Original Research Article

EVALUATION OF CHRONIC ADMINISTRATION OF THE CONCOMITANT COMBINATION OF ARTEMETHER-LUMEFANTRINE AND CIPROFLOXACIN ON REPRODUCTIVE HORMONES AND PROSTATE SPECIFIC ANTIGEN OF ADULT MALE WISTAR RATS

Abstract

Artemether/Lumefantrine is an artemisinin-based combination therapy recommended by World health organization for the treatment of uncomplicated malaria and ciprofloxacin is a second -generation fluoroquinolone antibiotics used to treat various bacterial infections. This work investigated the possible effect of the concomitant combination of artemether/lumefantrine and ciprofloxacin on selected male reproductive hormone such as Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Testosterone and Prostate Specific Antigen (PSA) in adult male Wistar rats. A total of twenty (20) rats were used for the study, and they were randomly allocated into four groups of five rats per group. Group 1 served as control and received distilled water. Group 2 and 3 was administered 20mg/120mg/kg body weight of artemether/lumefantrine and 125mg /kg body weight of ciprofloxacin respectively while group 4 was administered a combination of both. The drugs were administered orally, once daily for 14 days. The rats were sacrificed and blood was collected in plain bottles and allowed to clot, the serum obtained was used for the reproductive hormone assay. The testes were harvested and evaluated histologically for all groups. The obtained data was analyzed using the one-way analysis of variance (ANOVA) and followed by the post hoc turkey's test. Values at $p<0.05$ were considered statistically significant. Results showed that there was no statistically significant difference in the serum level of prostate specific antigen (PSA) in all treated groups when compared to the control group. However, in group 2,3 & 4, the serum concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH) significantly increased ($P <0.05$) while the serum concentration of testosterone in group 2,3 and 4 significantly decreased when compared to the control. No histological alteration was found in the treated group when compared to control. In conclusion, our findings suggest that the co-administration of artemether/lumefantrine and ciprofloxacin may adversely affect reproductive functions and lead to infertility but may not lead to prostate cancer in males**.**

Keywords: Artemether lumefantrine, ciprofloxacin, FSH, LH, Testosterone, prostate specific antigen, Histology

1. Introduction

Drugs are chemical substances, when introduced into the body, can cause physiological or psychological effect. It is used to prevent, control and cure diseases (Okwakpam et al., 2018). Malaria is one of the most common diseases and over the years it has been a major cause of death in Sub-Saharan Africa Africa. The disease proliferates in less developed areas with low awareness and poor health care systems (1). Malaria is an infectious disease caused by parasites that belongs to the Plasmodium group and transmitted by an infected female Anopheles mosquito. In Nigeria and most African countries, it is endemic causing high morbidity and mortality (Samuel et al., 2018). General symptoms associated with malaria include fever, headaches, fatigue, vomiting, joint aches, etc (Sabina 2017). There are five species of this Plasmodium. The most common in Nigeria is Plasmodium falciparum.

The major available intervention for the disease remains prompt treatment with effective antimalarial drugs. Artemisinin-based combination therapies (ACTs) have lately substituted chloroquine and sulfadoxine-pyrimethamine due to the progress and spread of resistance due to P. falciparium arising from drugs such as chloroquine and sulfoxide-pyrimethamine (Aprioku and Mankwe, 2017). The artemisinin-based

combination therapy (ACT) is therefore recommended by the world health organization for the treatment of uncomplicated cases of malaria; this involves the combination of artemisinin derivatives with another drug such as Lumefantrine, Amodiaquine, Mefloquine, etc. Artemisin and its derivatives are capable of reducing the number of Plasmodium found in the blood of malaria patients (Samuel et al., 2018).

Artemether–lumefantrine, an ACT which is a combination of artemether (C16H26O5) and lumefantrine (C30H32Cl3NO,) is a good candidate and is currently been used as a therapeutic agent of choice in the treatment of malaria (Samuel et al., 2018). It is usually used to treat uncomplicated cases of malaria and also an example of the artemisinin-based combination therapy (Samuel et al., 2018). In most African countries (Nigeria inclusive), Artemether–lumefantrine are readily obtainable over the counter, encouraging its use without proper parasitological diagnosis, especially in the poor rural communities The spread of the corona virus (COVID-19) has also led to the increase in the purchase of this anti-malarial drug because of the similarities in the symptoms experienced in patients that are infected with malaria parasite and the corona virus; these symptoms may include headache and fatigue.

