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RESEARCH ARTICLE

ANTIOXIDANT AND HISTOLOGIC RESPONSES OF ARTEMETHER-LUMEFANTRINE IN EXPERIMENTALLY COMPROMISED RATS' TESTES

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Abstract

Aim: The current study assessed the response of MSG and AL on rats' testes histomorphology and homogenate antioxidant markers.
Methodology: Thirty (30) male wistar rats were allotted to six groups(sample size, n=5) by random sampling and exposed to MSG and AL daily and *per* oral for 7 days thus: Groups A, distilled water (1 mL Kg^{-1}), B, therapeutic concentration of Artemether-lumefantrine, TAL, (1.14 mg Kg^{-1}AL), C, high dose Artemether-lumefantrine, HAL (5.7 mg Kg^{-1}AL), D, MSG (8000 mg Kg^{-1}), E, TAL (1.14 mg Kg^{-1}AL)+ MSG (8000 mg Kg^{-1}) and F, HAL (5.7 mg Kg^{-1}AL)+ MSG (8000 mg Kg^{-1}). Alterations in the testes histology and antioxidant markers were assessed while data were tested for statistical significance by acceptable protocols.
Results: MSG mono-therapy altered the rats' testes anti-oxidation mechanism and histology by increasing (p<0.05) glutathione peroxidase (GP_X), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), albumin (ALB), total protein (TP), and magnesium (Mg), but decreasing (p<0.05) zinc (Zn) levels and degenerating spermatids within the seminiferous tubules compared to control, and other treatments (except CAT activity in MSG + HAL-fed group that increased (p<0.05) above that of MSG–fed rats). These responses following TAL and HAL mono-treatments were inconsistent compared to control and MSG mono-therapies. These underscore the proclivity of MSG + HAL co-therapy to up-regulate the apparent dysfunction in CAT metabolism, and the inconsistency in the apparent modulatory responses by AL against effects by MSG mono-therapy in the rats' testes.
Conclusion: AL caused inconsistent modulation of alterations in rats' testes histology and antioxidant function markers due to MSG assault. The modulation may not be sustainable and the alterations may be instead being spiked warranting caution in co-feeding AL and MSG to rats.
Keywords: Artemether-lumefantrine, magnesium, monosodium glutamate, oxidative response, testes histomorphology, zinc.

INTRODUCTION

Monosodium glutamate (MSG) is the salt variant of glutamic acid formed by replacing hydrogen with a sodium moiety[1]. MSG is present in tomatoes and it is responsible for the characteristic tomatoes flavour and taste[2]. It is popularly used for enhancing food flavor for preparing varied local and intercontinental edible products[3,4]. It is similar to glutamic acid except that one proton (H^+) in the carboxylic group of glutamic acid is replaced with one sodium moiety (Na^+)[5]. Glutamic acid (glutamate), is an amino acid but it is not essential for the synthesis of proteins in plants and animals[6]. Glutamic acid is central to the metabolism of amino acids including the synthesis of arginine, proline and other important biomolecules[5,7]. MSG can dissociate easily in water to give out glutamic acid and free sodium ion[8]. This may implicate free sodium ion with the MSG-related toxicities. MSG impairs the histological and functional integrities of the liver, kidney, brain[3] and testes[2]. A recent report implicated MSG with significant dysfunction on male reproductive system[9]. These and other adverse responses due to MSG treatment were usually accompanied by increased oxidative stress. Various study reports were able to link MSG adverse outcomes with increased oxidative stress. Such increases in oxidative stress was equal to diminished antioxidants, compared to pro-oxidants, and the attendant collapse in antioxidant defense mechanisms[2,3,9].

