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Research Article

Changes in Liver Oxidative Stress Biomarkers, Biochemicals and Histological Assessment in Lactating Wistar Rats Following Oral Monosodium Glutamate (MSG) Administration

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Abstract

MSG consumption has been implicated in significant oxidative stress complications. Increased metabolic demand during lactation as well as increased consumption of food containing MSG by lactating mothers could predispose the liver to detrimental metabolic burden. This study evaluated the effects of MSG on oxidative stress biomarkers, albumin, total protein, liver enzymes and histology in lactating rats. At parturition, 24 adult female lactating rats were divided into four (4) groups of six animals each (n = 6). Group I served as normal control and was given distilled water (2 ml/kg BW), while group II was given metoclopramide (5 mg/kg BW). Groups III and IV were administered MSG at 1850 and 3700 (mg/kg BW) respectively for 14 days. Animals were sacrificed at the end of the experiment and samples obtained. Liver enzymes (ALT, AST and ALP), total protein and albumin were assayed from sera, whereas oxidative stress biomarkers (MDA, SOD, GSH and CAT) from liver homogenate. Histological assessment was also carried out. MDA was significantly higher ($P < 0.05$) in the MSG treated groups compared to control. Although the antioxidant enzymes were lower in all the treated groups compared to control, significant difference ($P< 0.05$) was observed in GSH and CAT only. Serum liver enzymes (ALT, AST and ALP), total protein and albumin were all significantly higher ($P < 0.05$) in the MSG treated groups compared to control. There was also a significant ($P < 0.05$) positive correlation between hepatic lipid peroxidation and all the serum liver enzymes. Hepatological alterations consisting of central vein dilation, necrosis of hepatocytes, lysis of red blood cells, sinusoidal dilatations were observed in the MSG treated groups. Thus, excessive consumption of MSG rich diets should be carried out with caution during lactation.

Keywords: *Liver; Lactation; Oxidative Stress Biomarkers; Histology; Monosodium Glutamate*

Introduction

The liver is known for its plethora of metabolic activities as well as its position as the first contact site of xenobiotics introduced orally into the system. It is multifunctional and biochemically diverse with the propensity for rapid response to insults and stimuli aimed

towards preservation of optimal function [1]. However, hepatic metabolism is not without some physiological burden. These metabolic interactions within the liver culminate into both detoxification as well as a possible bio-activation of reactive metabolites like the reactive oxygen and nitrogen species (RONS) [2]. Subsequently, increased production of RONS would precipitate oxidative imbalance and dysregulation of hepatic function. Thus, oxidative stress has been implicated in several liver diseases [3]. The relationship between oxidative stress and reproductive activities as posited by significant literatures is synonymous to that of a two-sided coin with oxidative stress serving as the cost for reproduction. Lactation characterized by increased daily energy expenditure and basal metabolic rate has been reported to be the most energetically demanding period of a female's life [4]. Consequently, some school of thoughts proposed oxidative stress generation during lactation unless antioxidant defences are sufficiently upregulated [5]. There have been reports of increased gluconeogenesis by 2-fold or more in response to increased demand for lactose synthesis during lactation [6]. This increased metabolic burden on the liver is placated by a corresponding increased fatty acid oxidation as well as lipolysis and blood flow to the liver [7]. Monosodium glutamate (MSG); a food additive is readily available in open markets in Nigeria as ajinomotto, white maggi or vidan [8]. Needless to say that MSG has generated significant controversy among its local and global consumers as well as in research community. There is a growing body of phobia on its usage particularly in places like Nigeria where non-conventional applications as bleaching agents for stain removal from clothes is well practised. MSG has been reported to cause metabolic changes [9,10] as well as detrimental impact on hypothalamic nerve cells [11]. Pregnant women and lactating mothers usually present with cravings involving delicacies peculiar to one's environment. In northern Nigeria, most of these locally made delicacies are prepared using MSG as vital condiments thus predisposing them to high consumption rate. Therefore, this study focused on the changes associated with oral MSG consumption on hepatic oxidative stress, hepatohistological changes and other biochemical parameters in adult lactating Wistar rats.

