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Research Article

Evaluation of Metoclopramide Hydrochloride-Induced Alterations in Prolactin Receptor Expression, Aquaporin-3, Oxytocin Receptors, and Other Key Biomarkers in the Mammary Glands of Lactating Wistar Rats

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Abstract

Galactagogues, which can be either synthetic or derived from plants, are utilized to stimulate, sustain, and enhance milk production. Metoclopramide, a powerful promoter of prolactin secretion, achieves this via dopamine receptors in the hypothalamus. This study focused on exploring the molecular mechanisms underlying metoclopramide's galactagogue effects in lactating Wistar rats. Twelve lactating Wistar rats at the time of parturition were randomly divided into two groups of six animals each. The first group, served as the control. The second group was administered metoclopramide hydrochloride (5 mg/kg) orally for fourteen days. At the conclusion of the experiment, the rats were anesthetized and sacrificed, with blood and tissue samples collected for molecular, biochemical, and histological analysis. Treatment with metoclopramide hydrochloride significantly increased milk production compared to the control group by elevating serum prolactin and oxytocin levels, mammary prolactin and oxytocin receptors, aquaporin-3, mRNA prolactin receptor gene, and pituitary gland SOD, while reducing mammary gland ROS production and pituitary gland MDA levels. These findings suggest that metoclopramide hydrochloride positively modulates hormonal and molecular pathways essential for lactation via upregulation of mammary prolactin and oxytocin receptors, aquaporin-3, and the mRNA prolactin receptor gene.

Keywords: *Metoclopramide; Galactagogues; Lactation; Receptors; Aquaporin-3; Mammary Gland*

Abbreviations

MDA: Malondialdehyde; SOD: Superoxide Dismutase; UNICEF: United Nations Children's Fund; PO: Per Os; EU: European Union; PCR: Polymerase Chain Reaction; PBS: Phosphate Buffer Solution; RNA: Ribonucleic Acid; USA: United State of America; ELISA: Enzyme Linked Immunosorbent Assay; ER: Estrogen Receptor; IMB: International Business Machines Cooperation; MHCL: Metoclopramide

Hydrochloride; NC: Normal Control; DW: Distilled Water; ROS: Reactive Oxygen Species; CPB: Chromophobe Cells; BPC: Basophilic Cells; CT: Connective Tissue; PRL: Prolactin; PVN: Periventricular Nucleus (PVN); PRF: Prolactin-Releasing Factor; GH: Growth Hormone; PRLR: Prolactin Receptor; OTR: Oxytocin Receptor; AQP: Aquaporin; JAK-STAT: Janus Kinase

Introduction

Breastfeeding is the optimal form of nutrition for the physical and neurological development of infants and is considered the most significant way to prevent child mortality [1]. UNICEF data released in 2021 show that worldwide, only 44% of newborns are exclusively breastfed for the first 5 months of life [2]. Studies report reasons for early cessation, such as cracked nipples, pain, or insufficient milk [3]. Thus, women have come to rely on galactagogues during breastfeeding exercises. Galactagogues, which can be either synthetic or derived from plants, are utilized to stimulate, sustain, and enhance milk production. These agents facilitate intricate processes that involve both physical and physiological interactions [4,5].

There are however contradictory results on the use of metoclopramide, which is also used mostly in experimental studies [1]. The drug has shown effectiveness in reducing post-operative vomiting and radiation sickness, as well as improving certain types of drug-induced vomiting [6]. However, additional controlled trials are necessary to validate the efficacy of metoclopramide in these suggested areas of application [7]. Metoclopramide is one such drug option, and clinical data shows its efficacy in promoting milk production in women. It is a dopamine antagonist that increases prolactin levels, initiating or augmenting milk production [8]. Metoclopramide is a potent stimulus of prolactin secretion and exerts this effect by blocking dopamine receptors in the hypothalamus and decreasing prolactin-inhibiting factors. A recent study has reported metoclopramide to increase milk production in primiparous sows [9]. Another study by Keller., *et al*. [10] concludes that it is likely bitches with insufficient or delayed milk production could benefit from metoclopramide treatment. However, metoclopramide's safety, efficacy, and underlying mechanisms in enhancing lactation remain inadequately explored. This research aims to investigate the molecular and physiological effects of metoclopramide on lactation, providing a comprehensive evaluation of its potential as a therapeutic option for addressing lactation insufficiency.

Materials and Methods

Materials

Monosodium glutamate, metoclopramide hydrochloride tablets 10 mg (NAFDAC REG NO: 04-6476), ELISA kits (prolactin, growth hormone, oxytocin and dopamine), ketamine, xylazine, distilled water, alcohol, hematoxylin and eosin. PCR grade water, forward and reverse primers for test and reference genes (stock 100 μM), DNA shield. Prolactin (ER7070 fine test, Wuhan China), oxytocin (ER1619 fine test, Wuhan China) and aquaporin-3 receptor (ER0743 fine test, Wuhan China) ROS commercial kit [Cat No: CK-bio-20410, Shanghai Coon Koon Biotech Ltd, China]. Malondialdehyde [NWK-MDA01 assay kit from Northwest Life Science Specialties], USA. Superoxide dismutase [NWLSS™ NWK-SOD02 assay kit].

