DRUG DISCOVERY

Alterations in testis histology, reproductive hormones and abnormal sperm morphology in mice treated with polyherbal antimalarials

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ABSTRACT

Introduction: Malaria remains a significant public health challenge in sub-saharan Africa. As a result, the high cost of conventional antimalarial drugs, poor quality drugs, and the emergence of drug resistance have necessitated the need for alternative sources of medicine to treat and prevent malaria. Safety concerns have also been raised in regard to herbal remedies, which have made it necessary for the screening of two antimalarial polyherbal remedies namely CtA and CtB prepared from a defined mixture of hot water extracts of 6 plants (*Cymbopogon citratus* Stapf, *Curcuma longa* L., *Enantia chlorantha* Oliv., *Mangifera indica* L., *Carica papaya* L., *Alstonia boonei* De Wild.). *Materials and methods:* Scientific justification was reported as to the assumed efficacy of the plant cocktails. On that basis, the reprotoxic impact of antimalarial treatments of CtA and CtB on the male reproductive system of mice is investigated in this study. This is done by evaluating testiculosomatic index, histopathological changes of testes, sperm morphology, and enzyme immunoassays for testosterone and luteinizing hormone. Analyses of data were done by using the software version 23 of SPSS. This was followed by Dunnett's multiple post hoc test with significance considered at $p<0.05$. *Results:* The data analyzed showed that testiculosomatic index significantly decreased in the suppressive group, but increased in the prophylactic and treated/unparasitized groups. Histology of the testes revealed interstitial oedema, erosion of the germinal epithelium. The number of abnormal sperm cells was significantly increased in the curative, suppressive, prophylactic and treated/unparasitized groups. Sperm cells with folded tail occurred more prominently, while knobbed sperm cells had fewer occurrence. Testosterone and luteinizing hormone concentrations were significantly decreased in the suppressive and prophylactic groups. In the curative groups, concentrations had a significant increase for testosterone, but there was a decrease in luteinizing hormone concentration. *Conclusions:* The results generally showed treatment-associated damage to the mice DNA. Therefore, it is noted in this study that excessive consumption of these antimalarial cocktail should be regulated. Further

studies in this area should focus on establishing appropriate means to use polyherbal antimalarials.

Keywords: Polyherbal antimalarials; Reproductive hormones; Testis histology; Abnormal sperm morphology; *Mus musculus*

1. INTRODUCTION

Malaria is the world's most important tropical disease, posing a significant global public health challenge. Malaria is a preventable and treatable disease. Nevertheless, it continues to affect about 87 countries and territories around the world, causing tremendous burden to mainly countries in Africa, Asia and Central and South America (World Health Organization, 2020). In an effort to curb the effect of malaria, scientists have made various efforts worldwide. Particularly, quinine, the first chemically purified effective drug for the treatment of malaria was isolated from *Artemisia annua* plant in 1820. Since then, a number of other natural and synthetic antimalarial compounds have been developed.

However, as time passed, Plasmodium strains began to resist these drugs, rendering them less effective (Tola et al., 2020; Ariey et al., 2014). This has made it necessary for their use to be ceased or restricted to particular situations, constituting a significant problem for effective malaria control (Menard and Dondorp, 2017; World Health Organization, 2011). In recent times, one of the major challenges in curbing malaria has to do with combating drug resistance. Halting the spread of drug-resistant malaria needs to be a global priority, and resources must be focused on those areas of the world where the burden from the disease is greatest.

Phytochemical screening is increasingly being pursued in both developing and developed countries to identify new antimalarials to treat and ultimaltely curb the disease (World Health Organization, 2008). A new antimalarial phytomedicine from the bark of the plant *Nauclea pobeguinii* for instance, was shown to substantially reduce parasitemia in mice infected with rodent malaria (Mesia et al., 2010). It has also proven to be efficacious in phase IIb clinical trials (Mesia et al., 2011). Another natural product from the *Argemone mexicana* aerial part decoction, has also been discovered to be effective in a Phase II clinical trial (Graz et al., 2010).

Unfortunately, one of the major challenges with herbal medicines is the dearth of information about their safety, especially as the health benefits or risks may increase when a combination of medicinal plants is presented as a polyherbal formulation (Orabueze et al., 2018). In order to ensure a thorough efficacy and toxicity assessment of herbal medicines, WHO encourages endemic countries to evaluate local antimalarial remedies for their efficacy and safety and support initiatives that would help to enhance standardized, quality-controlled preparations and products (World Health Organization, 2005). Reproductive toxicities on synthetic antimalarial drugs have been reported. Chloroquine was found to reduce fertility in male rats Trager and Polonsky, (1981), inhibit testosterone secretion in hCG-stimulated testis of pubertal rats Nduka and Dada, (1984), reduce sperm motility and a number of fetuses of cohabited female rats (Adeeko and Dada, 1998).

The disruption of spermatogenesis accompanied by a decline in serum testosterone levels in rats has also been reported (Okanlawon and Ashiru, 1998). Pyrimethamine, a prophylactic antimalarial drug has been known to cause spermatogenic arrest and male infertility in mice (Trager and Polonsky, 1981). A study by Tijani et al., (2010) has revealed that there is a reduction in mean sperm count, motility and viability in rats exposed to co-artesiane when compared to the group that received physic ologic saline as control. Some antimalarial medicinal plants have also been known to alter reproductive functions Ogbomade et al., (2014) including reversible suppressive effect of *Azadirachta indica* on male fertility Sathiyaraj et al., (2010), anti-infertility effects of *Phyllanthus amarus* Ogbomade et al., (2014), and *Alstonia boonei* Oze et al., (2008) in Wistar rats.

