopathology of control and artemisinin-treated rats. Black arrows indicates fibrillar pink staining materials and few fibrocytes in epididymis of rats treated with the overdose of artemisinin. 160×.

in the male reproductive organs. The toxicities resulting from chemotherapy is related to the type of drugs used, the total dose and the duration of therapy (Cherry *et al.*, 2004; Prahalathan *et al.*, 2006; Oyagbemi *et al.*, 2010). The results of the study showed that administration of therapeutic dose and overdose of artemisinin did not cause any significant changes in the body weight gain, absolute and relative organ weights in the treated rats. The lack of effect on the body weight gain in artemisinin-treated rats indicates that the general metabolic functions of the animals are in the normal range.

In mammalian male reproduction, the hormonal control of reproduction is based on the action of luteinizing hormone (LH) to stimulate the Leydig cells in the testes to produce testosterone, which is vital in the initiation and maintenance of spermatogenesis by affecting Sertoli cell androgen receptors. Follicle-stimulating hormone (FSH) is also required for quantitatively normal spermatogenesis in pubertal rats (Russell *et al.*, 1987; Sriraman *et al.*, 2005). The present study showed that there were no alterations in the plasma levels of LH, FSH and testosterone following therapeutic dose and overdose administration of artemisinin in rats. The normal gonadotrophin levels observed in artemisinin-treated rats in the present study possibly suggest the safety of artemisinin exposure on the anterior pituitary gland. The anterior lobe of the pituitary gland through thyrotropin regulates thyroid hormones which play a critical role in the physiological regulation of growth and development of the male reproductive organs as well as in the testicular basal metabolic activity (Jannini *et al.*, 1995; Maran, 2003; Adedara & Farombi, 2013). The lack of significant changes in the plasma concentrations of triodothyronine and tetraiodothyronine in all the experimental groups suggests that artemisinin administration had no effect on thyroid gland function within the time course of our investigation.

Although there were no observable alterations in the weights of reproductive organs and hormonal profile after artemisinin exposure in the present study, the increased oxidative stress in the epididymis appeared to be more closely related to the specific toxic effects of artemisinin in the epididymal milieu than in the testes. The first line of defence to the cells is provided by the mutually supportive relationship between metalloenzyme SOD, which accelerates the dismutation of endogenous cytotoxic superoxide radicals to H_2O_2, and CAT, which converts the deleterious peroxide radicals into water and oxygen (Adedara & Farombi, 2010). The increase in testicular SOD and CAT activities in rats treated with both therapeutic dose and overdose of artemisinin was accompanied by an insignificant alterations in the GSH, H_2O_2 and MDA levels which was comparable to the control rats. These observations indicate the ability of the testicular antioxidant systems to effectively mitigate induction of oxidative stress in the testicular milieu. However, increased sperm lipid peroxidation, evidenced by elevated MDA level, was accompanied by a concomitant decrease in GPx, and GST activities as well as GSH level in rats administered with overdose of artemisinin. The high level of H_2O_2 in spermatozoon of artemisinin-treated rats suggests inability of CAT to eliminate the H_2O_2 from SOD activity thereby allowing its observed deleterious effects. Excessive generation of hydrogen and lipid peroxides may cause over utilisation of GSH and inhibition of antioxidant enzymes (Pigeolet *et al.*, 1990).

5′-nucleotidase (5′-NTD) is an integral glycoprotein which hydrolyses extracellular adenosine 5′-monophosphate (AMP) to adenosine and free phosphate (Newby *et al.*, 1975; Oyagbemi *et al.*, 2010). The present study showed that overdose of artemisinin treatment increased testicular 5′-NTD. The increase in the activity of this microsomal marker enzyme may suggests an induction of testicular ribose-5-phosphatase activity to increase ribose synthesis. An increase in testicular nucleic acid synthesis may thus occur possibly to compensate microsomal damage resulting from oxidative stress during overdose of artemisinin therapy. The biochemical evidence of the artemisinin toxicity is well supported by the histological evaluation. The severe erosion of the epididymal lining by artemisinin compared with the mild effects observed in the testes may indicate the susceptibility of the epididymis to reactive oxygen species (ROS) damage than the testes.

