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SERUM LIPID PROFILE AND MALE REPRODUCTIVE PARAMETERS AFFECTED BY FREQUENT ADMINISTRATION OF ARTEMETHER-LUMEFANTRINE: AN INFLUENCE ON MALE INFERTILITY

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ABSTRACT

This study examined the effects of frequent administration of artemether-lumefantrine (Lokmal)^{R} on serum lipid profiles and male reproductive parameters, as well as the relationship between these parameters and male fertility. Thirty-five (35) male Wistar rats weighing between 230 and 330g were divided into five subgroups, each with seven animals. Oral gavage was used to provide graded doses of artemether-lumefantrine twice a day. Group I served as the control; they were not given any medication. Group II was given 8 mg/kg bodyweight (bw) for three days. 12 mg/kg bw was given to Group III for three days. For three weeks, Group IV was administered 8 mg/kg bw three days a week with a two-week recuperation interval, while Group V was administered 12 mg/kg bw three days a week with a two-week rest interval. Investigated parameters include semen parameters and serum lipid profile. A significant ($p<0.05$) decrease in sperm motility and concentration was observed in the treatment groups when compared to the control. No significant ($p>0.05$) changes in male reproductive hormone and organs were observed. HDL cholesterol concentration recorded a significant ($p<0.05$) reduction. Triglyceride, total cholesterol, LDL-cholesterol, VLDL-cholesterol recorded no significant increases or decreases except for the significant increase in triglyceride in Group V and a decrease in total cholesterol in Group V. The findings point to the declining consequences of indiscriminately administering ACTs repeatedly on semen characteristics of healthy adult male which may eventually affect fertility, and they should be discouraged.

Artemether-lumefantrine, Lipid Profile, Malaria, Semen, Sperm Motility.

1.0 INTRODUCTION

In recent past, artemisinin combination therapies (ACTs) have been of high interest in pharmacological research especially in the sub-Saharan African countries considering its wide usage. The World Health Organization recommended ACTs for the treatment of uncomplicated malaria caused by the *Plasmodium* parasite.[1] Malaria is endemic in sub-Saharan African countries and is ranked among the deadliest tropical diseases with a high rate of morbidity and mortality and remains a major public health distress regardless of efforts made to control and eliminate it; pregnant women and children under five are most affected.[2,3] By combining two active ingredients with different mechanism of action, ACTs is regarded as the most effective antimalarial medicines available today.[1,4] The World Health Organization also recommended parasitological confirmation for all suspected cases of malaria before treatment with ACTs.[4] However, 60% report of malaria in outpatient visitation in Nigeria was largely due to presumptive clinical diagnosis[5,6] and currently, unregulated purchase and misuse of these anti-malarial in the open market is a common practice. This misuse is brought on the by the continuous evolution of the malaria parasite as a result of the limitations of the current artemisinin combination therapies as and its resistance to available pyrethroids and insecticides which has reversed the progress made by public health initiatives to combat malaria. While there isn't an official report of ACT-resistant malaria in sub-Saharan Africa yet, more surveillance is desperately needed to track its development and re-evaluate the effectiveness and caliber of available ACTs.[7]

They have been reports of possible decline of semen quality in the past decades which have generated intense interest of researchers in risk factors for semen quality, such as biological and environmental factors.[8,9,10] The inability of a man to impregnate a fertile female is called the "male factor" infertility and accounts for 40 – 50%

