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Effect of Foeniculum Vulgare And Metoclopramide On The Carbohydrate And Protein Levels Of The Mammary Glands In Lactating White Mice

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ABSTRACT

Background: Galactagogues are chemical treatments, foods, or herbal supplements known to stimulate, support, and enhance breast milk production. Women can use these galactagogues to stimulate, increase, or maintain milk production.

Aim of the study: This study aimed to investigate the effect of both Foeniculum vulgare and metoclopramide on the development of mammary glands of lactating mice by using 2 type of staining Schiff's reagent (PAS) stain and Mercuric bromophenol blue (MBB)**.**

Material and method: The research ran from October 27, 2024, until January 27, 2025, at Tikrit University's College of Science and the animal house of the College of Veterinary Medicine. Eighty female mice were used in the experiment for 20 days and divided into four groups, each group contain 20 rats as follows: T1 (control group): lactating females were given normal food and water. T2: lactating females were given 10% Foeniculum vulgare. T3: lactating females were given 2 mg of metoclopramide. T4: lactating females were given 10% Foeniculum vulgare and 2 mg of metoclopramide.

Result: The Foeniculum vulgare used in the study contain the compounds Anethole, Fenchone, Estragole, Limonene, α-Pinene and Linalool at concentrations of 39.27, 9.30, 8.60, 5.43, 4.34 and 4.68 mg L-1, respectively. By using two type of staining, the histopatological section showed the group dosed with Foeniculum vulgare and metoclopramide, the reaction was positive, and high purple staining was observed, indicating the severity of the reaction, in addition, the purity of the stain was clearly evident in some sections of the alveoli, especially the large ones.

Conclusion: Foeniculum vulgare and metoclopramide have Synergic effect on mammary gland which increase alveolar secretions than control.

Keywords: *White mice, mammary glands, Foeniculum vulgare, metoclopramide Schiff's reagent stain, Mercuric bromophenol blue (MBB).*

1. INTRODUCTION

A mammary gland is an apocrine gland composed of alveoli encased in simple cuboidal epithelium, which is bordered by myoepithelial cells. The alveoli aggregate to create clusters referred to as lobules. Each lobule contains a lactiferous duct that empties into apertures in the nipple**[1].** It is essential to recognize that breastfeeding is a symbiotic process between mothers and infants, influenced by various factors, including maternal and neonatal health, health issues, improper breastfeeding techniques [2]. Many women initiate breastfeeding but do not continue for the duration as recommended by the World Health Organization. This is due to an insufficient milk supply [3,4]. When non-pharmacological measures fail to satisfactorily address milk production challenges, galactagogues can be the next alternative to facilitate increased milk production and ejection [5]. Galactagogues, drugs that stimulate and support sufficient milk production, can be categorized into two groups: chemotherapeutics and natural compounds, such as sweet seed[6].

Plants are a significant source of diverse secondary metabolites utilized as drugs, agrochemicals, flavorings, perfumes, colorants, insecticides, and food additives. Medicinal plants are Nature's boon to humanity, aiding in the pursuit of a disease-free, healthy existence. Plants exhibit a diverse array of pharmacological effects, including endocrine effects, including galactagogue activity[7]. Natural products, particularly those produced from plants, have historically served as a foundation for medicinal compounds, with an estimated 30–40% of contemporary medications originating directly or indirectly from these natural sources[8]. Foeniculum vulgare is a conventional and esteemed herb with an extensive history of medicinal usage. Pharmacological investigations have demonstrated that Foeniculum vulgare possesses antimicrobial, antioxidant, anti-inflammatory, and anti-estrogenic effects[9]. It also includes phytoestrogens that stimulate breast tissue growth and alveolar formation in the mammary glands, hence functioning as a galactagogue [10].

Metoclopramide is a dopamine inhibitor that elevates prolactin levels, hence stimulating or enhancing milk production[6]. It is used to treat nausea and vomiting and facilitate gastric emptying by stimulating the smooth muscles of the gastrointestinal tract[11]. Metoclopramide has several side effects, including hyperprolactinemia and (rarely) neuroleptic malignant syndrome[12], headache, drowsiness, dizziness, and diarrhea [13].