Materials and Methods

Transparent white plastic cages, water bottles and feeding troughs, syringes (1 and 5 ml), cotton wool, oral cannula, antiseptic, hand gloves, plain bottles, pipettes, electronic weighing machine, centrifuge (bench top), dissecting kit, ketamine and diazepam, monosodium glutamate, metoclopramide hydrochloride (10 mg) (NAFDAC REG NO: 04-6476), phosphate buffer solution, mortar and pestle (ceramic) digital weighing balance (0.01 sensitivity), 10% formalin solution, slides and distilled water, monosodium glutamate (Ajino motto).

Experimental animals

A total of twenty four (24) nulliparous adult female Wistar rats and twelve (12) adults male Wistar rats used for the study. The male rats were used only for copulatory purposes. Animals with body weight (130 – 200 g) were sourced from the Department of Human Physiology, Ahmadu Bello University animal house. These animals were housed in plastic cages (transparent) with adequate air vents. Soft sawdust material was utilized for bedding with free access to food and water throughout the period of study.

Ethical approval

Handling of laboratory animals during this study was carried in accordance with the guidelines of the National Institute of Health on care and use of laboratory animals. Local Institutional ethical approval for the use of laboratory animals for research was obtained from the Ahmadu Bello University ethical committee on animal use and care with approval number: ABUCAUC/2018/092.

Mating protocol

To achieve pregnancy, mating protocol was carried out in the ratio of 6:3 (female to male). Animals were allowed to cohabit until obvious signs of pregnancy were observed in the female Wistar. By day thirteen (13) of gestation the abdominal enlargement was visible alongside mammary gland development and nipple enlargement according to the method of [12].

Experimental design

At parturition, the dams were separated into different cages (four groups) consisting of six (n = 6) animals each: Group 1: distilled water (2 ml/kg BW), Group 2: metoclopramide (5 mg/kg BW) Group 3: MSG (1850 mg/kg BW), Group 4: MSG (3700 mg/kg BW). Animals were treated orally for 14 days using an oral cannula. Doses of MSG used were adopted from [13].

Preparation of drug

Monosodium glutamate (Ajinomotto) was sourced from Samaru local market of Sabon Gari LGA, Kaduna state Nigeria. A fresh stock concentration was prepared daily in distilled water thus; 100 mg/mL stock concentration was formed from 1000 mg of MSG dissolved daily in 10 ml of distilled water. Metoclopramide tablet (10 mg) was each dissolved daily in 10 ml of distilled water (1 mg/ml stock concentration). The volumes administered orally to each animal were calculated according to the relationship expressed below:

$$\text{Volume administered} = \frac{Required\ dosage\ (mg\backslash kg) \times Weight\ of\ animal\ (kg)}{Stock\ concentration\ (mg\backslash ml)}$$

Animal sacrifice and sample collection

Ketamine and diazepam at 75 and 5 (mg/kg) were administered intraperitoneally at the end of the experiment as the anaesthetic agent. Blood samples were collected via cardiac puncture using 5 ml syringes and emptied into plain tubes and the sera separated afterwards by centrifugation at 3,000 × g for 10 minutes. The liver was excised from the experimental animals and rinsed off blood. A

weighed portion was homogenized using mortar and pestle for biomarkers of oxidative stress analysis while the reminder preserved in 10% formalin solution followed by processing and staining using haematoxylin and eosin method then viewed under light microscope for histological changes.

Serum biochemical assessment of liver enzymes, total protein and albumin

Serum ALP was determined according to the method described by [14], while serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were assayed as described by International Federation of Clinical Chemistry, IFCC. Total protein and albumin were assayed according to the method described by [15].

Determination of malondialdehyde concentration (MDA)

A weighed portion of the liver tissue was homogenized in potassium phosphate buffer 10 mM pH (7.4) and used for MDA estimation according to the method of [16] and expressed in nmol/g tissue.

Determination of superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was measured based on inhibition of the formation of amino blue tetrazolium formazan according to the method of [17] and the result expressed in U/g protein.

Determination of reduced glutathione (GSH)

GSH was estimated in the liver homogenate using DTNB by the method of [18]. The absorbance was read at 412 nm and the results were expressed as nanomole GSH/mg of wet tissue.

Determination of catalase (CAT)

The CAT activity assay was carried out as described by [19] and expressed as nanomole/mg of wet tissue Oxidative stress index was calculated as ratio of MDA to SOD as described by [20].

Statistical analysis

Data obtained from the study were expressed as mean ± SEM and the statistical analysis was carried out using version 20 of SPSS with the aid of one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Values with $P < 0.05$ were considered statistically significant. Scatter plots were drawn using Excel and the linearity between variables observed. Pearson correlation was carried out for all variables with scatter points close to a straight line.