Ciprofloxacin, on the other hand, belongs to the second generation of fluoroquinolone antibiotics (Mokhimar et al., 2020) with the chemical name cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid (Jawad et al.,2020). It is used for the treatment of bacterial infections such as skin, bone, and joint infections, urinary tracts infections, prostatitis, typhoid fever, sexually transmitted infections, salmonellosis and others (Thai et al., 2020). The co-administration of artemisinin/artemisinin derivatives and ciprofloxacin is a common practice in Nigeria in the treatment of malaria and enteric fever co-infection (Amorha et al., 2018; Ayogu et al., 2016).

Follicle Stimulating Hormone is one of the gonadotrophic hormones. It is released into the blood stream by the pituitary gland. It is one of the hormones that is essential for the development and function of the ovaries in women and the testes in men. The follicle stimulating hormone stimulates the growth of the follicles in the ovaries of a woman before the egg is released from a follicle during ovulation and in men; the hormone stimulates the process of spermatogenesis by acting on the sterol cells. Like follicle stimulating hormone, LH is a gonadotrophic hormone produced and released by cells in the anterior pituitary gland. It is crucial in regulation of the function of the testes in men and ovaries in women.

In men, luteinizing hormone can be referred to as interstitial cell stimulating hormone because of its action on the Leydig cell to aid the production of testosterone (Dutta et al., 2019). LH stimulates Leydig cells in the testes to produce testosterone which acts locally to support sperm production (Obidike et al., 2020).

Testosterone is produced by the gonads (by the Leydig cells in testes in men and by the ovaries in women), although small quantities are also produced by the adrenal glands in both sexes. It is an androgen that it stimulates the development of male characteristics. Low testosterone levels can cause mood disturbances, increased body fat, loss of muscle tone, inadequate erections and poor sexual performance, osteoporosis, difficulty with concentration, memory loss and sleep difficulties. Current research suggests that this effect occurs in only a minority (about 2%) of ageing men. However, there is a lot of research currently in progress to find out more about the effects of testosterone in older men and also whether the use of testosterone replacement therapy would have any benefits.

Prostate Specific Antigen is the major protein in the semen, it is produced in the prostate gland by the epithelial cells (Crawford et al., 2019). It is protein produced in the prostate cells and specific for diagnosis of prostate cancer (Okwakpam et al., 2018). It is also known as gamma-seminoprotein or p-30 antigen. In men with healthy prostrate, P.S.A is present in minute amount in the serum but in elevated amount in men with prostrate disorders and cancer. Prostate cancer, being second most common cancer, is one of the leading causes of increased mortality rate in men worldwide. Prostate specific antigen helps in the detection of prostate cancer. (llic et al., 2018).

Although Artemether lumefantrine is considered a relatively safe antimalaria drug (Ashley et al., 2018), concerns are raised recently on their potential anti-fertility (Aprioku,and Obianime, 2011) and cancerous effects.

The aim of this study therefore, was to investigate the effect of treatment with concomitant combination of artemether/lumefantrine and ciprofloxacin on testicular weight and serum hormone levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) male reproductive hormone. The effect of the drug treatments on serum level of prostate specific antigen was equally evaluated.

2. Material and Methods

2.1 Drugs

The Artemether/Lumefantrine, brand name Lonart (Laborate Pharmaceutical Co. Ltd., India) and Ciprofloxacin (Sun Pharmaceutical Ind. Ltd., India) tablets were purchased from Ebus Pharmaceutical Company, Port Harcourt, Rivers State, Nigeria. The drugs were administered to the rats orally once daily for fourteen (14) days.

2.1 Experimental Animals

Twenty (20) male albino rats of average weight 100g -120g were used for this experimental study. They were obtained from animal house of the Department of Biochemistry, Rivers State University and kept in well ventilated cages. They had free access to food and water and allowed to acclimatize for a period of two weeks before the commencement of the experiment.

2.2 Experimental Protocol

The twenty (20) male albino rats used for this study were randomly grouped into four experimental groups of five (5) rats per group. The group one (1) served as control group and received distilled water throughout the period of the experiment. Group 2 was given Artemether/Lumefantrine, 20mg/120 mg/kg bw/daily, which is equivalent to its standard daily clinical dose for treatment of uncomplicated malaria. Group 3 received ciprofloxacin, 125 mg/kg/daily, which is equivalent to its standard daily clinical dose for control of uncomplicated infection and Group 4 received a standard daily clinical dose equivalent of Artemether/Lumefantrine + ciprofloxacin. The study lasted for fourteen (14) days.

