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Effects of Short Term Administration of Artemether –Lumefantrine on Testicular Functions and Antioxidant Defence in the Rat.

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Abstract: AIM: Malaria remains one the most common causes of illness in the world, particularly in Africa and malaria chemotherapy has been linked with infertility. This study was undertaken to examine the effects of Artemether – Lumenfantrine, a potent antimalarial, on testicular functions and its antioxidant defence system. METHODS: Male rats were divided into five (5) groups of 5 animals each and treated as follows: Group A, control (distilled water); group B, Artemether – Lumefantrin (4:24mg/kg BW for 3 days); group C, Artemether – Lumefantrin (8:48 mg/kg BW for 3 days); group D, Artemether – Lumefantrin (4:24mg/kg BW for 6 days); and group E, Artemether – Lumefantrin (8:48mg/kg BW for 6 days). Testicular weight; epididymal sperm counts, motility, viability and morphology; testicular malonhydialdehyde, reduced gluthathione and superoxide dismutase; serum testosterone levels were determined. RESULTS: The administration of Artemether-lumefantrine combination caused no significant ($P>0.05$) change in the weight of testis and other accessory reproductive organs. Similarly, there was no significant ($P>0.05$) change in the sperm count, motility, live/dead ratio, sperm abnormality and serum testosterone level. The level of malonhydialdehyde was significantly ($P<0.05$) increased only in group E (Artemether – Lumefantrin, 8 - 48mg/kg BW for 6 days) while reduced glutathione level was significantly reduced by Artemether-lumefantrine combination administration. CONCLUSION: The results suggest that the administration of Artemether-lumefantrine combination at the doses and durations employed in this study had no adverse effects on testicular functions in the rat.

Key words: Artemether - Lumenfantrine, testosterone, antimalarial, free radicals and sperm.

INTRODUCTION

Artemether – Lumenfantrine, an artemisinin – based combination therapy [ACT] is used as a therapeutic agent of choice in the treatment of malaria [WHO]. It is a new class of antimalarial indicated in the treatment of all forms of Plasmodium and multiple drug resistant strains of P. falciparum. Previous studies have shown that several classes of antimalarial drugs have been associated with adverse effects on reproductive functions in rodents. The aminoquinolines, chloroquine [1,2]; quinoline – alcohols, quinine [3]. aryl – alcohol, halofantrine [4] and folate antagonist, sulphadoxine / pyrimethamine [5] have all been demonstrated to impact negatively on male fertility. Recent experimental studies on artemisinin derivatives such as artemether and dihydroartemisinin also reported significant decrease in sperm count, motility and testosterone level [6,7]. Besides, a number of studies have indicated that antimalarial alter antioxidant status. Rats exposed to quinine, amodiaquinne, mefloquine and halofantrine exerts an increased incidence of lipid peroxidation in the liver microsomal cells [8,9]. Chloroquine has been equally been shown to impose oxidative stress to renal tissues [10], halofantrine causes increased lipid peroxidation and decreased enzyme and non-enzyme antioxidants [11,12]. The parasiticidal activity of Artemether has been associated with the presence of an endoperoxide bridge [13,14], however, the endoperoxide bridge is metabolically activated to generate free radicals [13,15]. Free radicals are oxidants and they have become a real concern because of their potential toxic effects at high levels on sperm function [16,18].

With the development, efficacy and widespread usage of artemisinin – based combination therapy [ACT] relative to the "artemisinin or artemisinin-derivative only" in the therapeutic management of malaria, it is desirous to evaluate the reproductive activities of the ACT drug in an animal model. More

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importantly, despite several studies on the effects of artemisinin-based antimalarial on male reproductive functions, there appears to be little or no information in the literature on the impact of an ACT drug, Artemether – Lumenfrantrine, on testicular functions and antioxidant defense system. This study was therefore undertaken to examine the effects of Artemether - Lumenfantrine on testicular functions and its antioxidant defence system.