Results

Effect of MSG on Liver homogenate lipid peroxidation (MDA) of lactating Wistar rats

MDA (nmol/g tissue) concentration in figure 1 below was significantly higher in all MSG treated groups compared to both control and metoclopramide treated groups [$F = (3, 20) = 62.733$; $P = 0.0001$]. Although it was also higher in the metoclopramide treated group compared to control, the difference was however not significant ($P > 0.05$).

The effect of MSG on antioxidant enzymes (SOD, CAT) and GSH of lactating Wistar rats

In table 1 below GSH (nmol/mg wet tissue) was lower significantly in MSG (1850 mg/kg) compared to the control [$F = (3, 20) = 4.921$; $P = 0.010$]. Although GSH (nmol/mg wet tissue) was lower in the other treated groups, these differences were however not significant ($P > 0.05$). There was no statistically significant difference in the result of SOD (U/g protein) in table 2 below [$F = (3, 20) = 2.058$; $P = 0.138$].

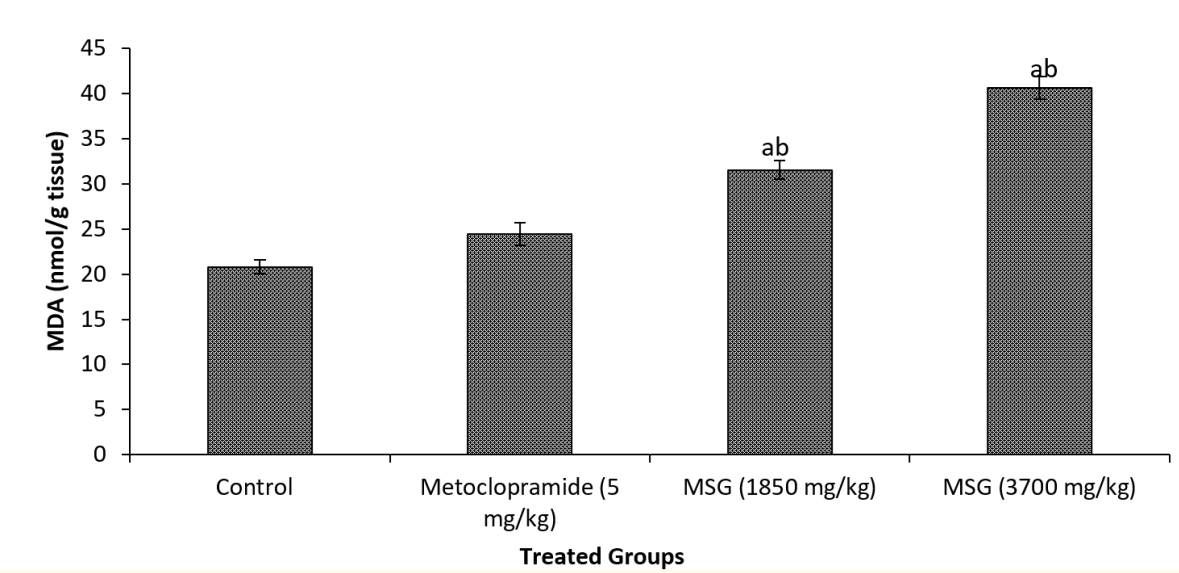

Figure 1: *MDA (nmol/g tissue) in lactating Wistar rats. Different superscripts (a, b) indicate statistically significant difference (P ≤ 0.05) compared to control and metoclopramide treated groups respectively. One way analysis of variance (ANOVA) was carried out followed by Tukey post hoc test.*

The result of CAT (nmol/g tissue) in table 1 below showed a statistically significant increase in CAT (nmol/g tissue) in all MSG treated groups compared to control as well as in MSG (3700 mg/kg) compared to the metoclopramide treated group [F = (3, 20) = 6.981; P = 0.002]. Oxidative stress index was significantly [F = (3, 20) = 24.296; P = 0.0001] higher in all the treated groups compared to control and in MSG (1850 mg/kg) compared to MSG (3700 mg/kg) in table 1 below.