Animal handling and grouping

Animal use in this study was in tandem with ethical approval obtained from the Ahmadu Bello University Committee on Animal Use and Care with ethical number: ABUCAUC/2018/092. Fourty animals; 20 males and 20 females (180 to 200 g body weight) were sourced from the Animal House of the Department of Human Physiology Ahmadu Bello University and used for this study. The male rats were used for mating protocol as described by Bronson., *et al*. [11] at a ratio of 1:1. Female rats were separated from the males after the pregnancy detection by manual palpation [12]. At parturition, the female rats were randomly grouped into two (2) of ten animals each [13]. Group 1 served as the normal control and was given 1 ml/kg distilled water while group 2 was given metoclopramide at 5 mg/kg body weight [14]. The administration was given orally from day three after parturition and lasted fourteen (14) days. At the end of the experiment, animals were anaesthetized with 75 mg/kg ketamine and 10 mg/kg xylazine [15].

Milk yield assessment

Milk yield was estimated daily 18 and 23 hours after gavage according to the method described by Sampson and Jansen [16], as a difference between pre-and post-suckling weights of the litters. Milk yield 18 hours after gavage was calculated as . Where w2 = pre-suckling weight of litters (11:00 am) and w3 = post-suckling weight of litters (noon). The correction for weight loss due to metabolic processes was calculated 18 hours after gavage as: where w2 = pre-suckling litters' weight, w1 = pre-isolation litters' weight.

Blood sample and tissue collection

Blood was collected via cardiac puncture at the end of the experiment after the animals were anaesthetized using ketamine and xylazine at 75 and 10 mg/kg respectively [15]. The pituitary gland was excised as described by Tzou., *et al*. [17] and homogenized in a phosphate buffer solution. Mammary gland tissue homogenates according to the method of Tolg., *et al* [18]. The mammary gland samples used for PCR were washed in PBS 02 M pH 7.4 [19], and preserved in RNAlater [thermofisher Scientific inc USA].

Biochemical assessment

Hormonal assays were carried out using the Fine Test ELISA kits from Wuhan, China; prolactin (ER-0076), growth hormone (ER-0993), oxytocin (ER-1723), and brain dopamine (EU0392), according to the manufacturer's manual. Prolactin (ER7070 fine test, Wuhan, China), oxytocin (ER1619 fine test, Wuhan, China), and aquaporin-3 receptor (ER0743 fine test, Wuhan, China) were assayed according to the manufacturer's manual. Reactive oxygen species (ROS) were assayed using the commercial kit [Cat No: CK-bio-20410, Shanghai Coon Koon Biotech Ltd, China] according to the manufacturer's manual. Malondialdehyde estimation was carried out using the NWK-MDA01 assay kit from Northwest Life Science Specialties, USA. Superoxide dismutase was measured using the NWLSS™ NWK-SOD02 assay kit. Total RNA was extracted using the RiboPure™ Kit, cDNA was synthesized using a reverse transcription kit, and gene expression assays were performed using TaqMan primers and probes. Beta-actin was used as the endogenous control. Quantitative PCR was carried out using the Light Cycler® system, and fold change in expression was calculated using the ΔΔCT method. Primer sequences for β-actin and PRLR were provided. The expression fold change was then calculated as (2-ΔΔCT) [20]. Primer sequences used (5' to 3'): β-actin (TTGTAA CCAACTGGGACGATATGG)-forward, (GATCTTGATCTTGATGGTGCTGCTAGG)-reverse. Prolactin receptor sequences: (CCTGAAGACAAGGAACAAGCC) forward and (TGGGAATCCCTGCGCAGGCA) reverse. Assays were carried out in triplicates.

Histological assessment

The mammary gland tissues were harvested, fixed in formalin, dehydrated in alcohol solutions, cleared in xylene, and embedded in paraffin wax. Sections were cut using a microtome and stained with hematoxylin and eosin for histological assessment [18,21].

Data analysis

Statistical analyses were conducted using SPSS version 23 (IBM, USA) with an independent sample T-test. A p-value of less than 0.05 was considered statistically significant. A repeated measure was carried out for the data of daily milk yields. Graphs were generated using GraphPad Prism 8 software (version 8.0.2 [263]; GraphPad Software, San Diego, USA).

Results

Serum prolactin, growth hormone, oxytocin and brain dopamine

Brain dopamine level in figure 1a was non-significantly ($p > 0.05$) higher in the MHCL-treated group compared to the normal control. In figure 1b, serum prolactin was significantly ($p < 0.05$) higher in the group treated with metoclopramide (MHCL 5 mg/kg) compared

to the normal control. In figure 1c, serum oxytocin hormone was significantly higher ($p < 0.05$) in the MHCL-treated group compared to the normal control group. Although the serum growth hormone level in figure 1d was higher in the MHCL-treated group compared to the control group, the change was however not statistically significant ($p > 0.05$).

