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Original Research Article

Histochemical study of the mammary glands of lactating albino mice treated with mentha spicata extract and metoclopramide

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Abstract: Plants are abundant in phytochemicals utilised for treating numerous ailments, with Mentha spicata L. serving as a significant source of polyphenols. These polyphenols have exhibited various bioactivities. This study seeks to examine the impact of mentha spicata extract and metoclopramide on mammary gland development and growth in lactating albino rats, utilising three types of staining: Mercuric bromophenol blue (MBB), Masson Trichrome stain (MTri), and Schiff's reagent (PAS). Between the dates of October 27, 2025 and November 27, 2025, the research was carried out at Tikrit University. Twenty-eight female mice were utilised in the experiment and were evenly distributed into four groups as follows: Control Group A1: Seven lactating females provided with standard food and water. Group A2: Seven lactating females administered 10% mint. Group A3: Seven lactating females administered 2 mg of metoclopramide. Group A4 consisted of 7 nursing mothers administered 2 mg of metoclopramide with 10% mint. The present study showed that Pyridic acid-Schiff (PAS) staining showed a positive reaction in the control group, characterized by a slight pinkish-violet staining in some lobule cavities. In contrast, the combined treatment with spearmint and metoclopramide resulted in the strongest positive PAS staining reaction. Mason's three-color staining revealed collagen fibers in all groups, while the combination treatment group showed heavy collagen deposition. Bromophenol mercury blue staining showed a positive proteolytic reaction in the control group. The combination treatment group exhibited the highest intensity of blue staining. This study illustrates that Mentha spicata extract with metoclopramide can enhance milk production in female rats, perhaps improving lactation in humans as well.

Keywords: Peppermint, Mercurial Bromophenol Blue dye, Mason's Trichrome dye, Schiff reagent, Collagen fibers.

INTRODUCTION

Breastfeeding is the ideal nutritional method for the physical and neurological development of children and is regarded as the most crucial means of preventing child mortality. Data from UNICEF published in 2021 indicate that globally, merely 44% of newborns are exclusively breastfed throughout the initial 5 months of life. Research indicates factors contributing to premature discontinuation, include fissured nipples, discomfort, or inadequate milk supply [3]. Consequently, women have evolved to depend on galactagogues during lactation activities. Galactagogues, whether synthetic or plant-derived, are employed to stimulate, sustain, and augment milk production. These chemicals enable complex processes that encompass both physical and physiological interactions [4,5].

Plants are a crucial source of various secondary metabolites employed as pharmaceuticals, agrochemicals, flavourings, fragrances, colourants, insecticides, and food additives. Medicinal herbs are a natural gift to humanity, facilitating the quest for a disease-free and healthy life. Plants provide a variety of pharmacological actions, encompassing endocrine activities and galactagogue activity. Several herbal plants, rich in chemically active compounds such as alkaloids with galactogenic characteristics, can be utilised as herbal medicine to facilitate milk let-down in lactating animals [7].
The Mentha genus of the Lamiaceae family is extensively found over Europe, Asia, Africa,

Australia, and North America. Extracts from this species are traditionally utilised in cuisine and are esteemed for their substantial content of antioxidants, including phenolic bioactive substances. A study by [12]shown that Mentha spicata leaves possess mammagenic activity without any adverse effects. This may be attributed to the anti-androgenic properties of spearmint, which elevate serum oestrogen levels, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Metoclopramide is a pharmacological option, and clinical evidence demonstrates its effectiveness in enhancing lactation in women. It is a dopamine antagonist that elevates prolactin levels, hence beginning or enhancing milk production [12,13,14]. The current investigation sought to ascertain the synergistic effects of menta and metoclopramide on the mammary glands and to compare the outcomes utilising specialised stains and reagents for these drugs.

Methdology

Experimental Design

From October 27, 2025, to November 27, 2025, the research was carried out at Tikrit University, specifically in the Veterinary Medicine Department as well as in the laboratories of the College of Science. During the course of the experiment, there were a total of twenty-eight mice that were each assigned to one of four groups, which were as follows:

1. Control Group A1: 7 lactating females given normal food and water.
2. Mint Group A2: 7 lactating females given 10% mint.
3. Metoclopramide A3 Group: 7lactating females given 2 mg of metoclopramide.
4. Metoclopramide and mint Group A4: 7 lactating females given 2 mg of metoclopramide with 10% mint.