Experimental Groups	GSH (nmol/mg wet tissue)	SOD (U/g protein)	CAT (nmol/g tissue)	Oxidative Stress Index
Control	10.50 ± 0.51	11.86 ± 0.83	1.65 ± 0.65	1.80 ± 0.14
Metoclopramide (5 mg/kg)	8.75 ± 0.77	9.72 ± 0.41	1.59 ± 0.06[d]	2.52 ± 0.12[ad]
MSG (1850 mg/kg)	7.13 ± 0.47[a]	10.97 ± 0.59	1.44 ± 0.05[a]	2.91 ± 0.14[ad]
MSG (3700 mg/kg)	8.96 ± 0.69	11.44 ± 0.68	1.34 ± 0.04[a]	3.61 ± 0.91[a]

Table 1: Results of antioxidant enzymes (SOD, CAT), GSH and oxidative stress index of lactating Wistar rats. *GSH: Reduced Glutathione; SOD: Superoxide Dismutase; CAT: Catalase. Superscripts a, and d indicate statistically significant difference (P < 0.05) compared to control and MSG (3700 mg/kg). MSG: Monosodium L-Glutamate.*

Effect of MSG on serum ALT, AST, ALP and AST/ALT ratio

In table 2 below, serum ALT (U/L) was significantly higher in all groups treated with MSG compared to control [F = (3, 20) = 7.963; P = 0.001]. Although serum ALT (U/L) was also higher in the metoclopramide treated group compared to control, as well as in the MSG treated groups compared to the metoclopramide treated group, these differences were however not statistically significant (P > 0.05).

Experimental Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	AST/ALT ratio
Control	57.00 ± 1.73	101.17 ± 1.05	99.83 ± 3.77	1.78 ± 0.05
Metoclopramide (5 mg/kg)	62.83 ± 2.37	113.17 ± 2.34[a]	115.83 ± 2.99[a]	1.82 ± 0.10
MSG (1850 mg/kg)	69.67 ± 1.43[a]	129.16 ± 0.79[ab]	123.67 ± 1.45[a]	1.86 ± 0.04
MSG (3700 mg/kg)	66.50 ± 2.04[a]	126.33 ± 1.93[ab]	132.50 ± 2.04[ab]	1.91 ± 0.07

Table 2: *Result of MSG on serum liver enzymes of lactating Wistar rats.*

ALT: Alanine Transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; U/L: Unit Per Litre; MSG: Monosodium L-Glutamate. Superscripts a/b indicates statistically significant differences (P < 0.05) compared to control and metoclopramide groups respectively.

Serum AST (U/L) in table 2 below was significantly higher in all treated groups compared to control as well as in the MSG treated groups compared to the metoclopramide treated group [F = (3, 20) = 60.968; P = 0.0001]. There was statistically significant increase in level of serum ALP (U/L) in all treated groups compared to control [F = (3, 20) = 26.097; P = 0.0001] as shown in table 2 below. Although the AST: ALT ratio was higher in all treated groups compared to control, these differences were however not statistically significant [F = (3, 20) = 0.643; P = 0.596].

Effect of MSG on serum total protein (g/L) of lactating Wistar rats

Total protein (g/L) level in figure 2 below was significantly higher in all the MSG treated groups compared to control and metoclopramide treated groups [F = (3, 20) = 22.907; P = 0.0001].

