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RESEARCH

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Preliminary monosodium glutamate-induced changes in mammary gland receptors and gene expression, water channel, oxidative stress, and some lactogenic biomarkers in lactating rats

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Abstract

Background Changes induced by monosodium glutamate (MSG) can negatively impact milk production and secretion, among other adverse effects. This study aimed to investigate the effects of MSG consumption on receptor gene expression and quantification of hormones and receptors, as well as oxidative stress biomarkers and other lactogenic parameters in lactating animals. Twenty-four female Wistar rats, nine weeks of age, were randomly assigned to four groups, each containing six rats, at parturition. The rats in groups II, III, and IV were given varying doses of monosodium glutamate (MSG); while, group I was given distilled water and served as the control. The experimental period lasted two (2) weeks.

Results The groups administered with MSG showed a significant decrease in mammary PRLR gene expression ($p < 0.05$), as well as a marked reduction ($p < 0.05$) in mammary PRLR, OXT receptor, AQP-3, brain antioxidant enzymes (SOD, GPx, and CAT), and pituitary SOD compared to the control group ($p < 0.05$). Furthermore, there was a significant increase ($p < 0.05$) in reactive oxygen species levels in the serum and mammary gland homogenates, erythrocyte osmotic fragility, and elevated ($p < 0.05$) brain and pituitary MDA levels in the MSG-administered groups compared to the control group. Daily milk yields were significantly decreased ($p < 0.05$) in the MSG-administered groups between days 10 and 14 of lactation.

Conclusion The findings of this study suggest that prolonged consumption of MSG could interfere with lactation-associated functions via increased ROS production, reduced antioxidants, decreased AQP-3, mammary prolactin and oxytocin receptors, and prolactin receptor mRNA in lactating Wistar rats.

Keywords Monosodium glutamate, Prolactin, Oxytocin, Growth hormone, Gene, Receptors, Aquaporin-3, Lactation

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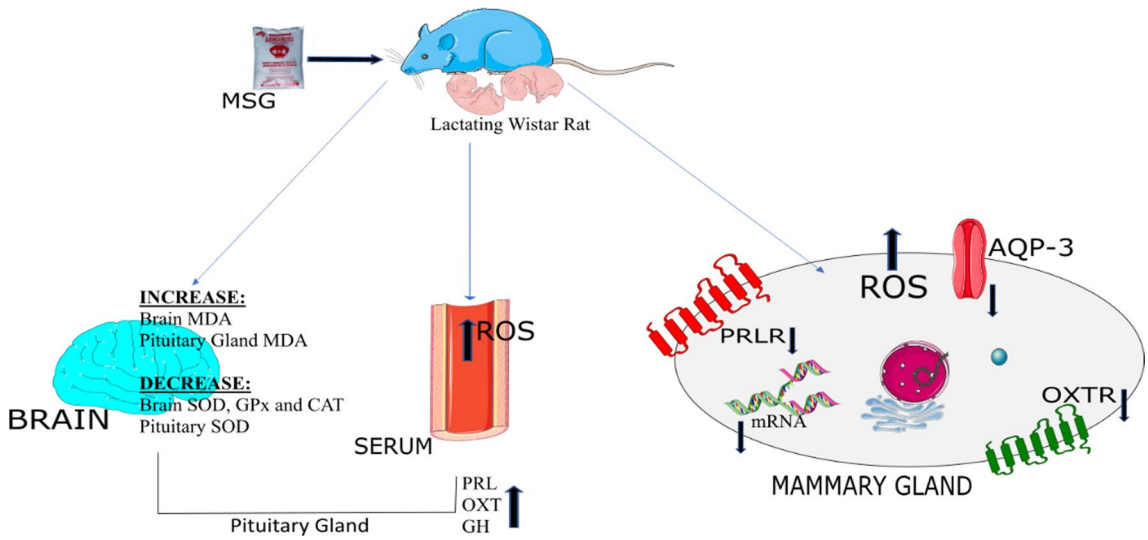
Graphical Abstract



Background

Monosodium glutamate (MSG) is frequently used in the global food industry to improve food properties such as flavor, color, taste, appearance, and texture (Maluly et al., 2017). MSG is widely used to create umami, brothy, and savory flavors (Silva et al., 2017). MSG consumption varies by region, with developed countries consuming approximately 3–10 g/day. The average intake in the UK is 0.58 g/day, in Germany it is 100 g, and in other European countries it is around 10 g/day (Rhodes et al., 1991). Nigerians consume 0.56–10 g per day on average (Henry-Unaeze, 2017). MSG is marketed in the open market and shops as Ajinomoto or Vedan by West African Seasoning Company Limited (Inuwa et al., 2011).

MSG is used in homes in Nigeria for its convenience, affordability, and unique taste (Henry-Unaeze, 2017); as a result, MSG was suggested by others as an implicit medium for perfecting nutritive quality in Nigeria (Nnanyelugo, 1997). The biosafety of MSG remains one of the most debated health-related issues in both scientific and public settings (Omogbiya et al., 2020). There is no fixed amount of MSG, which is generally considered safe. As a result of a deficient manufacturer's clarity, there is inordinate consumption (Wajiyasekara and Wansapala, 2017). The original average daily input estimate (0.3–1 g) per person in most developing and industrialized nations has significantly soared in recent times to as high as 14 g in some populations (Moneim et al., 2018; Onaolapo et al., 2016).

Breast milk is regarded as a vital source of nutrients and energy, but there are many deterrents despite its advantages (Lee et al., 1997). Factors like cultural traditions have been reported to affect lactation behavior (Gabriel et al., 1986; Osman et al., 2009). There are myths regarding maternal diet during breastfeeding that could lead to misguided dietary restrictions void of scientific evidence in breastfeeding mothers (Jeong et al., 2017). In Nigeria and other parts of the world, there is a growing phobia of the effects of MSG consumption on reproductive functions (Oladipo et al., 2015). Some of the widely generated perceptions could be fueled by MSG's local usage by most communities in Nigeria as a laundry bleaching agent, making consumers concerned about the possible detrimental effect it could have on different systems of the body (Inuwa et al., 2011). During nursing, the mother's energy demand and expenditure increase because she is expected to meet both her own and her offspring's needs. Among other things, oxidative stress (OS) has been proposed as one of the concessions during reproductive exercises.

This is, however, rivaled by the OS shielding hypothesis (Naaviaux, 2012), which suggests oxidative protection during reproduction. Some of the antioxidant protection provided during reproductive exercises in females is attributed to the antioxidant actions of hormones such as prolactin, as shown in other studies (Munoz-Mayorga et al., 2023; Thebault, 2017). According to the findings of Cunha et al. (2022), prolactin

protects against mercury-induced toxicity. However, Veena et al. (2008) reported increased lipid peroxidation in women with unexplained infertility and high serum prolactin levels. Diet can counteract oxidative balance by triggering a cascade of events that disrupt endocrine processes and the balance required to maintain normal reproductive function (Diamanti-Kandarakis et al., 2017). MSG suppresses reproductive function by interfering with ovarian and uterine functions (Mondal et al., 2018). It also causes metabolic changes (Wahyuni et al., 2014), with MSG having a negative impact on hypothalamic nerve cells, as reported by Igwebuike et al. (2011). This could lead to changes in the hypothalamic–pituitary–gonadal regulatory axis, resulting in hormonal dysregulation. MSG has the potential to cause endocrine disruption (Shannon et al., 2017), which could affect milk synthesis in the young. Therefore, this study was designed to evaluate preliminary monosodium glutamate-induced changes in mammary gland receptors and gene expression, water channels, oxidative stress, and some lactogenic biomarkers in lactating rats.