infertility in humans.[11] An alteration in sperm concentration, motility, or morphology in at least one sample of two sperm analysis taken one or four weeks apart can be considered as a "male factor" infertility.[11,12] To conceive a child, a man's sperm must combine with a woman's egg in order to fertilize it, and to properly fertilize an ovum, an ideal sperm should have an undamaged cell membrane, effective cellular motility, consistent fuel sources, and signal sensitivity and these mechanisms are influenced by the kind and quantity of lipid content in sperm.[13] A robust and stable cellular structure is necessary for sperm to be able to swim a great distance and still fertilize an ovum when it gets there. Sperm also need to be able to transfer energy from chemical stores in the blood and seminal fluid into continuous, controlled cellular movement and part of the energy produced by sperm cells comes from lipid metabolic pathways.[13] The biochemical constituents of the plasma membrane of the sperm cell are often an area of interest in the study of sperm physiology and etiology because of its crucial role in sperm fertilization capacity, adhering to the cell membrane of an ovum and in spermatozoon-oocyte exchange. The lipid composition of the sperm plasma membrane has been elucidated and are essential in the process of spermatogenesis and sperm maturation.[14,15,16]

Artemether-lumefantrine have been reported to be relatively safe but can be toxic under certain conditions.[17] Frequent repeated administration of artemether-lumefantrine may have some public health implications like drug resistance and expose the body to adverse effects that can critically affect vital body organs and impair important cellular functions.[18] Many anti-malarial drugs have been implicated to have anti-fertility actions, the anti-steroidogenic and anti-fertility actions of quinine and chloroquine have been well documented[19,20,21] and the toxic effects of artesunate and ACTs in males has also been reported.[22,23,24] Toxicity studies in guinea pigs and rats revealed dose dependent and potentially fatal toxic effects after the intake of artemether – lumefantrine at higher and multiple doses.[25,26,27] With numerous outstanding researches in this topic, the roles that lipids play in male infertility has come under increased scrutiny, it is therefore crucial to assess how frequent administration of artemether-lumefantrine, which has been the standard of care for the treatment of uncomplicated malaria in sub-Saharan Africa, affects male reproductive parameters and serum lipid profile and how it correlates with abnormal semen parameters in male infertility.

2.0. MATERIALS AND METHODS

2.1. Drugs and Chemicals

The Artemether + Lumefantrine (Lokmal)^{R}: Artemether 80 mg/lumefantrine 480 mg per tablet from Emzor Pharmaceutical Industries Limited, Nigeria used for this study was purchased at Quenthall Pharmacy in Uyo metropolis, Akwa Ibom State, Nigeria. All other chemicals and reagents used were of analytical grade.

2.2. Materials

Well-ventilated animal cages, Wood fillings, Feeders and drinkers, Artemether-lumefantrine (brand name; Lokmal^{R}), Needles and syringes, Microplate Reader [MayaMed BY010], Disposable micropipette tips, clean glass tubes and test tube racks, Micropipettes, Beakers (10 – 1000ml), Light Microscope, Oral cannula, Rubber gloves, Weighing scale, Serum bottles, Centrifuge and Pasteur pipette, ELISA kit, Semi-automatic Chemistry Analyzer [Medsinglong MSLBA50].

2.3. Experimental animals

Thirty-Five (35) male Wistar rats obtained from the animal house of the Department of Pharmacology and Toxicology, University of Uyo, Uyo, Akwa Ibom State, Nigeria, was used for the study. The animals were acclimatized for three weeks prior to the commencement of drugs administration. They were kept in well ventilated cages and were fed with standard rat pellets and water *ad libitum* throughout the experimental period.

2.4. Research Design

The Animals were randomly divided into five groups of seven (n = 7) animals each and administered graded doses of artemether-lumefantrine through oral gavage. The dose of artemether-lumefantrine (Lokmal)^{R} used for this study was calculated from the manufacturer's recommended dose for a man weighing at least 70kg and calculated in mg/kg body weights of the experimental animals. The experimental design is as follow:

1. Group 1: Control
2. Group 2: Therapeutic dose of 8 mg/kg bodyweight of artemether-lumefantrine (AL) for 3 days divided into equal parts twice daily. This is to mimic the normal therapeutic dose of AL and duration of the drug in human.
3. Group 3: Overdose of 12 mg/kg bodyweight of Artemether – Lumefantrine for 3 days divided into equal parts twice daily.
4. Group 4: Therapeutic dose of 8 mg/kg bodyweight of artemether-lumefantrine for 3 weeks divided into equal parts and administered twice daily for 3days each week with a 2 weeks' interval in-between dosage.
5. Group 5: Overdose of 12 mg/kg bodyweight of artemether-lumefantrine for 3 weeks divided into equal parts and administered twice daily for 3 days each week with a 2 weeks' interval in-between dosage.