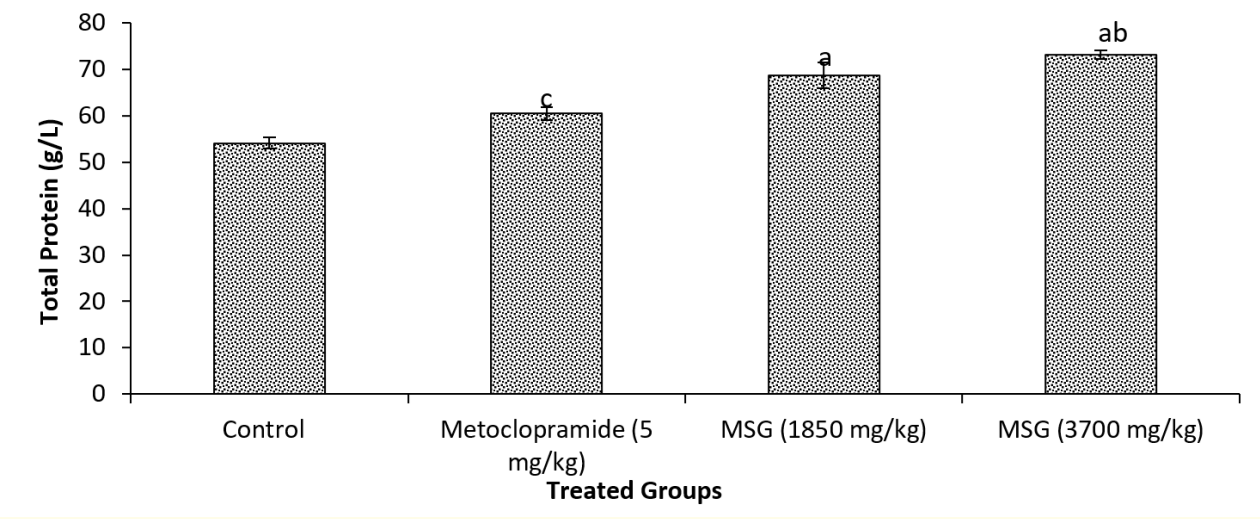

Figure 2: *Serum total protein (g/L) in lactating Wistar rats. Different superscripts (abc) indicate statistically significant difference (P ≤ 0.05) compared to control, metoclopramide and MSG (1850 mg/kg) respectively. One way analysis of variance (ANOVA) was carried out followed by Tukey post hoc test.*

Effect of MSG on serum albumin (g/L) of lactating Wistar rats

Serum albumin (g/L) level in figure 3 below was significantly higher in all the MSG treated groups compared to control group as well as in MSG (3700 mg/kg) compared to metoclopramide treated [F = (3, 20) = 11.180; P = 0.0001].

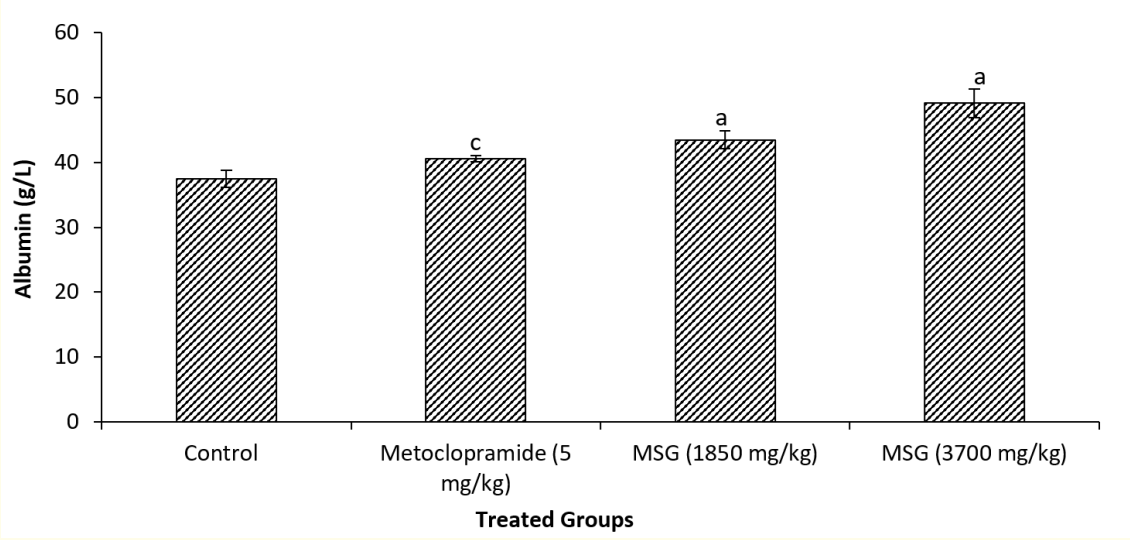

Figure 3: *Serum albumin (g/L) in lactating Wistar rats. Different superscripts (a, c) indicate statistically significant difference (P ≤ 0.05) compared to control and MSG (1850 mg/kg) respectively. One way analysis of variance (ANOVA) was carried out followed by Tukey post hoc test.*

Correlation between lipid peroxidation (MDA), liver enzymes (ALT, AST and ALP), total protein and albumin

Table 3 below shows the result of correlation between lipid peroxidation and some biomarkers of hepatic toxicity. There was a statistically significant positive correlation between lipid peroxidation (MDA) and ALT, AST, ALP, TP and albumin with the least relationship observed between MDA and ALT; while the strongest between MDA and total protein as shown in table 3 below.