These reports have led to the need to screen antimalarial-active cocktails popularly used to treat malaria locally and to inform patients and healthcare practitioners on the likely reproductive disorders posed by these therapies. According to Hermann et al., (2000), the male reproductive parameters are mainly measured in terms of blood level of testosterone, follicle-stimulating hormone, luteinizing hormone, and sometimes the weight and volume of the testis. The genetic consequences of two plant-based antimalarial cocktails on reproductive parameters of male mice following scientific validation of their acclaimed antimalarial efficacies in Ogun and Oyo States Nigeria Omagha et al., (2022) is therefore evaluated in this study.

These antimalarial-active herbal formulations, Cocktail treatment A (CtA) and Cocktail treatment B (CtB) were prepared from commonly used medicinal plants namely: *Cymbopogon citratus* Stapf, *Curcuma longa* L., *Enantia chlorantha* Oliv., *Mangifera indica* L., *Carica papaya* L., *Alstonia boonei* De Wild (names checked with "World Flora Online"). Antiplasmodial activities of these cocktail extracts showed parasite inhibition was dose dependent. At 800mg/kg, inhibition with CtA and CtB was respectively: 96.95 % and 99.13 % on established infection; 96.46 % and 78.62 % on early infection; 65.05 % and 88.80 % on residual infection (Omagha et al., 2022).

2. MATERIALS AND METHODS

Drugs, animals and parasite species for study

As discussed in Omagha et al., (2022), the plant parts used for this study were collected from medicinal plants growers at Oje, Ibadan. A plant taxonomist in the Department of Botany, University of Lagos, Nigeria did identification and authentication. Voucher specimens with LUT numbers 7817, 7818, 7819, 7820, 7821, 7822 for the specimens *Alstonia boonei* (stem bark), *Carica papaya* (fruits), *Cymbopogon citratus* (leaves), *Curcuma longa* (roots), *Magnifera indica* (stem bark) and *Enantia chlorantha* (stem bark) respectively were deposited at the herbarium unit of the Department of Botany, University of Lagos, Nigeria. Each of the plants parts were sorted, washed adequately under running tap water, cut in pieces and then dried separately at 38 0C. Powdered *E. chlorantha* (stem bark), *C. citratus* (leaves), *C. papaya* (unripe fruits), *M. indica* (stem bark), *C. longa* (roots) *and A. boonei* (stem bark) were then separately extracted with hot water, dried, labeled and stored. The extracts were combined in ratios in order to obtain Cocktail treatment A (CtA) and Cocktail treatment B (CtB). CtA was prepared by dissolving 5.70 g + 2.87 g + 1.43 g of *E. chlorantha, C. citratus and C. longa* in 200 mL distilled water equivalent to 50 mg/mL concentration.

In the same vein, CtB was prepared by dissolving 5.00 g + 2.33 g + 1.27 g + 1.27 g of *E. chlorantha, A. boonei*, *C. papaya* and *M. indica* in 200 mL distilled water equivalent to 50 mg/mL concentration. The results of the combinations were separately heated over a water bath for 30 minutes and left to cool. They were then labeled and refrigerated at 4 °C in air-tight bottles. Chloroquine phosphate (CQ) and Pyrimethamine (PY) manufactured by Vitabiotics Limited, and SKG-Pharma Limited, respectively, are the standard chemotherapeutic drugs for malarial control used in this study. Prior to use, the doses required for each of the standard drugs, 25 mg/kg and 5 mg/kg respectively Iwalokun, (2008), Alli et al., (2011) were prepared in distilled water (vehicular/negative control).

A total of 156 sexually matured Lanning et al., (2002) male mice of about 10 - 12 weeks old weighing between 18 – 26 g obtained from the Animal House, University of Lagos, Nigeria was used for this study. Chloroquine-sensitive *Plasmodium berghei berghei* were obtained from the Institute for Advanced Medical Research and Training, (IMRAT), University of Ibadan, Nigeria. This was done by intraperitoneal inoculation of uninfected mice with 0.2 mL of diluted blood from previously infected mice.

Antimalarial tests

As previously reported by Omagha et al., (2022), one hundred and twenty mice divided into 3 groups were used to evaluate antimalarial curative Ryley and Peters, (1970), 4-day suppressive Peters, (1965) and repository (prophylactic) tests. Thirty-six mice were also exposed as the unparasitized/treated. The body weight of each mouse for all the tests was taken before and after exposure. 0.2 ml of the prepared *P. berghei berghei* parasitized erythrocytes suspension in normal saline was injected intraperitoneally into each mouse. The drugs and plant cocktails were orally administered with different doses (200 mg/kg, 400 mg/kg and 800 mg/kg respectively) of CtA and CtB, and 25 mg/kg chloroquine phosphate.

At the end of treatments (curative = day 8, suppressive = day 7, Prophylactic = day 11, treated/unparasitized = day 5), the animals were maintained daily on a standard rodent diet, till they were fasted overnight, sacrificed on day 25, and samples collected for safety assessment studies. Each testis was surgically removed, weighed and fixed in 5 % Bouin fluid for histological analysis in accordance with the report of (Idowu et al., 2015). The caudal epididymis was removed into physiological saline for sperm morphology analysis.

Toxicity assessment on the male reproductive system

Estimation of testiculosomatic index

As previously reported by Madhubanti et al., (2014), testiculosomatic index was calculated from the weights of each testis recorded immediately after collection using the formula: Weight of the testes / final body weight of the mice x 100 % in accordance with (Ademola et al., 2020).

