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Original Research Article

Comparative effect of chronic administration of artemether/lumefantrine and chloroquine on selected male reproductive indices

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Abstract

Purpose: *To evaluate the effect of administration of artemether/lumefantrine and chloroquine on some male reproductive parameters.*
Methods: *Sixty adult male albino rats, aged 12 – 13 weeks and weighing between 186 – 199 g, were assigned into five groups: control group and four other groups administered 2/12 mg/mL of artemether/lumefantrine (ART/LUM), 4/24 mg/mL of ART/LUM, 10 mg/mL chloroquine (CHLQN) and 20 mg/mL CHLQN, respectively. Treatment was carried out at 48 h intervals for 28 days, followed by assessment of gonadosomatic index, testicular and epididymal sperm cell counts, testicular histology, testis epithelial thickness and tubular diameter according to standard methods.*
Results: *Artemether/lumefantrine exposure did not significantly ($p > 0.05$) affect testes relative weight, epididymis tail weights and sperm count per gram epididymis, but the sperm count per gram testis and sperm count per testis were increased. Chloroquine showed no significant ($p > 0.05$) impact on most parameters except for reduction in testis weight in the high-dose group. Histomicrographs of testicular tissue showed normal structure in control and ART/LUM-treated groups, while CHLQN-treated groups exhibited moderate testicular degeneration.*
Conclusion: *Artemether/lumefantrine does not adversely affect male reproductive health based on the parameters assessed, but chloroquine administration induces mild adverse histological changes. The study opens avenues for further research into mitigating potential reproductive side effects associated with antimalarial treatments.*

Keywords: *Antimalarial, Chloroquine, Artemether/lumefantrine, Albino rats, Reproductive indices, Testicular histology*

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INTRODUCTION

Malaria remains a significant global health concern, with over 200 million cases reported annually [1]. Artemether/lumefantrine (ART/LUM) and chloroquine (CHLQN) are widely used antimalarial drugs [2,3]. While their efficacy in treating malaria has been established, concerns have been raised about the potentially harmful effects of these antimalarials on various body organs and systems, including the reproductive system. Although exposure to antimalarial drugs

such as ART/LUM and CHLQN has been studied for potential impact in animal models, including albino rats, the impact of these antimalarials on the mammalian male reproductive system remains to be fully elucidated. The testis, a crucial component of the reproductive system in male animals, plays a pivotal role in spermatogenesis and hormone production. Therefore, investigating the effects of antimalarial drugs on the histomorphology of the testis and sperm-related parameters is essential to comprehensively understand the potential impact of such drugs on male fertility.

Artemether/lumefantrine and chloroquine belong to different classes of antimalarial drugs, but both have been associated with potential adverse effects on various tissues [4,5]. Studies on the effects of antimalarial drugs on testis histology are limited, but existing research suggests that some antimalarials disrupt the delicate balance of the testicular microenvironment, leading to alterations in histological architecture. For instance, chloroquine has been linked to degenerative changes in testicular tissues, including disruption of seminiferous tubules and reduction in germ cell populations [2,6,7]. However, the specific impact of Artemether/lumefantrine and chloroquine on rat testis histology remains underexplored.

The testis is a primary organ of the male reproductive system, in which the sperm (male gametes) are produced. The amount of sperm produced, known as the sperm count, is a crucial parameter for assessing male reproductive health and fertility potential. Alterations in sperm count may arise from disruptions in spermatogenesis or sperm maturation processes. Limited research on the impact of antimalarials such as chloroquine (CHLQN) and artemether/lumefantrine (ART/LUM) on sperm count, particularly within the testis and epididymis, exists. Some studies have reported reduced sperm counts in animals exposed to antimalarial drugs, attributing the effect to the drugs' interference with spermatogenesis [2,6,7]. Understanding whether these drugs influence sperm count in both the testis and epididymis could provide valuable insights into their impact on male reproductive function.

While the effects of the antimalarials CHLQN and ART/LUM on various organs have been studied, their specific impact on testis histology remains inadequately addressed. Given the importance of the testis in male reproductive function, this research gap warrants attention. Furthermore, current research frequently concentrates on clinical data, offering little insight regarding the cellular and histological alterations that underlie reported effects. A comprehensive investigation into the cellular alterations in testis histology and potential disruptions in sperm health induced by these antimalarial drugs is essential for a more thorough understanding of their implications for male fertility. Therefore, this study assessed the effects of ART/LUM and CHLQN on rat testis histology, as well as sperm counts within the testis and epididymis. Specifically, the study evaluated the histological changes in rat testes following exposure to ART/LUM and CHLQN and alterations in sperm count within the testis and epididymis in response to treatment with these antimalarial drugs, so as to identify the relative impacts of these antimalarials on male reproductive health.

