International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Histological study of the mammary gland of lactating albino Wistar rats treated with oil palm sap as galactagogue

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International Journal of Science and Research Archive, 2024, 11(02), 1816–1823

Publication history: Received on 10 February 2024; revised on 17 March 2024; accepted on 20 March 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.11.2.0484

Abstract

Background: Population of mothers' resident in Southeastern Nigeria often use the juicy oil palm sap from African (*Elaeis guineensis*) palm tree commonly called Palm Wine as galactagogue to boost milk production in lactating women. This study investigated the effects of oil palm sap on the histology of mammary gland in lactating rats.

Method: Twenty primiparous female albino wistar rats were used and randomly divided into four groups (n=5, each). The rats in the treated groups received a daily administration of oil palm sap through orogastric method at a dose of 10ml/kg. The positive and negative control groups received 5mg/kg of metoclopramide and 2.5mg/ kg of bromocriptine, respectively while the normal control group received 0.5 ml distilled water. The mammary glands of lactating rats were harvested and fixed with 10% formalin for routine histological procedures and stained with hematoxylin-eosin and viewed under light microscope.

Result: Histological analysis showed that oil palm sap induced the development of the lobuloalveolar system of the mammary glands.

Conclusion: This study scientifically validates the galactagogue properties of oil palm sap and justifies the traditional use of this plant extract to boost milk production in lactating women.

Keywords: Histology; Mammary gland; Oil palm sap; Metoclopramide; Galactagogue Effect

1. Introduction

Oil palm sap is the unfermented pale-yellow exudate from tapped unopened spathe of oil palm tree (*Elaeis guineensis*, *Raphia hookeri or Raphia vinifera*) widely consumed as a refreshing and nourishing beverage (Obahiagbon, 2012; Ikegwu et al., 2014) in West African countries (Ugboma et al., 2021). Fresh oil palm sap is sweet, as it has about 10% - 12% (w/v) sugar, mainly sucrose (Ugwoke et al., 2021).

Many health benefits have been attributed to the use of this folk juicy medicine. It has been demonstrated that fresh oil palm sap has low alcoholic content. It also contains yeast, lactic and acetic acids which confers probiotic properties to the drink. It is a good source of vitamins (Ugwoke et al., 2021), antioxidants and other beneficial chemicals (Nwaiwu and Chikezie, 2020).

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According to Okamkpa et al., (2022), fresh oil palm sap has also been used as a galactagogue for postpartum lactating women. Today, the interest in induced lactation stems from the desire of mothers to meet with the current World Health Organization (WHO) recommended breastfeeding practices, especially the early initiation of breastfeeding (EIBF) within 30 minutes of birth, which gives the best possible start in the life of a newborn baby (Okamkpa et al., 2022). In view of this, oil palm sap was employed by mothers mostly from Southeastern Nigeria to ensure an abundant milk supply or to rectify milk insufficiency. However, there is paucity of clinical/ scientific studies to support this age long traditional remedy.

Breast milk production (lactogenesis) is a complex neuro-endocrine activity that involves interaction of a number of physical, emotional and nervous factors along with the action of multiple hormones, mainly prolactin and oxytocin (Sani et al., 2019). Prolactin is synthesized and secreted by specialized cells of the anterior pituitary gland, known as the lactotrophs; and is necessary for the final preparation of mammary glands for the production and secretion of milk. Oxytocin acts on mammary glands by activating G-protein coupled oxytocin receptor which causes the contraction of myoepithelial cells that lines the mammary gland (Jonas and Woodside 2016). This leads to the ejection of milk from alveoli of the mammary gland (Gu et al., 2016). The production and discharge of milk are affected by the hormone prolactin and the hormone oxytocin that can stimulate the number of new alveoli formation. The process of formation of new alveoli is caused by suction of breast milk at the beginning of lactation (Utary et al., 2019).

From a histological point, the mammary gland is a compound tubulo-alveolar gland with lactiferous ducts. The smaller ducts are lined by columnar epithelium, the larger ducts have two or three layers of these cells. Near the openings of these ducts on the nipple the lining becomes stratified squamous columnar epithelium. The structure of the glandular elements of the mammary gland varies considerably at different periods of life with respect to the production and secretion of milk (Aranda-Gutierrez and Diaz-Perez, 2024).