Methods

Animals

Adult nulliparous female rats, nine (9) weeks old, were obtained from the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria, and housed in a well-aerated cubicle with free access to water and food (Guzman et al., 2006). The Ahmadu Bello University ethical committee on animal use and care granted local institutional ethical permission for the use of laboratory animals for this research with approval number: ABUCAUC/2018/092. Mating was performed with sexually experienced males, and female estrus was induced by exposing the grouped females to the bedding materials of the male rats for three days (Bronson et al., 1986). The adult female rats were mated with male Wistar rats in a 1:1 ratio around 6:00 p.m. on the third day. This cohabitation was allowed to continue until the female rats showed obvious signs of pregnancy, at which point the pregnant rats were removed and housed separately.

At parturition, the females were randomly divided into four groups of six animals each, and the litter size was limited to six pups per dam. Group 1 was given 1 ml/kg of distilled water as a control; while, groups 2, 3, and 4 were given MSG at varying doses of 925 mg/kg, 1850 mg/kg (Emmanuel et al., 2020), and 3700 mg/kg (Emmanuel et al., 2020), respectively. All administrations were given via oral gavage, began three days after parturition, and lasted 14 days.

Milk yield assessment

Milk yield was estimated daily between 18 and 23 h after gavage according to the method described by Sampson and Jansen (1984) as a difference between the pre- and post-suckling weights of the litters. Milk yield 18 h after gavage was calculated as $w3-w2$. Where w2 = pre-suckling weight of litters (11:00 am) and $w3$ = post-suckling weight of litters (12:00 pm). The correction for weight loss due to metabolic processes was calculated 18 h after gavage as $\frac{w2-w1}{4}$ where $w2$ = pre-suckling litters' weight, $w1$ = pre-isolation litters' weight. Milk yield 23 h was calculated as $w5-w4$ where w4 = pre-suckling weight of pups (4:00 pm) and $w5$ = post-suckling weight of pups (5:00 pm). Weight loss correction 23 h after gavage (Morag, 1970) was calculated as $\frac{[(w2-w1)]+(w4-w3)]}{8}$.

Blood sample and tissue collection

At the end of the experiment, animals were anesthetized with 75 mg/kg ketamine and 10 mg/kg xylazine (Abdulghani et al., 2022) and euthanized by cervical dislocation afterward (Donovan & Brown, 2005). Blood samples and tissues were collected for biochemical assessments in heparinized and plain tubes by cardiac puncture (Parasuraman et al., 2010). The pituitary gland was removed from the surrounding dura matter and scooped out of the sphenoid bone, weighed and stored in a plastic tube on dry ice (Tzou et al., 2010), and subsequently homogenized in phosphate buffer solution (PBS, pH 7.4). The remaining whole brain was rinsed thoroughly in ice-cold PBS, weighed, and cut into smaller fragments, then homogenized in PBS (Francik et al., 2014). Mammary tissues were excised as described by Tolg et al. (2018), and the samples used for the PCR were washed in PBS at pH 7.4 (Battagello et al., 2020) and preserved in RNAlater [thermofisher scientific, Inc., USA]. The remaining mammary tissue was reduced to tiny pieces and homogenized in PBS, and the supernatant obtained was used for biochemical assays.

Assessment of erythrocyte osmotic fragility

Erythrocyte osmotic fragility assessment was carried out according to the method described by Faulkner and King (1970), as modified by Oyewale (1992). The percentage of hemolysis was calculated using the given formula:

$$\text{Percentagehaemolysis} = \frac{\text{opticaldensityoftestsolution}}{\text{opticaldensityofstandardsolution}} \times 100$$

Hormonal assay

Serum prolactin, growth hormone, oxytocin, and brain dopamine assays were carried out according to the manufacturer's manual using their respective ELISA kits:

prolactin hormone (ER-0076 [fine test], Wuhan China), growth hormone (ER-0993 [fine test], Wuhan China), oxytocin hormone (ER-1723 [fine test], Wuhan China), and brain dopamine (EU0392 [fine test], Wuhan China).

Tissue receptor assay

Prolactin (ER7070 fine test, Wuhan China), oxytocin (ER1619 fine test, Wuhan China), and aquaporin-3 receptor (ER0743 fine test, Wuhan China) were carried out according to the manufacturer's manual.

Assay of oxidative stress biomarkers

The ELISA kits were used to estimate the concentrations of malondialdehyde, superoxide dismutase activity, glutathione peroxidation, and catalase activity. The malondialdehyde assay utilized the NWLSS™ NWK-MDA01 kit from the USA, with a sensitivity of 0.08 μM, and the result was expressed as nmol/mg protein according to the method of Janero (1990). The superoxide dismutase activity was measured according to the method described by Martin et al. (1987), using the NWLSS™ NWK-SOD02 kit from the USA, with a sensitivity of 5.0 U/ml, and the result was expressed as IU/L. The glutathione peroxidation assay was performed according to the method of Paglia and Valentine (1967), using the NWLSS™ NWK-GPX01 kit from the USA, with a sensitivity of 1.0 mU/mL, and the results were expressed as IU/L. The catalase activity was determined using the ER0264 kit from Wuhan, China, with a sensitivity of 18.75 mIU/ml based on the method of Beers and Sizer (1952). Reactive oxygen species (ROS) were estimated using the Rat reactive oxygen species ELISA kit [Cat No.: CK-bio-20410, Shanghai Coon Koon Biotech Ltd., China] according to the manufacturer's manual.

Analysis of gene expression by real-time PCR

Total RNA was extracted using the RiboPure™ Kit [AM1924] according to the manufacturer's instructions. Complementary deoxyribonucleic acid (cDNA) was synthesized using a high-capacity cDNA reverse transcription kit with an RNAase inhibitor (Applied Biosystems, USA). Gene expression assays were done using TaqMan-labeled primers and probes for the target genes: prolactin receptor and β-actin. The endogenous control was beta-actin (the housekeeping gene). The target cDNA was normalized using an endogenous control. The quantitative polymerase chain reaction was carried out based on the Light Cycler® system instructions using Ultra SYBR Mixture (with Rox) and the following cycling protocol: 10 min at 95 °C, followed by 40 cycles consisting of 15 s at 95 °C and 60 s at 60 °C. The fold change in expression was calculated using the relative quantification technique (ΔΔCT). To do this, the threshold cycle (CT) values of the target cDNAs were first normalized to the CT values of the internal control β-actin in the same samples (ΔCT = CTTarget—CTβ-actin). Using the control, it was further normalized (ΔΔCT = CT-CTControl). The expression fold change was then calculated as ($2^{-\Delta\Delta CT}$).

Primer sequences used (5' to 3'): β-actin (TTGTAACCAACTGGGACGATATGG)-forward, (GATCTTGATCTTGATGGTGCTGCTAGG)-reverse. That of the prolactin receptor is: (CCTGAAGACAAGGAACAAGCC)-forward and (TGGGAATCCCTGCGCAGGCA)-reverse.

Mammary gland histological assessment

The mammary gland tissue was harvested as described by Tolg et al. (2018) and fixed in 10% formalin for histological assessment. Tissues were dehydrated through a series of alcohol solutions of increasing concentrations for 15 min each. Subsequently, the tissues were cleared using xylene for 20 min and embedded in paraffin wax into blocks, then clamped into a microtome and cut into sections between 5 and 8 microns according to the method of Macsween (1977). These sections were then stained with hematoxylin and eosin in a series of steps as described by Yousif and Qasem (2016).