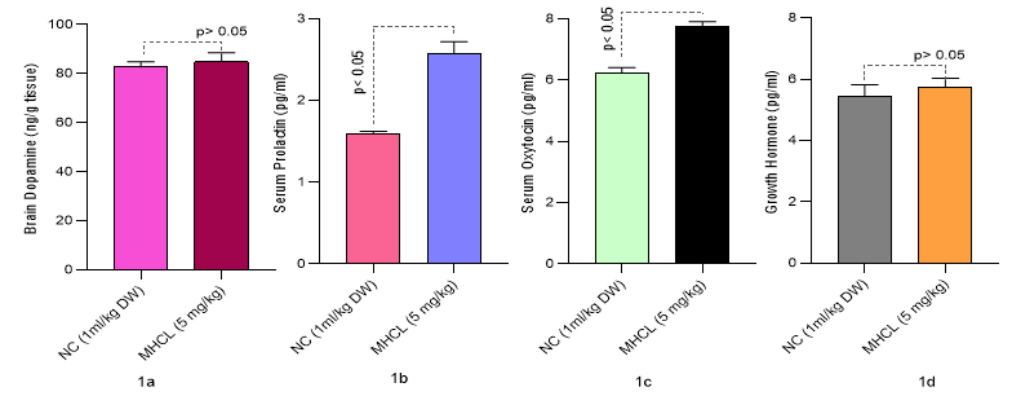

Figure 1: *Brain dopamine [1a], serum levels of prolactin hormone [1b], oxytocin hormone [1c] and growth hormone [1d]. NC: Normal Control; MHCL: Metoclopramide Hydrochloride; DW: Distilled Water. P < 0.05 = statistically significant difference. P > 0.05 = no statistically significant difference.*

Mammary gland ROS, pituitary gland MDA and SOD

The level of mammary gland ROS in figure 2a was significantly ($p< 0.05$) reduced in the MHCL-treated group compared to the normal control group. In figure 2b, pituitary gland MDA was non-significantly ($p > 0.05$) reduced in the MHCL-treated group compared to the normal control. Pituitary SOD in figure 2c was significantly higher ($p < 0.05$) in the MHCL-treated group compared to the normal control group.

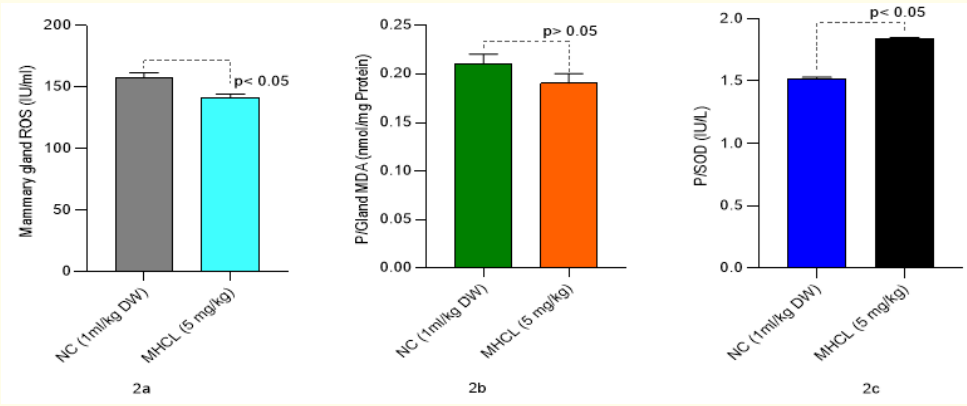

Figure 2: *Mammary gland ROS [2a], pituitary gland MDA [2b] and pituitary gland SOD [2c]. NC: Normal Control; MHCL: Metoclopramide Hydrochloride; DW: Distilled Water. P < 0.05 = statistically significant difference. P > 0.05 = no statistically significant difference.*

Mammary gland prolactin receptor, oxytocin receptor and aquaporin-3 (AQP-3)

In figure 3a, mammary gland prolactin receptor was significantly ($p < 0.05$) higher in the group treated with MHCL compared to the normal control. There was a non-significant ($p < 0.05$) increase in the mammary gland oxytocin receptor in the MHCL-treated group compared to the normal control in figure 3b. Mammary gland aquaporin-3 in figure 3c was significantly higher ($p < 0.05$) in the MHCL-treated group compared to the normal control.