Various studies have been carried out on conventional galactagogues as breast milk producing medications, but these drugs have been associated with unwanted side effects such as sedation, depression, weight gain, gastrointestinal disturbances, headache, nausea and dry mouth (Bazzano et al., 2016). Several studies have reported the proliferation of secretory mammary cells following administration of herbal galactagogues, (Al-Bazii et al., 2019; Sani et al. 2019). Al-Bazii and co reported that *Sessamum indicum* seeds induces the growth and development of mammary glandular tissues in female white rats while Sani and co-workers reported that *Launaea taraxacifolia* and resveratrol exhibited galactopoietic potentials individually by stimulating hyperprolactinaemia, while their combination increased milk production by increasing serum oxytocin activity.

A Thai herbal medicine called Tri-Than-Thip (Tri-TT) containing three main components: *Cassia fistula, Pithecellobium dulce, and Ficus benjamina* has been shown to significantly increase overall milk production in lactating rats compared the control group, while histological analysis showed a larger alveolar diameter of the mammary gland in comparison to the control group. The study concluded that these findings provide supportive evidence for the galactagogue activity of Tri-TT and that the observed enhancement in milk production associated with Tri-TT could potentially be attributed to its ability to widen the alveolar diameter of the mammary gland, thereby facilitating increased milk volume (Chonsut et al., 2023).

Having demonstrated the galactagogue effects of oil palm sap (Okamkpa el al., 2022), we designed this study to investigate the histological changes induced in the mammary glands following administration of fresh oil palm sap. We also compared these changes with that of conventional drugs using post-partum albino wistar rats.

2. Materials and methods

2.1. Plant Material

Fresh palm sap from African oil palm (E. gunieensis) trees was collected daily from a palm wine tapper in Abor, Udi, local government area of Enugu State. Abor is a town in southeastern Nigeria, located in Enugu State near the city of Enugu, Latitude: 6° 28’ 17.99” N Longitude: 7° 24’ 17.99” E of the Greenwich meridian.

2.2. Experimental animals

Twenty (20) primiparous female albino wistar rats weighing 180-250g were randomly selected and mated for this study. All mature female rats used weighed 180 - 220 g and were purchased from the Animal Facility Centre (AFC), Department of Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The animals were kept in a good, hygienic and favourable living condition. They were allowed free access to water and feed ad libitum

throughout the acclimation period which lasted for two weeks before the commencement of the experiment. Experimental procedures and protocols used in this study were approved by the research Ethics Committee of the Enugu State University of Science and Technology, College of Medicine, Enugu.

2.3. Chemicals and drugs

The substances/ drugs and chemicals used for this study were Metoclopramide hydrochloride tablets 5mg manufactured by Jiangsu Pengyao Pharmaceutical Inc. China, and Bromocriptine 2.5mg by Novartis pharmaceutical services.

All drugs were dissolved and/or diluted in distilled water on each day of our experiments.

2.4. Experimental design and protocols

The pregnant rats were then randomly divided into 4 groups, A, B, C and D each containing 5 animals in a cage based on the administration protocol and weight similarity. Following parturition, the dams received the plant substrate and the drug daily for 14 days through orogastric tube. The number of pups per dam was culled to 5 pups per litter.

Group A, the normal control group received distilled water. Group B, which received 5mg/kg of metoclopramide was used as positive control. Group C received 10ml/kg of oil palm sap while group D which received 2.5mg/ kg of bromocriptine was used as the negative control.

2.5. Animal Sacrifice and Tissue Processing

On day 15 of lactation, the lactating albino wistar rats were anaesthetized with chloroform, sacrificed and with the aid of a sharp surgical blade and scissors, the fur was trimmed and inguinal mammary gland tissues were harvested. The tissue samples were deposited in labeled test tubes containing physiological solution (10% formal saline) to prevent tissue autolysis and putrefaction. The tissues were processed and stained with Hematoxylin and Eosin to produce slides for microscopic examination.