Statistical analyses

Statistical analyses were carried out by ANOVA using SPSS version 23 [IBM, USA]. *Tukey*'s post hoc test was employed to show the differences between the groups. Values with a $p < 0.05$ were considered statistically significant. GraphPad Prism 8 software (version 8.0.2 [263]; GraphPad Software, San Diego, USA) was used for charts.

Results

Serum level of prolactin and growth hormone

Serum prolactin was higher nonsignificantly ($p > 0.05$) in all the MSG-administered groups compared to the controls [Fig. 1a]. The increase in prolactin in all the MSG groups was dose-dependent. There were no significant ($p > 0.05$) changes observed in the level of GH in the MSG-administered groups compared to the control. However, GH was higher in all the MSG-administered groups compared to the control [Fig. 1b].

Serum level of oxytocin and brain dopamine

There were no significant ($p > 0.05$) changes observed in serum oxytocin in the MSG-administered animals compared to the control [Fig. 2a]. Oxytocin was, however, higher in the MSG-administered animals compared to the control. There were no significant ($p > 0.05$) changes observed in brain dopamine in the MSG-administered animals compared to the control [Fig. 2b]. However, there was a non-significant decrease in the level of brain

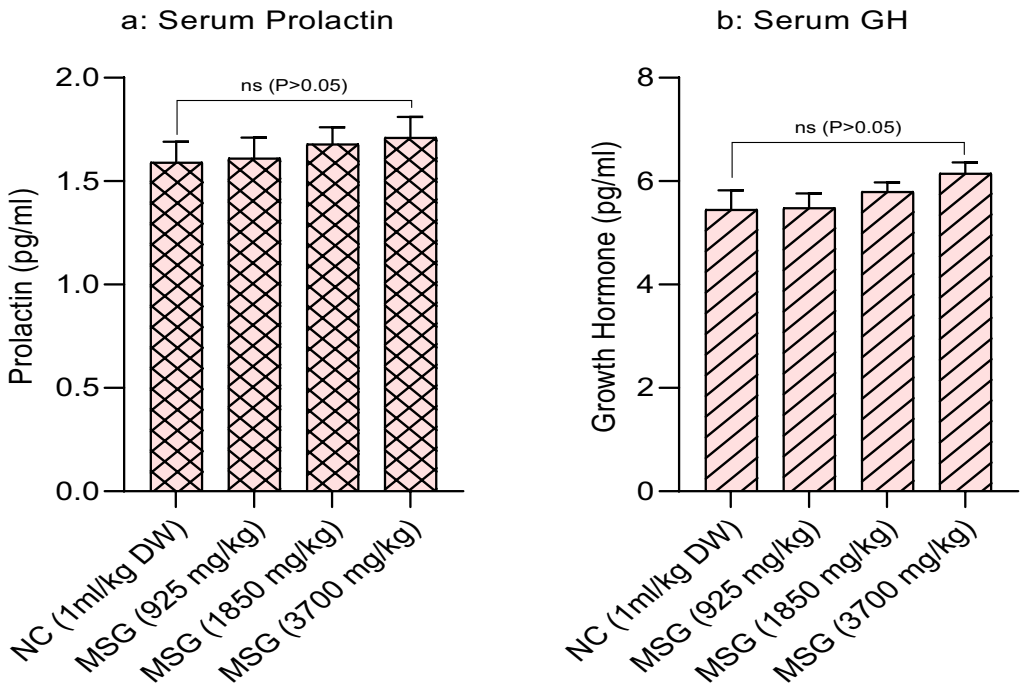


Fig. 1 Serum levels of prolactin [1a] and growth hormone [1b] of lactating Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water GH = growth hormone

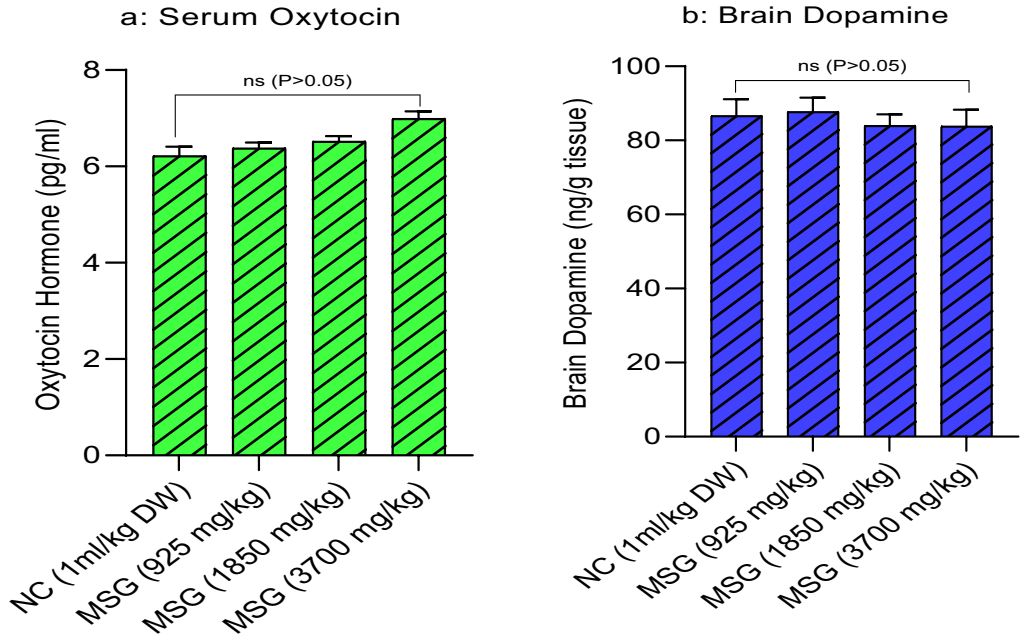


Fig. 2 Serum level of oxytocin hormone [2a] and brain dopamine [2b] of lactating Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water

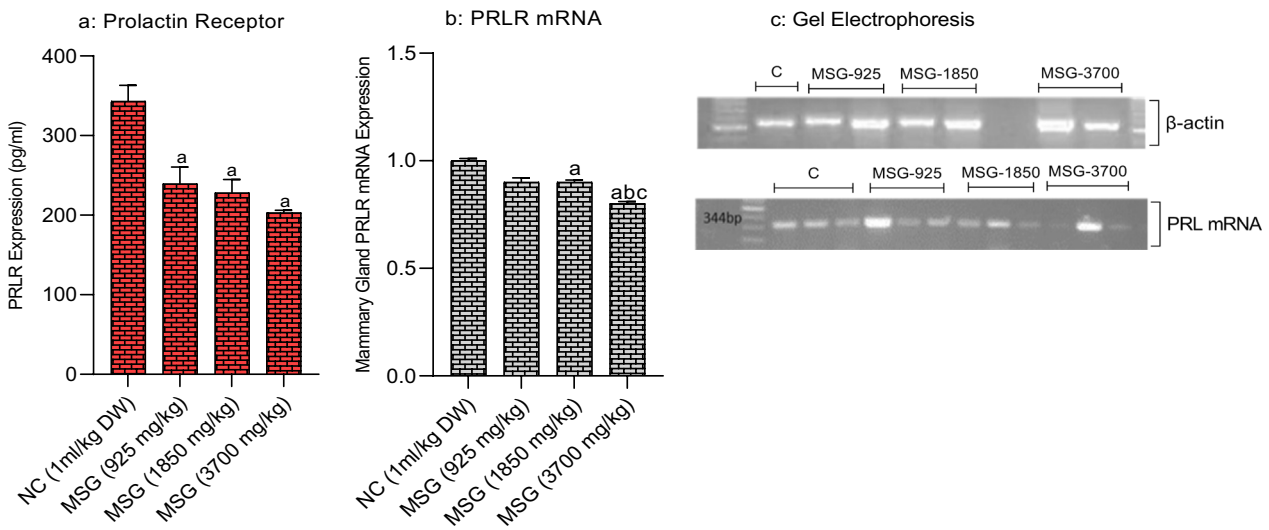


Fig. 3 Expression of mammary gland prolactin receptor [3a], mammary gland prolactin receptor mRNA [3b] and gel electrophoresis for housekeeping gene (β-actin), and gene of interest (GOI) [3c] of lactating Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water. PRL = prolactin, PRLR = prolactin receptor, C = control. Superscripts a, b and c indicate statistically significant difference ($p < 0.05$) compared to control, MSG 925 mg/kg and MSG 1850 mg/kg, respectively