2. MATERIALS AND METHODS

Experimental Design

The study was conducted in the animal house of the College of Veterinary Medicine and the laboratories of the College of Science at Tikrit University from October 27, 2024 to January 27, 2025. Eighty female mice were used in the experiment for 20 days and divided into several groups as follows:

T1 (control group): 20 lactating females were given normal food and water.

T2: 20 lactating females were given 10% Foeniculum vulgare.

T3: 20 lactating females were given 2 mg of metoclopramide.

T4: 20 lactating females were given 10% Foeniculum vulgare and 2 mg of metoclopramide.

Plant Collection and dosage determination

The plants used in the experiment were obtained from local sources in their raw form. Foeniculum vulgare was used as fodder by grinding it and mixing it with brown flour at a rate of 10% of the rats' daily diet, creating a plant paste that was dried and fed to the animals. Foeniculum vulgare were used as a 10% feed [14].

Dosage of metoclopramide

Metoclopramide was procured from local pharmacies as 2 mg hard tablets, which were subsequently broken and diluted in 1 ml of distilled water. Metoclopramide was administered orally at a dosage of 2 mg per day [15].

Animals Preparation

The animals were obtained from the College of Veterinary Medicine and were kept in an air-conditioned animal house at approximately 25°C and with a light cycle of 12 hours light and 12 hours dark. This room in the veterinary animal house was chosen to ensure optimal care and monitoring of the animals for as long as possible. They were housed in plastic cages with metal mesh lids (30 x 13 x 13 cm) manufactured by North Kent Plastic Cages LTD, England. The cage floor was covered with sawdust free of harmful substances and replaced every three days. Care was taken to ensure cleanliness and disinfection. The animals were selected at the age of 12-15 weeks, with an average weight of approximately 200 g, and were all in good health. They were fed a prepared ration specifically for rats with a metabolic energy value of 1213 kcal/kg, which is the standard ration.

Preparation of Microscopic Slides

Females were anesthetized with chloroform and dissected 21 days after weaning, i.e., after weaning. The abdomen was incised, and the mammary glands were removed. Microscopic slides were prepared according to [16,17], as follows: Schiff's reagent (PAS) stain was used to detect the presence of carbohydrates and sugars among the secreted substances in the lumen of the vesicles and canaliculi, where their interaction with the stain gives a pink or purple color. Also stained with Mercuric bromophenol blue (MBB).

Analysis of active compounds in plants

The study's active plant components were examined via high-performance liquid chromatography (HPLC) utilizing a ZORBAX Eclipse Plus C18 column, measuring 4.6 mm in diameter and 150 mm in length, at a temperature of 30°C and irradiated with a UV wavelength of 210 nm [18].

3. RESULT

As shown in Table (1) and Figure (1) that the Foeniculum vulgare used in the study contain the compounds Anethole, Fenchone, Estragole, Limonene, α-Pinene and Linalool at concentrations of 39.27, 9.30, 8.60, 5.43, 4.34 and 4.68 mg L-1, respectively.

Table (1) Active compounds and concentrations (mg/L-1) in Foeniculum vulgare plant

Compound name	Linaloo l	α-Pinene	Limonene	Estragole	Fenchone	Anethole
Concentration	**4.68**	**4.34**	**5.43**	**8.60**	**9.30**	**39.27**