Malaria is a disease resulting from the transmission of malaria parasites through the bites of female Anopheles mosquitoes infected with *Plasmodium*[10,11]. Based on a recent world health report on malaria, the global prevalence and associated death rate due to malaria remain high[12]. The burden due to malaria disease is reportedly highest in sub-Saharan countries[13]. Malaria is managed with antimalarials[14].The penchant for the high resistance by malaria parasites, notably *Plasmodium falciparium* and *P. vivax* against existing drug mono-therapies necessitated the formulation of combination therapies[10]. Artemether-lumefantrine (AL) is a potent and choice artemesinin-based antimalarial co-therapy widely used against uncomplicated malaria caused by *P. falciparium*[10,15]. AL comprises Artemether (20 mg) and Lumefantrine (120 mg)[15]. The overall bioavailability and pharmacologic action of AL can last for a long time as a result of the high stability of the second drug constituent, lumefantrine. The artemether component of AL initiates rapid clearance of malaria parasites for 2 hours while lumefantrine lasts for 5 days to mop up any remainder of the parasites not cleared by artemether thereby preventing any recrudescence[15,16]. AL is an over the counterdrug[17]. Over the counter drugs are not restricted and are obtained with ease but, as speculated earlier, these over the counter drugs may increase oxidative stress[18]. The mechanisms of pharmacologic action of artemether component of AL include an increased free radicals production and a reduction in blood antioxidants[10].

These mechanisms of Artemether action will lead to a spike in oxidative stress and, consequently, to oxidative damages. Oxidative stress is a situation that results when oxidants generated during cellular activities could not be cleared through the anti-oxidative reaction mechanisms mediated by natural antioxidants[19]. Antioxidants counteract oxidative stress and associated oxidative damages by quenching and reducing excess free radicals[20]. The knowledge of antioxidants status and metabolism will therefore provide significant understanding of the bio-functional state of animal and inherent organs. AL may be prone to abuse (and it could be consumed together with foods containing MSG) which could result to unknown and possibly untoward effects on animals' testes as on the liver reported earlier[21]. The testes are an important component of the male reproductive system and determinant of fertility[22]. Oxidative responses and damages are basic expressions in compromised organ, functional and health conditions and these oxidative responses were associated with male infertility[23,24]. Adverse effects on the reproductive system due to oxidative stress manifest easily on the testes[25]. This is because testes have low vascularity and oxygen content[26]. Possible concomitant effects of MSG and AL on the testes could be ascertained from alterations on the testes histology and antioxidant markers. These underscored the need for the current study with aim to assess the response of MSG and AL on rats' testes histomorphology and testes homogenate antioxidant markers.

MATERIALS AND METHODS

Drug and chemicals procurement

Certified analytical grade of chemicals were used in the current study. They were bought along with the kits which were products of Randox Laboratories Limited, United Kingdom. AL (in the ratio of A:L 20:120 mg per tablet) and MSG were obtained from appropriate sources near the host institution and used with no other purification.

Animals and experimental design

Thirty (30) male wistar rats were kept for 7 days to acclimatize in the animal facility of the host department. They were assigned to six groups and exposed to MSG and AL orally and daily for 7 days thus: Groups A (1 mL Kg^{-1} of distilled water), B, therapeutic concentration of Artemether-lumefan-trine, TAL (1.14 mg Kg^{-1} of AL), C high dose of Arthemether-lumfantrine, HAL (5.7 mg Kg^{-1} of AL), D MSG (8000 mg Kg^{-1} of MSG) as explained previously[27], E, TAL + MSG (TAL 1.14 mg Kg^{-1} of AL + MSG 8000 mg Kg^{-1}) and F HAL + MSG (HAL 5.7 mg Kg^{-1} of AL + MSG 8000 mg Kg^{-1}). All rats were allowed free access to feed (produced by Top feed limited, Nigeria) and clean water. The exposure was through gavages-assisted oral cavity. TAL concentration was calculated from the normal therapeutic dose of four tablets (80:480 mg of A:L) for an average weighing man of 70 Kg while HAL concentration was the product of TAL concentration multiplied by five[21].

Ethical consideration and approval

The study followed the animal use ethics of the host institution based on acceptable guidelines[28] with approval number: ACE-ODUS/14-215632019.

Testes tissues collection, homogenization and preparation for histological assessment

The rats' testes were excised after dissection following humane sacrifice after overnight fasting on day 8. A part was homogenized as described and referenced recently[29]. The other part of the testes tissue sample was fixed in 10% phosphate formalin buffer for histologic examination as described previously[30].