Histology of testes

Tissue sections of each preserved right testes were cut transversly and prepared on clean slides for Hematoxylin-Eosin staining before mounting in neutral DPX medium (Alimba et al., 2016; Adeoyea et al., 2015; Mebratu et al., 2013). Prepared slides were examined at X100 magnification.

Sperm morphology assay

Cauda epididymis was processed within 1 hour after collection by mincing the cauda in physiological saline, then stained with 1 % eosin Y (9:1, normal saline: eosin) for 45 minutes to obtain the sperm suspension. Dry smears prepared on slides were coded for microscopic examination at X1000 for morphological abnormalities according to standard procedures (Wyrobek et al., 1983; Otubanjo and Mosuro, 2007; Olatunji-Ojo et al., 2020).

Quantitative determination of testosterone and luteinizing hormone

Serum obtained from centrifuged blood samples was kept at −20 °C refrigerator until used for reproductive hormonal analysis of mice exposed till day 25. Enzyme-linked immunosorbent Assay (ELISA) reagent kits were used. The assays were carried out according to the manufacturer's instructions. The parameters were evaluated at all doses as similarly followed in (Asare et al., 2013).

Data analysis

Data were gathered and analyzed by using Microsoft Excel and Statistical Package for Social Sciences version 23.0. The differences between means among negative and positive controls as well as treatment groups were compared for significance using one way analysis of variance, followed by Dunnett's multiple post hoc tests. Differences were considered significant to negative control when $p<0.05$.

3. RESULTS

Effects of CtA and CtB on the reproductive system of mice

Results of testiculosomatic index

Following antimalarial suppressive test, testicular weight decreased significantly ($p<0.05$) in the CQ25 mg/kg, CtA200 mg/kg and CtA800 mg/kg groups. However, in the prophylactic groups, there was significant ($p<0.05$) increase in the testicular weights of CtA800 mg/kg, CtB400 mg/kg and CtB800 mg/kg groups. In the treated/unparasitized groups, testicular index was significantly ($p<0.05$) increased only in the CtA800 mg/kg group (Figure 1 a-d).

Outcome of histopathological analysis of animals' testes

Relative to control, testicular histology of the extract-treated prophylactic and treated/unparasitized groups was characterized by severe diffuse but mild testicular interstitium (interstitial oedema) with few sections showing erosion of the germinal epithelium). Plates 1 and 2 below show histopathological changes in testes on day 25.

Abnormal sperm morphology assessment

Table 1 and Plate 4 (a-j) show the different types of abnormal sperm cells observed following exposure of male mice to different concentrations of CtA and CtB. Sperm cells with folded tails were the most prominent, with a total of 320 (19.9 %), 304 (18.9 %), 461 (28.6 %) and 525 (32.6 %) seen in the curative, suppressive, prophylactic and treated/unparasitized groups respectively. In the same way, knobed sperm cells had fewer occurrences, with 2 (28.6 %), 2 (28.6 %), 2 (28.6 %) and 1 (14.3 %) seen in the curative, suppressive, prophylactic and treated/unparasitized groups respectively.

Relative to control, the increases in the percentage of abnormal sperm cells in the curative group were statistically significant ($p<0.05$) in the CQ25 mg/kg and CtB800 mg/kg groups. There was no significant difference in the changes seen in the suppressive groups. As compared to the control, a rise in percentage abnormalities in the prophylactic groups was statistically significant ($p<0.05$) in the CtA400 mg/kg and CtA800 mg/kg groups. Increased abnormal sperm cells seen in the treated/unparasitized groups was statistically significant ($p<0.05$) in the PY5 mg/kg, CtA200 mg/kg, CtA400 mg/kg, CtA800 mg/kg, CtB200 mg/kg and CtB400 mg/kg.