EXPERIMENTAL

Drug procurement

The two commercially available antimalarial formulations used, artemether/lumefantrine (80/480 mg) and chloroquine (250 mg), were acquired over the counter from a reputable Pharmacy in Nsukka, Nigeria.

Animal sourcing and handling

A total of sixty adult male (Sprague Dawley) albino rats, aged 12 – 13 weeks, with weights between 156 and 179 g, were procured from the Genetics and Experimental Animal Breeding Laboratory at the University of Nigeria, Nsukka. Ethical approval for the experimental protocol was received from the Veterinary Medicine Institutional Committee for Animal Use of the University of Nigeria, Nsukka (Ref no. FVM-UNN-IACUC-2023-1025), and the protocol was strictly adhered to as approved. The rats were housed in hygienic metal wire cages furnished with drinking troughs and trays for faecal droppings, and they had access to feed (commercial "growers' mash") and clean tap water *ad libitum*. A two-week acclimatization period was observed before the commencement of the experiment.

Study design

A randomized complete block design was used in the study, with each group comprising three replicates and involving four rats in each replicate. The experimental animals were divided into five groups designated A to E, with each comprising twelve rats. The rats were administered treatments as follows: Group A – distilled water (control group); Group B – 2/12 mg/mL of Artemether/lumefantrine (ART/LUM);

Group C – 4/24 mg/mL of ART/LUM; Group D – 10 mg/mL of chloroquine (CHLQN) and Group E – 20 mg/mL of CHLQN. The control group received distilled water (as a placebo). The body weights of the experimental animals were used to calculate the volume of treatment/drug administered, and the route of administration was oral using gavage. Treatment was administered daily for a duration of 28 days.

Gonadosomatic index of testes

On the twenty-eighth day of the experiment, the experimental rats were sacrificed by cervical dislocation, after which the testes were excised from the rats across different treatment groups, cleaned of adherent tissues and blood, and weighed. The whole body, testes, epididymis and epididymis tail weights of the animals were determined using a Metler digital sensitive balance. The relative gonad (testis) weights (RGW) were calculated using Eq 1 [8].

$$RGW = (GW/BW)100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where GW = gonad weight and BW = body weight

Sperm counts

Following the excision and determination of the weights of testes and epididymis, the tissues were ground in separate mortars to release the sperm cells. The numbers of sperm cells per testis (SPT) were counted using a haemocytometer, following the method outlined by [9]. The sperm count per gram of epididymis (SPGE) and sperm count per gram of testis (SPGT) were obtained by dividing the sperm counts by the epididymal and testicular weights (in grammes), respectively.

Histological studies

Testicular tissue samples collected from each treatment group were further processed for histological analysis. Briefly, the procedure involved placing the samples in 10 % formal saline for fixation, transferring the samples through increasing concentrations of ethanol for dehydration, sectioning and then mounting on slides for microscopy as described previously [10].

Statistical analysis

The datasets on sperm counts were processed using analysis of variance (ANOVA) with Duncan's comparison of means in IBM Statistics software (SPSS v20.0), while the datasets on epithelial thickness and tubular diameter were processed using ANOVA with Fisher's LSD comparison of means in GraphPad Prism 8.2.1 to distinguish the means. All analyses were performed at a 95 % level of significance, i.e., statistical differences were considered significant where the *p*-value was less than 0.05 ($p < 0.05$). The results are presented in tables and graphs as mean ± standard error (SE).

RESULTS

Gonadosomatic indices and sperm counts

Animals administered 2/12 mg/mL of ART/LUM recorded significantly ($p < 0.05$) lower testis and epididymis weights (g) compared to control group animals. However, testis relative weights (%) did not differ significantly ($p > 0.05$). While no significant ($p > 0.05$) difference was observed in the epididymis tail weights and sperm count per gram of epididymis, sperm count per gram of testis and sperm count per testis of the ART/LUM-treated rats significantly ($p < 0.05$) increased compared to those of the control group (Table 1). Furthermore, despite the observed lower testis weights (g) of animals administered CHLQN 20 mg/mL (Table 2), all other parameters – testis relative weight (g), epididymis weight (g), epididymis tail weight (g), sperm/g epididymis, sperm/g testis, sperm per testis – did not reflect significant differences between CHLQN-treated animals and control group ($p > 0.05$).