	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Protein (g/L)	Albumin (g/L)
MDA (nmol/g tissue)					
r	0.524**	0.763**	0.796**	0.834**	0.806**
P Value	0.009	0.0001	0.0001	0.0001	0.0001

Table 3: *Result of correlation between lipid peroxidation (MDA), (ALT, AST and ALP), total protein and albumin.*

ALT: Alanine Transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; IU/L: International Unit Per Litre. MSG: Monosodium L-Glutamate. Pearson correlation was carried out.

Correlation between lipid peroxidation (MDA), GSH and antioxidant enzymes (SOD and CAT)

Table 4 below shows the result of correlation between lipid peroxidation and some antioxidant enzymes. There was a statistically significant negative correlation between lipid peroxidation (MDA) and CAT. Table 4 below shows a weak positive correlation between MDA and SOD.

	GSH (U/g tissue)	SOD (U/g tissue)	CAT (nmol/g tissue)
MDA (nmol/g tissue)			
r	- 0.350	0.126	- 0.696**
P Value	0.094	0.557	0.0001

Table 4: *Result of correlation between lipid peroxidation (MDA), GSH, SOD and CAT.*

*GSH: Reduced Glutathione, SOD: Superoxide Dismutase; CAT: Catalase. **: Indicate statistical significant difference (P < 0.05).*

Effect of MSG on liver tissues histology of lactating Wistar rats

Plate 1 below shows normal liver architecture with central vein (A), normal vascular sinusoids (C) dividing the linear hepatic plates (D). It also shows normal epithelial cells (F) and Hepatocytes (E) containing prominent nucleus and preserved cytoplasm. Plate 2 below shows abnormally dilated central vein (A), slight vascular sinusoidal dilatations (C) with mild vascular necrosis and fatty deposits. Plate 3 present with slightly dilated central vein (A) containing lysed blood cells, abnormal sinusoidal dilatations (C), necrosis of hepatocytes (B), increased fatty deposits, decreased prominent nucleus and cytoplasmic vacuolization. Plate 4 below shows degenerative alterations around the central vein (A) containing lysed RBC, atrophy of hepatocytes, hepatic lobules and plates, increased fatty deposits and over all cytoplasmic vacuolization.