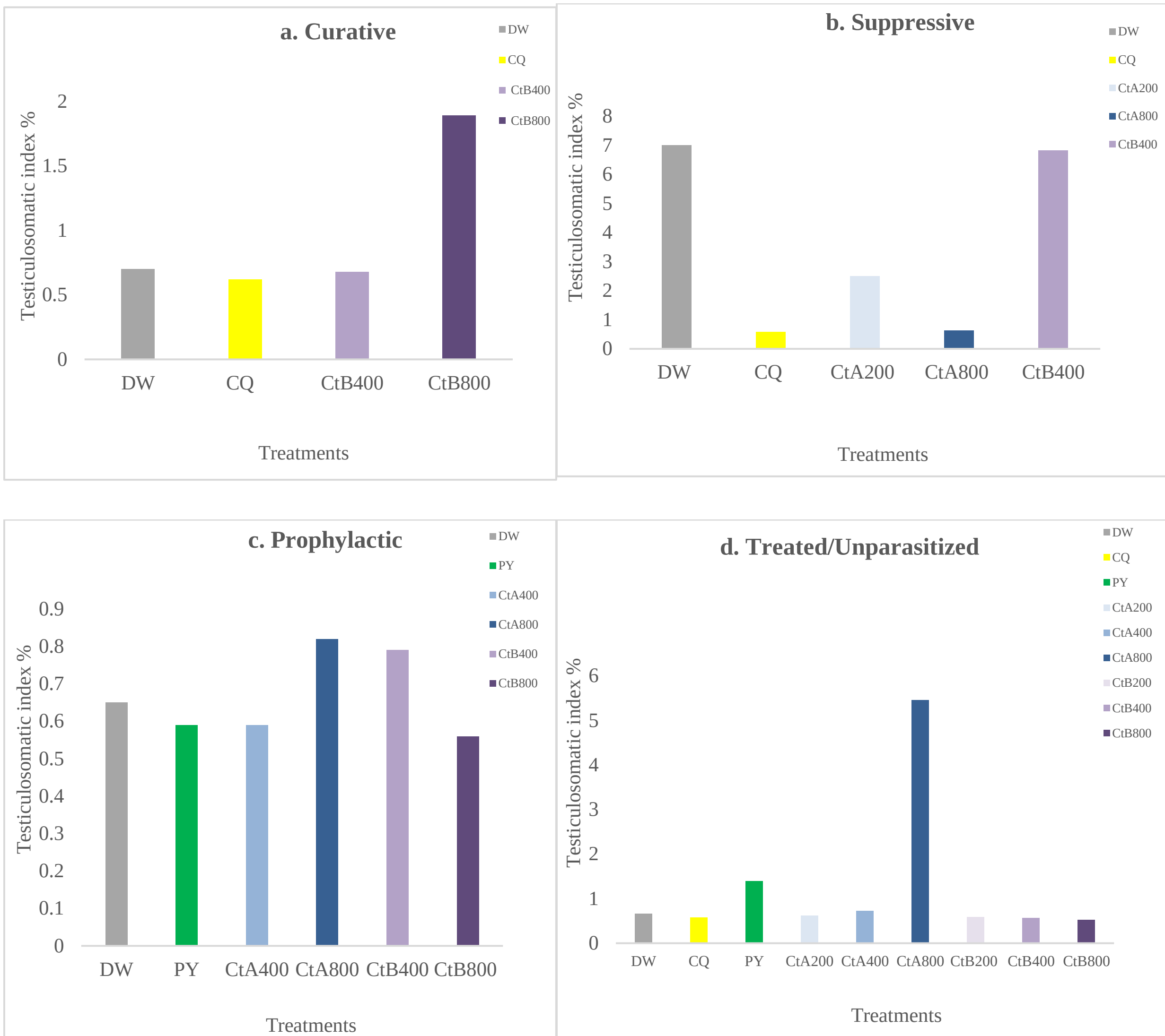

Figure 1 (a-d) Testiculosomatic index of mice post treatment with CtA and CtB. NB: DW (Distilled water), CQ (Chloroquine), PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment B).

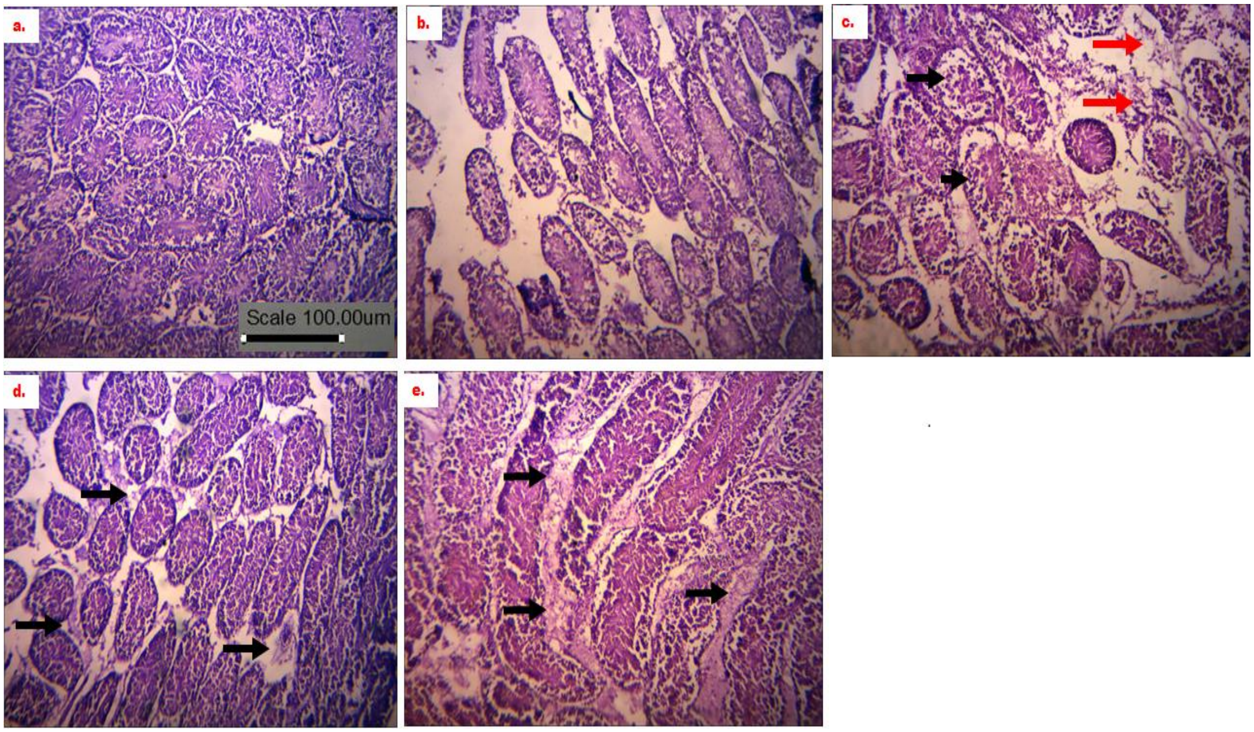

Plate 1 (a-e) Photomicrographs of transverse sections of the testes for prophylactic groups on day 25 (MAG. X 100). (a) DW: No visible lesions seen. (b) PY25 mg/kg: No visible lesions seen. (c) CtA400 mg/kg: There is a mild interstitial oedema (red arrows) with some sections showing erosion of the germinal epithelium (black arrows). (d) CtA800 mg/kg: There is a severe diffuse pink staining material in the testicular interstitium (interstitial oedema). (e) CtB400 mg/kg: There is a severe oedema of the testicular interstitium.