Following weaning, the females were put under anaesthesia with chloroform and then dissected 21 days later. An incision was made in the belly, and the mammary glands were removed for removal. The following is a description of the slides that were created using a microscope in accordance with references [15,16]: Mercuric bromophenol blue (MBB), Masson Trichrome stain (MTri), and Schiff's reagent (PAS) were utilised

RESULTS

A positive reaction was seen in the control group using Schiff's reagent stain (PAS), which targets carbohydrates, leading to positive secretion in certain lobule cavities. This was attributed to their staining with a purple or pink hue, but to a smaller degree than that observed in the experimental groups. As illustrated in Figures 1 and 2. In the mint group, the positive reaction or secretion of PAS The alveolar secretions exhibited a significantly higher concentration compared to the control group, with staining being more apparent in the presence of lipid droplets.As seen in Figures 3 and 4. Figures (5) and (6) illustrate that the metoclopramide-treated group exhibited a more prominent positive reaction or secretion compared to the control group. The alveoli exhibited significant purple staining, with some containing different amounts of lipid droplets. In group A4 administered mentha spicata with metoclopramide, the reaction exhibited a favourable response with pronounced purple staining. This demonstrates the severity of the reaction. As seen in Figures 7 and 8.
A positive reaction, indicated by blue-stained collagen fibres, was seen in certain lobule lumens of the control group (A1) using Masson Trichrome stain (MTri). As seen in Figures 9 and 10. In group A2, the positive reaction was less pronounced, exhibiting fewer fibres in the interlobular septa compared to the control group A1, as illustrated in Figures 11 and 12. In group A3, the positive reaction was more prominent than in the control group, with the fibres exhibiting a blue colouration. As seen in Figures 13 and 14. In group A4, a positive reaction was seen, with pronounced blue staining indicating a robust reaction in the fibres, as illustrated in Figures 15 and 16.
A favourable reaction was reported in the control group when employing mercuric bromophenol blue (MBB), leading to blue staining of the lobules and membranes due to protein interaction. As seen in Figures 17 and 18. The group administered mint (A2) exhibited a significantly enhanced positive response (MMB) in the alveolar secretions relative to the control group (A1). The stain was more pronounced, accompanied by lipid droplets, as illustrated in Figures 19 and 20. In group A3, the positive reaction was more prominent than in the control group, with several alveoli exhibiting considerable blue staining, as illustrated in Figures (21) and (22). In the group administered both mint and metoclopramide (A4), a favourable response was found, accompanied by a significantly high degree of blue staining, as illustrated in Figures (23) and (24).

40X

Control(A1)

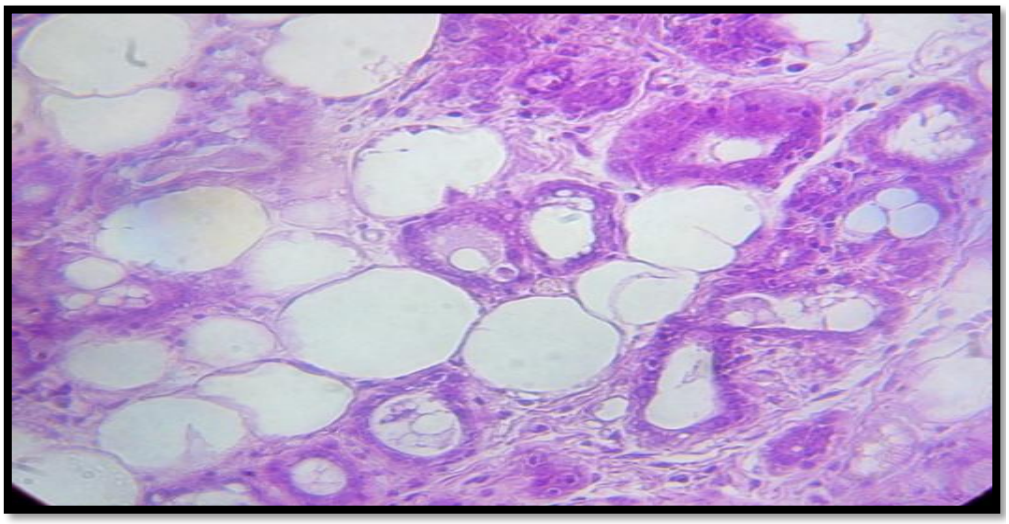

Figure (2) A histological segment of a breastfeeding female mouse's mammary gland(the control). It was found that the PAS stain had a positive reaction in the alveoli that contained secretory material, 40x

.