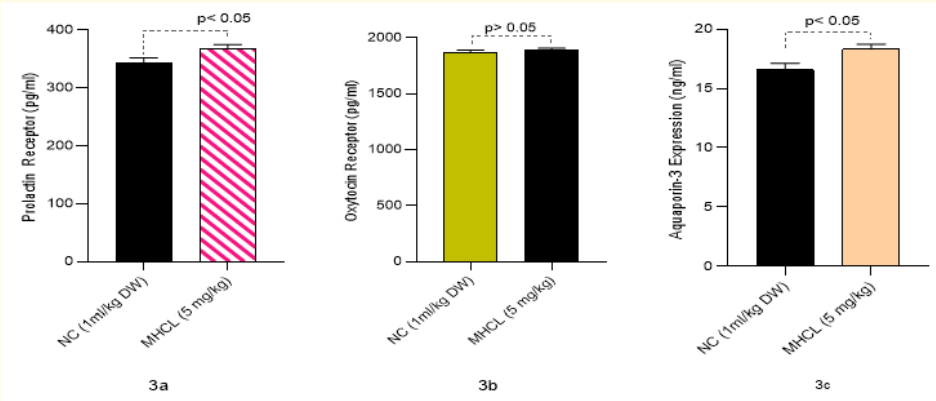

Figure 3: *Mammary gland prolactin receptor [3a], Oxytocin receptor [3b] and Aquaporin-3 [3c]. NC: Normal Control; MHCL: Metoclopramide Hydrochloride; DW: Distilled Water. P < 0.05 = statistically significant difference. P > 0.05 = not statistically significant difference.*

Mammary gland prolactin receptor mRNA, Milk yield 18 and 23 hours post gavage

In figure 4a, the was a significant fold ($p < 0.05$) increase in the expression of the prolactin receptor gene in the group that was given MHCL compared to the normal control group. Total milk yield at 18 hours in figure 4b and at 23 hours in figure 4c following oral administration of MHCL was significantly higher in the MHCL-treated groups compared to the normal control group.

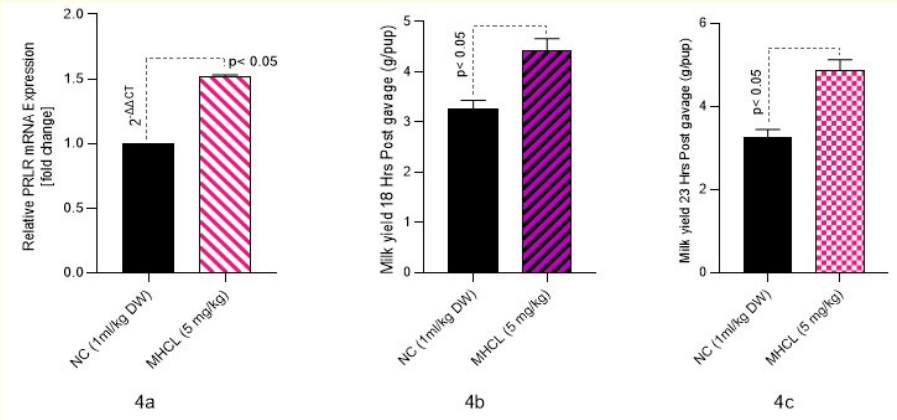

Figure 4: *Relative prolactin receptor mRNA expression [4a], Milk yield 18hrs post gavage [4b] and Milk yield 23hrs post gavage [4c]. NC: Normal Control; MHCL: Metoclopramide Hydrochloride; DW: Distilled Water. P < 0.05 = statistically significant difference.*

Daily milk yield at 18 and 23 hours post gavage

Figure 5a shows the result of daily milk yield 18 hours after oral administration of MHCL. The daily milk yield in the MHCL-treated group was significantly ($p < 0.05$) higher on days 4 to 11 compared to the normal control. Milk yield distribution was uniform in the MHCL-treated group between days 3 and 7 compared to the normal control group. The daily milk yield on days 13 and 14 was non-significantly lower ($p > 0.05$) in the MHCL-treated group compared to the normal control. At 23 hours after gavage, milk yield in the MHCL-treated group was significantly lower on day 3 compared to the normal control. However, between days 5 and 12, there was a significant ($p < 0.05$) increase in the MHCL-treated group compared to the normal control.

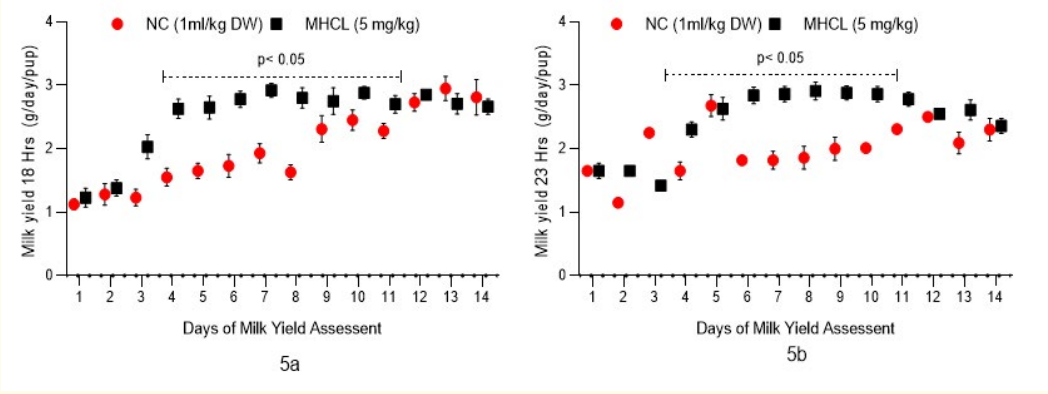

Figure 5: *Relative prolactin receptor mRNA expression [5a], Milk yield 18hrs post gavage [5b]. NC: Normal Control; MHCL: Metoclopramide Hydrochloride; DW: Distilled Water. P < 0.05 = statistically significant difference.*