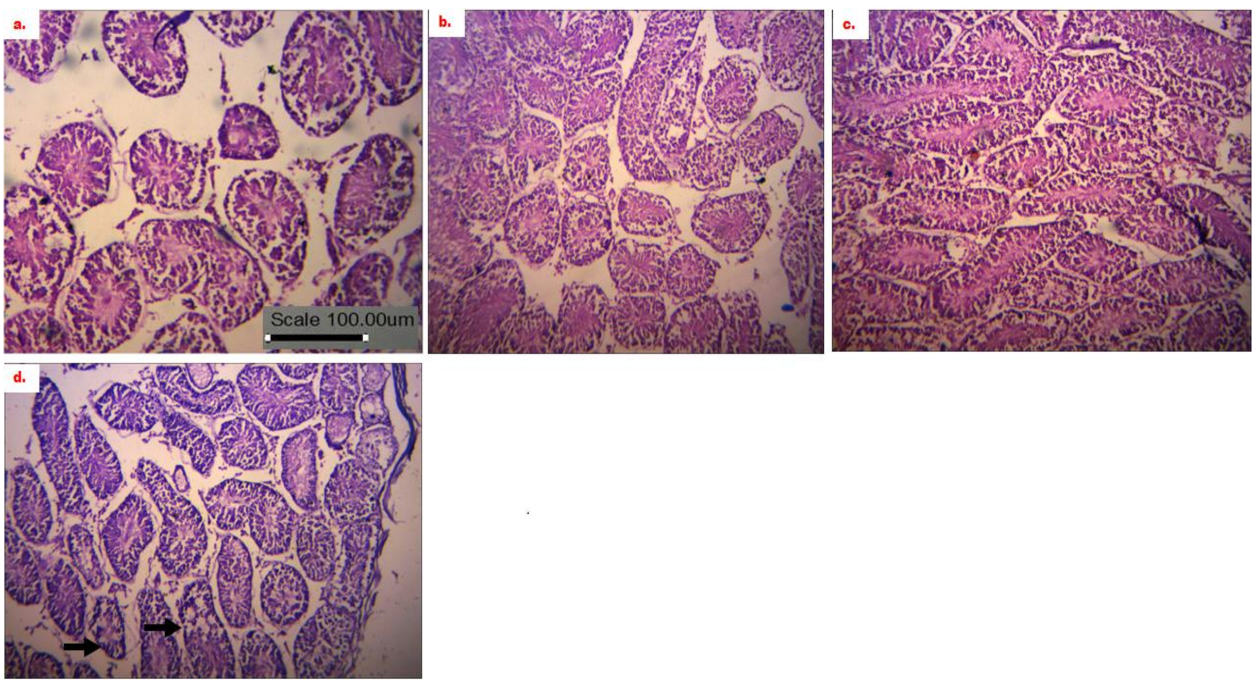

Plate 2 (a-d) Photomicrographs of transverse sections of the testes for treated/unparasitized groups on day 25 (MAG. X 100). (a) DW: No visible lesions seen. (b) PY: No visible lesions seen. (c) CQ: No visible lesions seen. (d) CtA800: There is mild interstitial oedema, with some sections showing erosion of the germinal epithelium (arrows).

Table 1 Sperm abnormalities post treatment

Groups	Treatments mg/kg	Types of sperm abnormalities													
		Amorphous head	Banana head	Pin head	No hook	Short hook	Wrong hook angle	Long and sickled hook	Knobed	Folded tail	Double tail	Wrong tail attachement	Total	Mean	% abnormalitie
CURATIVE	DW	10	0	1	7	3	5	3	0	36	0	17	82	82	8.2
	CQ	54	2	3	18	22	29	1	2	112	6	27	276	92^	9.2
	CtB400	24	0	0	12	18	14	2	0	84	0	19	173	86.5	8.65
	CtB800	27	2	0	9	16	14	1	0	88	0	19	176	88^	8.8
SUPRESSIVE	DW	12	0	0	5	5	4	2	1	44	0	12	85	85	8.5
	CQ	29	1	2	11	16	20	5	1	138	0	23	246	82	8.2
	CtA 200	19	0	0	14	21	12	1	0	61	0	36	164	82	8.2
	CtA 800	12	0	0	8	17	9	0	0	22	0	21	89	89	8.9
	CtB400	9	0	0	8	6	8	1	0	39	0	16	87	87	8.7
PROPHYLACTIC	DW	8	0	0	9	5	8	1	2	37	1	12	83	83	8.3
	PY	57	0	1	19	23	38	2	0	203	12	76	431	86.2	8.62
	CtA 400	21	0	0	31	14	29	1	0	117	1	56	270	90^	9
	CtA 800	15	0	0	9	13	10	0	0	26	0	22	95	95^	9.5
	CtB400	6	0	0	9	4	10	0	0	40	0	19	88	88	8.8
	CtB800	9	0	1	11	3	9	1	0	38	1	16	89	89	8.9
TREATED / UNPARASITIZED	DW	48	0	0	9	21	29	2	0	172	0	49	330	82.5	8.25
	CQ	37	0	1	15	19	13	0	0	212	0	51	348	87	8.7
	PY	44	1	0	16	12	27	0	0	189	1	68	358	89.5^	8.95
	CtA 200	51	0	0	33	38	18	2	0	197	1	54	394	98.5^	9.85
	CtA 400	84	5	1	48	27	34	6	1	206	0	60	472	118^	11.8
	CtA 800	66	2	0	55	31	28	3	0	237	0	59	481	120^	12.025
	CtB200	28	0	0	41	31	39	1	0	153	1	66	360	90^	9
	CtB400	33	1	0	41	14	36	0	0	171	1	67	364	91^	9.1
	CtB800	42	0	0	37	12	42	0	0	164	0	51	348	87	8.7