2.4 Sample Collection/Preparation

Prior to animal sacrifice, the weight of each rat was obtained using a bench top sensitive scale. The rats were sacrificed by cervical dislocation and blood samples were obtained from each animal and immediately poured into plain specimen bottles. The sera were centrifuged and preserved at − 20 °C until used for biochemical analysis. The testes were isolated carefully and weighed and the relative organ weight (organ to-body weight ratio) was obtained weighed. Thereafter, the testis was fixed in 10% formaldehyde for preservation prior to histopathological examination.

2.5 Relative organ weight

The relative organ weights for the testes = organ weight/ final body weight x 100.

2.6 Biochemical analysis

The serum levels of follicle stimulating hormone, luteinizing hormone, testosterone and prostate specific antigen were determined using the enzyme-immunoassay (E.I.A.) technique. The E.I.A. kits were produced by Accubind (London, UK) and obtained from Nzemat (Lagos, Nigeria). Accubind Elisa microwells kits were used according to the manufacturer's instructions. The optical density was read using a

spectrophotometer (Jen- way, 6300 Spectrophotometer, UK) that was sensitive at wavelengths between 492−550 nm.

2.6 Histological Analysis

Testes of the animals was harvested and fixed immediately in 10% formaldehyde for preservation purpose, prevention of bacteria and subsequent damage prior to the histological examination.

After 24 hours of fixation, the testes were processed via dehydration in order to embed the tissues in paraffin wax. The dehydration process involves putting the testes in ascending concentrations of alcohol. Sections were cut and mounted on slides and stained with hematoxylin and Eosin technique. The slides were cleaned and rounded using DPX oxidant, labelled with stickers and incubated at 37℃ for 12-24 hours. An Olympus microscope (BX51) was used to observe the stained tissue sample and photomicrographs was taken using a charge -coupled camera.

2.7 Data Analysis

The collected data were expressed as mean ± standard error of mean (S.E.M) for all parameters. GraphPad prism version 9.0 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 $P < 0.05$.

3. RESULTS

Biochemical findings

Changes in testicular weight (g) of adult male Wister rats treated with Artemether/Lumefantrine and ciprofloxacin

The results revealed that there was no significant (p value <0.05) change in relative organ weight of rats administered 20mg/120mg/kg body weight of Artemether/Lumefantrine or 125mg/kg body weight of ciprofloxacin when compared to the control group however administration of a combination of Artemether-Lumefantrine and ciprofloxacin once daily for 14 days significantly(p value <0.05) decreased relative reproductive organ weight when compared to the control group (Figure 1). Testicular weight was measured and the relative weight (organ-to-body weight ratio) was obtained.

Changes in serum level of reproductive hormones and prostate specific antigen of adult male Wister rats treated with Artemether/Lumefantrine and ciprofloxacin

From table 2, the results revealed that administration of 20mg/120mg/kg body weight of Artemether/Lumefantrine, 125mg/kg body weight of ciprofloxacin or a combination of Artemether-Lumefantrine and ciprofloxacin, orally once daily for 14 days significantly(p value <0.05) increased the serum level of FSH and LH when compared to the control group(Table 1) while serum testosterone level was significantly decreased when in all the treatment groups when compared to the control group (Table 2). However, there was no significant (p value <0.05) change in the serum PSA level in all treatment groups when compared to the control group (Figure 1)