Photomicrograph of the pituitary gland in adult female lactating wistar rats

The histological examination of the pituitary gland from adult female lactating Wistar rats was conducted to assess the cellular changes induced by metoclopramide hydrochloride (MHCL) treatment. Plate A: Normal Control (NC) The photomicrograph of the pituitary gland in the normal control group shows a well-organized structure. The chromophobe cells (CPB) are evenly distributed, while the basophilic cells (BPC) are identifiable. The connective tissue (CT) appears normal, providing structural support to the gland. Plate B: Metoclopramide Hydrochloride (MHCL) Treatment In the MHCL-treated group, notable histological changes were observed. The chromophobe cells (CPB) show a marked increase in number, indicating potential cellular hyperplasia. The basophilic cells (BPC) also exhibit morphological alterations, with some cells appearing hypertrophic. The connective tissue (CT) demonstrates signs of increased density, which may be indicative of a reactive fibrotic process in response to the treatment.

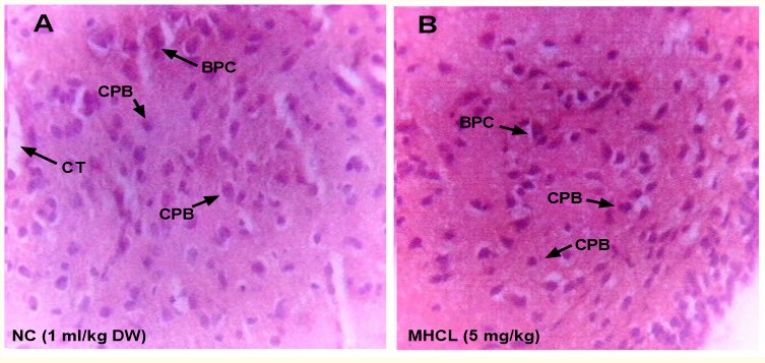

Figure 6: *Photomicrograph of the pituitary gland in adult female lactating Wistar rats. CPB: Chromophobe Cells; BPC: Basophilic Cells; CT: Connective Tissue. Plate A: Normal Control (NC); Plate B: MHCL = Metoclopramide Hydrochloride.*

Discussion

In this study, there was a non-significant increase in brain dopamine levels ($p > 0.05$) in the group treated with metoclopramide (MHCL). Previous research has indicated that metoclopramide can inhibit cerebral dopamine receptors, causing a buildup of dopamine in the synaptic cleft due to the inability of dopamine to bind and be cleared from the receptors. This accumulation of dopamine in the synaptic cleft may contribute to the observed elevation in brain dopamine levels [22].

The higher serum prolactin (PRL) level ($p < 0.05$) observed in the MHCL-treated group in this study can be attributed to MHCL's action on dopamine receptors, impacting the synthesis and release of prolactin. MHCL has been shown to inhibit the dopamine (D2) receptor [23]. By blocking the D2 receptor, adenyl cyclase is activated, leading to the production of cyclic adenosine monophosphate, which in turn stimulates prolactin release [24]. The effect of MHCL on serum prolactin in this study aligns with the results of Teixeira Gomes., *et al*. [25], demonstrating that MHCL not only boosted the number of lactotrophs but also enhanced their metabolic function, leading to larger nuclear volumes and heightened prolactin secretion. In the present study, the higher oxytocin levels observed with MHCL treatment could potentially be attributed to the direct influence of the elevated circulating prolactin levels on oxytocin. Prolactin has been shown to stimulate the release of oxytocin by activating neuronal nitric oxide synthase in the supraoptic and paraventricular nuclei [26,27]. The observed rise in serum oxytocin levels in animals treated with metoclopramide MHCL reinforces the idea that prolactin supports milk production and facilitates milk ejection by enhancing oxytocin release. The ability of MHCL to elevate both prolactin and oxytocin levels suggests its potential as a therapeutic agent for addressing lactation insufficiency. By leveraging the hormonal crosstalk between prolactin and oxytocin, MHCL could improve both the quantity and ease of milk delivery in lactating individuals. These findings pave the way for further research into the molecular mechanisms underlying prolactin and oxytocin interaction. Exploring the exact pathways and additional factors involved in this hormonal regulation can help optimize treatments for lactation challenges and enhance our understanding of neuroendocrine control in lactation.