(P<0.05)^ was considered significant to negative control using Dunnett's multiple post hoc test. NB: DW (Distilled water), CQ (Chloroquine), PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment B).

Effects of CtA and CtB on Male Reproductive Hormonal Concentration

As presented in Figure 2 (a-d), testosterone levels in the curative groups were statistically ($p<0.05$) increased at the CtB400 mg/kg and CtB800 mg/kg treated groups, while luteinizing hormone concentrations were significantly ($p<0.05$) decreased in CQ25 mg/kg, CtB400 mg/kg and CtB800 mg/kg exposed groups. In the suppressive groups, the reduced testosterone level was statistically different ($p<0.05$) only in the CtA200 mg/kg group. In the prophylactic groups, luteinizing hormone levels were statistically ($p<0.05$) reduced in PY5 mg/kg treated group, CtA800 mg/kg, CtB400 mg/kg and CtB800 mg/kg group, but increased in CtA400 mg/kg. In the unparasitized/treated groups, testosterone and luteinizing hormone levels did not significantly change compared to the control group.

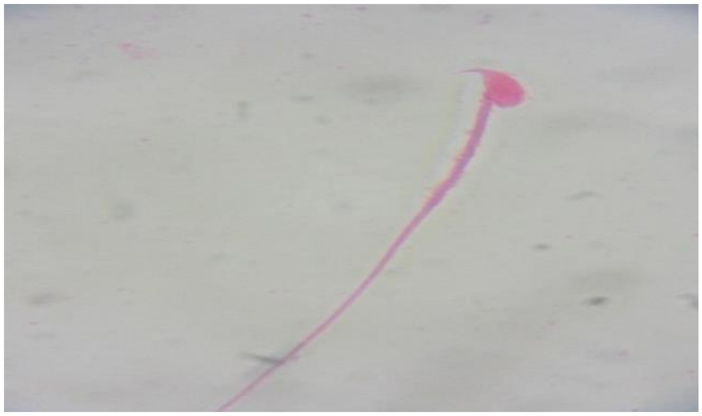

Plate 3 Sperm cell showing normal features in mice. MAG. X 1000

NB: Normal features including: Smooth, oval-shaped head, long tail, no visible abnormality of neck, midpiece or tail.