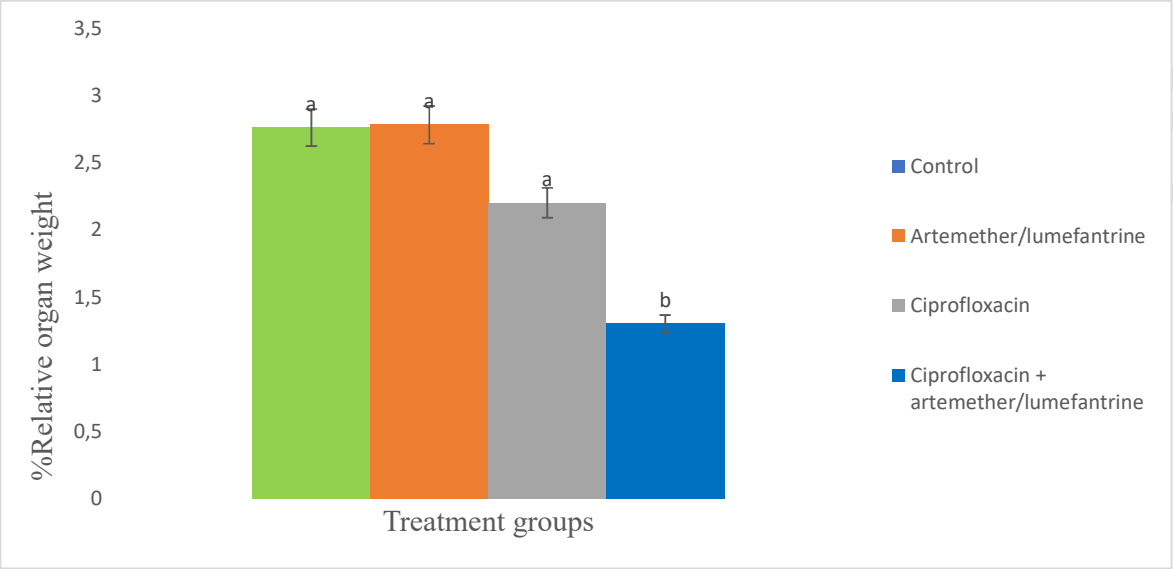

Figure 1: Changes in testicular weight (g) of adult male Wistar rats treated with Artemether/Lumefantrine and ciprofloxacin

Values are expressed as Mean ± Standard error of mean (SEM) n=5. Values with the same superscript are not significantly different at ($p<0.05$).

Table 1: Changes in serum levels of FSH, LH and testosterone of adult male Wistar rats treated with Artemether/Lumefantrine and ciprofloxacin.

Groups	FSH (mIU/ml)	LH (mIU/ml)	TESTOSTERONE (ng/ml)
1(control)	7.7±0.26 ^{a}	9.3± 0. 15^{a}	4.9 ± 0. 18^{a}
2	9.9 ± 0.46^{b}	10.9± 0. 23^{b}	3.7 ± 0. 18^{b}
3	10.0 ± 0.17^{b}	12.6 ± 0. 35^{b}	3.1 ± 0. 40^{b}
4	12.1 ± 0.35^{b}	12.8± 0. 35^{b}	3.5 ± 0. 15^{b}

Values are expressed as Mean ± Standard error of mean (SEM) n=5. Values with the same superscript within a column are not significantly different at ($p<0.05$).

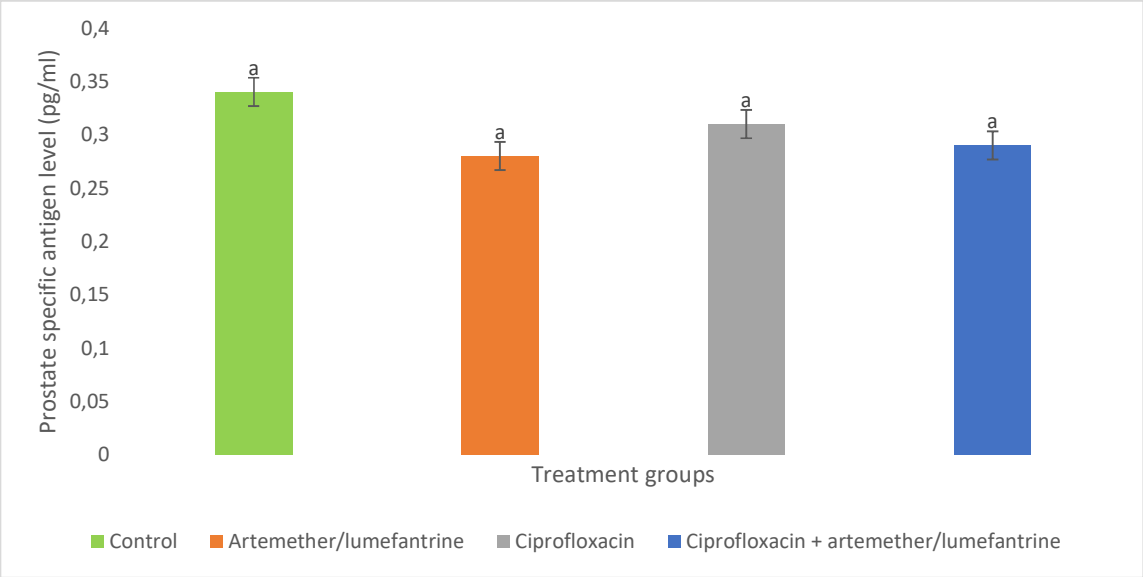

Figure 2: Changes in serum level of Prostate specific antigen of adult male Wistar rats treated with Artemether/Lumefantrine and ciprofloxacin.