Determination of testes homogenate antioxidant markers

CAT, SOD and GP_X activities, were determined following the instructions on the manual accompanying the respective Randox kits which were respectively based on the methods of Sinha[31], Xin *et al.*,[32] and Paglia and Valentine[33]. MDA concentration was determined according to Wallin *et al.*,[34] while Zn concentration was determined according to Johnsen and Eliasson[35]. Mg concentration was estimated according to Farrell[36] while TP and ALB concentrations were respectively determined according to methods explained previously[37].

Statistical analysis

Data analysis of variance (ANOVA) and test for statistical significant difference in mean followed Duncan's multiple range test principle. These were processed with Windows SPSS (20.0) set at $p<0.05$ confidence level. Results obtained were represented as the mean ± standard deviation (SD) for 5 rats.

RESULTS

Exposing rats to MSG mono-therapy caused an overriding significant increase (p<0.05) in the rats' testes GP_X activity in contrast to other treatments. Exposure of rats to TAL mono-treatment (followed by HAL-fed, MSG + TAL-fed and MSG + HAL-fed) caused the least increase (p<0.05) compared to the control but significantly decreased (p<0.05) the most compared to MSG-treatment (Figure 1) (Values are mean±SD; n=5. Different letters a, b, c, d, e, f (arranged from a=least to f =highest) are significantly different at p<0.05. MSG=8000 mg Kg^{-1}, TAL=1.14:6.85 mg Kg^{-1} of A: L. HAL=5.7:34.25 mg Kg^{-1} of A: L).

In Figure 2, the study outcome revealed that exposing rats to MSG mono-therapy caused a significant increase (p<0.05) in the rats' testes SOD activity above that of the control and other treatments. Exposure of rats to TAL mono-treatment (followed by HAL-fed) decreased significantly (p<0.05) below that of the control and the other treatments while exposure to MSG + HAL (followed by MSG + TAL) co-therapy increased significantly (p<0.05) above that of the control but decreased significantly (p<0.05) below that of the MSG-treatment.

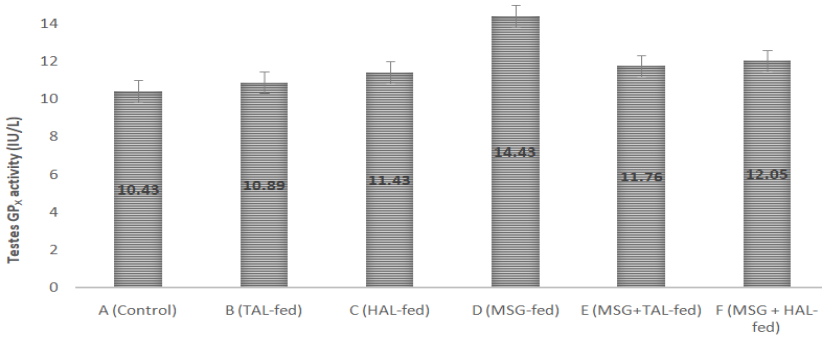

Figure 1: Response of AL and MSG on GP x activity (IU/L) in rats' testes homogenate.

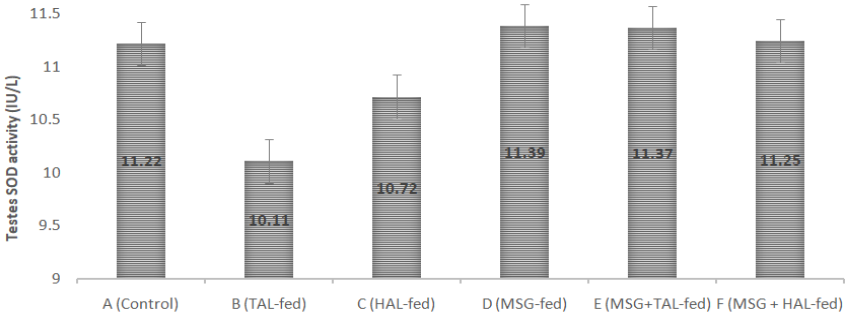

Figure 2: Response of AL and MSG on SOD activity (IU/L) in rats' testes homogenate.