2.5. Drug Preparation and Administration

The artemether-lumefantrine was ground to a powdered form, mixed with distilled water and administered as an aqueous suspension. The drug suspension was continuously agitated during the administration in order to deliver the drug homogenously to the animals. The treatment was administered to all the rats twice daily using a 1.0 mL syringe by oral gavage.

2.6. Collection and Treatment of Samples

At the end of the treatment, all rats were anaesthetized by chloroform inhalation in a closed chamber and thereafter, sacrificed. Blood was collected by cardiac puncture into a plain serum bottle and later centrifuged at 3500 rev/min at room temperature for 15 min to obtain serum. The serum was kept frozen until it was used for biochemical and hormonal analysis. The reproductive organs (testis and epididymis), were carefully harvested and immediately cleared of adhering tissues and weighed and sperm samples were gotten from the cauda epididymis.

2.7. Assay of biochemical parameters

Serum Lipid Profile including total cholesterol, high-density lipoprotein cholesterol, triglyceride and low-density lipoproteins, were assayed using Aggape assay kit. Very low-density lipoprotein cholesterol was calculated using Friedwald's formula.[28] The male reproductive hormones were assayed using Enzyme-linked immunosorbent assay (ELISA) technique, using their respective ELISA kits from Monobind Incorporation, USA,[29] the manufacturer's manuals were strictly followed. Absorbance at 450nm (using a reference wavelength of 620 - 630nm to minimize well imperfection) was read within 30 minutes with a microplate reader.

2.8. Estimation of Semen Parameters

Sperm motility and morphology, were assessed as an aliquot of a solution placed on a slide and observed under light microscope at x 400 magnification. Sperm concentration was assessed using the counting chamber of the hemocytometer and placed under a binocular light microscope using an adjustable light source. The sperm viability was determined using Eosin/Nigrosin stain.

2.9. Statistical Analysis

Data obtained from the study were expressed as mean ± standard error of mean (SEM.) Statistical analysis was done using version 17 of SPSS with the aid of one-way analysis of variance (ANOVA) and Tukey post-hoc test. Differences between experimental groups are considered statistically significant at $p < 0.05$.

3.0. RESULT

The main semen parameters of the groups as illustrated in Table 1. were observed to be significantly reduced in treatment groups compared to the control. Assessment showed a significant ($p<0.05$) reduction in the percentage of sperm motility and active sperm cells of the treatment groups when compared to the control group. However, the group 5 was observed to be significantly ($p<0.05$) lower in percentage sperm motility when compared to the other treatment groups. The concentration of sperm cells is seen to be significantly ($p<0.05$) lower in treatment groups when compared to the control and other treatment groups. The sperm morphology illustrated in Table 2. presented no significant ($p>0.05$) changes in the percentage of normal sperm cells across the treatment groups when compared to the control group. No significant ($p>0.05$) decrease in the percentage of bent neck was observed in all the treatment groups when compared to the control group. There were also no significant ($p>0.05$) changes in the percentage curved sperm cells of all the treatment groups when compared to the control group.

Serum lipid profile of male Wistar rats at therapeutic and overdose artemether-lumefantrine for 3 days and 3 weeks respectively is presented in Table 3. Observations revealed a significant decrease in high density lipoprotein cholesterol in treatment groups compared to the control. Triglycerides, total cholesterol, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol recorded some increase and decrease which were not significant when compared to the control except for the significant increase in triglycerides in group V and a significant decrease in total cholesterol of group V rats.