Histopathological effects

Testis histomicrographs of the rats in Groups A (Figure 1), B (Figure 2), and C (Figure 3) showed normal histological morphology. Normal, multi-layered seminiferous tubules (St) having normal seminiferous epithelium (Se) were observed, with optimal presence of Sertoli cells and all the cells of the spermatogenic lineage, including spermatogonia, spermatocytes, spermatids and spermatozoa. In addition, there was a marked presence of spermatozoa (black arrowhead) in the lumen (Lm) of most tubules, as well as a normal presence of interstitial or Leydig cells (white arrowhead) in the interstitium (Is). However, in the testis histomicrographs of rats in Groups D (Figure 4) and E (Figure 5), there was evidence of moderate seminiferous tubular degeneration, a slight decrease in tubular numbers and loss of epithelial cells, resulting in a marked increase in interstitial spaces.

Table 1: Effect of artemether/lumefantrine on gonadosomatic indices and sperm counts of male albino rats

Treatment	Rat weight (g)	Testis weight (g)	Testis relative weight (%)	Epididymis weight (g)	Epididymis tail weight (g)	SPGE ($x10^8$)	SPGT ($x10^7$)	SPT ($x10^7$)
D.W. (Control)	155.33±7.62[a]	1.40±0.02[b]	0.90±0.01[a]	0.22±0.01[b]	0.17±0.02[a]	2.34±0.16[a]	1.79±0.18[a]	2.50±0.23[a]
ART/LUM 2/12 (mg/mL)	142.00±5.13[a]	1.17±0.08[a]	0.83±0.08[a]	0.18±0.00[a]	0.15±0.01[a]	3.06±0.77[a]	2.68±0.14[b]	3.13±0.22[a, b]
ART/LUM 4/24 (mg/mL)	157.33±3.28[a]	1.23±0.04[a,b]	0.78±0.02[a]	0.22±0.02[b]	0.19±0.02[a]	3.18±0.99[a]	3.13±0.23[b]	3.83±0.20[b]

Note: *****D.W. = Distilled water; ART/LUM = artemether/lumefantrine; SPGE = Sperm count per gram of epididymis; SPGT = Sperm count per gramme of testis; SPT = Sperm count per testis; values are presented as mean ± standard error; different superscripts signify significant difference ($p < 0.05$) within columns

Table 2: Effect of chloroquine on gonadosomatic indices and sperm counts of male albino rats

Treatment	Rat weight (g)	Testis weight (g)	Testis relative weight (%)	Epididymis weight (g)	Epididymis tail weight (g)	SPGE ($x10^8$)	SPGT ($x10^7$)	SPT ($x10^7$)
D.W. (Control)	155.33±7.62[a]	1.40±0.02[b]	0.90±0.01[a]	0.22±0.01[a]	0.17±0.02[a]	2.34±0.16[a]	1.79±0.18[a]	2.50±0.23[a]
CHLQN 10 (mg/mL)	168.67±8.95[a]	1.45±0.08[b]	0.86±0.00[a]	0.22±0.01[a]	0.18±0.01[a]	3.38±0.76[a]	2.60±0.56[a]	3.68±0.59[a]
CHLQN 20 (mg/mL)	155.33±7.54[a]	1.24±0.01[a]	0.80±0.36[a]	0.21±0.01[a]	0.18±0.01[a]	3.16±1.47[a]	2.60±0.14[a]	3.23±0.18[a]

Note: *****D.W. = Distilled water; CHLQN = Chloroquine; SPGE = Sperm count per gram of epididymis; SPGT = Sperm count per gram of testis; SPT = Sperm count per testis; values are presented as mean ± standard error; different superscripts signify significant difference ($p < 0.05$) within columns