Exposing rats to MSG mono-therapy caused an increase (p<0.05) in the rats' testes CAT activity in contrast to control and other treatments except MSG + HAL-fed. Exposure of rats to MSG + TAL co-therapy decreased (p<0.05) testes CAT activity compared to the control and the other treatments (Figure 3). In Figure 4, the study outcome revealed that exposing rats to MSG mono-therapy caused an increase (p<0.05) in the rats' testes MDA concentration above that of the control and other treatments. Exposure of rats to HAL mono-treatment (followed by TAL-fed) decreased (p<0.05) below that of the control and the other treatments while exposure to MSG + HAL (followed by MSG + TAL) co-therapy increased (p<0.05) above that of the control and others except MSG mono-therapy. Rats that were exposed to MSG mono-therapy had an increased (p<0.05) testes ALB concentration above that of others and control. Exposure of rats to TAL mono-treatment (followed by HAL-fed, MSG + TAL-fed and MSG + HAL-fed) decreased (p<0.05) testes ALB concentration compared to the control and MSG mono-treatment (Figure 5).

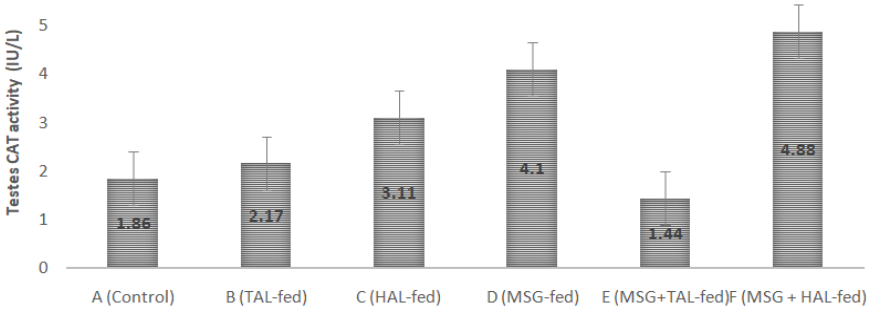

Figure 3: Response of AL and MSG on CAT activity (IU/L) in rats' testes homogenate.

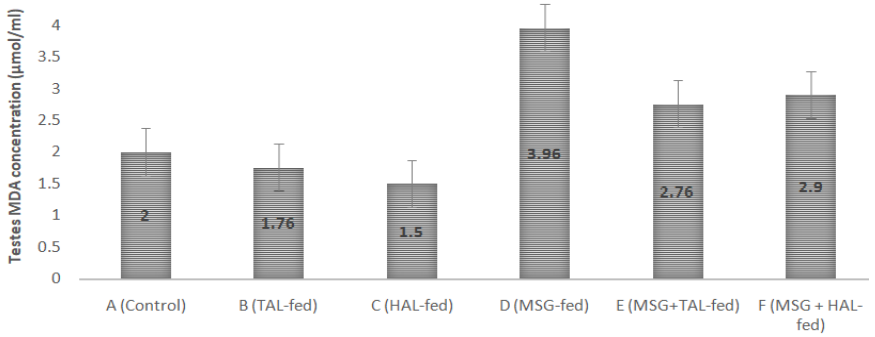

Figure 4: Response of AL and MSG on MDA concentration (µmol/ml) in rats' testes homogenate.

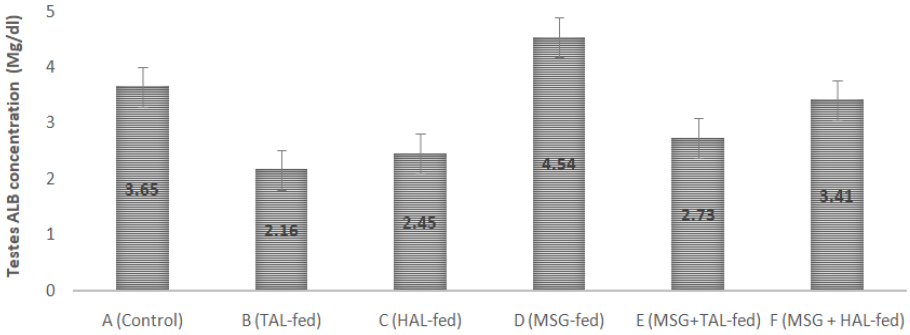

Figure 5: Response of AL and MSG on ALB concentration (Mg/dl) in rats' testes homogenate.