MATERIALS AND METHODS

Experimental Protocol: Twenty - five male rats (mean weight 150 - 180g) were maintained under standard laboratory conditions and were allowed free access to food and water ad libitum. Animals were divided randomly into five groups. Group A, control (distilled water); group B, Artemether – Lumefantrin combination (4 - 24mg/kg BW for 3 days); group C, Artemether – Lumefantrin combination (8 – 48 mg/kg BW for 3 days); group D, Artemether – Lumefantrin combination (4 - 24mg/kg BW for 6 days); and group E, Artemether – Lumefantrin combination (8 - 48mg/kg BW for 6 days).

Drug, Route And Duration Of Treatment: The drug was prepared with a known mass of the Artemether - Lumefantrine powder suspended in distilled water to yield a suspension of 4mg Artemether/24mg Lumefantrine per ml. The doses selected were based on the recommended dosage (4mg Artemether/24mg Lumefantrine/kg body weight) and "(8mg Artemether/48mg Lumefantrine/ kg body weight). The suspension was administered by oral syringe for a period of 3 and 6 days before sacrifice.

Organ And Blood Collection: Animals were weighed at autopsy; blood sample was collected from each animal via cardiac puncture into plain sample bottles and used for hormonal estimation. Testes were excised from the rats, cleared of adherent tissue and weighed immediately.

Sperm Analysis: Sperm analysis was done on sample derived from the cauda epididymis. Briefly, sperm motility was assessed immediately after autopsy by conventional methods; motile spermatozoa were calculated per unit area and expressed as percent motility. Epididymal sperm counts were made using the haemocytometer and were expressed as million/ml of suspension. The sperm viability was also determined using Eosin/Nigrosin stain [19]. Semen was smeared on microscopic slides and two drops of stain was added. Live (motile) sperm cells were stained while dead sperm cells remained unstained. Hundred sperm cells were counted from a well prepared uniform smear per slide in order to estimate the percentage live / dead ratio. Sperm morphology was determined from a count of 400 spermatozoa in sperm smear prepared with Wall and Ewas stain and examined under the microscope using X100 objectives under oil immersion. The abnormal sperm cells were counted and expressed as percentage.

Lipid Peroxidation Assay: The level of lipid peroxidation was estimated as the concentration of thiobarbi-turic acid reactive product malondialdehyde* (MDA), after the method of Ohkawa *et al.,* [20]. 1ml of the tissue homogenate was thoroughly mixed with 2ml of TCA-TBA-HCl solution and heated for 15 minutes in a water bath. After cooling, the precipitate is removed by centrifugation and the absorbance measured at 532nm using a spectrophotometer.

Reduced Glutathione: Glutathione (GSH-reduced) was determined using 5,5- Dithio-bis 2- Nitrobenzoic acid (DTNB) and Tris- EDTA buffer. 100µl the sample was added to 1ml of 0.2ml Tris-EDTA buffer (pH 8.2) followed by 0.9ml of 20mM EDTA (pH 4.7) and 20µl of DTNB. The sample was incubated at room temperature for 30 minutes. The mixture was centrifuged and absorbance of the supernatant read at 412nm.

Hormonal Assay: Blood samples were spun at 2500rpm for 10 minutes in a centrifuge. Serum samples obtained were assayed for testosterone using the tube-based enzyme immunoassay (EIA) method. The EIA kits were manufactured by Immunometrics (UK) Limited and meet the WHO standards in research programme for human reproduction. The intra and interassay variations were 10.06 and 10.01 for testosterone. The optical density was read at wavelength of between 492 and 550 nm using a spectrophotometer.

Statistical Analysis: Data are expressed as mean ± SEM and analysed using the Student's *t*-test and ANOVA where necessary. $P < 0.05$ was accepted as significant.

RESULTS AND DISCUSSION

Effects Of Artemether – Lumefantrine On Organ Weight: Administration of Artemether - Lumefantrine caused no significant changes in the weight of the testis, epididymis, seminal vesicle and prostate gland after 3 and 6 days treatment when compared with the

controls (Table 1). However, the testes of group D and E showed a decrease in weight which is not significantly different from the control counterparts.