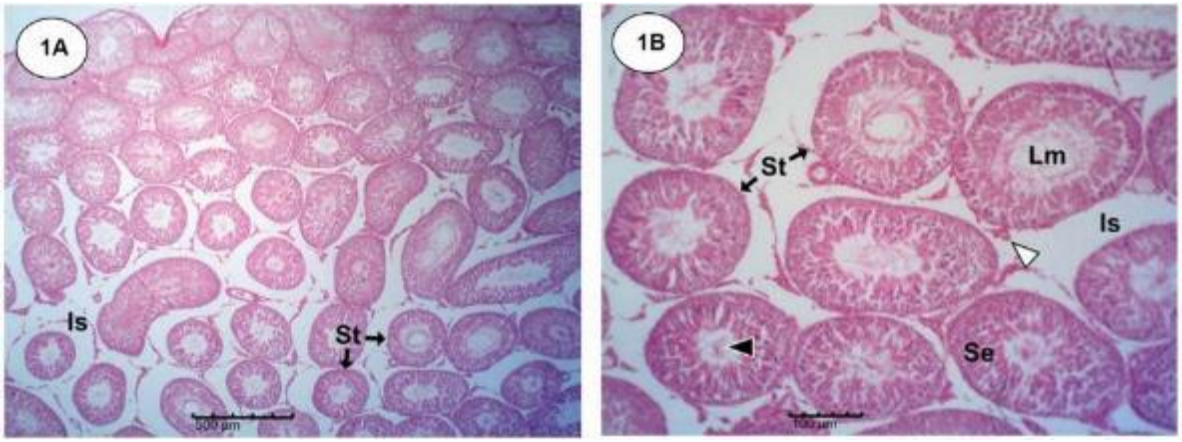

Figure 1: Testis histomicrographs of untreated rats (Control). The histomicrographs presented seminiferous tubules (St) with normal seminiferous epithelium (Se), normal spermatozoa (black arrowhead), lumen (Lm), interstitial or Leydig cells (white arrowhead) and interstitium (Is). Scale bars: 1A = 500 µm; 1B = 100 µm; H & E

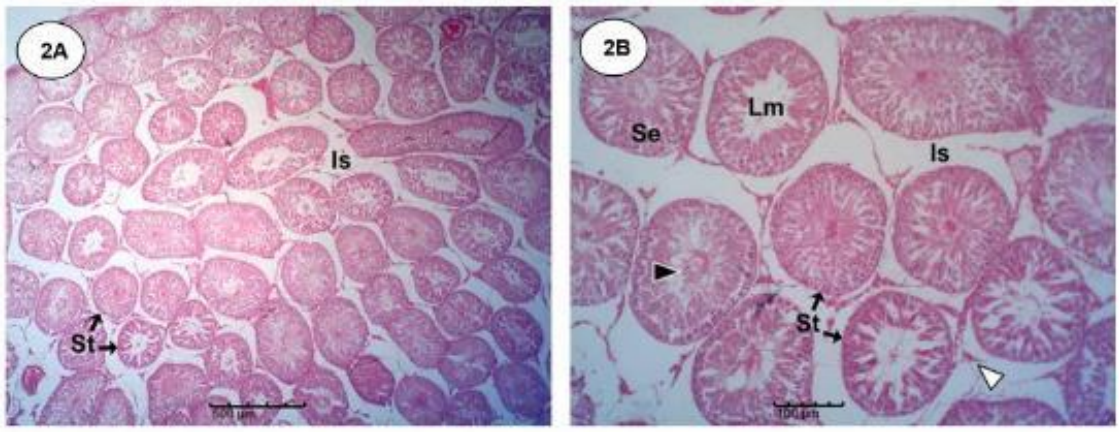

Figure 2: Testes histomicrographs of rats treated with 2/12 mg/mL artemether/lumefantrine (Group B). Normal histoarchitecture was observed, showing normal seminiferous tubules (St) with normal seminiferous epithelium (Se), spermatozoa (black arrowhead), lumen (Lm), interstitial or Leydig cells (white arrowhead), in a compacted interstitium (Is). Scale bars: 2A = 500 µm; 2B = 100 µm; H & E