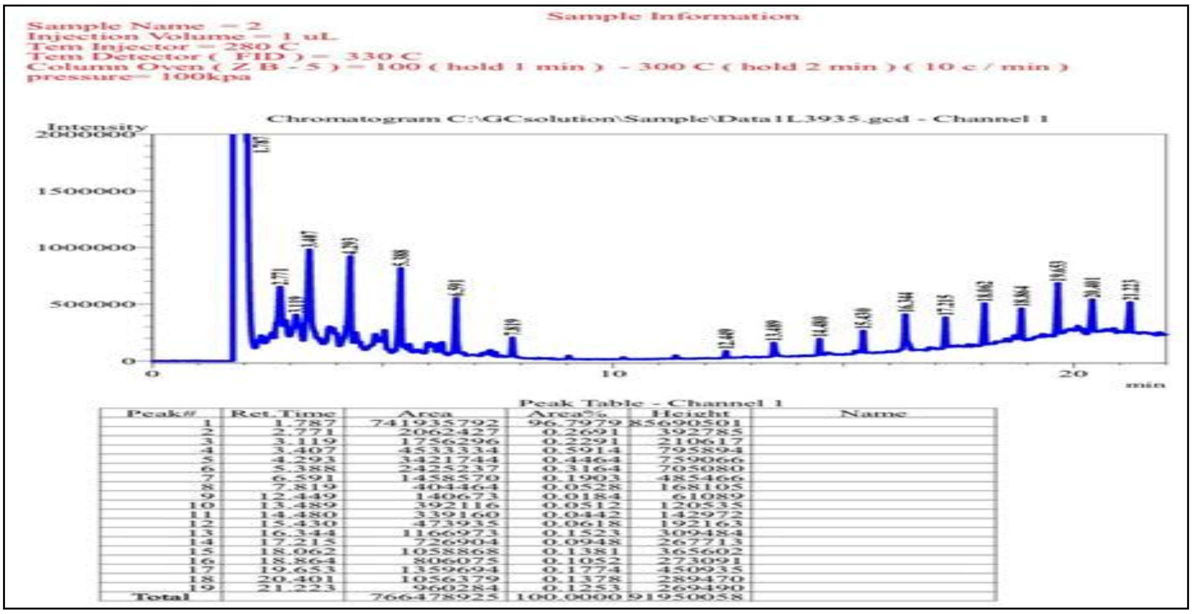

Figure (1) Retention time and peak area of the active ingredients in the volatile oil of Foeniculum vulgare.

Table (2) and Figure (2) shows the chemical analysis of Foeniculum vulgare that contain saponins, namely Diosgenin, Hecogenin and Yamogenin, at concentrations of 341.13, 300.54 and 212.78 micrograms g^{-1}.

Table (2) Saponins compounds and their concentrations (micrograms g-1) in Foeniculum vulgare plant.

Compound	Estragole	Fenchone	Anethole
Concentration	**8.60**	**9.30**	**39.27**

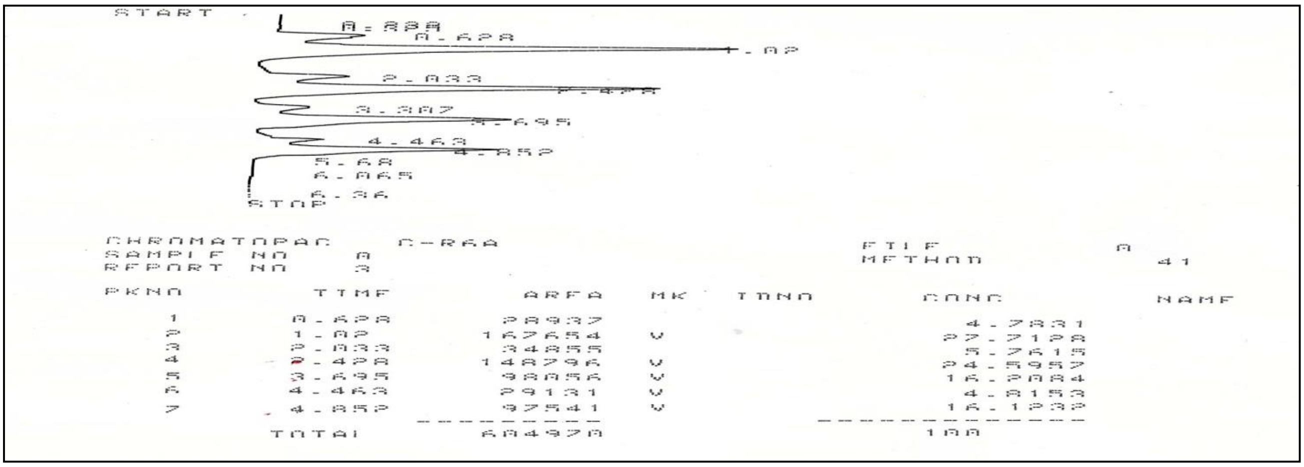

Figure (4-2) Retention time and peak area of saponins in Foeniculum vulgare plant

Using Schiff's reagent (PAS), which stains carbohydrates by reacting with them, a positive reaction to the dye, was observed in the control group (T1) in some of the alveolar cavities, due to their pink or purple staining. However, this was less than what was observed in the experimental groups (3). In the group T2, was significantly higher positive reaction to the PAS reagent in the alveolar secretions than in the control group. The stain was more intense, with the presence of lipid droplets. In the group (T3), the secretion or positive reaction was more evident than in the control group, as some alveoli appeared highly stained with purple. In the group (T4), the reaction was also positive, and high purple staining was observed, indicating the severity of the reaction.