Effects Of Artemether - Lumefantrine On Sperm Function: The effects of artemether-lumefantrine treatment on sperm function is depicted in Table 2. Caudal epididymal sperm count for the treated group B, C, D, and E (79.0, 78.4, 80.2 and 79.6 million/ml respectively) were not significantly different from the compared controls after 3 and 6 days treatment. Sperm motility values of 82.8%, 81.6%, 83.8% and 83.2% for treated group B, C, D, and E respectively were not significantly different from the control counterparts. Percentage viable sperm (live/dead) level of 83.4%, 84.6, 82.2% and 83.0% for groups B, C, D, and E respectively were within control value of 82.2%. Abnormal sperm value of the control group (9.6%) was not significantly different from the treated group B, C, D, and E (9.0%, 8.0%, 8.8% and 9.4% respectively).

Table 1: Effects of Artemether – Lumenfantrine on weight of the testis, epididymis, prostate and seminal vesicle after 3 days and 6 days treatment.

WEIGHT(gm)	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
TESTIS	1.28±.02	1.30±0.02	1.26±0.05	1.23±0.04	1.24±0.03
EPIDIDYMIS	0.52±0.01	0.53±0.02	0.51±0.02	0.52±0.02	0.51±0.01
PROSTATE	0.18±0.01	0.17±0.01	0.18±0.01	0.18±0.02	0.19±0.01
SEMINAL VESICLE	0.73±0.03	0.75±0.03	0.71±0.04	0.72±0.02	0.76±0.02

Group A, control (distilled water); group B, Artemether – Lumefantrin (4 - 24mg/kg BW for 3 days); group C, Artemether – Lumefantrin (8 – 48 mg/kg BW for 3 days), group D, Artemether – Lumefantrin (4 - 24mg/kg BW for 6 days); and group E, Artemether – Lumefantrin (8 - 48mg/kg BW for 6 days). Values are expressed as mean± SEM.

Table 2: Effects of Artemether – Lumenfantrine on sperm count, motility, viability and abnormal morphology after 3 days and 6 days treatment.

	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
COUNT (10^6/ml)	80.8±1.56	79.0±1.41	78.4±1.29	80.2±1.53	79.6±1.50
MOTILITY (%)	82.6±1.47	82.8±1.50	81.6±0.98	83.8±1.53	83.2±2.03
LIVE/DEAD (%)	82.2±1.06	83.4±0.98	84.6±1.81	82.2±2.29	83.0±1.58
% ABNORMAL SPERM	9.6±0.68	9.0±1.14	8.0±1.26	8.8±0.86	9.4±0.93

Group A, control (distilled water); group B, Artemether – Lumefantrin (4 - 24mg/kg BW for 3 days); group C, Artemether – Lumefantrin (8 – 48 mg/kg BW for 3 days), group D, Artemether – Lumefantrin (4 - 24mg/kg BW for 6 days); and group E, Artemether – Lumefantrin (8 - 48mg/kg BW for 6 days). Values are expressed as mean± SEM.

Effects Of Artemether - Lumefantrine On Lipid Peroxidation: Treatment with Artemether - Lumefantrine significantly increases ($P<0.01$) the level of malonhydialdehyde in the experimental group E (7.08nmol/ml) rats compared with the control (5.13nmol/ml). The malonhydialdehyde levels in the groups B, C and D (5.35, 5.48 and 5.52 nmol/ml respectively) were however not significantly different from the control group (Fig. 1).

Effects Of Artemether - Lumefantrine On Glutathione: The measured level of reduced glutathione in the group C (0.65µmol/ml), D (0.77 µmol/ml) and E (0.32 µmol/ml) were significantly lower ($P<0.001$, $P<0.01$, $P<0.0001$ respectively) compared with the control (1.18 µmol/ml) while the level in group B (1.16 µmol/ml) was not significantly different from the control values of group A.

Effects Of Artemether - Lumefantrine On Serum Testosterone Level: The effects of artemether-lumefantrine on serum testosterone level was depicted in Figure 3. Administration of artemether-lumefantrine had no significant impact on serum testosterone level as evidenced by the measured values of 29.9, 28.3, 29.6, 28.9 and 27.6 µmol/ml for group A, B, C, D and E respectively.