Values are expressed as Mean ± Standard error of mean (SEM) n=5. Values with the same superscript are not significantly different at ($p<0.05$).

2 Histology results

The testicular sections prepared from the control and treated groups showed normal maturing spermatocytes within the seminiferous tubules The tubules were lined with germinal cells and supporting sertoli cells. The germinal cells were arranged in several layers from the basement membrane towards the lumen of the tubules. These layers are formed of a series of spermatogenic cells at different stages of maturation including spermatogonia, primary spermatocytes, secondary spermatocytes, sper- matids and mature sperms. The interstitial tissue stroma between the seminiferous tubules contained the interstitial cells of Leydig (Plate 1,2, 3 and 4).

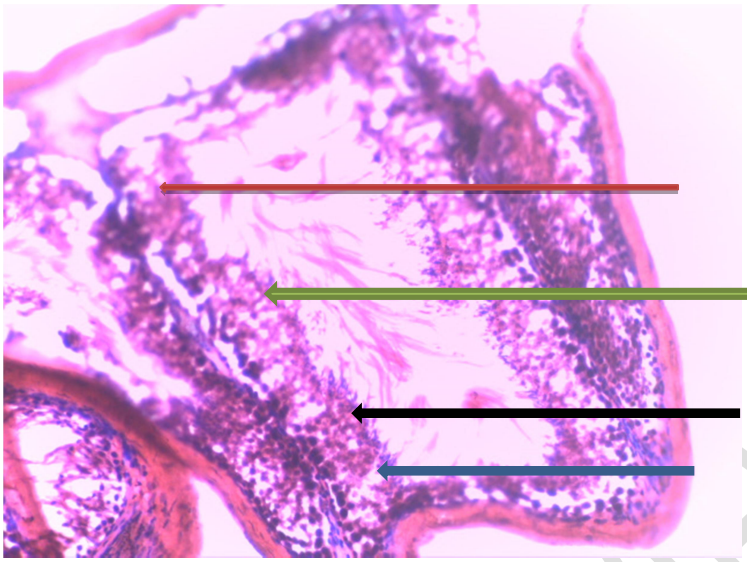

Plate 1: photomicrograph of histology of testicular tissue of control rat shows normal maturing spermatocytes within the seminiferous tubules. Present is the spermatogonia (blue), primary spermatocytes (black), secondary spermatocytes (green) and spermatids (red).

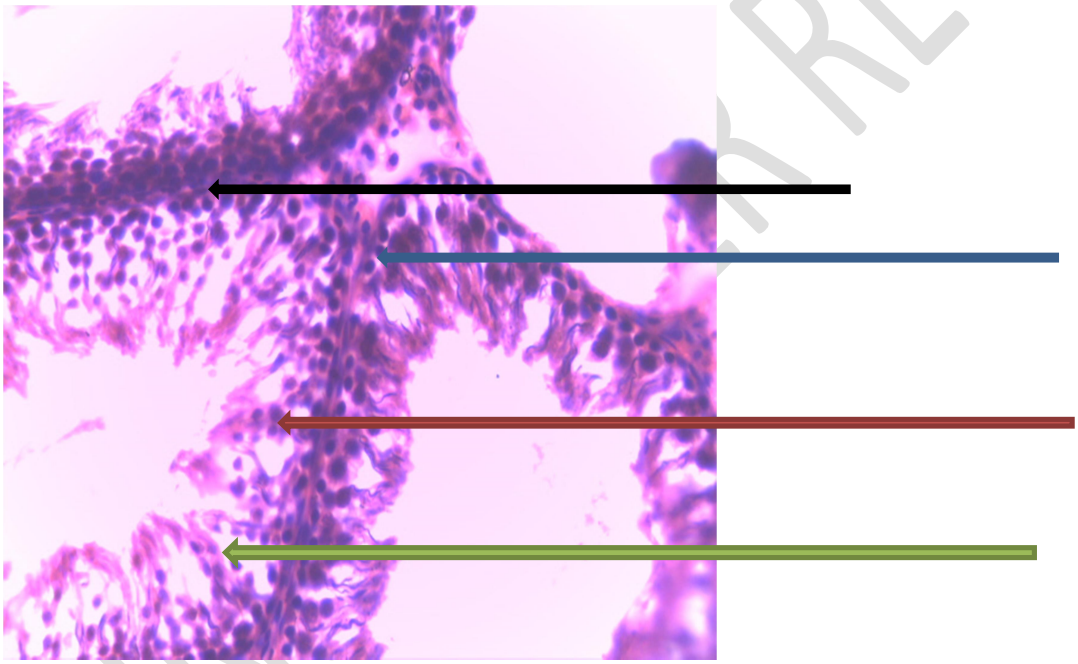

Plate 2: photomicrograph of histology of testicular tissue of group 2 rat after 14 days of oral administration of 20/120mg/kg/b.w of artemether/lumefantrine shows normal maturing spermatocytes within the seminiferous tubules. Present is the spermatogonia (blue), primary spermatocytes (black), secondary spermatocytes (green) and spermatids (red).