Treatment of male rats with artemether-lumefantrine for 3 and 3 weeks did not cause any statistically significant difference($p>0.05$) in the serum levels of male reproductive hormones (testosterone, follicular stimulating hormones and luteinizing hormones) in the treatment group when compared with the control presented in Table 4. The weight of the testis and epididymis are presented in Table 5. revealed no statistically significant ($p < 0.05$) changes in the weight of the reproductive tissues when compared with the control group. This pattern is consistent with the male reproductive hormones.

Table 1: Effect of repeated administration of AL on sperm motility and concentration.

Grouping/Dosage	% Motile	% Non-Motile	% Active	% Sluggish	Concentration (Cell × 106)
Group I Normal control	80.00 ± 7.071	23.00 ± 7.517	76.00 ± 3.674	24.00 ± 3.674	29.80 ± 3.304
Group II 8 mg/kg/bw AL for 3 days	58.00 ± 8.155[a]	42.00 ± 8.155	43.00 ± 9.823[a]	57.00 ± 9.823[a]	30.38 ± 1.750
Group III 12mg/kg/bw AL for 3 days	49.00 ± 1.871[ab]	81.00 ± 1.871[ab]	33.00 ± 5.385[ab]	67.00 ± 5.385[ab]	20.94 ± 2.467[ab]
Group IV 8 mg/kg/bw AL every 3 days for 3 weeks	67.00 ± 7.517[ab]	80.00 ± 7.071[ab]	38.00 ± 7.348[ab]	62.00 ± 7.348[ab]	20.48 ± 2.688[ab]
Group V	19.00 ± 2.449[abcd]	81.00 ± 2.449[abcd]	10.00 ± 2.236[abcd]	90.00 ±2.236[abcd]	22.52±2.621[abcd]

12 mg/kg/bw AL every 3 days for 3 weeks					

Values are presented as Mean ± Standard Error of Mean (SEM); n= 7; AL = Artemether-Lumefantrine, a = p<0.05 relative to control; b= p<0.05 relative to group 2; c= p<0.05 relative to group 3; d= P<0.05 relative to group 4.

Table 2: Effect of repeated administration of AL on sperm morphology.

Grouping/Dosage	% Normal	% Bent Neck	% Curve
Group I Normal control	71.00 ± 4.301	8.00 ± 2.000	21.00 ± 2.915
Group II 8 mg/kg/bw AL	73.00 ± 1.225	6.00 ± 1.000	21.00 ± 1.000
Group III 12 mg/kg /bw AL	71.00 ± 1.871	7.00 ± 1.225	22.00 ± 1.225
Group IV 8 mg/kg/bw AL every 3 days for 3weeks	72.00 ± 3.742	7.00 ± 1.225	21.00 ± 2.915
Group V 12 mg/kg/bw AL every 3 days for 3weeks	71.00 ± 3.317	6.00 ± 1.000	23.00 ± 3.391

Values are presented as Mean ± Standard Error of Mean (SEM); n = 7, AL = Artemether-Lumefantrine

Table 3: Serum Lipid Profile of Male Wistar rats on repeated administration of AL.

Grouping/Dosage	TC (mg/dl	TG (mg/dl	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Group I Normal control	138.0 ± 3.31	40.01 ± 2.22	30.67 ± 1.75	96.84 ± 2.974	8.001 ± 0.844
Group II 8 mg/kg/bw AL	138.7 ± 3.90	40.39 ± 1.97	28.02 ± 2.48*	98.69 ± 5.243	8.078 ± 0.795
Group III 12mg/kg/bw AL for 3 days	136.8 ± 4.67	41.73 ± 1.80	26.21 ± 4.05*a	98.72 ± 1.348	8.346 ± 0.423
Group IV 8 mg/kg/bw AL every 3 days for 3weeks	136.9 ± 2.20	41.97 ± 1.95	28.18 ± 3.16*a	98.45 ± 2.097	8.395 ± 0.011
Group V 12 mg/kg/bw AL every 3 days for 3 weeks	125. 6 ± 2.23*a	42.42 ± 1.81*a	24.97 ± 1.521*a	99.28 ± 4.041	8.485 ± 0.362