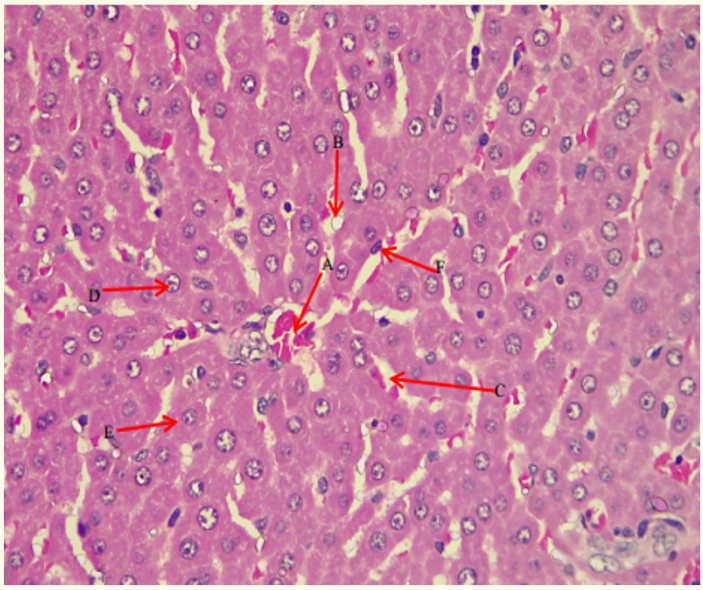

Plate 1: *Normal control (H&E ×400).*

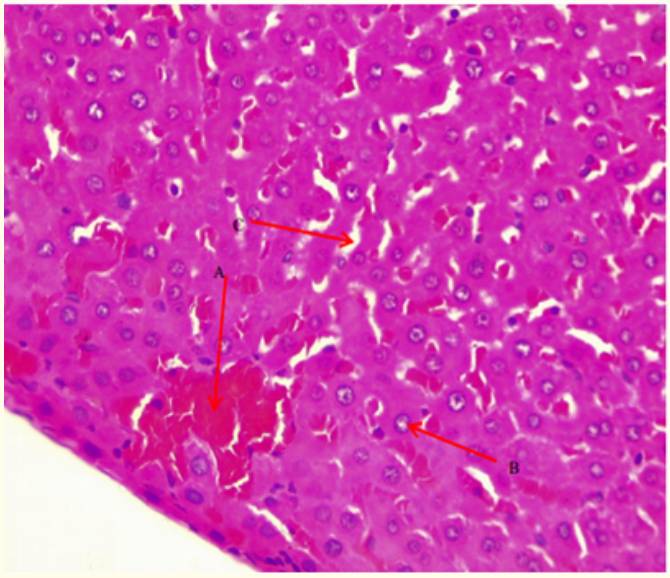

Plate 2: *Metoclopramide (5 mg/kg, H&E ×400).*

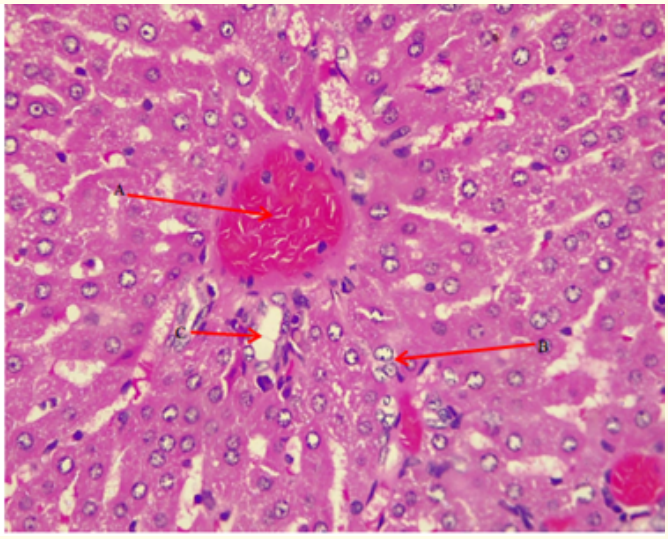

Plate 3: *MSG (1850 mg/kg, H&E ×400).*

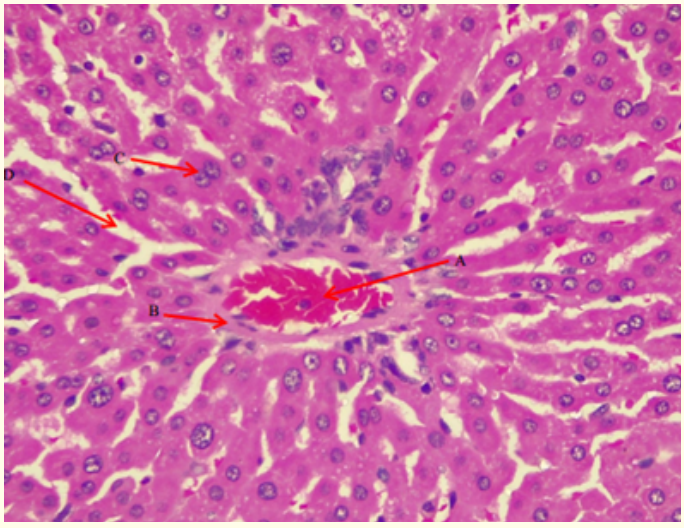

Plate 4: *MSG (3700 mg/kg, H&E ×400).*

Discussion

Previous studies have revealed the profound effects of reproductive exercises like lactation on hepatic metabolic capacity [6,21] with activities like gluconeogenesis increasing in the liver to complement mammary gland requirements. Some of the complementary responses to such needs are increased fatty acid oxidation, lipolysis in adipose tissues as well as increased hepatic blood flow among others [7]. The liver, adipose tissues and mammary gland are the major sites of fatty acid metabolism during lactation which makes any detrimental insult on the liver a threat to optimal lactational performance.

