Evaluation of prolonged administration of artemether-lumefantrine on sperm indices and testicular testosterone concentration in adult male wistar rats

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ABSTRACT

Objective: In the present study, the effect of prolonged administration of artemether-lumefantrine (AL) and the ameliorative effect of vitamin E (VE) on sperm indices and testicular testosterone concentration was evaluated.
Methods: Thirty five (n = 35) adult male Wistar rats used for this study were divided into five (5) groups of seven (7) rats each and treated as follows: Group I (control): 1ml/kg distilled water; group II: 1ml/kg olive oil; group III: 2.29 mg/13.74 mg AL; group IV: 2.29 mg/13.74 mg AL + 100 mg/kg of VE and group V: allowed to recover from treatment with AL for 2 weeks before sampling (2.29 mg/13.74 mg AL + two weeks recovery). Administration was done orally twice daily for a period of two (2) weeks. At the end of the experiment, animals were sacrificed and the right testis with its epididymis was collected for analysis of sperm indices, while the left was homogenised in phosphate buffered saline for testicular testosterone concentration assay.
Results: A remarkable but insignificant ($P > 0.05$) decrease in all sperm indices (concentration, motility, vitality and morphology) and testicular testosterone level in the AL group when compared with the control group was obtained from the study. However, a significant ($P < 0.05$) increase in abnormal sperm morphology was observed in the recovery group.
Conclusion: It is concluded from the present study that prolonged administration of artemether-lumefantrine does not significantly affect most sperm indices (except sperm head morphology) and testicular testosterone concentration.

KEY WORDS: Artemether-lumefantrine, Sperm indices, Oxidative stress, Testosterone, Vitamin E

Introduction

Infertility affects approximately 15% of all couples trying to conceive, and the male factor is the sole or contributing factor in roughly half of these cases. It is estimated that the male factor in couple infertility is between 25% to 50% [1,2]. This occurs when an "alteration in sperm concentration, motility, and/or morphology is present in at least one sample of two sperm analyses, collected 1 to 4 weeks apart" [3]. This problem becomes further compounded when no identifiable reason can be found [4]. Malaria illness imposes great burden on the society as it adversely affects the physical, mental and social wellbeing of the people as well as on the economic development of the nation [5]. Malaria control requires an integrated approach, including prevention (primarily vector control) and prompt treatment with effective anti-malarials, especially artemisinin-based combination therapies (ACTs) recommended by the World Health Organisation [6]. Artemether-lumefantrine is one of such ACTs. Artemether-lumefantrine like other artemisinin derivatives, cause malaria parasite death through the generation of free radicals. Excessive generation of free radicals such as reactive oxygen species (ROS) depletes the antioxidant defence system causing oxidative stress [7, 8].

Aside the well documented conventional risk factors of male fertility (male accessory gland infection, mumps, orchitis, varicocele, and cryptorchidism etc), poor lifestyle choices, including prolonged exposure to xenobiotics and other chemical substances can adversely affect the male reproductive system, thereby causing impaired fertility.

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Mechanisms of impaired fertility include direct disruption of sperm production and quality; effects on the delicately balanced hypothalamo-pituitary-testicular axis; effects on erectile or ejaculatory function and libido [9,10]. Moreover, oxidative stress mediated by reactive oxygen species (ROS) is increasingly being implicated as a causative factor in male infertility [11]. This is because mature spermatozoa, encased in a polyunsaturated lipid membrane, are vulnerable to the effects of ROS, which at high levels can impair spermatogenesis and decrease sperm quality [3, 12,13].Okorosobo *et al.*, reported that in Nigeria, 53% of malaria cases are treated by self-medication, with 7% and 40% treated by herbalist/spiritualists and clinics/hospitals, respectively. The possibility of prolonged and overdose administration as well as other forms of misappropriation in the usage of anti-malarial agents could lead to undesirable effects of the drugs [14].

The widespread use of artemether-lumefantrine as a result of recurrence in malaria endemic countries like Nigeria necessitates this study. Therefore, evaluating the reproductive changes associated with the negligent possibility of prolonged usage of artemether-lumefantrine could fill the knowledge gap that currently exists in this area of malaria treatment. The present study evaluated prolonged administration of artemether-lumefantrine (coartem®) on sperm indices and testicular testosterone concentration in male Wistar rats.