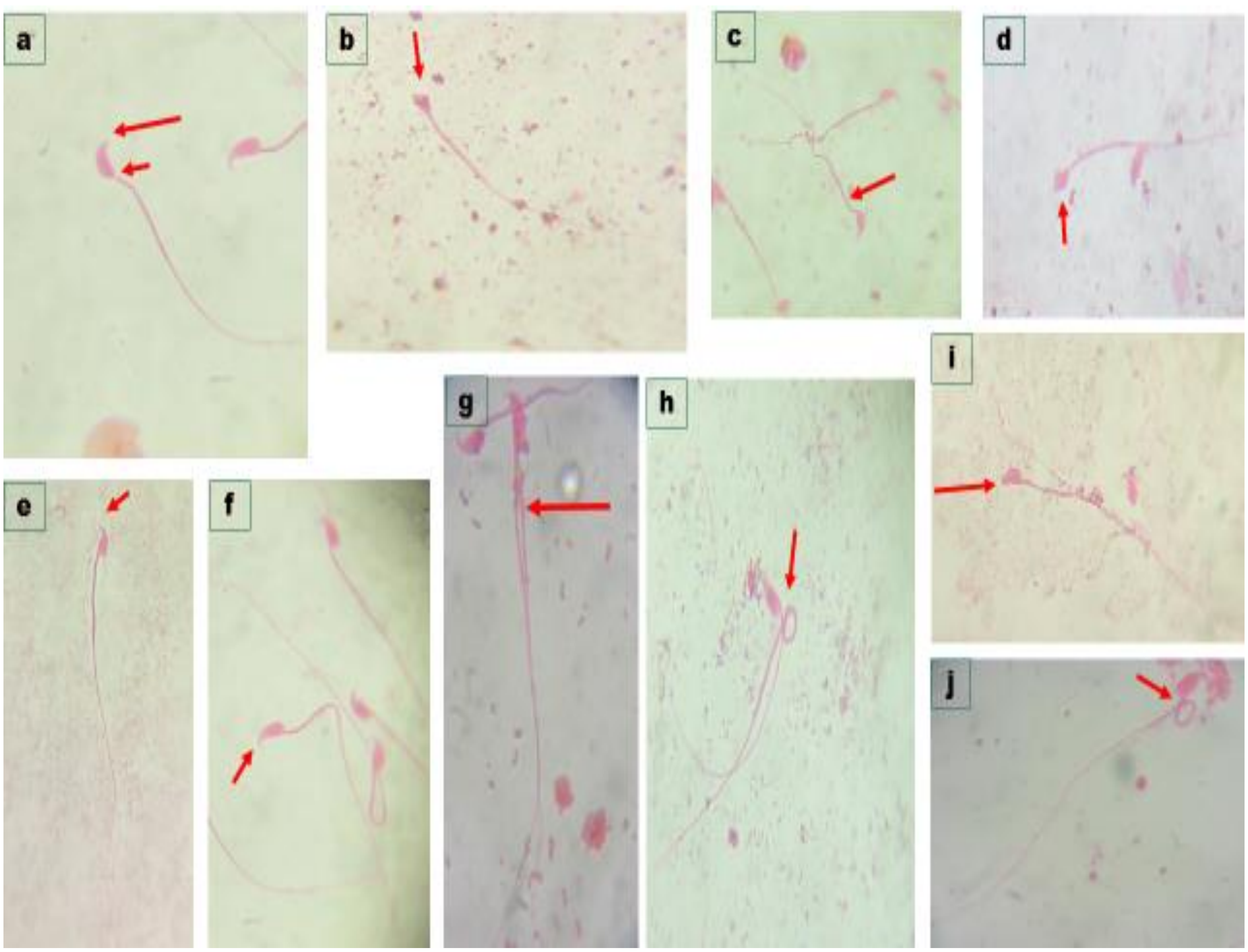

Plate 4 (a-j) Abnormal sperm cells post treatment with CtA and CtB: (a) Sperm cell with short hook, wrong tail attachment and banana head in mice treated with CtA800 mg/kg; (b) Sperm cell with a pin head in mice treated with CtA400 mg/kg; (c) Sperm cell with wrong tail attachment in mice treated with CtB800 mg/kg; (d) Sperm cell with an amorphous head in mice treated with CtB200 mg/kg; (e) Sperm cell with wrong hook angle in mice treated with CtA400 mg/kg; (f) Sickled sperm cell in mice treated with CtB800 mg/kg; (g) Sperm cell with double tail in mice treated with CtA200 mg/kg; (h) Double tail and folded sperm cell in mice treated with CtA800 mg/kg; (i) Sperm cell with no hook in mice treated with CtA400 mg/kg; (j) Folded sperm cell in mice treated with CtB400 mg/kg. Magnification X 1000.

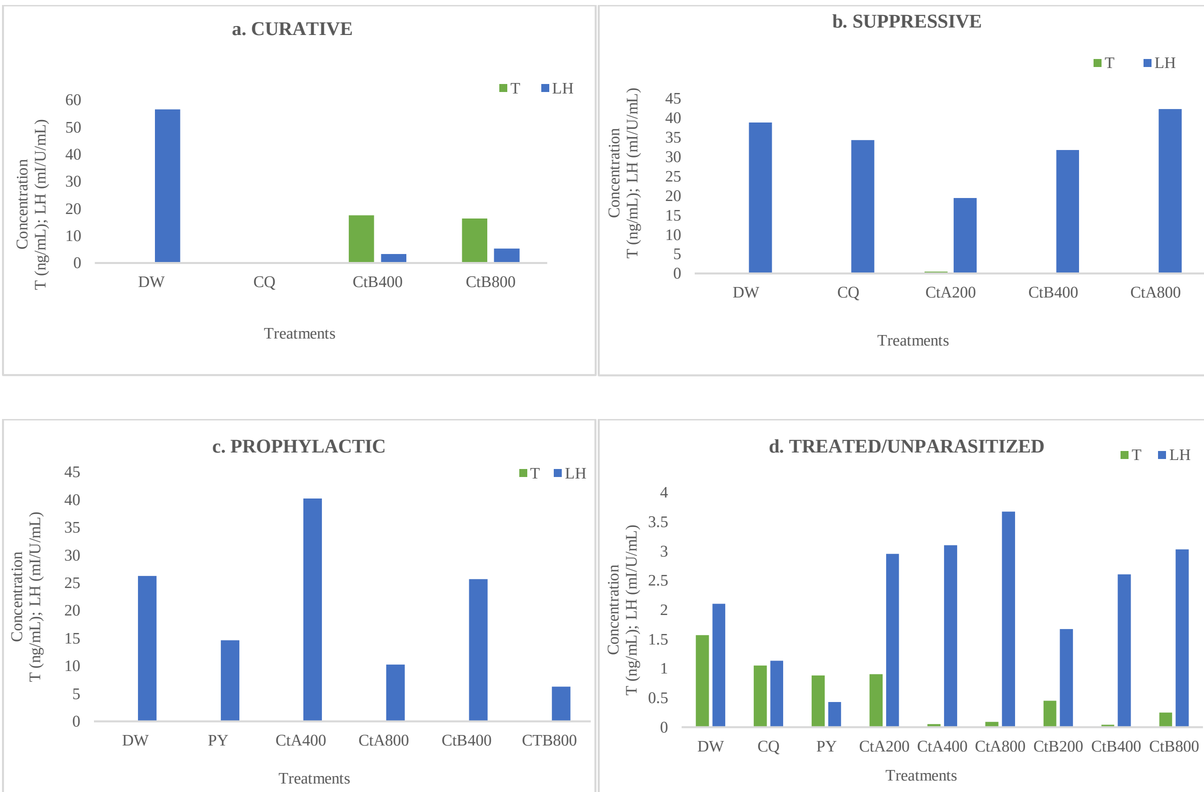

Figure 2 (a-d) Effects of treatment on testosterone and lieutenizing hormone concentration of exposed mice. NB: T (Testosterone), LH (Lieutenizing Hormone), DW (Distilled water), CQ (Chloroquine**)**, PY (Pyrimethamine), CtA (Cocktail treatment A), CtB (Cocktail treatment B).