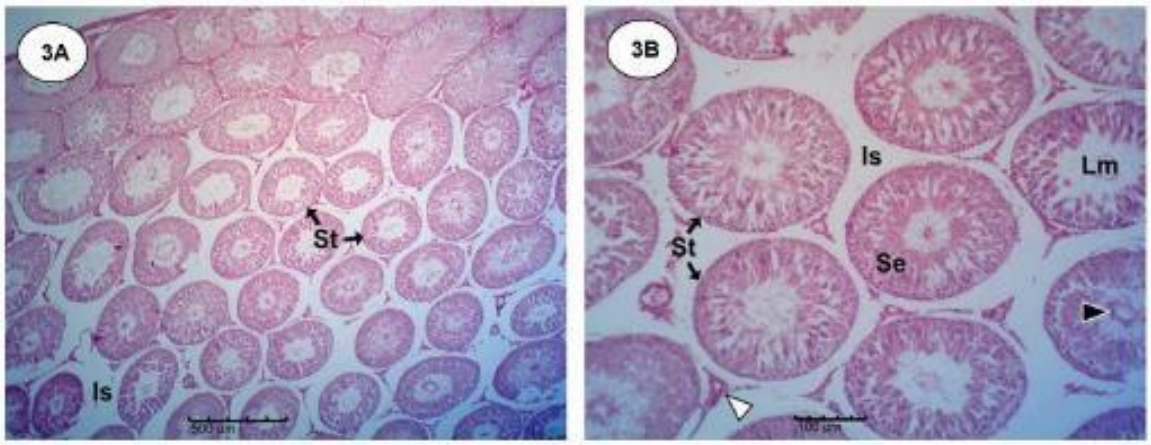

Figure 3: Testes histomicrographs of rats treated with 4/24 mg/mL artemether/lumefantrine (Group C). Normal histoarchitecture was observed, showing normal seminiferous tubules (St) with normal seminiferous epithelium (Se), spermatozoa (black arrowhead), lumen (Lm), interstitial or Leydig cells (white arrowhead), in a compacted interstitium (Is). Scale bars: 3A = 500 µm; 3B = 100 µm; H & E

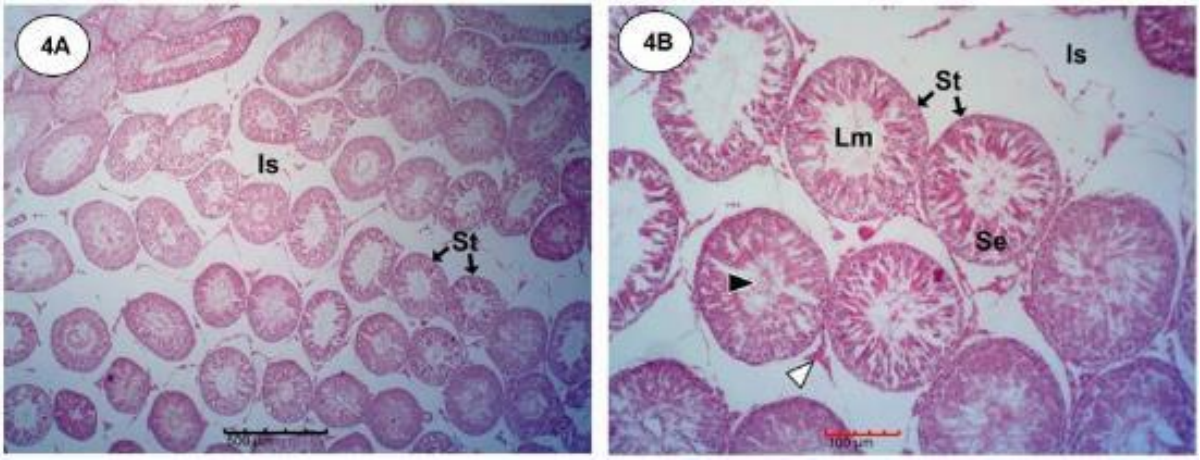

Figure 4: Testes histomicrographs of rats treated with 10 mg/mL chloroquine (Group D). Moderate testicular and tubular degeneration was seen, with slight decreases in the sizes of seminiferous tubules (St) and seminiferous epithelium (Se), resulting in wider interstitial spaces/Interstitium (Is); but normal spermatozoa (black arrowhead) in the tubular lumen (Lm), and normal interstitial or Leydig cells (white arrowhead). Scale bars: 4A = 500 µm; 4B = 100 µm; H & E