Exposing rats to MSG mono-therapy increased (p<0.05) testes TP concentration in contrast to control and other treatments. Exposure of rats to MSG + HAL followed by MSG+TAL co-therapy decreased (p<0.05) testes TP concentration below that of the control and others (Figure 6).

Exposing rats to MSG mono-therapy decreased (p<0.05) testes Zn concentration in contrast to control and other treatments. Exposure of rats to MSG + TAL co-therapy increased (p<0.05) testes Zn concentration above that of the MSG-mono-treatment and others, including the control (Figure 7). In Figure 8, rats that were exposed to MSG mono-therapy had an increased (p<0.05) testes Mg concentration above that of others and control. Exposure of rats to TAL mono-treatment (followed by HAL-fed) decreased (p<0.05) testes Mg concentration in comparison to other treated groups and the control while exposure of rats to MSG + HAL followed by MSG + TAL co-therapy decreased (p<0.05) testes Mg concentration compared to MSG-treatment. Testes histomorphology of the rats was represented in Figure 9. Photomicrograph of the testes from rats in the control group (Slide A) showed normal seminiferous tubules with normal lamina propria (LP), spermatogonia (SC), spermatids (SP) and leydig cells (LC).

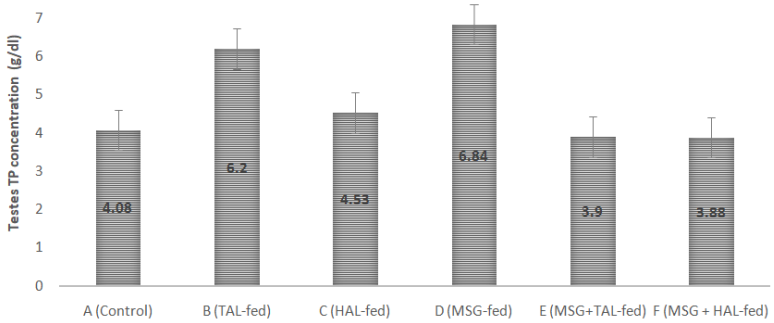

Figure 6: Response of AL and MSG on TP concentration (g/dl) in rats' testes homogenate.

MSG mono-therapy (group D, slide D) caused a severely degenerated spermatids (white arrows) within seminiferous tubules in the rats' testes. Photo-micrograph of the testes from rats in group C (slide C) as compared to group D, showed mild degeneration of spermatids (dSp) in the seminiferous tubules while photomicrograph of testes from rats in group B (slide B), showed numerous immature spermatocytes (black arrows). Photomicrograph of the testes from rats in group E (slide E) showed multifocal degeneration and necrosis of spermatids (white arrows) within the seminiferous tubules while that from rats in group F (slide F) showed severely proliferated spermatocytes and immature spermatids (black arrows) in the seminiferous tubule.

DISCUSSION

This study evaluated the responses of AL on rats' testes histomorphology and antioxidant markers compro-

mised by MSG as the testes are prone to adverse responses due to oxidative stress caused by extraneous chemical agents[25]. MSG mono-therapy caused an increase (p<0.05) in the rats' testes homogenate GP_X (Figure 1), SOD (Figure 2), CAT (Figure 3), MDA (Figure 4), ALB (Figure 5), TP (Figure 6), and Mg (Figure 7) levels but a decrease (p<0.05) in the rats' testes homogenate Zn level (Figure 8) and degenerated spermatids within the seminiferous tubules (Figure 9) compared to control. These were in line with recent review report[9]. The study outcomes demonstrated that MSG caused a significant adverse outcome on the testes histology and testes homogenate markers of antioxidant metabolism in the rats.