dopamine in MSG 1850 mg/kg and MSG 3700 mg/kg compared to the control group.

Mammary gland prolactin receptor and prolactin receptor gene expression

The mammary gland prolactin receptor was decreased significantly ($p < 0.05$) in the MSG-administered animals compared to the control [Fig. 3a]. Additionally, the prolactin receptor decreased in the groups given MSG with increasing MSG doses. However, these changes between the groups were not statistically significant ($p > 0.05$). The expression of prolactin receptors in the mammary gland was significantly ($p < 0.05$) decreased in the groups given MSG at 1850 mg/kg and 3700 mg/kg compared to the control. The decrease in the MSG 3700 mg/kg given group was significant ($p < 0.05$) compared to the MSG 925 mg/kg and MSG 1850 mg/kg groups [Fig. 3b].

Mammary gland oxytocin receptor and aquaporin-3 levels

The mammary gland oxytocin receptor was significantly decreased ($p < 0.05$) in all the groups' given MSG compared to the normal control [Fig. 4a]. There was also a significant ($p < 0.05$) dose-dependent decrease in oxytocin receptor expression with MSG administration. Aquaporin-3 was significantly ($p < 0.05$) decreased in all the MSG-administered groups compared to the control group [Fig. 4b]. Among the MSG-administered groups, there was a significant ($p < 0.05$) dose-dependent decrease.

Serum and mammary gland homogenate ROS level

Serum ROS was significantly increased ($p < 0.05$) in all the groups given MSG compared to the control [Fig. 5a]. Additionally, the increase in ROS between the MSG-administered groups was dose-dependent. In Fig. 5b, the mammary ROS level in all the MSG-administered groups compared to the control was significantly ($p < 0.05$) increased. With increasing MSG doses, mammary ROS levels increased nonsignificantly.

Brain homogenate levels of MDA, SOD, CAT and GPx

The brain homogenate level of MDA was significantly ($p < 0.05$) increased in all the groups given MSG compared to the control [Fig. 6a]. In the group given MSG at 3700 mg/kg, MDA was significantly ($p < 0.05$) higher compared to all the other MSG-given groups. The activity of SOD [Fig. 6b] and CAT [Fig. 6c] was significantly decreased ($p < 0.05$) in the MSG groups at 1850 mg/kg and 3700 mg/kg compared to the control and MSG 925 mg/kg groups. More so, in the 3700 mg/kg group, SOD and CAT significantly ($p < 0.05$) decreased compared to the MSG 1850 mg/kg group. GPx was reduced significantly ($p < 0.05$) in all the MSG-administered groups compared to the control. Additionally, GPx in the MSG 1850 mg/kg and 3700 mg/kg groups was significantly decreased ($p < 0.05$) compared to the MSG 925 mg/kg group.

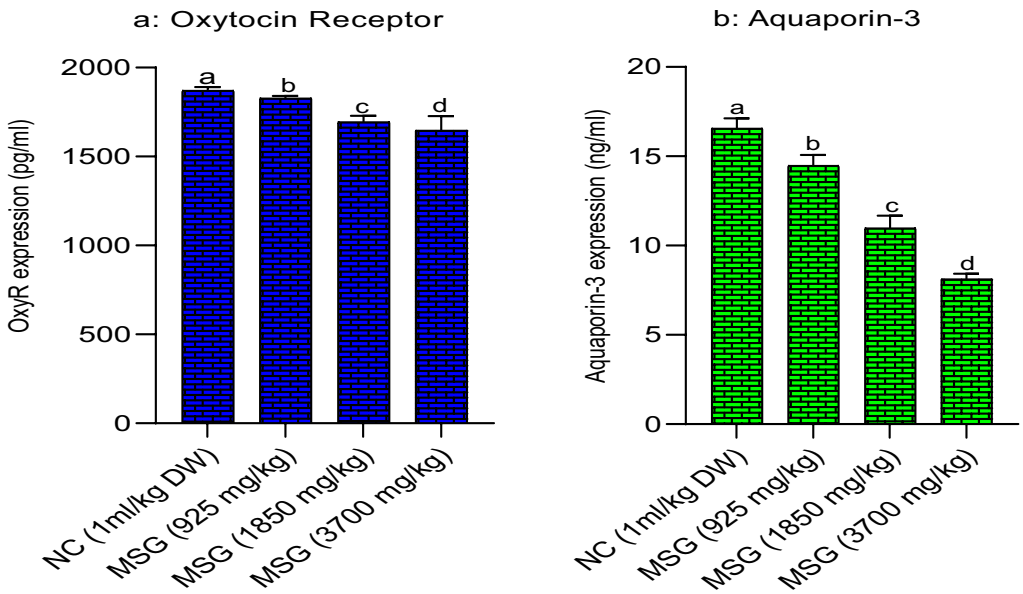


Fig. 4 Mammary gland oxytocin receptor [4a] and aquaporin-3 [4b] expressions in lactating Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water. Superscripts a, b and c indicate statistically significant difference ($p < 0.05$) compared to the normal control, MSG 925 mg/kg and MSG 1850 mg/kg, respectively