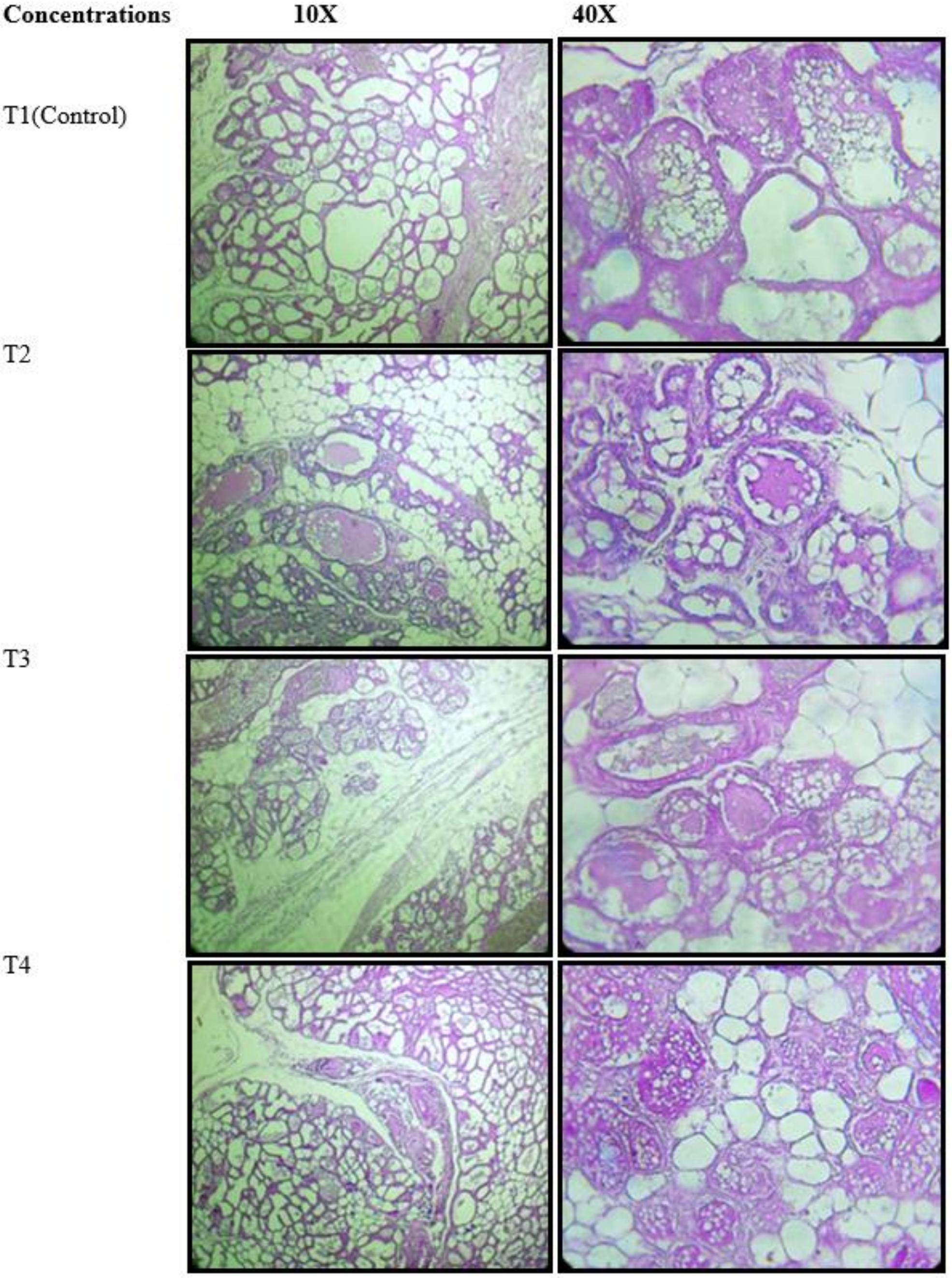

Figure (3) Histological section of the mammary gland of a lactating female mouse, T1 positive reaction to PAS stain. T2 showing higher positive reaction or secretion to the PAS reagent in the alveolar secretions. T3 demonstrated that the secretion or positive reaction was more evident than in T1, as some alveoli appeared highly stained with purple.T4 showed that the reaction was also positive, and high purple staining was observed, indicating the severity of the reaction.

Histopathologicl section of mammary glands showed a positive reaction to the dye was observed in the control group in some lobules and membranes by using mercuric bromophenol blue (MBB), which stains proteins by interacting with them. In the group T2, there was a significantly higher positive reaction or secretion of the MBB dye in alveolar secretions than in the control group. The staining was denser, with the presence of lipid droplets. In the group (T3), the positive secretion or reaction was more pronounced than in the control group, as some alveoli appeared to be stained to a moderate degree of blue. In the group (T4), the reaction was positive and a very high degree of blue staining was observed, which is evidence of the intensity of the reaction with the purity of the staining of the materials inside the vesicles.