Discussion: The study revealed an insignificant reduction in caudal epididymal sperm count in the Artemether-lumefantrine combination administered rats as compared with controls. The percentage motility and live/dead ratio exhibited insignificant increases and decreases. The percentage abnormal sperm was also within the control value range. These results suggest that Artemether-lumefantrine combination had no adverse effect on testicular functions following short term treatment in rats. The normal

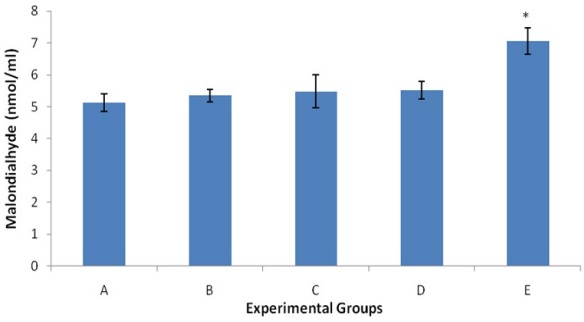

Fig. 1: Effects of *Artemether - Lumefantrine* on testicular malondialdehyde level in rats after 3 days and 6 days treatment. *P<0.05, values expressed as mean±sem, n= 6.

Group A, control (distilled water); group B, Artemether – Lumefantrin (4 - 24mg/kg BW for 3 days); group C, Artemether – Lumefantrin (8 – 48 mg/kg BW for 3 days), group D, Artemether – Lumefantrin (4 - 24mg/kg BW for 6 days); and group E, Artemether – Lumefantrin (8 - 48mg/kg BW for 6 days). Values are expressed as mean± SEM. Significant *P<0.05 compared with control values.

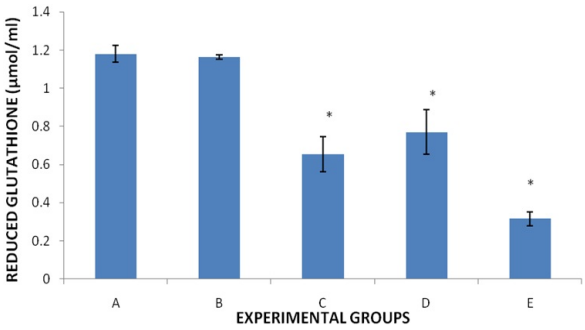

Fig. 2: Effects of *Artemether - Lumefantrine* on testicular reduced glutathione level in rats after 3 days and 6 days treatment. *P<0.05, values expressed as mean±sem, n= 6.

Group A, control (distilled water); group B, Artemether – Lumefantrin (4 - 24mg/kg BW for 3 days); group C, Artemether – Lumefantrin (8 – 48 mg/kg BW for 3 days), group D, Artemether – Lumefantrin (4 - 24mg/kg BW for 6 days); and group E, Artemether – Lumefantrin (8 - 48mg/kg BW for 6 days). Values are expressed as mean± SEM. Significant *P<0.05 compared with control values.

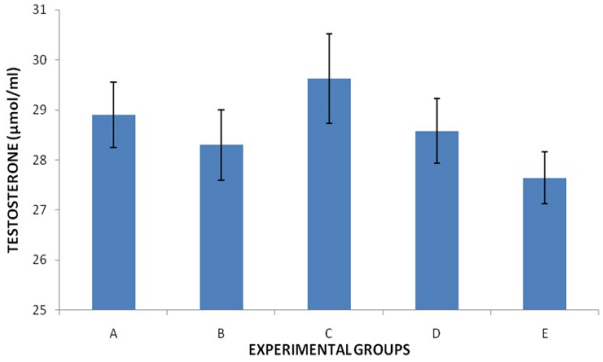

Fig. 3: Effects of Artemether - Lumefantrine on serum testosterone level in rats after 3 days and 6 days treatment. *P<0.05, values expressed as mean±sem, n= 6.