Materials and Methods

Experimental animals

Thirty-five (35) male Wistar rats (130–150 g) obtained from the animal house of the Department of Human Physiology, Ahmadu Bello University Zaria, Nigeria were used for the study. They had free access to food and water throughout the experiment. All procedures pertaining the use of animals were approved by Animal Ethical Committee at Ahmadu Bello University.

Animal grouping and dosage of administration

The thirty-five (n = 35) male Wistar rats used for the study were divided into five groups of seven (n = 7) rats each and treated as follows:

Group I (Control): Each rat was administered with distilled water at 1ml/kg body weight

Group II: Each rat was administered with olive oil at 1ml/kg body weight

Group III: Each rat was administered with 2.29 mg/13.74 mg/kg Artemether/Lumefantrine dissolved in distilled water.

Group IV: Each rat was administered with 2.29 mg/13.74 mg/kg Artemether/Lumefantrine + 100 mg/kg of Vitamin E dissolved in olive oil.

Group V: Each rat was administered with 2.29 mg/13.74 mg/kg Artemether/Lumefantrine and subsequently allowed to recover for 2 weeks during which no treatment was given.

The dose of artemether/lumefantrine (Coartem®) used for this study was calculated from the manufacturer's recommended dose for a man weighing at least 35 kg, a modification of Otuechere et al. [15].

Drugs and chemicals

The Coartem® (Artemeter/lumefantrine: 20 mg/120 mg; Novartis Pharmaceuticals Corporation – Switzerland), Vitamin E (α-tocopherol; Gujarat Liqui Pharmacaps Pvt. Ltd. – India, NAFDAC Reg. No. A4-8322) and olive oil used for this study were purchased from a reputable pharmaceutical store in Zaria, Kaduna State, Nigeria. The Vitamin E (1000 mg) was aspirated into a syringe and then reconstituted with olive oil (vehicle) prior to daily administration.

Drug preparation and administration

The coartem was ground to a powdered form, mixed with distilled water and administered as an aqueous suspension. The drug suspension was continuously agitated during administration in order to deliver the drug homogeneously to the animals. Vitamin E was aspirated using a 1 ml syringe and reconstituted in olive oil. The respective treatments were administered to all rats in all groups twice daily for a period of two weeks using a 1.0 mL syringe by oral gavage. However, rats in group V were allowed to recover for 2 weeks after the last treatment before they were sacrificed. The choice of dose and route of administration was done as recommended for humans, but the 2 weeks duration of treatment was to expose the animals to treatment beyond the recommended duration, to mimic the extended duration of treatment associated with malaria relapse due to incomplete treatment or endemicity.

Sample collection

At the end of the treatment and recovery periods (group V), all rats were anaesthetised by chloroform inhalation in a closed chamber and thereafter, sacrificed. The right testis was carefully exposed and removed together with its epididymis [16]. The epididymis was separated and the epididymal fluid collected from the caudal portion for the assessment of sperm motility, concentration, morphology and live/dead ratio (vitality). The left testis was homogenised in a physiological solution (7.4 pH phosphate buffered saline) and the homogenate centrifuged at 1788 × *g* for 10 mins. The supernatant was removed and used for assessment of intratesticular testosterone concentration.

Estimation of sperm indices

All sperm indices were estimated following procedures described in the laboratory manual for the examination and processing of semen [17].

Assay of testicular testosterone concentration

Testicular testosterone assay was carried out using rat Testosterone enzyme-linked immunosorbent assay (ELISA) kit. The procedures for the assay as contained in the manufacturer's manual were strictly followed.

Statistical analysis

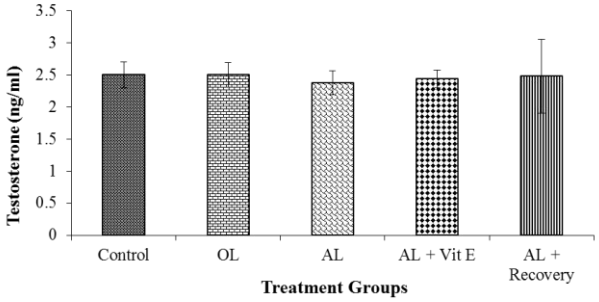
SPSS version 17 was used for the statistical analysis. The data obtained were calculated by one-way analysis of variance (ANOVA) and compared using the Tukey's post hoc test. Differences were considered statistically significant at $P < 0.05$.