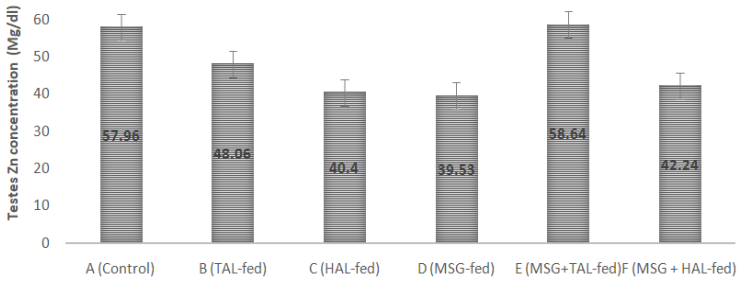

Figure 7: Response of AL and MSG on Zn concentration (Mg/dl) in rats' testis homogenate.

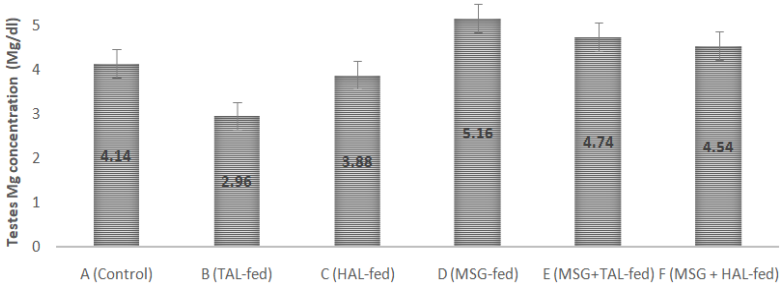

Figure 8: Response of AL and MSG on Mg concentration (Mg/dl) in rats' testes homogenate.