There is an interplay between dopamine and oxytocin hormones [28], where stimulation of dopamine neurons in the periventricular nucleus (PVN) can activate oxytocinergic neurons [29,30]. Prolactin promotes oxytocin release by activating neuronal nitric oxide synthase in the supraoptic and paraventricular nuclei [26]. Dopamine normally inhibits the release of growth hormone-releasing hormone (GHRH) from the hypothalamus. By blocking dopamine receptors, metoclopramide reduces this inhibitory effect, leading to increased GHRH and a consequential rise in growth hormone [31]. This is consistent with the rise in GH observed in this study. The rise in GH observed in this study suggests that metoclopramide could enhance milk production by stimulating the mammary glands through increased GH levels. The increase in GH, along with elevated prolactin levels, can have synergistic effects on lactation. While prolactin is directly responsible for milk production, GH can enhance the overall capacity of the mammary glands to produce and secrete milk. This dual hormonal enhancement can lead to improved lactation outcomes. Lactation insufficiency, where milk production is inadequate to meet the infant's needs, is a common challenge. By increasing GH levels, metoclopramide may offer a therapeutic approach to mitigate lactation insufficiency, providing a hormonal boost that enhances milk yield. This suggests a promising avenue for addressing lactation challenges and improving breastfeeding outcomes. Further research could explore the full extent of these benefits and the mechanisms involved.

The increased energy demand and oxygen requirements during lactation can result in oxidative stress [32]. Mammary glands are actively involved in anabolism and may experience imbalances in redox status due to the continuous production of reactive oxygen species (ROS), leading to oxidative stress [33]. In this study, treatment with MHCL was found to decrease ROS levels in the mammary gland ($p < 0.05$) and malondialdehyde (MDA) levels in the pituitary gland ($p > 0.05$). This effect could be attributed to the antioxidant properties of prolactin, which has been shown to enhance endogenous antioxidant defences rather than directly interacting with free radicals in other tissues [34]. Consistent with this, an increase in endogenous superoxide dismutase (SOD) levels was observed in the pituitary gland of animals treated with MHCL in this study. Prolactin has also been reported to reduce ROS and oxidative stress in ovarian tissues [35]. While

there is limited literature on the impact of MHCL on mammary gland ROS levels during lactation, MHCL has been shown to scavenge reactive oxygen species directly [36]. Furthermore, prolactin has been found to regulate total antioxidant capacity in seminal plasma [37], and dopamine has antioxidant properties as well [38]. Therefore, the antioxidant effects observed in the pituitary gland of animals treated with MHCL in this study may be attributed to changes in dopamine levels in the same group.

According to Uvnäs Mober., *et al*. [39], oxytocin demonstrates potent antioxidant effects through its actions in both central and peripheral tissues. Erbas., *et al.* [40] have noted that oxytocin can decrease lipid peroxides and enhance glutathione levels. Therefore, in this study, the rise in oxytocin levels may have played a role in decreasing reactive oxygen species (ROS) in the mammary gland and reducing malondialdehyde (MDA) in the pituitary gland, owing to its combined central and peripheral antioxidant properties.

The rise in prolactin levels stimulates the prolactin receptors on target cells, including those in the mammary gland. The increased stimulation of prolactin receptors leads to an upregulation of prolactin receptor mRNA, enhancing the expression of these receptors [41]. This could explain the prolactin-induced increased prolactin receptor mRNA observed in this study. This finding adds to the broader knowledge of molecular pathways involved in lactation. It highlights the role of prolactin not just in stimulating milk production directly, but also in enhancing the cellular machinery needed for an effective lactation response. The upregulation of prolactin receptor mRNA could serve as a biomarker for prolactin activity in the mammary glands. This can be useful in both research settings to study prolactin dynamics and in clinical settings to monitor and diagnose lactation-related conditions.

In this study, we examined the expression of oxytocin receptors (OTR) in the mammary glands. Typically, OTR levels are elevated in the mammary glands, facilitating lactation [42]. There is no prior research on the effects of MHCL administration on mammary gland OTR during lactation. The non-significant changes in OTR indicate that the circulating oxytocin in the MHCL group had a minimal impact on the expression of mammary gland OTR during lactation ($p < 0.05$). The precise mechanism behind this observation remains unknown.

Aquaporins (AQP) are essential membrane proteins for water movement across cellular membranes that help regulate water balance [43], and are crucial for milk synthesis and secretion [44]. AQP3 is present in the basolateral membrane of secretory epithelial cells and intralobular and interlobular duct epithelial cells [45]. In this study, the increased AQP3 expression in the mammary gland could have been from increased prolactin binding activity to its receptor, which has been shown to trigger signalling pathways which lead to the upregulation of AQP3 [46-48]. Additionally, the prolactin hormone has been shown to play a role in osmoregulation [47-49]. This finding provides a mechanistic insight into the activity of AQP3 in the mammary gland during lactation. The upregulation of AQP3 suggests a positive feedback loop where increased prolactin levels lead to more AQP3 expression, further enhancing the mammary gland's ability to produce milk. This finding adds to the body of knowledge on the molecular mechanisms involved in lactation and osmoregulation. It opens up new avenues for research into how hormonal regulation affects water transport and milk secretion. Treatment with metoclopramide hydrochloride (MHCL) resulted in significant histological alterations. A notable increase in the number of chromophobe cells (CPB) was observed, suggesting potential cellular hyperplasia. Basophilic cells (BPC) also displayed morphological changes, including hypertrophy in some cells. Furthermore, the connective tissue (CT) demonstrated increased density, potentially indicative of a reactive fibrotic response to the MHCL treatment. These have contributed to the higher milk yield observed in the group given MHCL by increasing the activity of prolactin and GH-producing cells.