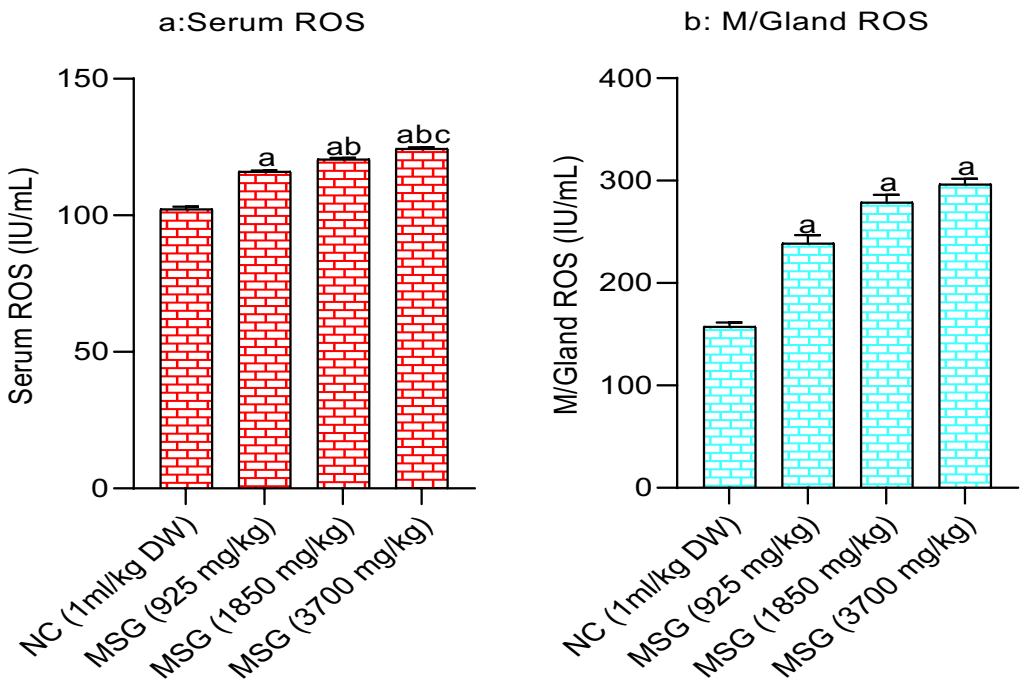


Fig. 5 Serum [5a] and mammary gland homogenate [5b] level of ROS in lactating Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water. Superscripts a, b and c indicate statistically significant difference ($p < 0.05$) compared to the normal control, MSG 925 mg/kg and MSG 1850 mg/kg, respectively

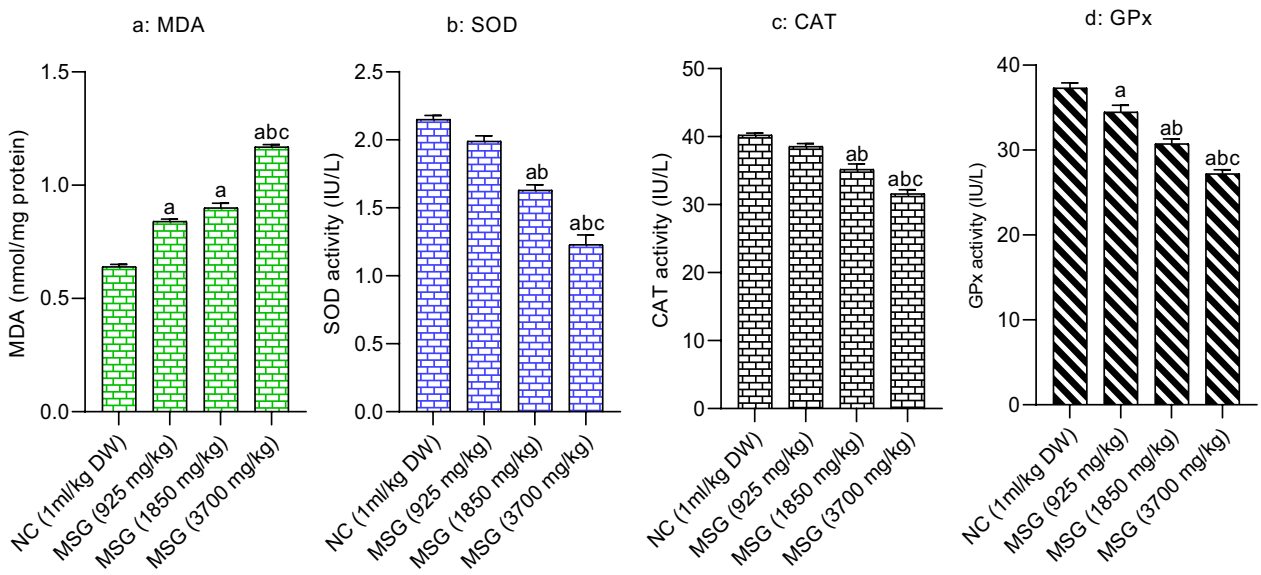


Fig. 6 Brain oxidative stress biomarkers in lactating Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water. Superscripts a, b and c indicate statistically significant difference ($p < 0.05$) compared to the normal control, MSG 925 mg/kg and MSG 1850 mg/kg, respectively

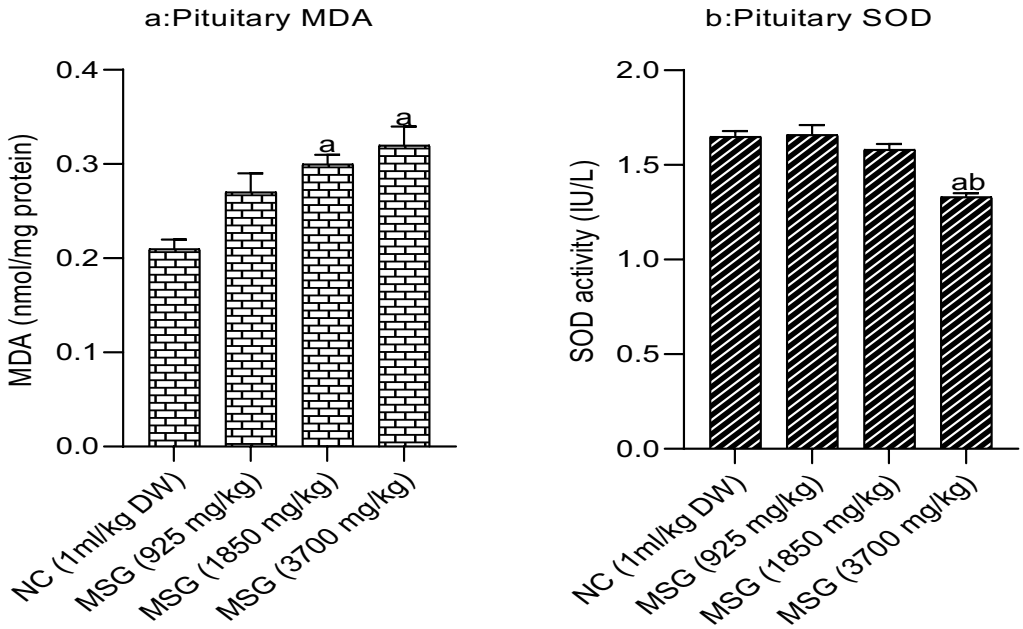


Fig. 7 Pituitary homogenate MDA [7a] and SOD [7b] of lactating Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water. Superscripts a, and b indicate statistically significant difference ($p < 0.05$) compared to the normal control and MSG 925 mg/kg, respectively

Pituitary gland homogenate levels of MDA and SOD

Pituitary MDA was significantly ($p < 0.05$) increased in the groups given MSG at 1850 mg/kg and 3700 mg/kg compared to the control [Fig. 7a]. At the highest MSG dose, SOD was significantly ($p < 0.05$) decreased only in the group given MSG at 3700 mg/kg compared to the control [Fig. 7b].