A2

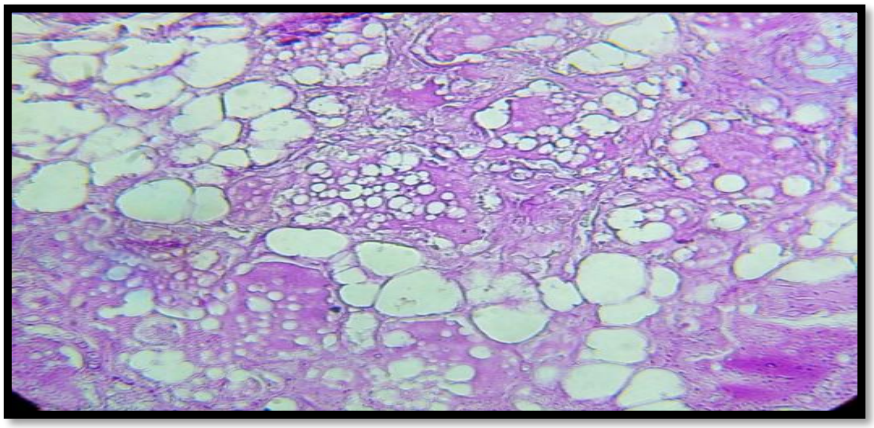

Figure (4) A histological segment of a breastfeeding female mouse's mammary gland (mint). Significantly positive reactivity of PAS stain in alveoli and secretory material, with a 40x increase in intensity

.

A3

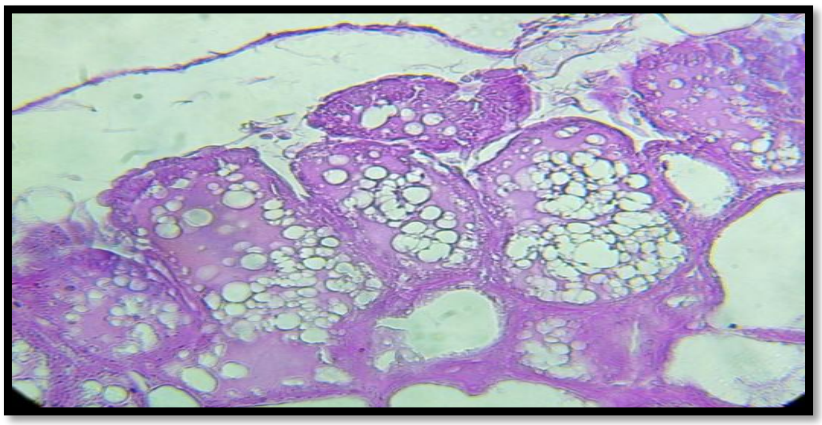

Figure (6) A histological segment of a breastfeeding female mouse's mammary gland **(metaclopramide). Positive reaction of PAS dye in alveoli containing a large amount of secretory material, 40x.**

A4

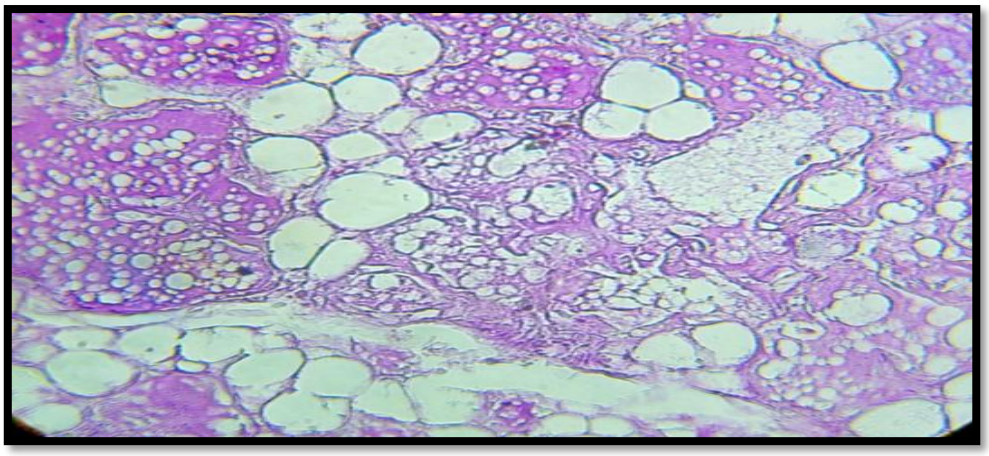

Figure (8) (metaclopramide and mint). Positive reaction of PAS stain in alveoli and secretory material, significantly 40X.