Values are expressed as Mean ± Standard Error of Mean (SEM); n= 7. TC= Total cholesterol; TG= Triglyceride; HDL-c= High density lipoprotein cholesterol; LDL-c= Low density lipoprotein cholesterol; VLDL-c= Very low-density lipoprotein cholesterol. * = p<0.05 relative to control Group 1, a = p<0.05 relative to Group II (p < 0.05).

Table 4: Effect of repeated administration of AL on male hormones.

Grouping/Dosage	FSH (mIU/ml)	TEST (ng/ml)	LH (mIU/ml)
Group I Normal control	1.577 ± 0.340	1.780±0.350	2.002 ± 0.273
Group II 8 mg/kg/bw AL	0.979 ± 0.216	0.784±0.126	1.058 ± 0.170
Group III 12mg/kg/bw AL for 3 days	1.173 ± 0.159	0.954±0.189	1.288 ± 0.255
Group IV 8 mg/kg/bw AL every 3 days for 3weeks	0.458 ± 0.130	0.866±0.219	1.169 ± 0.295
Group V 12mg/kg/bw AL every 3 days for 3 weeks 8 mg/kg/bw	1.000 ± 0.405	0.871±0.274	1.176 ± 0.370

Values are expressed as Mean ± SEM; N= 7. FSH= Follicle stimulating hormone; TEST= Testosterone; LH=Lutenizing hormone.

Table 5: Effect of Repeated Administration of Artemether-lumefantrine on Organ Weight.

Grouping/Dosage	Testis Weight	Epididymis Weight
Group I Normal control	2.764 ± 0.1234	2.418 ± 0.3366
Group II 8 mg/kg/bw AL	2.618 ± 0.1322	1.628 ± 0.1104
Group III	2.320 ± 0.1453	1.956 ± 0.1609

12 mg/kg/bw AL for 3 days		
Group IV 8 mg/kg/bw AL every 3 days for 3 weeks	2.470 ± 0.1094	1.798 ± 0.1787
Group V 12 mg/kg/bw AL every 3 days for 3 weeks	2.566 ± 0.0672	1.994 ± 0.2535

Values are expressed as Mean ± SEM; N= 7

4.0. DISCUSSION

The male reproduction parameters are mainly measured in terms of blood levels of male reproductive hormones (testosterone, follicle-stimulating hormone, luteinizing hormone), sperm parameters (motility, concentration, viability and morphology) and sometimes the weight and volume of the testis and epididymis. At least 15 x 10^6 mL sperm concentration, 40 % sperm cell motility, 58 % vitality and 4 % normal morphology are essential for effective fertilization.[30] Also, sperm count, motility and viability are critical indicators of sperm quality and fecundity in the males.[31] This acute and sub-acute study revealed that the administration of artemether-lumefantrine for 3 days and an extended duration of 3 weeks did not cause any significant effect on the blood levels of male reproductive hormones (testosterone, FSH and LH). However, the study showed a dose-dependent decrease in the testicular and epididymal weights of all the treated animals when compared to the control group that were not significant. The study also revealed a significant decrease in sperm motility, concentration and viability, this observation is in agreement with previous studies that testicular cell functions are highly sensitive and compromised by a wide range of compounds[32,33] including antimalarials.[34,35] This observed significant reduction in sperm motility, concentration and viability in treated groups, suggests that the metabolism of artemether-lumefantrine may produce metabolites that adversely affects testicular function which may cause infertility and this can be attributed to oxidative stress mediated by reactive oxygen species (ROS) which has been recognized as a more common causative factor in male infertility. In males, developing germ cells, spermatogonia and spermatocytes of the testis are seen to be highly sensitive and undergo apoptosis in conditions that cause oxidative stress.[36]