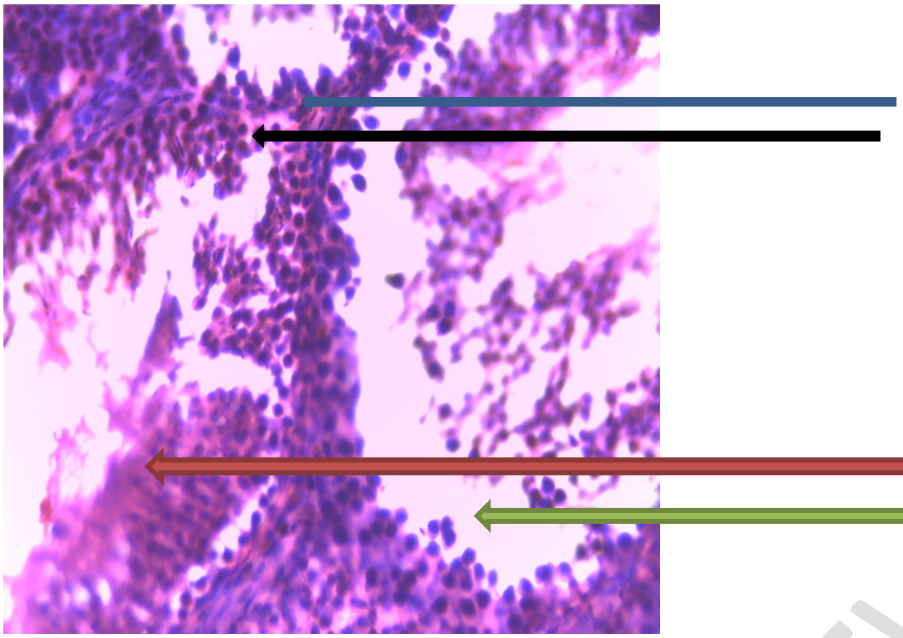

Plate 3: photomicrograph of histology of testicular tissue of group 3 rat after 14 days of oral administration of 125mg/kg/b.w of ciprofloxacin shows normal maturing spermatocytes within the seminiferous tubules. Present is the spermatogonia (blue), primary spermatocytes (black), secondary spermatocytes (green) and spermatids (red).