Control (T1)

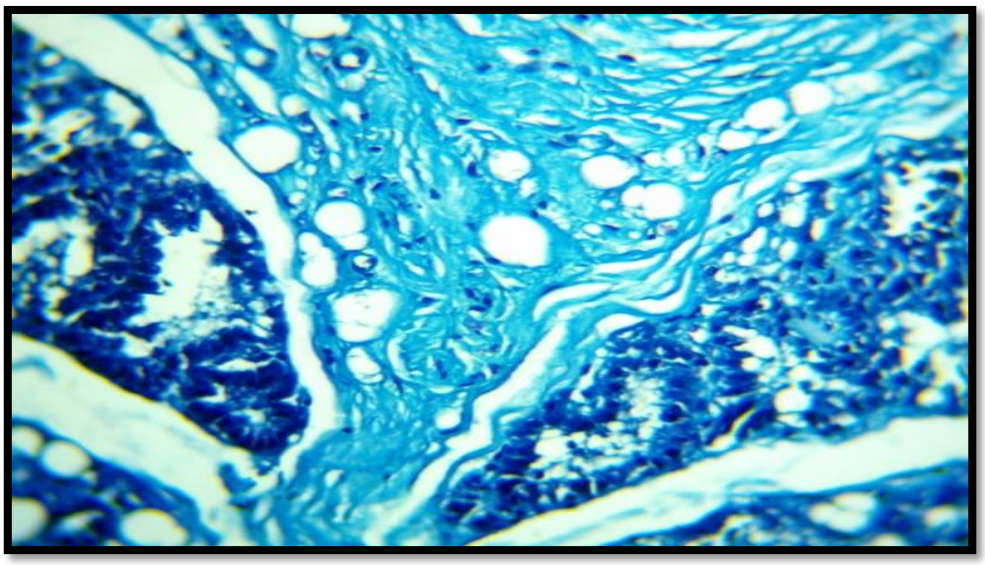

Figure (10) A histological segment of a breastfeeding female mouse's mammary gland **(control) Large fibers in the septa between lobules (MTri)) 40X.**

T2

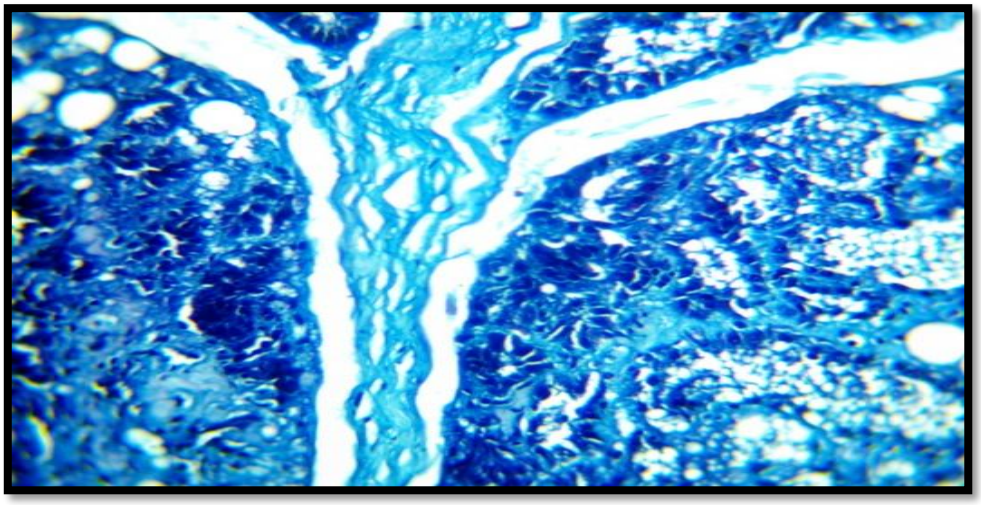

Figure (12) A histological segment of a breastfeeding female mouse's mammary gland **(mint). Large fibers in the septa between lobules (MTri) 40X.**