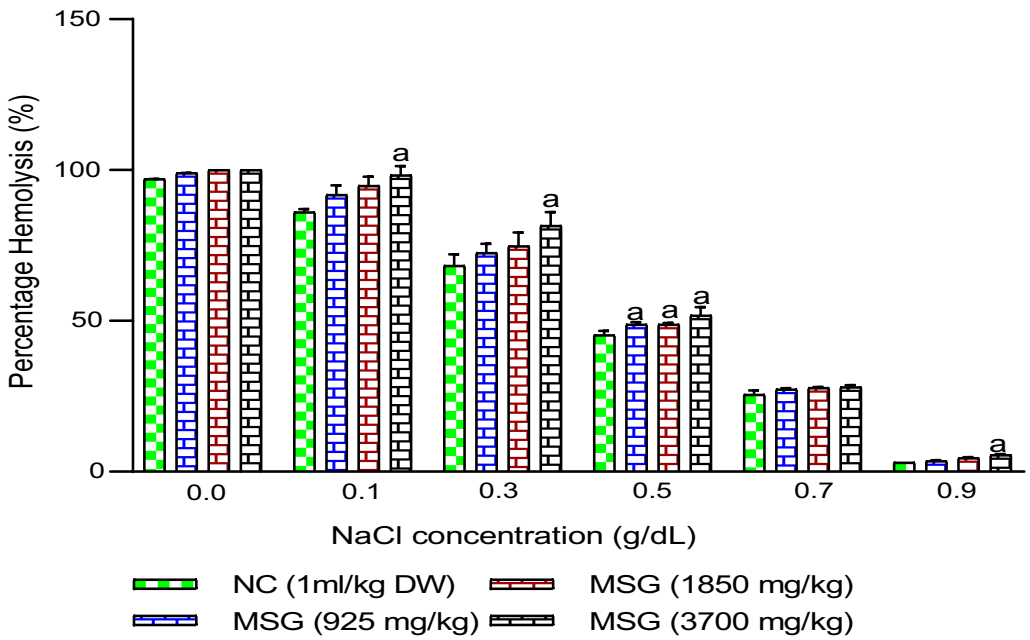


Fig. 8 Percentage hemolysis of red blood cells of breastfeeding Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water. Superscripts a indicates statistically significant difference ($p < 0.05$) compared to the normal control

Erythrocyte osmotic fragility

The percentage of hemolysis was significantly ($p < 0.05$) higher in the MSG 3700 mg/kg administered group compared to the control at 0.1 g/dL NaCl concentration solution in Fig. 8. At 0.3 g/dL NaCl concentration, the percentage of hemolysis was significantly higher in the MSG 3700 mg/kg group compared to the control. Although it was higher compared to the remaining MSG-treated groups, it was not significant ($p > 0.05$). The percentage of hemolysis was significantly higher ($p < 0.05$) in all the MSG-treated groups compared to the normal control group. A non-significant ($p > 0.05$) increase was

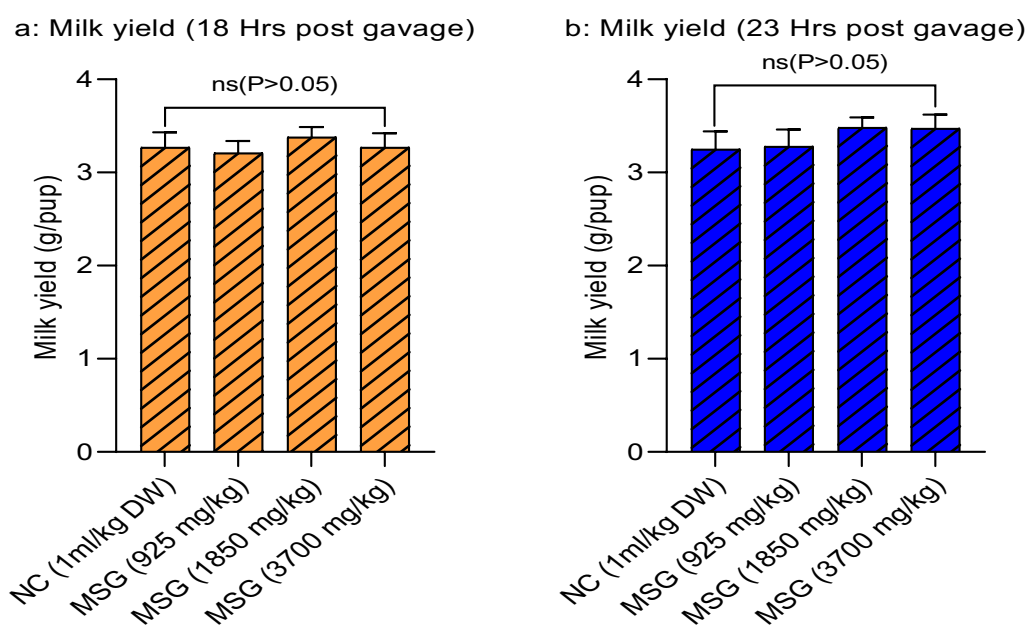


Fig. 9 Milk yield 18 [9a] and 23 [9b] hours post-gavage in lactating Wistar rats. NC = normal control, MSG = monosodium glutamate, DW = distilled water

observed in all the MSG-administered groups compared to the normal control group at 0.7 g/dL NaCl concentration. At 0.9 g/dL NaCl concentration, there was a significant increase ($p < 0.05$) in percentage hemolysis observed in the MSG 3700 mg/kg treated groups compared to the normal control group.