Results

Effect of prolonged administration of artemether-lumefantrine on intra-testicular testosterone concentration.

The effect of prolonged administration of artemether-lumefantrine on intra-testicular testosterone concentration is shown in figure 1. Intra-testicular testosterone concentration (ng/ml) for the five groups were as follows: control, 2.50 ± 0.20; OL, 2.50 ± 0.19; AL, 2.38 ± 0.19; AL + Vit E, 2.44 ± 0.14 and AL + Recovery, 2.48 ± 0.58, respectively. There was no statistical difference in testicular testosterone concentration between the groups.

Figure 1. Intratesticular testosterone concentration following 14 days of oral administration of artemether-lumefantrine, vitamin E and subsequent withdrawal of the drug for 14 days.



AL= artemether-lumefantrine; OL = Olive oil; Vit E= Vitamin E.

Effect of prolonged administration of artemether-lumefantrine on sperm concentration.

The effect of prolonged administration of artemether-lumefantrine on sperm concentration is shown in figure 2. Sperm concentration ($\times 10^6$/ml) for the five groups was as follows: control, 37.20 ± 2.71; OL, 37.00 ± 2.61; AL, 36.80 ± 1.46; AL + Vit E, 37.00 ± 1.45 and AL + Recovery, 37.20 ± 6.30, respectively. There was no statistical difference in sperm concentration between the groups.

Figure 2. Sperm concentration following 14 days oral administration of artemether-lumefantrine, vitamin E and subsequent withdrawal of the drug for 14 days.

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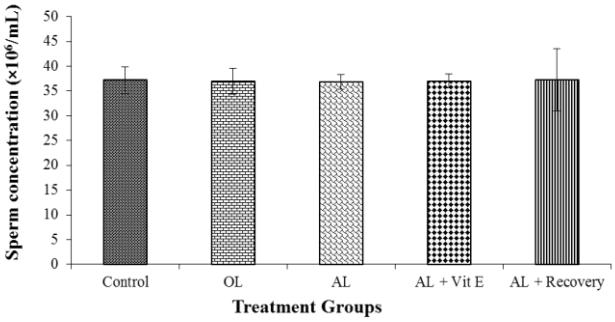
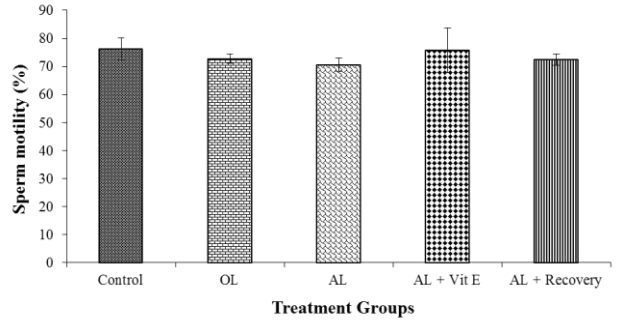


AL = Artemether-lumefantrine; OL = Olive oil; Vit E = Vitamin E

Effect of prolonged administration of artemether-lumefantrine on sperm Motility.

The effect of prolonged administration of artemether-lumefantrine on sperm motility is shown in figure 3. Sperm motility (%) for the five groups was as follows: control, 76.20 ± 3.93; OL, 72.80 ± 1.62; AL, 70.60 ± 2.44; AL + Vit E, 75.60 ± 1.93 and AL + Recovery, 72.40 ± 2.01, respectively. There was no statistical difference in sperm motility between the groups.

Figure 3. Sperm motility following 14 days oral administration of artemether-lumefantrine, vitamin E and subsequent withdrawal of the drug for 14 days.