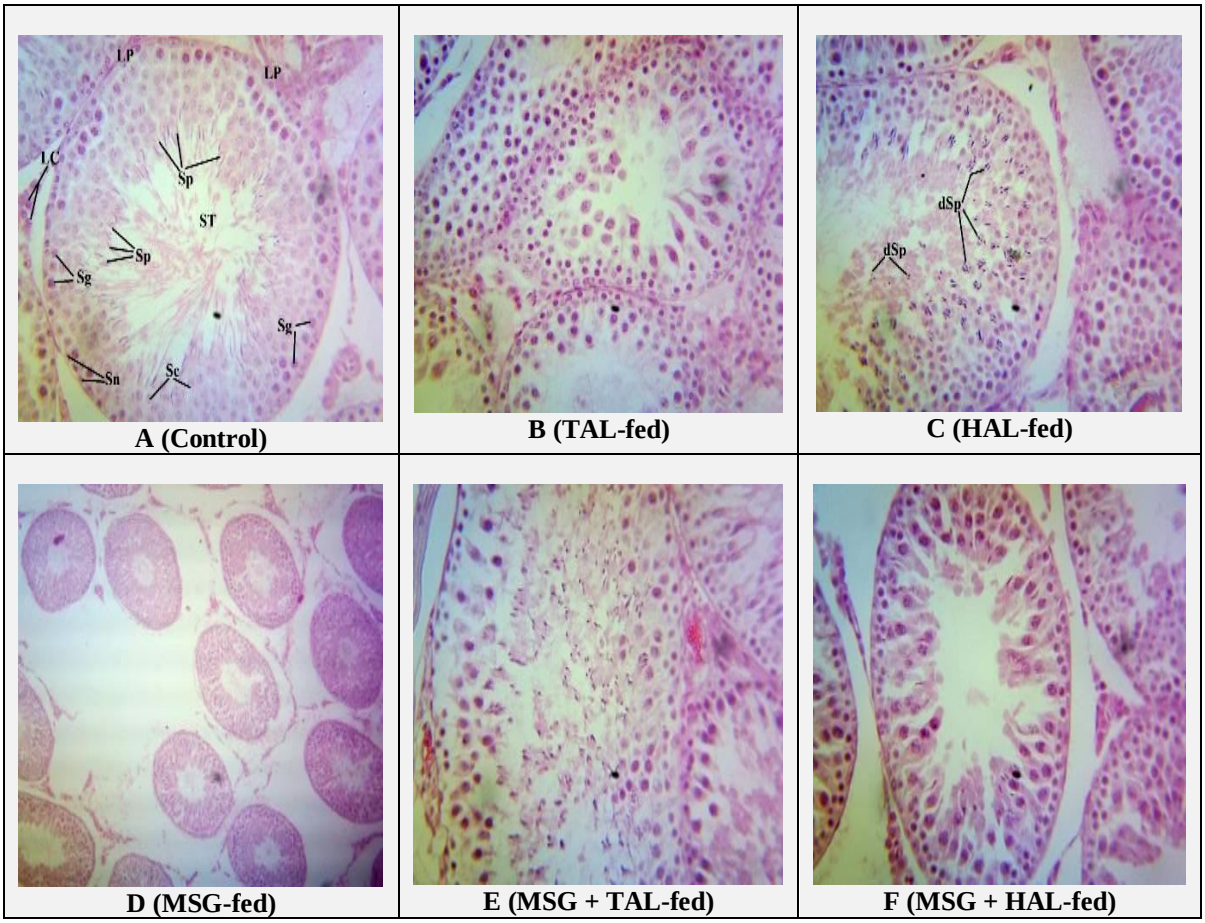

Figure 9: Photomicrograph of rats' brain and testes sections (Hematoxylin & Eosin) stained × 400).
(MSG = 8000 mg Kg^{-1}. TAL = 1.14:6.85 mg Kg^{-1} of A:L. HAL = 5.7:34.25 mg Kg^{-1} of A:L)

In accord with the present study, MSG mono-treatment altered testes histomorphology and bio-functional integrities in a previous study[2]. Consistent with this study, an increase in oxidative stress but a decrease in antioxidants as suggested from the results of this study were implicated with MSG outcomes in recent studies[3,9]. Other reports supported the present study that increased MDA concent-ration[26,38], SOD activity[39], ALB concentration[40], TP concentration[40,41], Mg concentration[42] but decreased Zn concentration[43] and degenerated spermatids within the seminiferous tubules[44,45] indicated oxidative stress and associated oxidative damage in the rats' testes. Collaboratively, ALB, TP and Mg which are immunological markers usually involved in the reduction of oxidative stress[40,42] increased in this study following MSG mono-treatment (Figure 5, Figure 6, and Figure 8). These are pointers to apparent surge in immune response and heightened attempt to reduce oxidative stress and attendant oxidative damage in rats' testes due to MSG assault[40,42].

Zinc protects cells against oxidative stress by inducing the synthesis of specialized protein (metallothionein) involved in reducing hydroxyl radicals and by acting as a co-factor to antioxidant enzymes metabolisms[43]. Zn concentration decreased as in this study in an apparent attempt to carry out the noted antioxidative roles in response to increased oxidative stress due to MSG mono-treatment. In contrast, however, several studies[26, 38,46,47] reported that decreased, instead of increased, activities of the determined enzymatic antioxidants (GP_X, SOD and CAT) indicated oxidative stress and associated oxidative damage in animal models. As this is an acute study that lasted for seven days, it is probable that the activities of these antioxidant enzymes were merely increased as in this study in readiness to mop up the excessive concentration of oxidants produced following MSG assault. This is in concord with the submission herein on the outcome of MSG assault on ALB, TP and Mg concentrations in rats' testes. It is plausible that, with adequate time duration, these enzymatic antioxidants would have decreased after mopping up the excess oxidants associated with increased oxidative stress due to MSG assault. In support of this hypothetical scenario, GP_X which is known to reduce hydrogen peroxide to water thereby reducing its harmful oxidant effects[47] may have been mobilized in readiness to combat the oxidant effect of hydrogen peroxide produced following MSG assault leading to the observed increase. Intriguingly, CAT activity in MSG + HAL-fed group increased (p<0.05) above that of MSG–fed rats (Figure 3). This may be underscoring an overriding adversity or up-regulated dysfunction on CAT metabolism on rats' testes due to MSG + HAL co-therapy compared to MSG monotherapy and other treatments which deserves follow-up studies. MSG can dissociate easily in ionic, including physiologic, media to give out, aside glutamic acid which is a harmless amino acid, a free sodium ion[8]. This study, therefore, speculates that the free sodium ion (Na^+) may, by unknown mechanisms, act either as an opportunistic free radical or to initiate series of reactions leading to the production of a free radical. In either cases, activities of a free radical could lead to excess generation of other free radicals and oxidative stress in the rats' testes suggested by the observations herein. The validation of this speculation which was not incorporated in the design of the present study is a significant limitation that deserves follow-up in subsequent studies aimed to evaluate the mechanistic roles of the dissociation components of MSG, notably the sodium ion moiety, in general MSG metabolism and toxicology.