In this study, oral administration of MSG precipitated lipid peroxidation as depicted by MDA concentration shown in figure 1 above. Hyperglycaemia and hyperlipidemia are associated with MSG consumption [22]. Thus, the result of MDA in this current study could have been from increased glucose oxidation, causing a marked formation of thiobarbituric acid reactive substances. This result is complemented by the hepatocytes necrotic changes in the liver tissues of the treated groups. With reports of lipid peroxidation from ROS/RNS generation during lactation [5] this current study presents a possible augmentation of hepatocytic lipid peroxidation during lactation as liver parenchymal cells are said to be highly susceptible to oxidative stress [23]. Although the result of MDA in this study suggests a detrimental interaction between MSG and the liver this result is however in contrast to Torri., *et al*. [24] who reported insignificant delivery of dietary consumed glutamate to the liver from higher extra-hepatic metabolism [25]. More so, metabolic products of glutamate like alanine, arginine, proline and ornithine have been reported to be disposed of through conversion to glutamate in the liver [26]. Therefore, this provides the possibility of increased glutamate presence within the liver from as might be the case in our study. As shown in plate 3 and 4 above, the increased RBC lysis in the MSG treated groups could also have contributed to glutamate presence within the liver by releasing the RBC bound glutamate into the liver parenchyma which originally was bound for the muscles. Liver glutamate dehydrogenase (GDH) is the bridge which connects ammonia detoxification and gluconeogenesis in the liver [27]. The cellular necrosis observed in the liver could have resulted in decrease GDH thus causing toxic effects from accumulated ammonia. The result of lipid peroxidation from metoclopramide treatment in this study suggests generation of ROS from mitochondrial activities coupled with the metabolism of the said

drug through phases I, II and III thus producing a transient active metabolite through Cytochrome P-450 enzymatic actions [28]. Phases I and II enzymes have been reported to manifest tangible inter-individual variability thus, culminating into different levels of exposure to reactive metabolites [29]. Metoclopramide was used as a standard galactagogue for its inhibitory activity on prolactin inhibiting hormone (dopamine) although the results on milk analysis is not captured in this study.

The decrease in antioxidants GSH, SOD and CAT in this study as shown in table 1 would be consequence of the prevailing lipid peroxidation as discussed above. The result of oxidative stress index in the treated groups is indicative of relatively increased lipid peroxidation than SOD activity. This is also supported by the negative correlation between GSH, CAT and MDA. However, the deviation observed with MSG treatment at a higher dose in this study having increased GSH and SOD relative to the lower dose is a paradoxical. This observation could have been a sort of unique defensive response at higher MSG concentrations. More so, the role glutamate in the synthesis of glutathione could also have contributed to the aforementioned behaviour of GSH from MSG treatment [30]. The result of this study on antioxidants from metoclopramide treatment could be explained by the lipid peroxidation provoked from oxidation reaction followed by a possible conjugation with glutathione [28] thus the reason for the depletion of GSH as observed in this study. Higher oxidative stress index is associated with increased lipid peroxidation with a corresponding decreased SOD activity. Oxidative stress index establishes more depletion of SOD relative to MDA in the all the MSG treated groups.

The function of detoxification, metabolism and storage by the liver makes it prone to damage. Liver enzymes have been implicated in glutamate metabolism [31]. ALP, ALT, AST, total protein and albumin among others have been routinely used as non-invasive biomarkers for diagnosing and monitoring liver disease [32]. The elevated serum liver enzymes in this study would be due to lipid peroxidation resulting in disintegration of hepatocytic cell membrane as shown from the histological evaluation. Disintegration of hepatocytes has been known to result in release of the aforementioned biomarkers of hepatic toxicity. These hepatocellular damages evident from the results of the liver enzymes, total protein and albumin alongside the histology in this study would be due to the sustained lipid peroxidation throughout the lactation period as represented by the result of correlation between lipid peroxidation and all these biomarkers which showed a strong significant positive correlation. However, the decrease in serum ALT and AST in the MSG higher dose group relative to the other could be a reflection of their depletion due to prolonged activities on sustained glutamate metabolism in the liver thus resulting in less quantity released following lipid peroxidation of the hepatocytes. ALT has been implicated in degradative and biosynthetic roles in glutamate cycling and cellular nitrogen metabolism [33]. GDH in the liver is found throughout the lobule although higher in perivenous cells with relatively high periportal expression [34]. Thus, in this study, the destruction of cells observed from the liver histology could have significantly influenced GDH activity as all glutamine must inevitably go through glutamate as a means of metabolism [35].

Conclusion

The findings of this study demonstrate decrease in liver integrity and optimal performance from oral consumption of MSG during lactation through

1. Development of hepatic lipid peroxidation
2. Increased liver enzymes (ALT, ALP and AST) alongside total protein and albumin and
3. Reduced tissue antioxidants (SOD, GSH and CAT).

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