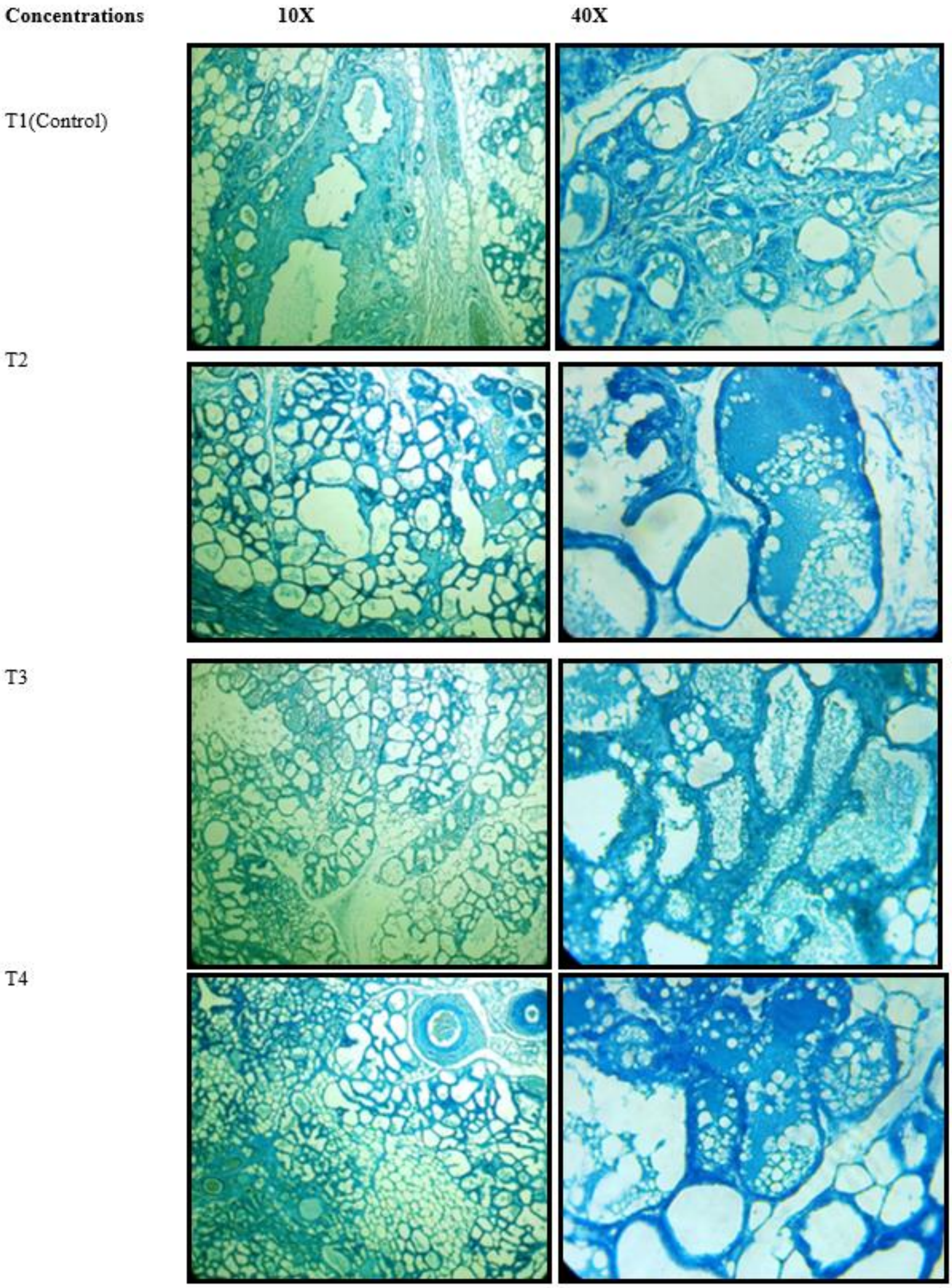

Figure (4) Histological section of the mammary gland of a lactating female mouse in group (T1,T2,T3, and T4)showing a positive reaction to BMM stain.

4. DISCUSSION

Saponins constitute a substantial category of secondary metabolites present in significant amounts throughout numerous plant species. The saponin content in plants is variable and can be affected by the surrounding environment. The local geographic climate, seasonal variations, and external factors like as light, temperature, humidity, soil fertility, and cultivation methods affect the quantity and qualitative content of saponins. This variation markedly influences the quality and characteristics of both wild and domesticated plants utilized for medicinal, nutritional, and industrial purposes [19]. The findings of this study align with those of [20,21], which demonstrated that fennel comprises numerous active compounds, notably fenchone, trans-anethole, estragole, para-anisaldehyde, alpha-phellandrene, nerol, alpha-pinene, gamma-terpinene, ocimene, d-limonene, and beta-myrcene. The concentration of these chemicals significantly fluctuates based on the phenological stage and geographical origin of the plant[22].

Using PAS stain, the appearance of a purple color indicates the presence of carbohydrates containing glycol groups that combine with Schiff's reagent to form dialdehydes, giving a purple-colored compound. The results of this study are consistent with the findings of [14]. In their study, which included the use of PAS stain, they found that positive secretion in the alveoli or vesicles ranged from medium to high, depending on the amount of sugars present in the secreted substances. This is evidence of increased milk production, the effect of which is similar to that of prolactin. Another study done by [23] also studied various types of dyes, including Schiff's reagent, in which they proved that aldehyde groups are responsible for the positive reaction of Schiff's reagent. As confirmed by [24] through