T3

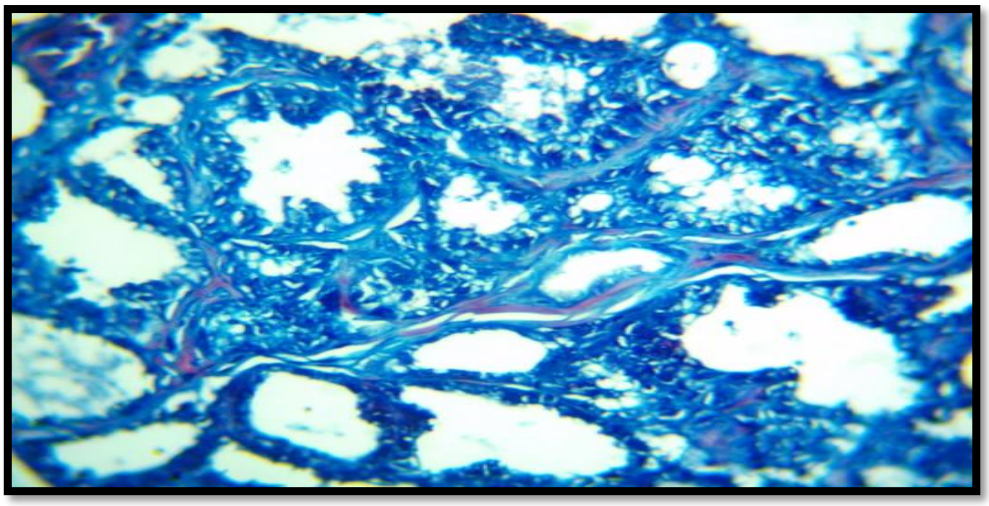

Figure (14) A histological segment of a breastfeeding female mouse's mammary gland **(metaclopramide) Small fibers in the septa between lobules (MTri) 40X.**

T4

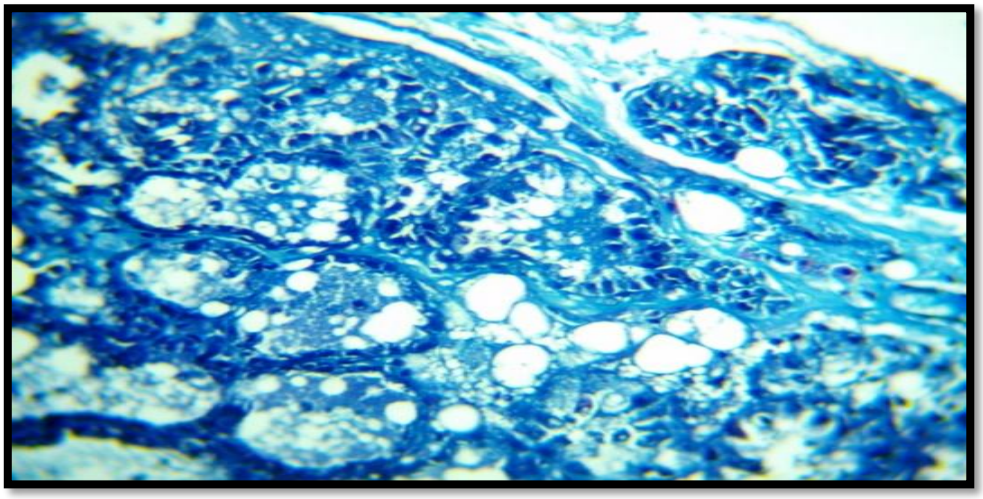

Figure (16) A histological segment of a breastfeeding female mouse's mammary gland**(metoclopramide and mint). Small fibers in the septa between lobules (MTri) 40x.**

Control (A1)

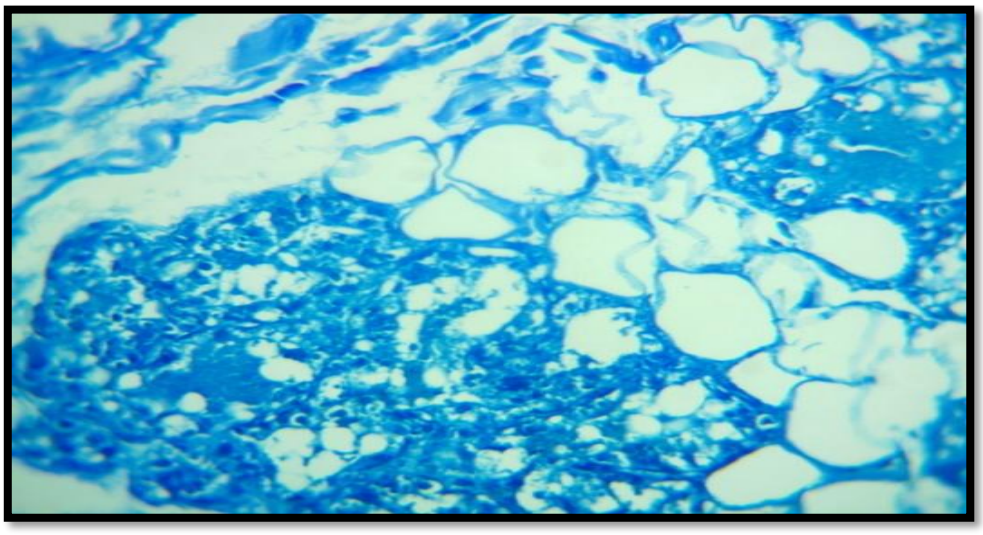

Figure (18) A histological segment of a breastfeeding female mouse's mammary gland **(control). The reaction is positive for the MBB stain in the lobules 40X.**