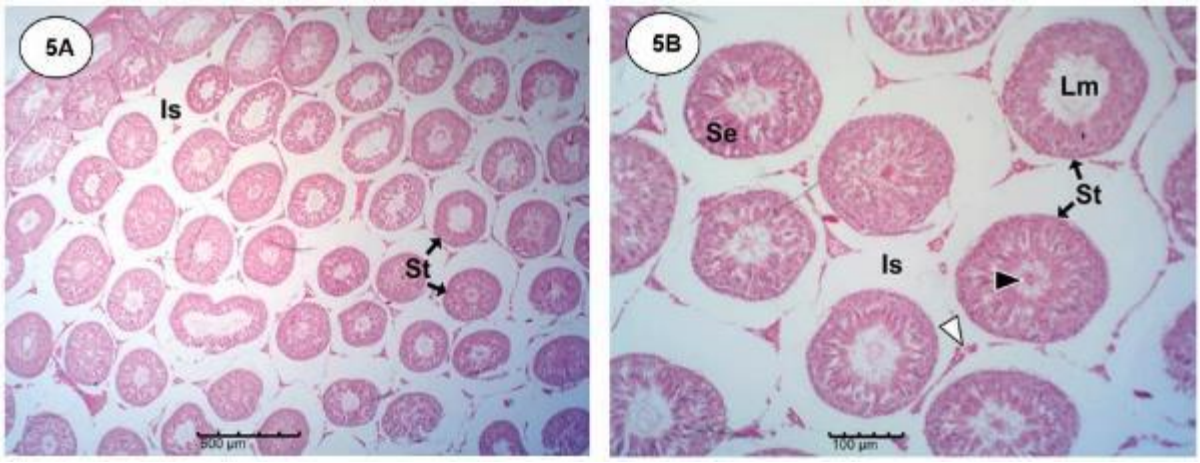

Figure 5: Testes histomicrographs of rats treated with 20 mg/mL chloroquine (Group E). Moderate testicular and tubular degenerations were seen, with a slight decrease in sizes of seminiferous tubules (St) and seminiferous epithelium (Se), resulting in wider interstitial spaces/Interstitium (Is); but normal spermatozoa (black arrowhead) in the tubular lumen (Lm), and normal interstitial or Leydig cells (white arrowhead). Scale bars: 5A = 500 µm; 5B = 100 µm; H & E

Seminiferous epithelial thickness and tubular diameter

The testes of rats exposed to a high dose of artemether/lumefantrine (4/24 mg/mL) recorded a significant increase ($p < 0.05$) in seminiferous epithelial thickness in comparison with rats in the control group (Figure 6 A). Rats administered low dose of artemether/lumefantrine (2/12 mg/mL) presented a significant decrease ($p < 0.05$) in the seminiferous tubular diameter (Figure 6 B) in comparison with the control group. Although decrease in the numbers of seminiferous tubules and loss of epithelial cells were observed in the testes of rats administered chloroquine, actual measurements revealed no significant difference ($p > 0.05$) in seminiferous epithelial thickness (Figure 7 A) and tubular diameter (Figure 7 B) of chloroquine-exposed rats in comparison with rats in the control group.

DISCUSSION

In the context of the effects of artemether/lumefantrine and chloroquine on sperm counts in the testis and epididymis, observations from this study unveiled some distinct outcomes that were contingent on dosage. Artemether/lumefantrine administration augmented sperm count per gram of testis (SPGT) and sperm count per testis (SPT), suggesting enhanced spermatogenesis.