their study of liver, stomach, and intestinal tissue, they found that the amount of carbohydrates in the liver showed a significant decrease in carbohydrate content after 10 days. Therefore, the purple color of the stain was lighter, indicating a decrease in carbohydrate content after 20 days, indicating a direct relationship between the pigment and carbohydrate content. When using MBB staining, positive secretion was observed in the alveolar cavities, where the intensity of staining ranged from medium to high, depending on the amount of proteins present in the secreted substances. This is evidence of increased milk secretion, similar to the effect of prolactin. This study is consistent with [24] who demonstrated that the intensity of staining is directly proportional to the amount of protein content in the tissue. When staining tissues with mercury bromophenol blue (MBB), the histochemical observations were entirely dependent on the intensity of the protein chemical components. After 10 and 20 days of cadmium sulfate treatment, liver protein reduced dose-dependently with increasing concentration, with lighter hepatocyte staining. Due to increased protein content, the control fish's gut had good blue color intensity around endothelial cells and villi. As cadmium concentration increased, protein reduced dose-dependently while blue color intensity remained light.

5. CONCLUSION

Foeniculum vulgare extract and metoclopramide had positive effects on the mammary glands, increasing their secretion. The substances used in the study affected the composition of the mammary glands, as an increase in the size of the gland's secretory vesicles and an increase in the thickness of their walls was observed, along with a clear increase in the branching of these vesicle walls, as shown in the histological sections of the study. An increase in sugar and protein contents within the secretory contents of the mammary vesicles was observed using Schiff's reagent and bromophenol mercury blue staining of the histological sections.