A2

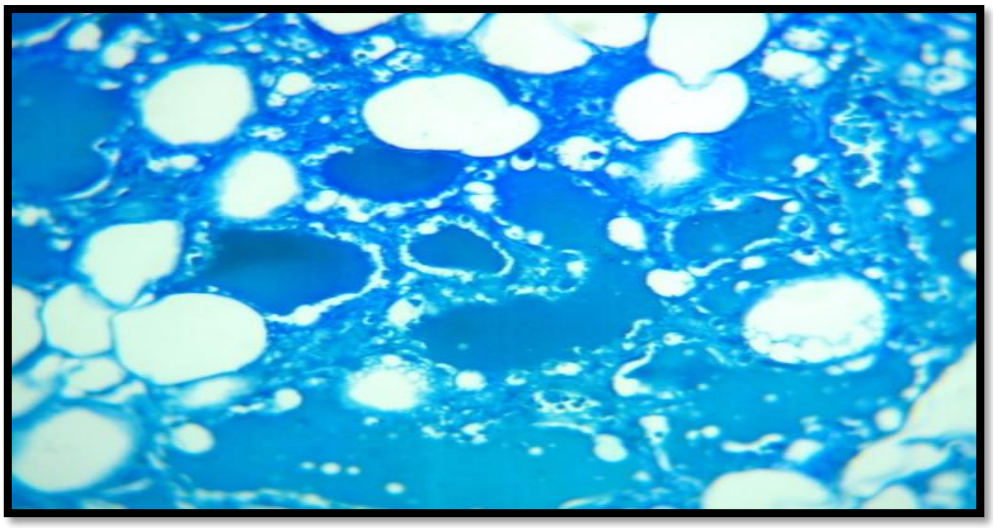

Figure (20) A histological segment of a breastfeeding female mouse's mammary gland **(mint). The reaction is positive for the stain MBB in the lobules and alveoli 40X.**

A3

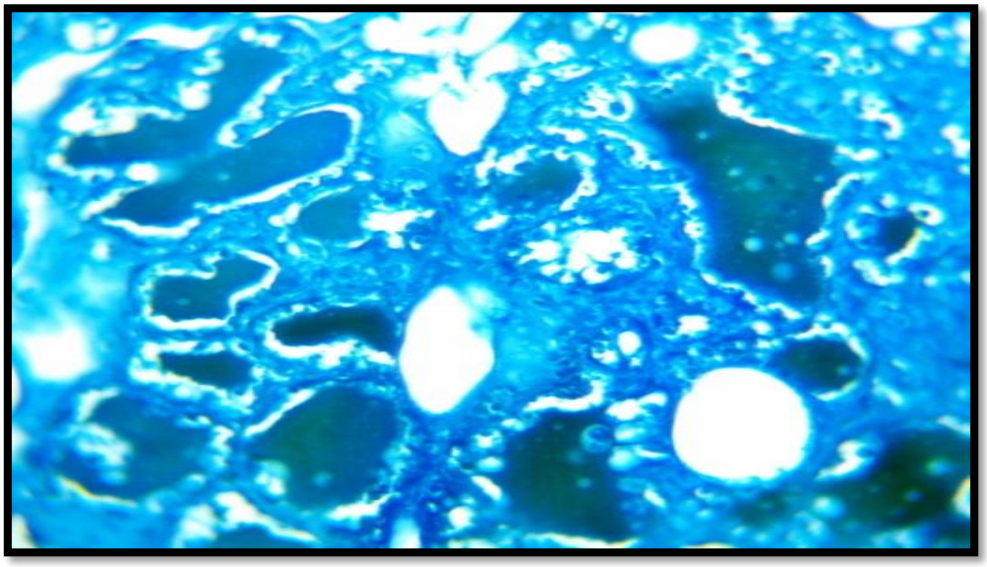

Figure (22) A histological segment of a breastfeeding female mouse's mammary gland **(metaclopramide). The reaction is highly positive for the stain MBB in the lobules and alveoli 40X.**