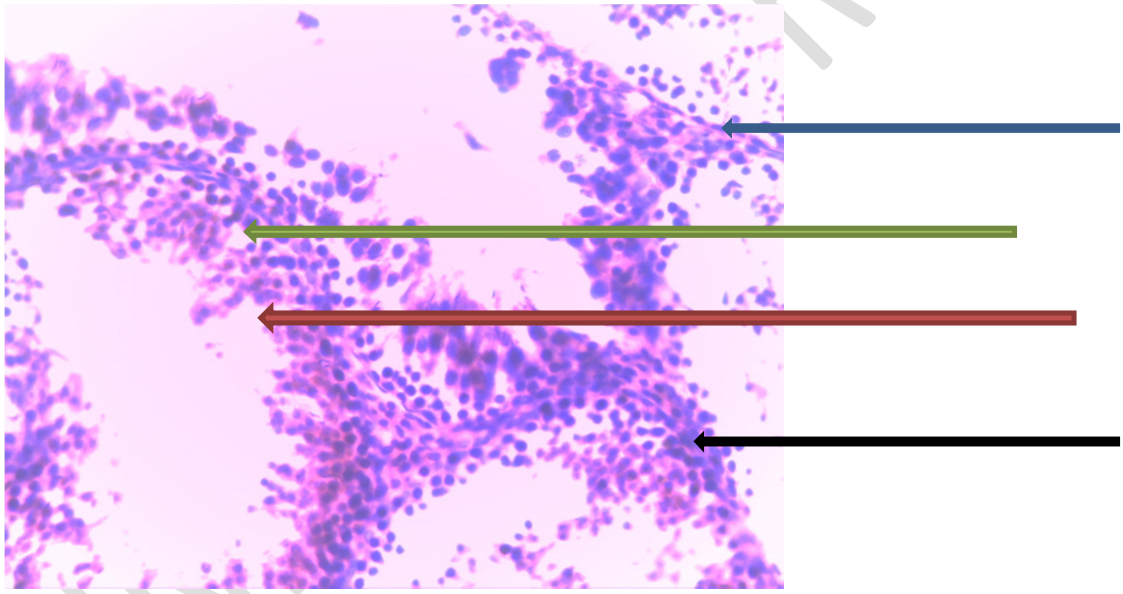

Plate 4: photomicrograph of histology of testicular tissue of group 4 rat after 14 days of oral combination administration of 20/120mg/kg/b.w of artemether/lumefantrine and 125mg/kg/b.w of ciprofloxacin shows normal maturing spermatocytes within the seminiferous tubules. Present is the spermatogonia (blue), primary spermatocytes (black), secondary spermatocytes (green) and spermatids (red).

Discussion

The dose and route of administration of the drugs used in this study was done as recommended for humans and the duration of administration (2 weeks) was used to imitate the frequent or chronic administration of the drugs which can be associated with the reoccurrence of malaria as a result of incomplete treatment or

endemicity. Again, due to the endemicity of malaria in Africa and rising case of the covid-19 pandemic, treatment of malaria with a combination of antimalarial drugs and antibiotics has been on the increase. The male reproductive organ functions with regards to creation of gamete (sperm)essential for insemination of the female ova resulting in progeny and continuity of life. Any contrary effect on the testis can disturb next generations which if not control can lead to extermination. Therefore, there is a need to investigate the safety of concomitant combination of artemether-lumefantrine and ciprofloxacin on male fertility.

This study has shown that chronic exposure to Artemether-lumefantrine showed no significant decrease in relative testicular weight of treated rats when compared with the control. This report agrees with the works of Morakinyo et al., (2009) who also reported no significant decrease in relative testicular weight when compared with the control after a short-term administration of Artemether-lumefantrine once daily using gavage for 3 ad 6 days. This suggests that long-term oral administration of this drug had no negative effects on somatic growth and the testis, which may be associated with the insensitivity of these organs to Artemether-lumefantrine combination. The report of this present study disagrees with works of Mofio et al., 2020, who reported a significant decrease in relative testicular weight of rats when compared with the control after Artemether-lumefantrine was administered ad libitum for 21 days. The differences between our observation and the reports of Daikwo and Kawu, 2015; Aprioku and Mankwe, 2018 and Mofio et al., 2020 on testicular weight could be due to the study design. This discrepancy may be related to different animal ages or species and study design. Administration of 125mg/kg body weight of ciprofloxacin daily for 14 days, did not induce significant decrease in testicular weight when compared with the control. This report disagrees with the report of Mokhimar et al., 2020 who stated that administration of 156.46mg/kg ciprofloxaxin for 14 days induced significant decrease in testis weight. However, result obtained from this study showed that the testis weight of the rats treated with a combination of Artemether-Lumefantrine and ciprofloxacin (group 4) showed a significant reduction in relative testicular weight when compared with the control. It has been reported that a change in relative weight of an organ after drug administration is an indication of the toxic effects of the drug ((Edagha et al., 2019). Moreover, the weight of male reproductive organs usually provides a useful fertility/reproductive risk assessment in experimental studies (Mokhimar et al., 2020). Testicular size is the best primary assessment for spermatogenesis since the tubules and germinal elements account for approximately 98% of the testicular weight (Edagha et al., 2019).

Follicle stimulating hormone, luteinizing hormone and testosterone are male major reproductive hormones and they all play a role to enhance spermatogenesis (Samuel et al., 2018). FSH and LH co-ordinates to synthesize testosterone and aids in the maintenance of a normal sperm health, density and spermatogenesis (Darbandi et al., 2018),

The result obtained from this present study showed that Artemether/Lumefantrine administered to the male rats caused a significant increase in the serum concentration of FSH and LH and a significant decrease in the serum concentration of testosterone in the experimental animals in group 2 when compared to the control. This finding agrees with the works of Daikwo and Kawu, 2015; Aprioku and Mankwe, 2018 and Edagha et al., 2019 who reported that Artemether/Lumefantrine decreases serum testosterone in non-parasitized and parasitized animal models. However, our result does not agree to that of Samuel et al., (2018) who reported that the 3- and 6-days administration of Artemether-Lumefantrine and artesunate-amodiaquine showed no significant effect on FSH, LH and testosterone levels in male Wistar rats. Our findings, also disagree with the works of Aprioku and Mankwe (2017) who reported that a decreased luteinising hormone and testosterone serum levels, without affecting follicle-stimulating hormone in guinea pigs.