The responses following TAL and HAL mono-treatments or co-treatments with MSG (Figure 1 to Figure 9) were inconsistent compared to control and MSG mono-therapies. These underscore the inconsistency in AL responses and in the apparent modulatory responses by AL against effects by MSG mono-therapy on the rats' testes. AL either alone or combined with MSG probably mediated novel oxidative response beyond the modulatory capacity of the natural antioxidative defense mechanisms as highlighted in the outcome of this study on CAT activity in MSG + HAL- co-treated rats' testes which evokes needs for further studies. Response to oxidative stress as a way to drug action was muted in earlier study, though, with some antihypertensives[18]. The underlying mechanisms for the pharmacologic actions of artemether component of AL include an increase in the production of free radicals and a reduction in blood antioxidants[10]. The capacity of lumefantrine to lasts for 5 days in the circulation may provide required duration for it to establish toxic outcome on the oxidant-antioxidant balance in the rats' testes[15,16]. These could concert to predispose AL to mediate oxidative responses leading to inconsistent outcomes recorded herein. In particular, the inconsistent responses following TAL and HAL mono-treatments or co-treatments with MSG on rats' testes histology compared to control and MSG mono-treatment (Figure 9) reflected and collaborated the testes homogenate chemistry results demonstrating varied adverse responses on the rats' testes in the other treatment groups.

Adverse effects due to oxidative stress manifest easily on the testes because testes have low vascularity and oxygen content[25,26]. MSG mono-therapy compromised the testes histology, antioxidants metabolism and reproductive integrity in a recent study[44]. Inconsistent results as obtained herein, therefore, predict unpredictability in possible outcomes from co-consumption of AL and MSG. Caution needs to be exercised in the concomitant treatment of AL and MSG in rats' especially against dysfunctions in rats' testes histology and antioxidant metabolism pending further clarification studies. The speculations or hypotheses and other perspectives highlighted in the discussion, however, need to be collaborated in further studies using similar but longer-duration study designs.

Limitations of the study

The study was based on a sample size of five rats and did not assess testicular function bio-indicators in the rats' testes homogenate or in the serum. This study did not explore the possible contributions from the dissociation components of MSG notably sodium ion

moiety on the rats' testes histology and antioxidant markers. Malaria disease model group was not included in this study to compare the study outcomes with a malaria disease model. The acute study design may not have allowed for a complete induction and evaluation of the determined responses. These concert to limit this study and warrant detailed address in subsequent studies.

CONCLUSIONS

Thus, AL caused inconsistent modulation of alterations in rats' testes histology and antioxidant function markers due to MSG assault. The modulation may not be sustainable and the alterations may instead be spiked warranting caution in co-feeding AL and MSG to rats notably as an intervention against dysfunctions in rats' testes histology and antioxidant metabolism pending further clarification studies.

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AUTHORS' CONTRIBUTIONS

Egbuonu ACC: formulated the concept; designed, supervised the study and reviewed the draft copy. **Alaebo PO:** writing, review, and editing. **Achi NK:** methodology, data curation. **Njoku CJ:** editing, data curation. **Eze OB:** prepared the draft copy of the manuscript and edited the statistics. All authors read and accepted to publish this version of the article.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

None to declare.

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