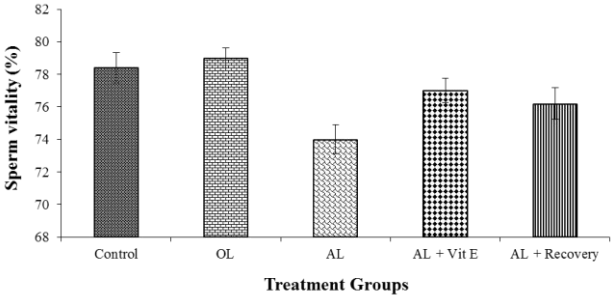


AL = Artemether-lumefantrine; OL = Olive oil; Vit E = Vitamin E.

Effect of prolonged administration of artemether-lumefantrine on sperm vitality.

The effect of prolonged administration of artemether-lumefantrine on sperm vitality is shown in figure 4. Sperm vitality (%) for the five groups were as follows: control, 78.40 ± 1.92; OL, 79.00 ± 1.61; AL, 74.00 ± 1.73; AL + Vit E, 77.00 ± 2.54 and AL + Recovery, 76.20 ± 2.20, respectively. There was no statistical difference in sperm vitality between the groups.

Figure 4. Sperm vitality following 14 days oral administration of artemether-lumefantrine, vitamin E and subsequent withdrawal of the drug for 14 days.

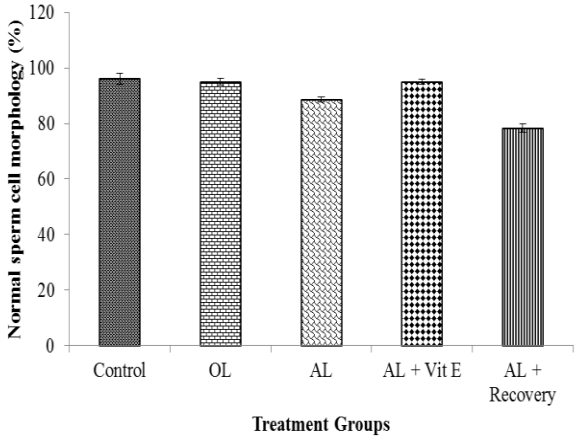


AL = Artemether-lumefantrine; OL = Olive oil; Vit E = Vitamin E.

Effect of prolonged administration of Artemether-Lumefantrine on normal sperm cell morphology.

The effect of prolonged administration of artemether-lumefantrine on normal sperm morphology is shown in figure 5. Normal sperm cell morphology (%) for the five groups were as follows: control, 96.20 ± 1.94; OL, 95.00 ± 1.20; AL, 88.75 ± 0.91; AL + Vit E, 95.00 ± 0.91 and AL + Recovery, 78.40 ± 1.46, respectively. There was no statistical difference in normal sperm morphology between the groups.

Figure 5. Normal sperm morphology following 14 days oral administration of artemether-lumefantrine, vitamin E and subsequent withdrawal of the drug for 14 days.

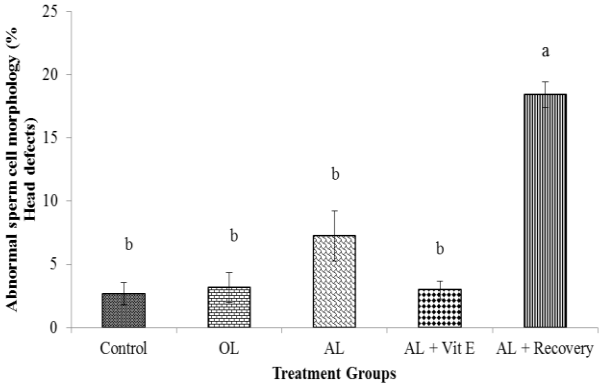


AL= artemether-lumefantrine; OL = Olive oil; Vit E = Vitamin E.

Effect of prolonged administration of artemether-Lumefantrine on abnormal sperm cell morphology (Head Defect).

The effect of prolonged administration of artemether-lumefantrine on abnormal sperm cell morphology (% head defect) is shown in figure 6. Rats that had a 2 – week recovery period post-treatment had significantly ($P < 0.05$) higher percentage sperm head defects than other groups (AL + Recovery: 18.40 ± 1.03 % vs control: 2.67 ± 0.88 %; OL, 3.17 ± 1.20; AL, 7.25 ± 1.97; AL + Vit E, 3.00 ± 0.71, respectively).