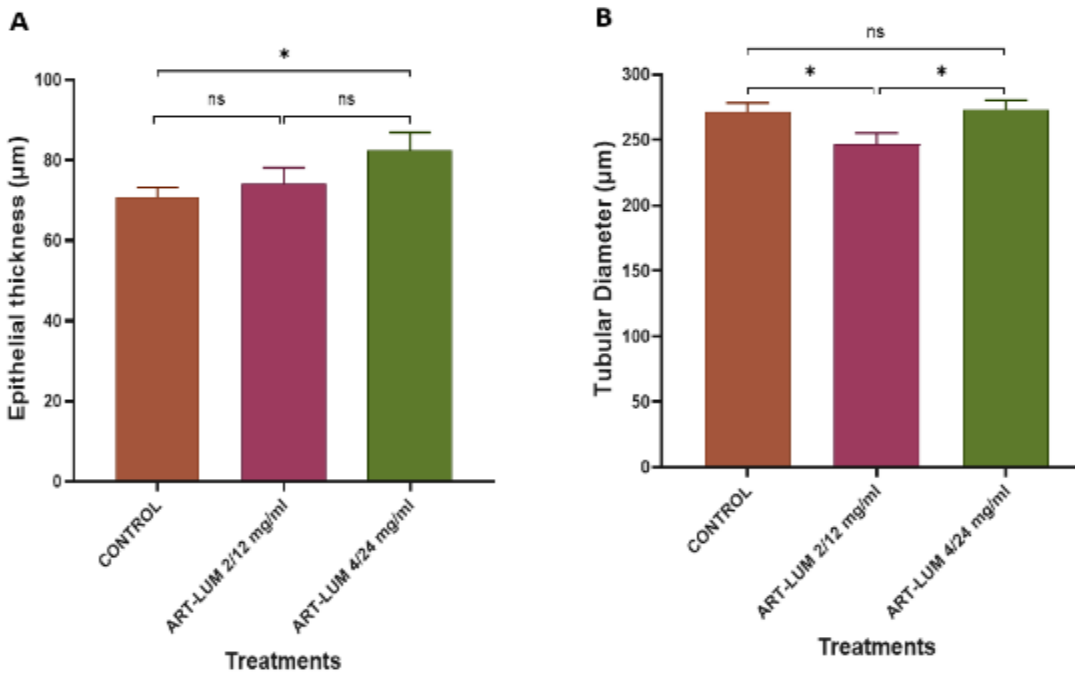

Figure 6: Effect of artemether/lumefantrine (ART/LUM) on seminiferous epithelial thickness (A) and tubular diameter (B) of male rats. The bar graphs illustrate the mean ± standard error; ns = not significant; *p < 0.05 vs control

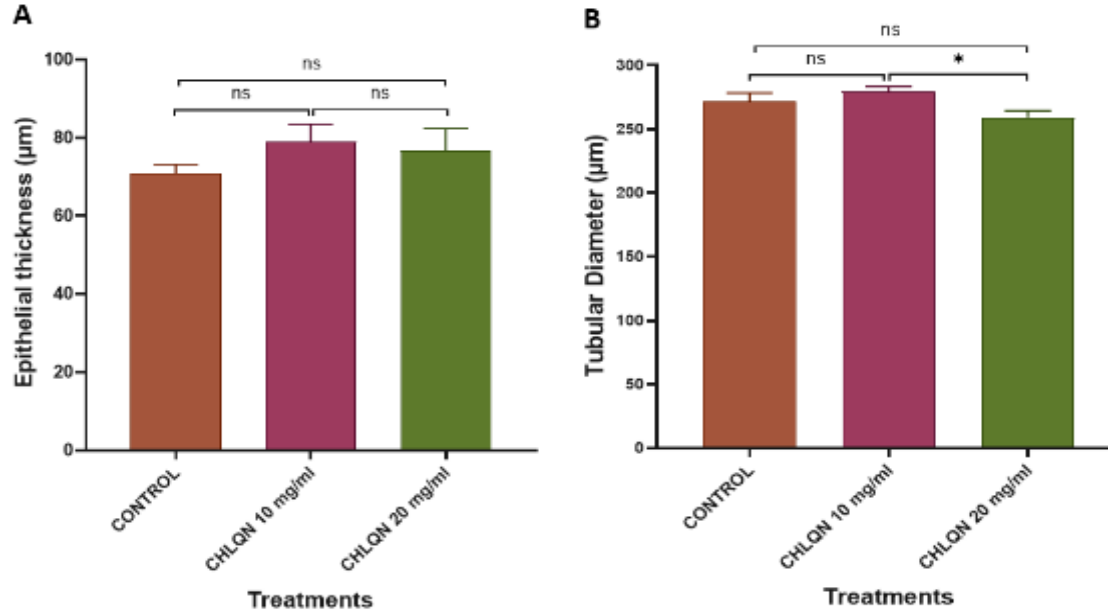

Figure 7: Effect of chloroquine (CHLQN) on seminiferous epithelial thickness (A) and tubular diameter (B) of male rats. The bar graphs illustrate the mean ± standard error; ns = not significant; *p < 0.05 vs control

However, the observed lower testis weight and testis relative weight hint at potentially toxic effects of artemether/lumefantrine on testicular health, leading to a marked reduction in testis weight, especially in the lower dose group. This aligns with the findings of Mofio *et al* [11] Concerning testicular weight reduction and agrees with the report of Morakinyo *et al* [6] In terms of SPGT and SPT in rats exposed to artemether/lumefantrine.