Our data indicated that overdose of artemisinin induced oxidative stress in epididymis resulting in decreased sperm quality and quantity. The susceptibility of spermatozoon to damage by ROS is attributed to their high content of polyunsaturated fatty acids. The destruction of sperm lipid matrix structure by lipid peroxidation is associated with loss of motility, defects of membrane integrity and has been implicated in male infertility (Griveau *et al.*, 1995; de Lamirande *et al.*, 1997; Sanocka & Kurpisz, 2004; Henkel, 2005). Administration of overdose of artemisinin caused a marked decrease in the epididymal sperm number, progressive sperm motility and viability, whereas sperm abnormalities increased without alteration in the testicular sperm number and daily sperm production in the treated rats. The reproductive toxicity of artemisinin derivatives in male guinea pig and rats has been reported (Raji *et al.*, 2005; Obianime & Aprioku, 2009). The observations from the spermiogram in the present study further revealed that artemisinin induced an epididymis specific effect on sperm characteristics without affecting the normal spermatogenesis by the testes. The impaired sperm motility observed in artemisinin-treated rats may result in infertility due to failure of the

spermatozoon to reach the site of fertilisation and to penetrate zona pellucida.

In conclusion, induction of oxidative stress due to compromised antioxidant defence system in the epididymis, but not in the testes, could be a mechanism for the manifestations of artemisinin-mediated reproductive toxicity. Results of the present study highlight adverse effect of artemisinin, which may result in male reproductive deficits and subfertility in populations unduly exposed to artemisinin therapy. The noxious effect of artemisinin on reproduction may not decline in as much as abuse of this drug by men continue. It is recommended therefore that healthcare givers in fertility clinics should advise patients to adhere to artemisinin prescription to reduce the risk of infertility especially in mosquito-endemic countries.

Acknowledgement

The technical assistance of Messrs Omoko Ejiro of the Department of Veterinary surgery and Reproduction, University of Ibadan, is appreciated.

References

Adedara IA, Farombi EO (2010) Induction of oxidative damage in the testes and spermatozoa and hematotoxicity in rats exposed to multiple doses of ethylene glycol monoethyl ether. *Hum Exp Toxicol* 29:801–812.

Adedara IA, Farombi EO (2012) Chemoprotection of ethylene glycol monoethyl ether-induced reproductive toxicity in male rats by kolaviron, isolated biflavonoid from *Garcinia kola* seed. *Human Exp Toxicol* 31:506–517.

Adedara IA, Farombi EO (2013) Chemoprotective effects of Kolaviron on ethylene glycol monoethyl ether-induced pituitary-thyroid axis toxicity in male rats. *Andrologia* 45:111–119.

Adjuik M, Babiker A, Garner P, Olliaro P, Taylor W, White N (2004) International Artemisinin Study Group. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* 363:9–17.

Agarwal A, Makker K, Sharma R (2008) Clinical relevance of oxidative stress in male factor infertility: an update. *Am J Reprod Immunol* 59:2–11.

Aultman KS, Gottlieb M, Giovanni MY, Fauci AS (2002) Anopheles gambiae genome. Completing the malaria triad. *Science* 298:13–14.

Boareto AC, Muller JC, Bufalo AC, Botelho GG, de Araujo SL, Foglio MA, de Morais RN, Dalsenter PR (2008) Toxicity of artemisinin [*Artemisia annua* L.] in two different periods of pregnancy in Wistar rats. *Reprod Toxicol* 25:239–246.

Cherry SM, Hunt PA, Hassold TJ (2004) Cisplatin disrupts mammalian spermatogenesis, but does not affect recombination or chromosome segregation. *Mutat Res* 564:115–128.

Clairborne A (1995) Catalase activity. In: Handbook of Methods for Oxygen Radical Research. Greewald AR (ed). CRC Press, Boca Raton, FL, pp 237–242

Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO (2000) Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a *Garcinia kola* seed extract. *Food Chem Toxicol* 38:353–541.

Giwa-Osagie OO (2003) Nigeria has twelve million infertile persons. *Pharmanews* 25:48–49.

Goodlad GA, Clark CM (1982) Alteration in hepatic 5′-nucleotidase in the tumor bearing rat. *Enzyme* 27:11–123.

Griveau JF, Dumont E, Renard P, Callegari JP, Le Lannou D (1995) Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa. *J Reprod Fertil* 103:17–26.

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 249:7130–7139.

Haynes RK (2001) Artemisinin and derivatives: the future for malarial treatment? *Curr Opin Infect Dis* 14:719–726.