Artemether and lumefantrine are both highly lipophilic compounds, and this study revealed that some serum lipid parameters recorded some degree of increase and decrease that were significant when compared to the control. A significant decrease in high density lipoprotein cholesterol was observed in treatment groups when compared to the control, this finding is in agreement with Nkereuwem *et al.*[37] who reported a reduction in serum HDL-cholesterol concentration following the administration of Artemether. However, triglycerides, total cholesterol, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol recorded some increase and decrease which were not significant when compared to the control except for the significant increase in triglycerides and decrease in total cholesterol in group V rats treated with 12 mg/kg bodyweight of artemether-lumefantrine for 3 weeks. These observations suggest that the misuse and frequent administration of artemether-lumefantrine may indeed alter the lipid profile in males which may impair some cellular functionality. Lipids have been reported to be significant in sperm cell maturation and functionality[38, 39] and it has been mooted in scientific literature that a balance in lipid metabolism is essential for sperm maturation, motility, capacitation and acrosome fusion.[12] Studies are also suggestive of a significant correlation between lipid profile and semen parameters.[40,41,42] A complex arrangement of signaling pathways is involved in the regulation of sperm motility and they are reports of negative association of increased serum lipid parameters with sperm motility.[41,12] Therefore, a reduction in the cholesterol levels or an increase in the triglyceride level as observed in treatment groups may have an effect on sperm maturation and motility. Cholesterol sulfate serves as a stabilizer in sperm maturation in the epididymis[43] and a study conducted to investigate the effect of cholesterol lowering agents such as pravastatin, demonstrated reduced sperm motility as a result of reduced total cholesterol and LDL levels with its 6 - 12 months of use.[44] Ventimiglia *et al*.[45] reports that higher levels of serum total cholesterol, free cholesterol and phospholipids is associated with a significantly lower percentage of sperm with intact acrosome and smaller sperm head area and perimeter implicating that elevated cholesterol levels in the blood may adversely affect sperm morphology, specifically the acrosome integrity and sperm head size. Ergun *et al*.[41] also demonstrated a significant correlation between plasma lipid concentrations and sperm motility, and reported that hypertriglyceridemia may have deleterious effects on spermatogenesis, implying that the levels of lipids in the blood are closely linked to how well sperm can move, which is crucial for fertilization. This present study depicts a negative correlation between imbalances in lipid levels and sperm maturation and motility and this aligns with the findings of other reports. However, the result of this study was not wholly consistent with the conclusion from an earlier report by Grizzard *et al*.[46] comparing sperm and seminal content of cholesterol and phospholipids between hypercholesterolemic and normocholesterolemic individuals and concluded that hypercholesterolemia does not affect these levels in spermatozoa and seminal plasma. Although there are strong correlations that indicates that changes in plasma lipid levels could directly affect sperm motility, a clear mechanism of the relationship between blood lipid profiles and semen lipid profiles has not been defined. So, it can be suggested that imbalances in the lipid

profile of blood serum can adversely affect the lipid contents of semen but this hypothesis has not been confirmed by this study. While there is no significant direct correlation established by the study, the hypothesis that blood lipid imbalances could affect semen lipids remains plausible but unconfirmed as no clear biological mechanism has been identified to explain these potential effects. This underscores the need for further research to elucidate the pathways through which lipid imbalances might impact male fertility.

5.0 CONCLUSION

This in-vivo study offers evidence which demonstrates that frequent administration and abuse of artemether-lumefantrine can cause an imbalance in serum lipids, affect semen parameters specifically sperm motility, concentration and viability highlighting the importance of cholesterol and lipid homeostasis for male fecundity.

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Disclosure of conflict of interest

There is no conflict of interest among the authors.

Statement of ethical approval

Ethical approval for the study was obtained from the Research Ethical Committee of the Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria.

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