REFERENCES

[1] MONSEFI, Malihezaman, et al. Salvia officinalis L. induces alveolar bud growing in adult female rat mammary glands. *Avicenna Journal of Phytomedicine*, 2015, 5.6: 560.

[2] Amir LH. Breastfeeding: managing ‘supply’ difficulties. Aust Fam Physician. 2006;35:686–9.

[3] Stuebe AM. Enabling women to achieve their breastfeeding goals. Obstet Gynecol. 2014;123:643–52. doi: 10.1097/AOG.0000000000000142. [DOI] [PubMed] [Google Scholar]

[4] Wagner EA, Chantry CJ, Dewey KG, Nommsen-Rivers LA. Breastfeeding concerns at 3 and 7 days postpartum and feeding status at 2 months. Pediatrics. 2013;132:e865–75. doi: 10.1542/peds.2013-0724.

[5] Academy Of Breastfeeding Medicine Protocol Committee ABM Clinical Protocol #9: use of galactogogues in initiating or augmenting the rate of maternal milk secretion (first revision January 2011) Breastfeed Med. 2011;6:41–9. doi: 10.1089/bfm.2011.9998. [DOI] [PubMed] [Google Scholar]

[6] Gabay MP. (2002) Galactogogues: medications that induce lactation. J *Hum Lact*.;18:274–9. [PubMed]

[7] 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.

[8] 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

[9] Xue G. Q., Liu Q., Han Y. Q., Wei H. G., and Dong T(2006)., Determination of thirteen metal elements in the plant Foeniculum vulgare Mill. by flame atomic absorption spectrophotometry, Guang Pu Xue Yu Guang Pu Fen Xi. 26, no. 10, 1935–1938

[10] Kaur, G.J., Arora, D.S., (2008). In-vitro antibacterial activity of threeplants belonging to the family

Umbelliferae. Int. *J. Antimicrob.Agents* 31, 393–395.

[11] ALBIBI, R., & McCALLUM, R. W. (1983). Metoclopramide: pharmacology and clinical application. Annals of Internal Medicine, 98(1), 86-95.

[12] Moos and Hansen, 2008 D.D. Moos, D.J. (2008) Hansen Metoclopramide and extrapyramidal symptoms: a case report J. Perianesth. Nurs., 23, pp. 292-299

[13] Hale T.W. Hale, K. Kendall-Tackett, Z. Cong (2018) Domperidone versus metoclopramide. Clin. Lact., 9 pp. 10-18.

[14] 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

[15] ROMAGNOLI, Stefano. Practical use of hormones in small animal reproduction. *Rev. bras. reprod. anim*, 2017, 41.1: 59-67.

[16] 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.

[17] 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.

[18] Huijun L.and Ping L.(2000) determination of jujuboside A and jujuboside B in spine date seed by HPLC-ELSD, Chinese Journal of Pharmaceutical Analysis, 20(2)82-84.

[19] Rai, S., Acharya-Siwakoti, E., Kafle, A., Devkota, H. P., & Bhattarai, A. (2021). Plant-derived saponins: a review of their surfactant properties and applications. Sci, 3(4), 44

[20] Vella, F.M.; Calandrelli, R.; Cautela, D.; Fiume, I.; Pocsfalvi, G.; Laratta, B. Chemometric screening of fourteen essential oils for their composition and biological properties. Molecules 2020, 25, 5126. [CrossRef]

[21] Cautela, D.; Vella, F.M.; Castaldo, D.; Laratta, B. Characterization of essential oil recovered from fennel horticultural wastes. Nat. Prod. Res. 2019, 33, 1964–1968. [CrossRef]

[22] Diaz-Maroto, M.C.; Perez-Coello, M.S.; Esteban, J.; Sanz, J. Comparison of the volatile composition of wild fennel samples (Foeniculum vulgare Mill.) from Central Spain. J. Agric. Food Chem. 2006, 54, 6814–6818. [CrossRef]

[23] 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.

[24] 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.