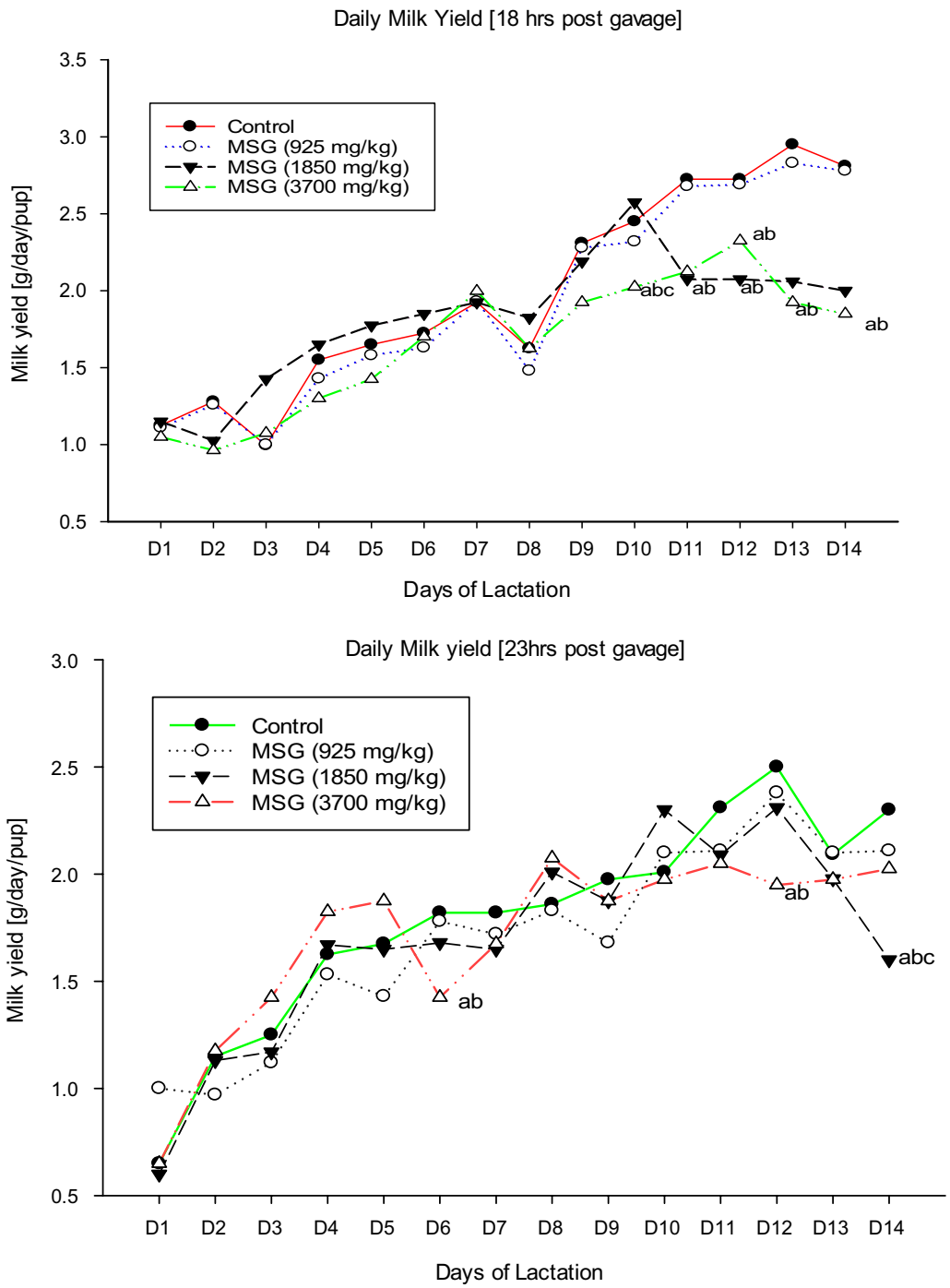


Fig. 10 Daily milk yield 18 h post-gavage in lactating Wistar rats. MSG = monosodium glutamate, DW = distilled water. Superscripts a, and b indicate statistically significant difference ($p < 0.05$) compared to the normal control and MSG 925 mg/kg, respectively

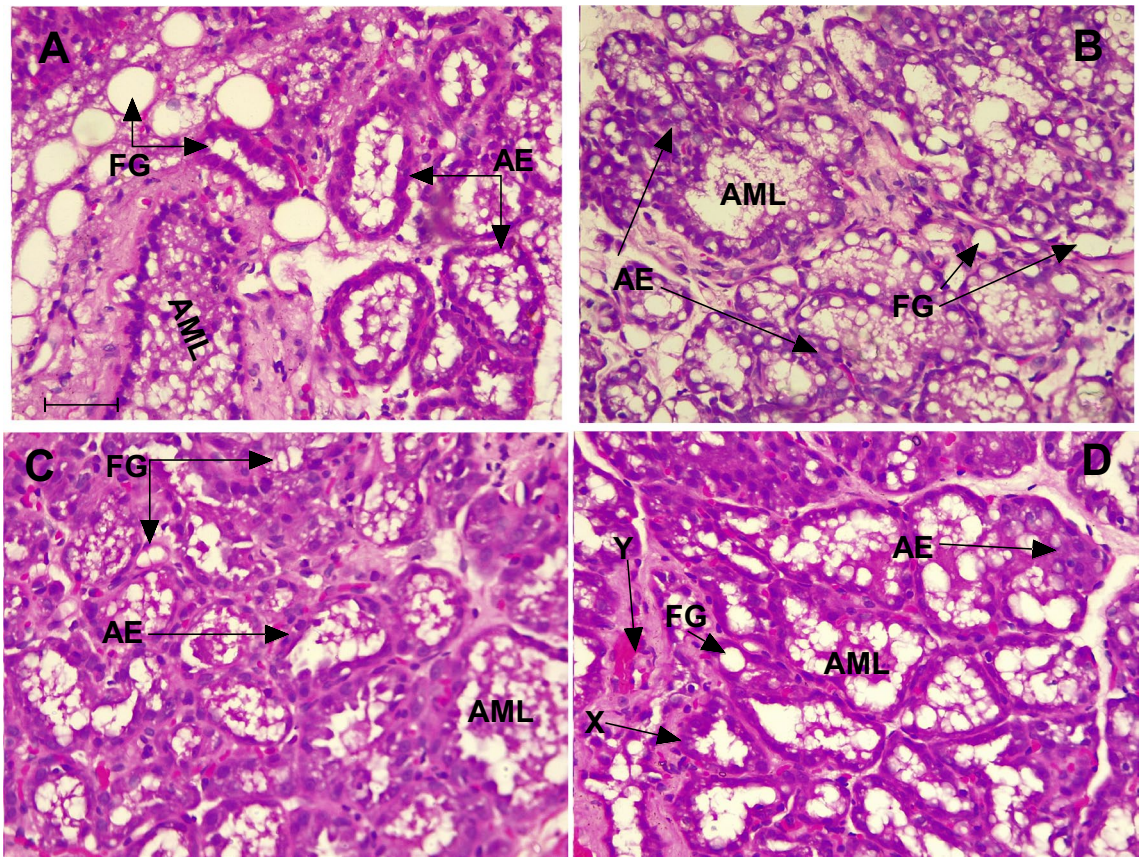


Fig. 11 Daily milk yield 23 h post-gavage in lactating Wistar rats. MSG = monosodium glutamate, DW = distilled water. Superscripts a, b and c indicate statistically significant difference ($p < 0.05$) compared to the normal control, MSG 925 mg/kg and MSG 3700 mg/kg, respectively

Milk yield at 18 and 23 h post-gavage

The milk yield at 18 h post-gavage was nonsignificantly ($p > 0.05$) higher in the groups given MSG at 1850 mg/kg and 3700 mg/kg compared to the control and MSG at 925 mg/kg in Fig. 9a. At 23 h post-gavage, the milk yield in all the MSG-administered groups was nonsignificantly higher ($p > 0.05$) compared to the control, as shown in Fig. 9b.

Daily milk yield distribution at 18 h post-gavage

Figure 10 is the daily distribution of the milk yield at 18 h post-gavage. Daily milk yield was significantly reduced ($p < 0.05$) in the MSG-administered groups at 1850 and 3700 mg/kg compared to the control and MSG 925 mg/kg groups from day 10 to day 14.

Daily milk yield distribution at 23 h post-gavage

Figure 11 is the daily distribution of the milk yield at 23 h post-gavage. A non-significant rise was observed in the MSG 3700 mg/kg group compared to the other groups between days 1 and 5. However, there was a significant decrease ($p < 0.05$) in the daily milk yield in the MSG 3700 mg/kg group compared to the control and MSG 925 mg/kg groups on days 6 and 12. On day 14, there was a significant ($p < 0.05$) decrease in the daily milk yield of the MSG group (1850 mg/kg) compared to all the other groups.

Plate [A] Control: normal mammary gland histoarchitecture with fat globule [FG], showing normal alveolar milk lumen [AML], and alveolar epithelium [AE] with normal epithelial cells. Plate B (MSG 925 mg/kg) shows normal mammary gland histoarchitecture with irregular alveoli branching lined with fat globules, numerous alveolar milk lumens containing milk, and a defined alveolar epithelium. Plate C (MSG 1850 mg/kg) shows irregular alveoli branching with fat globules. It also shows less defined epithelial cells with a smaller number of alveolar milk lumens. Plate D (MSG 3700 mg/kg) shows fat globules with a smaller number of distended alveoli. The alveoli of this group present irregularly disintegrating alveolar epithelium [X] and epithelial cells in the blood vessel [Y].

Discussion

Regions such as the pituitary gland, called periventricular organs (CVO), have been reported to allow molecules such as glutamate to pass in and out of the ECF (Hawkins & Viña, 2016). Functional glutamate receptors are involved in the release of prolactin (Login, 1990), growth hormone (Tena-Sempere et al., 2000), and oxytocin (Stern et al., 2000). These may be responsible for the changes observed using MSG in this study. In the current study, serum levels of prolactin, growth hormone, and oxytocin were not significantly increased in MSG-administered animals compared to controls. These results indicate that orally ingested MSG may exert minimal effects on both the adenohypophysis and the neurohypophysis of the pituitary gland. In addition, the significant pituitary and whole-brain lipid peroxidation in the MSG-administered group could have contributed to decreased pituitary function in the MSG-administered group. MSG did not significantly influence brain dopamine in this study. However, brain dopamine levels decreased with an increasing dose of MSG as opposed to the initial increase observed in the group given MSG at 925 mg/kg. The reduction in brain dopamine with MSG administration in this study is in concert with the findings of Wallace and Dawson (1990) and Abu-Taweel et al. (2014). However, the exact mechanism underlying the effect of MSG on brain dopamine is not clear.