A4

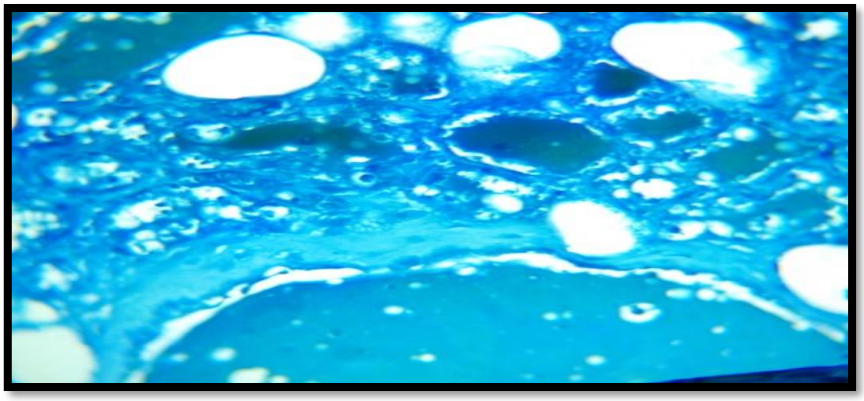

Figure (24) A histological segment of a breastfeeding female mouse's mammary gland **(metaclopramide and mint). The reaction is highly positive for the MBB stain in the lobules and alveoli 40X.**

DISCUSSION

The application of PAS stain reveals a purple hue, signifying the presence of carbohydrates with glycol groups that react with Schiff's reagent to produce dialdehydes, resulting in a purple molecule. The current study concurs with [17], which demonstrated that mint extract promotes mammary gland development, as evidenced by PAS staining, concluding that Mentha extract positively influences mammary gland growth and development. A further study conducted by [18] examined many types of dyes, including Schiff's reagent, demonstrating that aldehyde groups are accountable for the positive reaction observed with Schiff's reagent.

Utilising MBB labelling revealed positive secretion throughout the alveolar cavities, with staining intensity varying from mild to high, contingent upon the protein concentration in the released compounds. This indicates heightened milk secretion, akin to the influence of prolactin. This work aligns with [19], which indicated that staining intensity is exactly proportional to the protein quantity in the tissue. The histochemical observations during MBB staining of tissues were solely reliant on the intensity of the protein chemical constituents.

The specific component in spearmint responsible for these effects is unidentified. The production of ovarian steroid hormones is regulated by the hypothalamic-pituitary-gonadal axis, which is stimulated during puberty. The hypothalamus synthesises and releases luteinizing hormone-releasing hormone (LHRH), which interacts with its receptors in the pituitary gland, initiating a cascade of biochemical events that results in the production of FSH and LH. Both FSH and LH are then released into the systemic circulation and bind to receptors on the ovary, prompting ovulation and stimulating the synthesis of oestrogen and progesterone. A study by (21) indicated that the synthesis of oestrogen by the ovaries was markedly reduced in concentration due to the growth of the mammary gland during adulthood.

Metoclopramide is regarded as a safe and efficacious stimulator of prolactin secretion. It accomplishes this by obstructing dopamine receptors in the hypothalamus and diminishing the prolactin inhibitory factor. Metoclopramide (5 mg/kg) markedly enhanced milk production and serum prolactin concentrations [23].

Metoclopramide is a recognised galactagogue, mostly utilised as an antiemetic, which promotes mild hyperprolactinemia to enhance milk production while promoting mammary gland development [24]. A recent systematic review and meta-analysis indicated that metoclopramide did not enhance milk volume in the intervention groups relative to the control groups. Nonetheless, there was a notable elevation in serum prolactin levels following the administration of metoclopramide to the mothers [25].

CONCLUSION

These findings concluded that the mentha spicata extract and metoclopramide can stimulate milk production in the female rats which may be consequently effective in increasing the lactation of human too.