Group A, control (distilled water); group B, Artemether – Lumefantrin (4 - 24mg/kg BW for 3 days); group C, Artemether – Lumefantrin (8 – 48 mg/kg BW for 3 days), group D, Artemether – Lumefantrin (4 - 24mg/kg BW for 6 days); and group E, Artemether – Lumefantrin (8 - 48mg/kg BW for 6 days). Values are expressed as mean± SEM.

sperm characteristics in our study indicates that the drug possibly have no adverse effects on sperm production, testicular function or male fertility. This result agrees with the findings of Akinlolu *et al.,* [21] who reported that rats exposed to recommended doses of Artemether for 7 days showed normal morphological appearance of the testis with evidence of spermatogenesis. A contrary report indicated that exposure to Artemether for a period of 3 days at a very high dose level caused impairment to reproductive activity exhibited by reduced sperm count, motility, normal morphology; testosterone level and fertility index [6]. It therefore appears that effect of Artemether on reproductive functions in the rat is both dose and duration dependent with the dose factor being more pronounced. It should be noted that sperm production, development and maturation are processes that are vulnerable to interferences in the internal *millieu* of the reproductive organs [22].

The weight of male reproductive organs usually provides a useful reproductive risk assessment in experimental studies [23,24]. The observed weight of the testis, epididymis, seminal vesicle and prostate gland in both control and experimental rats suggest the administration of Artemether-lumefantrine combination at the doses and duration used had no toxic effect on these organs. It is therefore plausible to note that the weight of the testis and these accessory reproductive organs may be associated with the in-sensitivity of these organs to Artemether-lumefantrine combination *in vivo* which is likely due to the short duration of the treatment.

The serum testosterone level in this study remained normal. Reproductive functions are controlled by complex interactions involving these hormones and reproductive organs. These relationships are very critical to the spermatogenesis, sperm development, maturation and sexual performance among other reproductive functions. Rephrase as follow:
negligible effect of artemether-humenfantine on testicular steroielo genesis found in this study support the observed normal sperm quality and functions testicular weight.

Artemether has been reported to generate free radicals due to presence of an endoperoxide bridge [13.14]. Free radicals are oxidants that have become a real concern because of their potential toxic effects, at high levels, on sperm function [17,25]. A balance normally exists between free radicals [oxidants] production and antioxidant scavenging enzymes activities in the male reproductive system. As a result of such balance, only minimal amounts of oxidants remain, and they are needed for the regulation of normal sperm functions, such as sperm capacitation, the acrosome reaction, and sperm–oocyte fusion [26-27]. The production of excessive amounts of oxidant in the testis / semen can overwhelm the antioxidant defence mechanisms and causes oxidative stress [17,25]. Oxidative stress causes damage to spermatozoa thereby compromising sperm quality and functions.

Lipid peroxidation [indexed by MDA] which is a biomarker of oxidative stress was increased in the rat given higher doses of Artemether - lumenfatrine for longer period and antioxidant glutathione levels were greatly depressed with higher doses and prolonged treatment. The development of oxidative stress in the experimentally treated rats was therefore shown to be dose and duration dependent, although it appears that longer duration of treatment may play an important role in the development of oxidative stress.

Relating the observed normal sperm functions and the development of oxidative stress, we speculate that free radicals were not pathologically produced or elevated in the Artemether-lumefantrine combination administered rats during the acute exposure period to have made their testis and or sperm cells susceptible to damage as evidenced by the measured malondialdehyde level. This might have been possible due to the efficient protective mechanisms of the anti-oxidants enzyme, “reduced glutathione” against excessive accumulation of free radicals as reflected by the decreased enzyme level.

In conclusion, the short term administration of Artemether-lumefantrine combination at the doses and durations employed in this study had no adverse effects on testicular functions in the rat. However, there is indication that high doses and prolonged treatment cause greater oxidative stress. It is therefore to be recommended that prolonged treatment with Artemether-Lumefantrine should be discouraged. A study to access the effect of prolonged treatment should be undertaken.

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