Henkel R (2005) The impact of oxidants on sperm functions. *Andrologia* 37:205–206.

Jannini EA, Ulisse S, D'Armiento M (1995) Thyroid hormone and male gonadal function. *Endocr Rev* 16:443–459.

Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR (1974) Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology* 11:151–169.

Joyce KL, Porcelli J, Cooke PS (1993) Neonatal goitrogen treatment increases adult testes size and sperm production in the mouse. *J Androl* 14:448–455.

de Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C (1997) Reactive oxygen species and spermphysiology. *Rev Reprod* 2:48–54.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. *J Biol Chem* 193:265–275.

Maran RRM (2003) Thyroid hormones: their role in testicular steroidogenesis. *Arch Androl* 49:375–388.

Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 247:3170–3175.

Mulenga M (1998) Facing drug resistance: therapeutic option for treatment of uncomplicated Plasmodium falciparum malaria in adult Zambians. *J Med Health Sci* 2:11–20.

Newby AC, Luzio JP, Hales CN (1975) The properties and extracellular location of 5′-nucleotidase of the rat fat-cell plasma membrane. *Biochem J* 146:625–633.

Nosten F, White NJ (2007) Artemisinin-based combination treatment of falciparum malaria. *Am J Trop Med Hyg* 77:181–192.

Obianime AW, Aprioku JS (2009) Comparative study of artesunate, ACTs and their combinants on the spermatic

parameters of the male guinea pig. *Niger J Physiol Sci* 24:1–6.
Oyagbemi AA, Adedara IA, Saba AB, Farombi EO (2010) Role of oxidative stress in reproductive toxicity induced by co-administration of chloramphenicol and multivitamin-haematinics complex in rats. *Basic Clin Pharmacol Toxicol* 107:703–708.
Pant N, Srivastava SP (2003) Testicular and spermatotoxic effects of quinalphos in rats. *J Appl Toxicol* 23:271–274.
Pigeolet E, Corbisier P, Houbion A, Lambert D, Michels C, Raes M (1990) Glutathione peroxidase, superoxide dismutase, catalase inactivation by peroxides and oxygen derived free radicals. *Mech Ageing Dev* 51:283–297.
Prahalathan C, Selvakumar E, Varalakshmi P (2006) Lipoic acid modulates adriamycin-induced testicular toxicity. *Reprod Toxciol* 21:54–59.
Raji Y, Osonuga IO, Akinsomisoye OS, Osonuga OA, Mewoyeka OO (2005) Gonadotoxicity evaluation of oral artemisinin derivative in male rats. *J Med Sci* 5:303–306.
Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179:588–590.
Russell LD, Alger LF, Naquin LG (1987) Hormonal control of pubertal spermatogenesis. *Endocrinology* 120:1615–1632.
Sanocka D, Kurpisz M (2004) Reactive oxygen species and sperm cells. *Reprod Biol Endocrinol* 2:1–7.
Sriraman V, Anbalagan M, Rao AJ (2005) Hormonal regulation of Leydig cell proliferation and differentiation in rodent testis: a dynamic interplay between gonadotrophins and testicular factors. *Reprod Biomed* 11:507–518.
Wellems TE, Plowe CV (2001) Chloroquine-resistant malaria. *J Infect Dis* 184:770–776.
Wells ME, Awa OA (1970) New technique for assessing acrosomal characteristics of spermatozoa. *J Dairy Sci* 53:227.
Wolff SP (1994) Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol* 233:182–189.
World Health Organization (1995) The role of artemisinin and its derivatives in the current treatment of malaria. Geneva.
World Health Organization (1999) Collection and examination of human semen. In: WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction, 4th edn. Aitken RJ, Comhaire F, Baker HWG, Cooper TG, Barratt CLR, De Jonge C, Behre HM, Elasson R (eds). Cambridge University Press, UK, pp 4–33.
Yeung S, Pongtavornpinyo W, Hastings IM, Mills AJ, White NJ (2004) Antimalarial drug resistance, artemisinin-based combination therapy, and the contribution of modeling to elucidating policy choices. *Am J Trop Med Hyg* 71:179–186.
Zemjanis R (1970) Collection and evaluation of semen. In: Diagnostic and Therapeutic Technique in Animal Reproduction, 2nd edn. Zemjanis R (ed). William and Wilkins Company, Waverly Press, Inc, Baltimore, Maryland, USA, pp 139–153.