Conclusion

MHCL has a multifaceted impact on lactation, making it a promising candidate for addressing lactation insufficiency. Its ability to enhance milk yield through hormonal regulation and oxidative stress reduction positions MHCL as a potentially valuable therapeutic agent for improving lactation outcomes in lactating individuals.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Bibliography

1. Hussain NHN., *et al*. "Metoclopramide for milk production in lactating women: A systematic review and meta-analysis". *Korean Journal of Family Medicine* 42.6 (2021): 453-463.

2. Oberfichtner K., *et al*. "Breastfeeding in primiparous women - expectations and reality: a prospective questionnaire survey". *BMC Pregnancy and Childbirth* 23.1 (2023): 654.

3. Gianni ML., *et al*. "Breastfeeding difficulties and risk for early breastfeeding cessation". *Nutrients* 11.10 (2019): 2266.

4. Mortel M and Mehta SD. "Systematic review of the efficacy of herbal galactogogues". *Journal of Human Lactation: Official Journal of International Lactation Consultant Association* 29.2 (2013): 154-162.

5. Penagos Tabares F., *et al*. "Pharmacological overview of galactogogues". *Veterinary Medicine International* (2014): 602894.

6. Liu M., *et al*. "Neurokinin-1 receptor antagonists in preventing postoperative nausea and vomiting: a systematic review and meta-analysis". *Medicine* 94.19 (2015): e762.

7. Abdelmonem H., *et al*. "The efficacy and safety of metoclopramide in relieving acute migraine attacks compared with other anti-migraine drugs: a systematic review and network meta-analysis of randomized controlled trials". *BMC Neurology* 23.1 (2023): 221.

8. Gabay, M. P. "Galactogogues: Medications that induce lactation". *Journal of Human Lactation: Official Journal of International Lactation Consultant Association* 18.3 (2002): 274-279.

9. Mathews AT., *et al*. "Metoclopramide induces preparturient, low-level hyperprolactinemia to increase milk production in primiparous sows". *Domestic Animal Endocrinology* 74 (2021): 106517.

10. Keller SR., *et al*. "Effect of metoclopramide treatment of bitches during the first week of lactation on serum prolactin concentration, milk composition, and milk yield and weight gain of their puppies". *American Journal of Veterinary Research* 79.2 (2018): 233-241.

11. Bronson FH and Whitten WK. "Oestrus-accelerating pheromone of mice: assay, androgen-dependency and presence in bladder urine". *Journal of Reproduction and Fertility* 15.1 (1986): 131-134.

12. Agematsu Y., *et al*. "Early detection of pregnancy of the rat". *Jikken Dobutsu. Experimental Animals* 32.4 (1983): 209-212.

13. Charan J and Kantharia ND. "How to calculate sample size in animal studies?" *Journal of Pharmacology and Pharmacotherapeutics* 4.4 (2013): 303-306.

14. Emmanuel NS., *et al*. "Body weight changes and serum growth hormone comparative assessment in female lactating wistar rats (*Rattus norvegicus*) treated with metoclopramide and some atypical antipsychotics". *Journal of Advances in Medical and Pharmaceutical Sciences* 18.2 (2018): 1-6.

15. Abdulghani MAM., *et al*. "Effect of monosodium glutamate on serum sex hormones and uterine histology in female rats along with its molecular docking and in-silico toxicity". *Heliyon* 8.10 (2022): e10967.

16. Sampson DA and Jansen G R. "Measurement of milk yield in the lactating rat from pup weight and weight gain". *Journal of Pediatric Gastroenterology and Nutrition* 3.4 (1984): 613-617.

17. Tzou SC., *et al*. "Preparation of mouse pituitary immunogen for the induction of experimental autoimmune hypophysitis". *Journal of Visual Experiment* 46 (2010): 2181.

18. Tolg C., *et al*. "Mouse mammary gland whole mount preparation and analysis". *Bio-Protocol* 8.13 (2018): e2915.

19. Battagello DS., *et al*. "The rat mammary gland as a novel site of expression of melanin-concentrating hormone receptor 1 mRNA and its protein immunoreactivity". *Frontiers in Endocrinology* 11 (2020): 463.

20. Livak KJ and Schmittgen TD. "Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method". *Methods* 25.4 (2001): 402-408.

21. Alturkistani HA., *et al*. "Histological stains: A literature review and case study". *Global Journal of Health Science* 8.3 (2015): 72-79.

22. Olguín HJ., *et al*. "The role of dopamine and its dysfunction as a consequence of oxidative stress". *Oxidative Medicine and Cellular Longevity* (2016): 9730467.

23. Gürgen SG., *et al*. "Metoclopramide use to induce lactation can alter DRD2 and BDNF in the prefrontal cortex of offspring". *Journal of Chemical Neuroanatomy* 109 (2020): 101844.

24. Yawata S., *et al*. "Pathway-specific control of reward learning and its flexibility via selective dopamine receptors in the nucleus accumbens". *Proceedings of the National Academy of Sciences of the United States of America* 109.31 (2012): 12764-12769.