The generation of massive ROS results in persistent changes in signal transduction and gene expression, as well as oxidative modification of proteins and nucleic acids (Das et al., 2022; Leone et al., 2017; Turpaev, 2002). Therefore, the significant increase in serum and mammary ROS levels in the MSG group in this study may be responsible for the significant reduction in mammary gland receptors for prolactin, oxytocin, and PRLR gene expression observed in the MSG-administered groups. The decreased prolactin receptor in this study corroborates the significant decrease in the expression of its gene.

In this study, MSG decreased mammary gland AQP-3. Aquaporins play an important role in the movement of water and solutes across epithelial and endothelial membranes (Verkman, 2002; Yde et al., 2021). Recent studies have shown the presence of several aquaporins in the mammary gland, including AQP-3 (Mobasheri and Barrett-Jolley, 2014). In this study, higher mammary ROS may have caused or contributed to the reduction of mammary AQP-3. There is an inverse relationship between AQP-3 and oxidative stress in other tissues (Xie et al., 2013). However, the exact mechanism remains unknown. The current study is one of the first to report the preliminary activity of AQP-3 in the mammary gland of lactating animals administered MSG.

MSG administration significantly increased serum and mammary gland ROS levels in this study. The red blood cell is primarily involved in the maintenance of redox balance, with hemoglobin being a major factor in initiating oxidative stress within the erythrocyte (Rifkind and Nagababu, 2013). Thus, in this current study, the significant increase in serum ROS could have been due to the autoxidation of hemoglobin and activation of nicotinamide adenine dinucleotide phosphate oxidase in the MSG-administered groups, leading to the generation of superoxide radicals and their dismutation to hydrogen peroxides within the RBC (Wang and Zennadi, 2020). RBCs can be destroyed by ROS coming from external sources as well as from other cells that are in circulation. (Gwozdzinski et al., 2021). The results of the osmotic fragility test in the MSG-administered group in this current study reveal a significant increase in the percentage of hemolyzed RBCs, which corroborates with the serum ROS findings and suggests that MSG consumption could have weakened the membrane integrity of the RBCs, making them more vulnerable to destruction through ROS production (Mohanty et al., 2014). Several studies have demonstrated that MSG administration increases ROS production in the kidney (Omogbiya et al., 2020; Sharma, 2015; Sharma et al., 2014), thymocytes (Vucic et al., 2018), and leucocytes (Delibashvili et al., 2018). Moreover, according to Mukhajee et al. (2023), MSG consumption is associated with heightened ROS production. All of these could have served as exogenous contributors to ROS production in this study.

The significant increase in mammary gland ROS levels in the animals' given MSG in this study could be linked to the role of AQP-3. Miller et al. (2010) found that AQP-3 mediates hydrogen peroxide uptake into cells for intracellular signaling regulation. Wang et al. (2007) noted that aquaporins are associated with gas permeability across membranes. Therefore, AQP-3 may have enabled the transfer of ROS from exogenous sources into the mammary gland in this study. Additionally, Sarwar et al. (1998), Matsumoto et al. (2013), and Park and Choi (2016) found that the mammary gland selectively synthesizes glutamine for protein synthesis and the milk amino acid pool. Monosodium glutamate supplementation has been reported to increase glutamine synthesis (Boutry et al., 2011), and increased glutamine synthesis is associated with ROS generation (Yang et al., 2021). However, the exact mechanism of action was not ascertained in this study.

In this study, MSG administration significantly caused brain and pituitary gland lipid peroxidation. Some of the factors that increase the brain's susceptibility to oxidative stress include unsaturated lipid enrichment and increased circulating glutamate, among other things (Cobley et al.,

2018). The findings on brain oxidative stress in this study are in concert with those of Formobi and Onyema et al. (2006) and Omogbiya et al. (2020). A possible action of MSG in this study could have been via excitotoxicity, which leads to the over-activation of postsynaptic glutamatergic (NMDA) receptors in neurons, resulting in the influx of sodium and calcium and the production of ROS (Meldrum, 2000; Dong et al., 2009; Wang and Qin, 2010). A concomitant decrease in tissue antioxidants was observed in the brain and pituitary gland homogenates of the MSG-administered animals in this current study. SOD is utilized for the catalytic conversion of superoxide anions to oxygen and hydrogen peroxide (Scandalios, 1993). The GPx and catalase families of enzymes are involved in the termination reaction of the ROS pathway by converting hydrogen peroxide to water (Hasanuzzaman et al., 2020). Thus, the significant decrease in the tissue antioxidants in this study with MSG administration corroborates the higher ROS production in the said tissues.

There were no significant differences in total milk output found following gavage at 18 and 23 h in this study at the end of day 14. The daily milk outputs at 18 and 23 h in the MSG-administered group, however, showed significant differences. The MSG-administered group's daily milk yield initially increased between days 1 and 7, but it significantly decreased between days 10 and 14. These findings mimic a "poisoned chalice" concept where MSG first seemed advantageous by increasing daily milk output but was eventually countered by its harmful effect with sustained administration, as demonstrated by the significant decrease in the daily milk output between days 10 and 14. The non-significant increase in lactogenic hormones, the possible low ROS production at the time, and the potential optimal sensitivity of the mammary gland to these hormones due to the presence of their receptors could all be contributing factors to the initial increase in daily milk output in the early days of MSG administration. The opposite result, which was obtained between days 10 and 14, might be explained by the system's excessive ROS production, decreased AQP-3, which reduced the amount of water that could enter the mammary gland, as well as the decline in hormone genes and receptors with prolonged MSG administration. Changes in the mammary gland histology were consistent with lactation-induced adjustments in lactating rats, with toxicological alterations observed in the highest MSG-administered group.

Conclusions

The findings of this study suggest that prolonged consumption of MSG could interfere with lactation-associated functions via increased ROS production, reduced antioxidants, decreased AQP-3, mammary prolactin and oxytocin receptors, and prolactin receptor mRNA in lactating Wistar rats.

Abbreviations

ANOVA	Analysis of variance
AQP-3	Aquaporin-3
CAT	Catalase
CVOs	Circumventricular organs
DW	Distilled water
ELISA	Enzyme linked immunosorbent assay
GH	Growth hormone
GPx	Glutathione peroxidase
IBM	International business machines
MDA	Malondialdehyde
MSG	Monosodium glutamate
NC	Normal control
OS	Oxidative stress
OXT	Oxytocin
OXTR	Oxytocin receptor
PBS	Phosphate buffer solution
PCR	Polymerase chain reaction
PRL	Prolactin
PRLR	Prolactin receptor
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SPSS	Statistical package for social sciences

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Author contributions

NSE carried out conceptualization and design of the study, as well as drafting of the manuscript under the supervision of IBG, TY and AM. ISM, EDE, HAU and AM reviewed the manuscript and participated in literature review. NES and MA carried out the statistical analyses of the data.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

The Ahmadu Bello University ethical committee on animal use and care granted local institutional ethical permission for the use of laboratory animals for this research with approval number: ABUCAUC/2018/092.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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