In this study, the male rats treated with Ciprofloxacin in group 3 showed a significant reduction in the level of serum testosterone which agrees to the findings of Mokhimar et la., (2020). Ciprofloxacin also caused a significant increase in FSH and LH when compared to the control, and this is in agreement to the report of

Ahmadi et al., (2020) where levofloxacin, one of the fluoroquinolone antibiotic, caused a significant increase in the level of FSH and LH after a 60-day administration of the drugs to evaluate the effect of levofloxacin on spermatogenesis and testis tissue in male rats.

The co-administration of Artemether-Lumefantrine and ciprofloxacin to the experimental animals in group 4 showed a more predominant significant increase in the concentration of serum FSH and LH. Follicle stimulating hormone regulates reproduction qualities, pubertal maturation, sexual development and growth by enhancing the induction and maintenance of the normal sperm production (Orieke et al., 2019). FSH enhances the sertoli cells to produce androgen binding protein which binds to the FSH receptors and this is critical for spermatogenesis to occur (Samuel et al., 2018). Though, an excessive elevation in the concentration of FSH may affect reproduction by adversely affecting spermatogenesis. Luteinizing hormone enables the promotion of male reproduction function by stimulating testosterone production from the leydig cells of the testis (Orieke et al., 2019). The gonadotropin-releasing hormone controls the release of LH from the pituitary gland, low level of testosterone signals the release of GnRH by the hypothalamus which stimulates the pituitary gland to release LH while high testosterone level sends a negative feedback to the hypothalamus and pituitary gland and inhibits the release of LH and GnRH (Samuel et al., 2018).

The co-administration of ciprofloxacin and artemether/lumefantrine caused a significant reduction in testosterone level when compared to that of the experimental animals in the control group. Testosterone is naturally occurring and important hormone in maintaining testis weight through maintenance of the spermatogenic process and inhibition of germ cell apoptosis Inadequate or extremely low level of testosterone causes infertility in men (Samuel et al., 2018). A decreased testosterone level with an elevated FSH level leads to hypogonadism (Lindsay et al., 2015). Therefore, the decrease in testosterone level caused by the co-administrations of ciprofloxacin and artemether/lumefantrine after the 14 days oral administration is an affirmation of testicular toxicity production and may be due to the inhibition of spermatogenesis, decreased elongated spermatids which are associated to infertility (Halpern et al., 2019). The serum prostate specific antigen (PSA) showed no significant change in all treatment groups when compared to the control. PSA is a specific and sensitive marker for the prostatic carcinoma and elevated PSA levels can be linked to prostatic cancer (Okwakpam et al., 2018). From this study, there was no significant change in the serum level of PSA in the treated groups when compared to that of the control group. Hence, the co-administration of ciprofloxacin and artemether/lumefantrine do not affect PSA role in detecting prostate cancer and shows that it is safe for administration in healthy male population and males with prostate cancer.

Histological studies carried out on the testis tissue of the rats showed no evident pathology in the group 2, 3 and 4 when compared to the control group. Normal maturing spermatocytes were found in the histology studies which portrays that co-administration of ciprofloxacin and artemether/lumefantrine did not alter the structure of the testis. This result is not in concert with the report of Aprioku, 2013; Aprioku and Mankwe, 2018, who reported that artemether/lumefantrine caused deleterious effect on the testicular tissue, and these effects are reversible (Aprioku, 2013; Aprioku and Mankwe, 2018).

The testicular weight reduction reported in this study could be attributed to reduction in the size of seminiferous tubules and spermatogenic cells. The significant reduction in serum testosterone in the group that received co-administration of ciprofloxacin and artemether/lumefantrine compared to the control group suggests that co-administration of ciprofloxacin and artemether/lumefantrine did affect Leydig cells which secretes testosterone, but might have not affected spermatogenic cells that utilized the testosterone for spermatogenesis which was affirmed in the histological study.

Therefore, from the findings of this study, chronic the administration of concomitant combination of artemether- lumefantrine and ciprofloxacin ciprofloxacin may induce decrease in testicular weight and testosterone levels hence affecting reproduction and leading to infertility but do not affect the PSA levels, hence does not cause prostatic cancer.

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