Figure 6. Abnormal sperm cell morphology (% Head defect) following 14 days oral administration of artemether-lumefantrine, vitamin E and subsequent withdrawal of the drug for 14 days.



a,b = statistically different; AL = artemether-lumefantrine; OL = Olive oil; Vit E = Vitamin E.

Discussion

The production of spermatozoa requires high intra-testicular concentration of testosterone, produced by the Leydig cells of the testis [18, 19, 20]. The intra-testicular testosterone level in this study did not significantly differ in all the groups. This may suggest that the dose of artemether-lumefantrine used in this study does not have adverse effect on the steroidogenic function of the testis. It could also be that the array of antioxidant enzymes found within the testis were adequate to nullify any negative effect of artemether-lumefantrine on the testes. This finding is in concert with that of Shittu *et al*. [21] who reported that therapeutic dose of artesunate did not considerably alter testosterone level in male rats. However, this finding contradicts an earlier report of a significant decrease in testosterone level after artesunate administration in rats [22,23]. It equally does not agree with the increase in testosterone level after chronic administration of chloroquine as reported by Salman *et al.* [24]. The negligible effect of artemether-lumefantrine on testicular steroidogenesis as seen in this study supports the observed normal sperm parameters. Sperm indices such as concentration, motility, vitality and morphology are commonly used to determine the fertilization potential of sperm. Spermatozoa are produced in the seminiferous tubules of the testis from where they are transported to the epididymis. There is a gradient of concentration, maturation and increased motility as the spermatozoa move through the corpus epididymis and continues to improve through the cauda epididymis and vas deferens [25].

In this study, the epididymal sperm indices (concentration, motility, vitality and normal morphology) were investigated with a resultant insignificant decrease in the AL treated group compared to others. The mean abnormal sperm morphology was significantly higher in the recovery group with the most common abnormality being 'head defect'. However, the values obtained for all the indices were higher than the least normal values needed to achieve fertilization. At least 15×10^6/mL sperm concentration, 40% sperm cell motility, 58% vitality and 4% normal morphology are essential for effective fertilization [6, 26]. This finding agrees with an earlier report of insignificant reduction in sperm quality of rats administered artemether [27] and artesunate [21]. However, the report that artemether caused a significant reduction in sperm function of male rats by Raji *et al.* [28], contradicts this finding. Another contradictory report is the spermatoxicity/decreased sperm quality effect of artesunate in male rats [22, 29] and male guinea pigs [30, 31]. A significant decrease in all sperm indices in rats [32], and male guinea pigs [33] treated with dihydroartemisinin has also been reported.

These differences could however, be attributed to the dose and duration of the drugs, and animal species employed in the respective studies. Furthermore, the idea of combining two drugs of different generic origin is to achieve synergy of the therapeutic effect and reduce toxicity caused by the use of each drug alone. Therefore, the combination of artemether and lumefantrine could be more effective as an anti-malarial treatment and less toxic to tissues as seen in the present study. The Vitamin E treated and recovery groups gave promising results as most of the parameters investigated in this study were comparable to the baseline levels obtained in the control group. A remarkable but insignificant increase in testosterone concentration and sperm indices were observed in these groups. Although results in these groups were similar to the baseline levels obtained in the control group, it should be noted that animals in the control group were not administered artemether-lumefantrine. This is suggestive of the fact that Vitamin E assumed its antioxidant function, sparing the endogenous antioxidant enzymes, and thereby buffering the negative effect of free radicals generated by artemether-lumefantrine.

The restoration to normalcy observed for most sperm parameters in the recovery group of this study suggests that the deleterious effects of artemether-lumefantrine on sperm parameters could be spontaneously corrected with time after cessation of administration. However, the significantly higher sperm head defects observed in this group calls for attention as the fertilising capacity of the sperm cells could be jeopardised. The reversal of adverse effects of long-term administration of artesunate [34] and artemether alone [28] have earlier been reported. Based on the findings of this study, it is concluded that prolonged administration of artemether-lumefantrine in male Wistar rats does not affect most sperm indices (except sperm

head morphology) and testicular testosterone concentration.

Conflict of Interest

We declare that we have no conflict of interest.

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