REFERENCES

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. AL-SNAFI, A. E.; KHORSHEED, S. H.; FARJ, A. H. Mammary gland stimulating effects of the crude phenolic extracts of green tea (Camellia sinensis). International Journal of Biological & Pharmaceutical Research, 2015, 6.7: 573-576.
7. Garga, C., Khan, S., Ansari, S., Suman, A., & Garg, M. (2009). Chemical composition, therapeutic potential and perspectives of Foeniculum vulgare. Pharmacognosy Reviews, 3(6), 346
8. Mamadalieva, N.Z.; Akramov, D.K.; Ovidi, E.; Tiezzi, A.; Nahar, L.; Azimova, S.S.; Sarker, S.D. Aromaticmedicinal plants of the Lamiaceae family from Uzbekistan: Ethnopharmacology, essential oils composition,and biological activities. Medicines 2017, 4, 8.
9. Salehi B; ´c-Radi´c Z; Mateji´c J; Sharopov F; Antolak H; Kr˛egiel D ID; Sen S; Sharifi-Rad M, Acharya K; Sharifi-Rad K; Martorell M; ID, Sureda A, Martins N ID and Sharifi-Rad J. Plants of Genus Mentha: From Farm to Food Factory. Plants.2018, &,70.2-36.
10. Al bazii s, J; Al-Tu’ma, F J and Al-Obaidi. S R . Study the effects of Spearmint Mentha Spicata Extracts on Mammary Gland in Female Rats

2013 . Journal of Kerbala University, Scientific .11, 3 198- 206
11. 13. Akdogan, M.; Tamer, E.; Cure, B.; Koroglu, B. and Delibas, N. (2007). Effect of Mentha spicata Labiate teas on Androgen level in women with hirsutism. Phytother. Res., 21: 444-447
12. SOLOMON, Emmanuel Nachamada, et al. 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. EC Pharmacology and Toxicology, 2025, 13: 01-11.
13. Abdul Jabbar, Al-Fatt Qais, Al-Sudani, Wari Muhammad, Al-Ubaidi, Salem Rashid (2014) Histochemical study of the effect of sweet seed (fennel) Foeniculum vulgare seeds on the mammary glands of female rats. Journal of Al-Nahrain University, 17 (2), 45-53.14
14. ROMAGNOLI, Stefano. Practical use of hormones in small animal reproduction. Rev. bras. reprod. anim, 2017, 41.1: 59-67.
15. Al-Hajj, Hamid Ahmad (1998) Light Microscopic Preparations, First Edition, Department of Life Sciences, University of Jordan, Jordanian Book Center, Amman, Jordan, pp. 121-140, 149-186, 221, 232.
16. Muhammad, Rehab Ghaleb, Radhi, Fadhel Abbas, (2012) Guide to Microscopic Preparations, Ministry of Higher Education and Scientific Research, University of Babylon, College of Agriculture, Hillah, Iraq, pp. 55-59, 82-113.
17. AL-BAZII, Sinaa Jaboury; OBEID, Ashwaq Kadhem; HAMEED, Rana Majeed. Mammogenesis Effect of Hot Aqueous Extract of Mentha spicata Leaves on Mammary Tissue of Ovariectimized Rabbits. Prof.(Dr) RK Sharma, 2021, 21.1: 1677.
18. Scott, H. R., & Clayton, B. P. (1953). A comparison of the staining affinities of aldehyde-fuchsin and the Schiff reagent. Journal of Histochemistry & Cytochemistry, 1(5), 336-352.
19. Jain, S., & Shrivastava, S. B. Zubair Azad, 2018.“. Histochemical studies on the liver and intestine of oreochromis mossambicus (cichlid fish) induced by cadmium sulphate” International Journal of Current Research in Life Sciences, 7(03), 1397-1402.
20. Tamm,K; Suhorutshenko, M; Rõõm,M ; Simm,J and Metsis,M, (2012).steroid -basic science. Steroid hormones ..intech, Open Access Publisher. Tallinn University of Technology , Faculty of Science, Centre for Biology of Integrated Systems.
21. Dessauge F, Finot L, Wiart S,Aubry J, Ellis, S.Effect of ovariectomy prepubrtal goat . J. physiol. and Pharma.,2009,60:127-133.
22. Judd SJ, Lazarus L, Smythe G. Prolactin secretion by metoclopramide in man.J Clin Endocrinol Metab. 1976; 43(2): 313-7.
23. OLAIMAT, Tamadur, et al. Caraway Seed Extract and Lactation: in vivo study. Jordan Journal of Pharmaceutical Sciences, 2025, 18.3: 842-852.
24. Umur, N., Ildan, C.S., Yazici, G.N., Gurgen, S.G. (2022). Investigation of the effect of metoclopramide on proliferation signal molecules in breast tissue. Int J Exp Path. 103:83-89.
25. Hussain, N.H.N., Noor, N.M., Ismail, S.B, Zainuddin, N.A., Sulaiman, Z. (2021). Metoclopramide for Milk Production in Lactating Women: A Systematic Review and Meta-Analysis. Korean J Fam Med.;42(6):453-463. doi: 10.4082/kjfm.20.0238. Epub 2021 Nov 20. PMID: 34871486; PMCID: PMC8648493.