The high and low doses of chloroquine administered caused no significant differences in body weight, testis relative weight, epididymis weight, epididymis tail weight, sperm count per gramme of epididymis (SPGE), SPGT and SPT in the treated rats compared with the control. However, decreased testis weights were observed and this reduction may stem from

protein deficiency linked to diminished appetite and reduced caloric intake [12,13].

The administration of artemether/lumefantrine at varying doses did not elicit any detrimental effects on the architectural integrity of testicular histology in the treated rat subjects. In contrast to this finding, Mofio *et al* [11] reported severe disruption, vacuolar degeneration and necrosis of testicular tubules of mice exposed to artemether/lumefantrine for 21 days, speculating that chronic administration of artemether/lumefantrine could lead to infertility. Other reports have also suggested that antimalarials such as ART/LUM induce toxic effects on testicular tissues and male reproductive function via oxidative stress, altered steroidogenesis, reduction in testosterone levels, reduction of the spermatogenic cell layers and degeneration of seminiferous tubules [14-16]. The discrepancy in findings might indicate a differential in sensitivity to artemether/lumefantrine in the different animal models used, as findings from this study aligned with the report by Morakinyo *et al* [6] who used a similar animal (rats) model.
Furthermore, this study revealed that chloroquine administration led to slightly toxic effects on the histological structure of the testes among the treated rats. Mild testicular degeneration manifested in the form of tubular degeneration, a non-significant reduction in seminiferous tubular dimensions, epithelial cell loss and a notable increase in interstitial spaces. Similar findings were made by Asuquo *et al* [17] and Akin *et al* [18]. According to Asuquo *et al* [17] and Akin *et al* [18], histotoxic impact of chloroquine on the testes could potentially be attributed to its direct influence on cellular cytoskeleton and structure, culminating in disrupted cell-cell contacts, alterations in tubular dimensions and augmented interstitial spaces. They further suggested that chloroquine might induce apoptotic pathways, triggering seminiferous tubule degeneration and epithelial cell loss.

Further investigation into the effects of artemether/lumefantrine and chloroquine on testis epithelial thickness and tubular diameter revealed distinct outcomes. The higher artemether/lumefantrine dosage induced a considerable increase in epithelial thickness, whereas the lower dosage triggered a significant reduction in tubular diameter. These variations might be attributed to inherent biological diversity in individual responses among the subjects. Interestingly, these findings also contrast with those of Mofio *et al* [11]. No substantial differences were observed in terms of epithelial thickness and tubular diameter among rats subjected to chloroquine treatment, relative to the control group. These findings suggest that the doses and duration of chloroquine administration employed in this study did not exert any notable effects on these male testicular components. This outcome is inconsistent with the findings reported by Asuquo *et al* [17], which indicated significant decrease in tubular diameter and necrospermia in chloroquine-treated male Wistar rats.

This study holds both scientific and clinical significance. Scientifically, the study contributes to the understanding of the cellular mechanisms through which artemether/lumefantrine and chloroquine may affect male reproductive health. Clinically, the findings could provide valuable information for healthcare practitioners and policymakers when considering the use of these antimalarial drugs, especially in populations where fertility is a critical concern.

CONCLUSION

Artemether/lumefantrine administration does not adversely affect testicular histology and sperm parameters, but chloroquine administration induces mild adverse histological changes, signifying contrasting impacts of the two antimalarial drugs on male reproductive function. The study opens avenues for further research into mitigating potential reproductive side effects associated with antimalarial treatments.

DECLARATIONS

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Ethical approval

Ethical approval was received from the Veterinary Medicine Institutional Committee for Animal Use of the University of Nigeria, Nsukka (ref no. FVM-UNN-IACUC-2023-1025).

Use of Artificial intelligence/Large language models

We declare also that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that the work embodied in this article was carried out by the authors named in the article, and all liabilities pertaining to claims relating to the content of this article will be borne by them. Conceptualization and design – Emmanuel Ikechukwu Nnamonu, Edmund Chidiebere Mbegbu; Investigation – Emmanuel Ikechukwu Nnamonu, Edmund Chidiebere Mbegbu, and Chiemekam Samuel Ezechukwu; Formal analysis – Emmanuel Ikechukwu Nnamonu and Edmund Chidiebere Mbegbu; Initial manuscript drafting – Emmanuel Ikechukwu Nnamonu; Editing manuscript – Edmund Chidiebere Mbegbu and Chiemekam Samuel Ezechukwu. All authors approved the manuscript for publication.

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