25. Gomes RC., *et al*. "Effects of metoclopramide on the mouse anterior pituitary during the estrous cycle". *Clinics (Sao Paulo)* 66.6 (2011): 1101-1104.

26. Vega C., *et al*. "Prolactin promotes oxytocin and vasopressin release by activating neuronal nitric oxide synthase in the supraoptic and paraventricular nuclei". *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 299.6 (2010): R1701-R1708.

27. Augustine RA., *et al*. "Prolactin regulation of oxytocin neurone activity in pregnancy and lactation". *The Journal of Physiology* 595.11 (2017): 3591-3605.

28. Baskerville TA and Douglas AJ. "Dopamine and oxytocin interactions underlying behaviors: potential contributions to behavioral disorders". *CNS Neuroscience and Therapeutics* 16.3 (2010): e92-e123.

29. Melis MR., *et al*. "Oxytocin induces penile erection when injected into the ventral subiculum: role of nitric oxide and glutamic acid". *Neuropharmacology* 58.7 (2010): 1153-1160.

30. Succu S., *et al*. "Oxytocin injected into the hippocampal ventral subiculum induces penile erection in male rats by increasing glutamatergic neurotransmission in the ventral tegmental area". *Neuropharmacology* 61.1-2 (2011): 181-188.

31. Bioletto F., *et al*. "Central and peripheral regulation of the GH/IGF-1 axis: GHRH and beyond". *Reviews in Endocrine and Metabolic Disorders* (2024).

32. Ozgur, R., *et al*. "Reactive oxygen species regulation and antioxidant defence in halophytes". *Functional Plant Biology: FPB* 40.9 (2013): 832-847.

33. Tan C., *et al*. "Effects of dietary supplementation of nucleotides from late gestation to lactation on the performance and oxidative stress status of sows and their offspring". *Animal Nutrition (Zhongguo Xu Mu Shou Yi Xue Hui)* 7.1 (2021): 111-118.

34. Thébault S. "Potential mechanisms behind the antioxidant actions of prolactin in the retina". *Experimental Eye Research* 160 (2017): 56-61.

35. Yang R., *et al*. "Effect of prolactin on cytotoxicity and oxidative stress in ovine ovarian granulosa cells". *PeerJ* 11 (2023): e15629.

36. Jeding I., *et al*. "Characterization of the potential antioxidant and pro-oxidant actions of some neuroleptic drugs". *Biochemical Pharmacology* 49.3 (1995): 359-365.

37. Mancini A., *et al*. "Hormonal regulation of total antioxidant capacity in seminal plasma". *Journal of Andrology* 30.5 (2009): 534-540.

38. Yen GC and Hsieh CL. "Antioxidant effects of dopamine and related compounds". *Bioscience, Biotechnology, and Biochemistry* 61.10 (1997): 1646-1649.

39. Uvnäs Moberg K., *et al*. "Oxytocin is a principal hormone that exerts part of its effects by active fragments". *Medical Hypotheses* 133 (2019): 109394.

40. Erbas O., *et al*. "Oxytocin provides protection against diabetic polyneuropathy in rats". *Neurological Research* 39.1 (2017): 45-53.

41. Brunton PJ and Russell JA. "Maternal brain adaptations in pregnancy". *Knobil and Neill's Physiology of Reproduction (Fourth Edition)* (2014): 1957-2026.

42. Petraglia F., *et al*. "Neuroendocrine mechanisms in pregnancy and parturition". *Endocrine Reviews* 31.6 (2010): 783-816.

43. Day RE., *et al*. "Human aquaporins: regulators of transcellular water flow". *Biochimica et Biophysica Acta* 1840.5 (2014): 1492-1506.

44. Mobasheri A and Barrett-Jolley R. "Aquaporin water channels in the mammary gland: from physiology to pathophysiology and neoplasia". *Journal of Mammary Gland Biology and Neoplasia* 19.1 (2014): 91-102.

45. Matsuzaki T., *et al*. "Expression and immunolocalization of water-channel aquaporins in the rat and mouse mammary gland". *Histochemistry and Cell Biology* 123.4-5 (2005): 501-512.

46. Breves JP., *et al*. "Prolactin and cortisol in tilapia gill". *The Journal of Endocrinology* 230.3 (2022): 325-337.

47. Kaihoko Y., *et al*. "Distinct expression patterns of aquaporin 3 and 5 in ductal and alveolar epithelial cells in mouse mammary glands before and after parturition". *Cell and Tissue Research* 380.3 (2020): 513-526.

48. Emmanuel NS., *et al*. "Haematological changes, oxidative stress assessment, and dysregulation of aquaporin-3 channel, prolactin, and oxytocin receptors in kidneys of lactating wistar rats treated with monosodium glutamate". *Naunyn-Schmiedeberg's Archives of Pharmacology* (2024).

49. Emmanuel NS., *et al*. "Preliminary monosodium glutamate-induced changes in mammary gland receptors and gene expression, water channel, oxidative stress, and some lactogenic biomarkers in lactating rats". *The Journal of Basic and Applied Zoology* 85.1 (2024): 1-15.

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