4. DISCUSSION

Our findings show that the general perception that herbal remedies are very safe and devoid of adverse effects is misleading. The results herein reported are corroborated by previous studies to suggest that plant herbal conctions, particularly at high concentrations, exhibit various toxicities to the male reproductive organ. A study by Raji et al., (2005) reported that oral artemisinin derivative, artemether, caused a significant reduction in progressive sperm motility, viability, sperm count and serum testosterone levels in a dose-dependent fashion during an acute administration of the drug in male rats. It has been reported that Chloroquine reduces sperm motility and fertility, by a reduction in the average number of fetuses, of cohabited female rats (Adeeko and Dada, 1998).

Furthermore, a study on the potential effect of some local antimalarial herbs on the reproductive functions of male albino Wistar rat showed that extract of *Cylicodiscus gabunensis*, *Nauclea latifolia* and *Araliposis soyauxii* significantly reduced testosterone concentration (Ikpeme et al., 2013). Antimalarial activities of CtA and CtB have been previously reported by (Omagha et al., 2021). In the present study, reproductive parameters of male mice (testiculosomatic index, sperm morphology, testosterone and luteinizing hormone) have been assessed in order to determine reprotoxic effects associated with the administration of CtA and CtB antimalarials. The results suggested damage to the mice DNA, thus posing potential health risk in humans. Testicular weight is useful for assessing reproductive risk in experimental studies (Morakinyo et al., 2009).

In this study, decreased testicular weight in the suppressive groups implies that the antimalarial remedies studied caused degeneration of tubules and loss of germinal elements. The increase in weight of the testis in the prophylactic and treated/unparasitized groups suggests toxic effect of CtA and CtB antimalarials on the testes. Histology of the testes to assess the reproductive safety of the treatments also indicated that CtA and CtB might have toxic potential on testes, particularly at high concentrations of the tested polyherbal concotions. Sperm morphological evaluation to provide a measure of the quality of sperm production in sexually mature male mice Lanning et al., (2002) post-treatment with CtA and CtB showed different types of abnormal sperm cell, including sperm cells with no hook, those with double tail, wrong tail attachment, sickled sperm cells, and folded sperm cells. Findings agree with earlier report (Chukwurah et al., 2015; Aduloju et al., 2008). They equally suggest that the effects of CtA and CtB in malarial treatment altered sperm morphology are capable of interacting with the genetic processes involved in spermatogenesis in mice.

Furthermore, sperms with abnormal morphologies are tended towards harbouring abnormal chromosome complements in their nuclei. This suggests that when they succeed in fertilizing normal oocytes, it can lead to the production of individuals with various chromosomal disorders (Ademola et al., 2020; Asare et al., 2013). Testosterone and lieutenizing hormones are essential for normal reproductive functioning of the testes and healthy spermatogenesis (Morakinyo et al., 2009). Hormonal changes due to malaria have been studied. Malaria chemotherapy has been associated with adverse effects of reproductive function (Raji et al., 2006; Okanlawon and Ashiru, 1998). Anti-fertility effects of artemisinin in male rats have been reported (Morakinyo et al., 2009).

Previous studies similarly reported that extract of *Cylicodiscus gabunensis*, *Nauclea latifolia* and *Araliposis soyauxii* significantly reduced the testosterone concentration of male albino Wistar (Ikpeme et al., 2013). Following oral administration of CtA and CtB antimalarials in this study, the results showed that serum levels of testosterone and LH concentrations were significantly reduced in the suppressive and prophylactic groups. In the curative groups, concentrations of testosterone were significantly increased, but LH was decreased. The findings show malaria treatment with CtA and CtB altered sex and reproductive hormones in male mice, an effect that can directly affect the reproductive health of the population who abuse these remedies in an attempt to treat malaria.

5. CONCLUSION

Malaria control continues to rely upon antimalarial plant remedies commonly used as combination therapies. The ultimate goal is to find and produce the desired antimalarial agents with exquisite levels of safety and tolerability that can combat persistent parasite resistance. In that regard, new antimalarials also need to be affordable and available to poor populations who are mostly at risk of malaria morbidity. This study has shown that *Enantia chlorantha* + *Cymbopogon citratus* + *Curcuma longa* (CtA) and *Enantia chlorantha* + *Alstonia boonei* + *Carica papaya* + *Magnifera indica* (CtB) at high concentrations are capable of resulting in genotoxicity of the germ cells and pathological abnormalities of the testes. Therefore, there is need to create more awareness in order to address address concerns regarding the use of herbal antimalarials prepared traditionally.

Ethics approval

The Ethics Committee at the Nigerian Institute of Medical Research Institutional Review Board (NIMR IRB) reviewed the use of animals in this study and granted approval (assigned number IRB/17/036). The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

Author's contributions

RO conceptualized the idea, drafted the proposal. Under ETI, CGA, AOO, EOA, WAO's supervision, RO carried out the research work, wrote and coordinated editing of the manuscript. ETI, CGA, AOO, EOA, WAO provided guidiance, contacts for resources, and contributed to reviewing of the manuscript. RO edited and implemented the changes in the final draft. All authors approved the manuscript for publication.

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Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interest.

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Data and materials availability

All data associated with this study are present in the paper.

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