3. Results

3.1. Histology of mammary gland Tissue

Haematoxylin-Eosin-stained sections of control Wistar mammary glands showed interlobular connective tissue (ILCT) with numerous lobules (L), little alveolar development and the abundance of mammary adipose cells (Figure A).

Figure B represents the photomicrograph of the lactating Wistar rats that were treated with Metoclopramide (5mg/kg). It showed a well-defined alveolar development with moderate increase in the size of lobules and the abundant mammary adipose cells.

Figure C showed the photomicrograph of the lactating Wistar rats treated with oil palm sap. It revealed a highly proliferating lobulo-alveolar system with lobular dilatation (LD), ducts branching into ductile, with well-defined alveolar development and fewer adipose cells.

Figure D shows a photomicrograph of mammary glands of lactating Wistar rats treated with bromocriptine (2.5mg) showed little alveolar development with moderate to severe degeneration of the breast tissue, cystic dilatation (CD) of the breast duct, atypical lobular necrosis (ALN) and very little adipose cells (Figure D2).

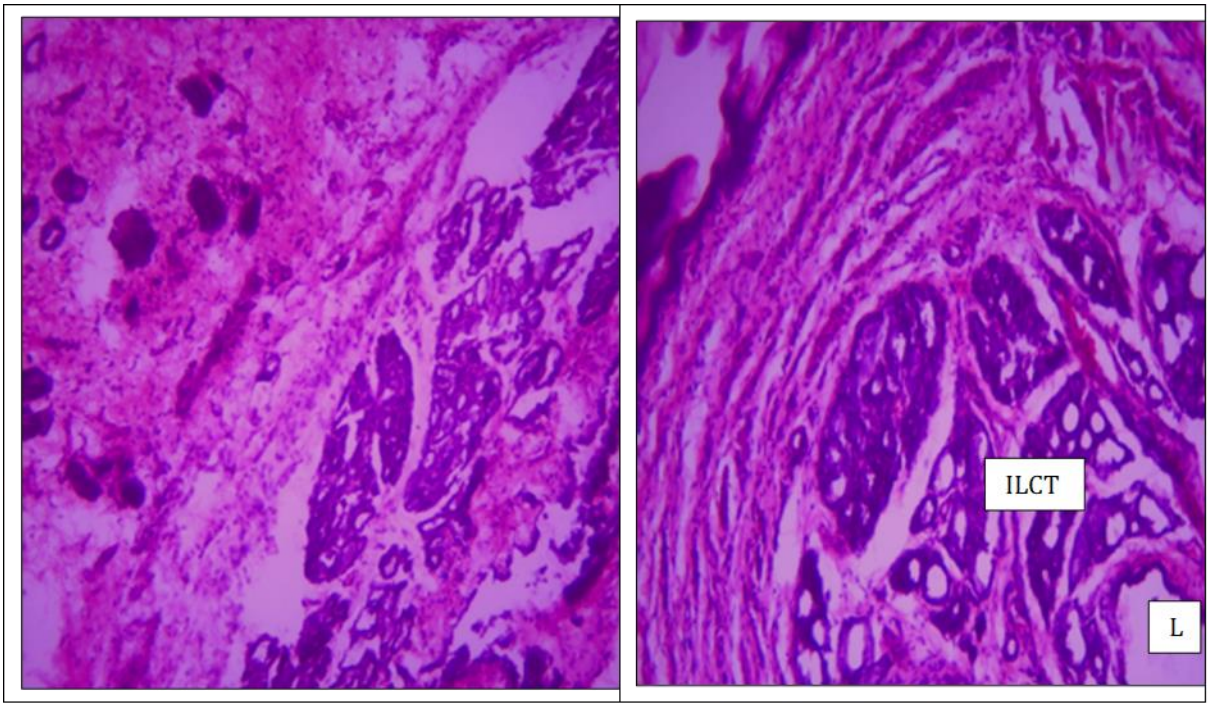


Figure A1	Figure A2

Figure A Photomicrograph section of the mammary glands from lactating rats treated with 0.9 % NaCl, showing interlobular connective tissue (ILCT) with numerous lobules (L), little alveolar development and the abundance of mammary adipose cells. H & E × 400

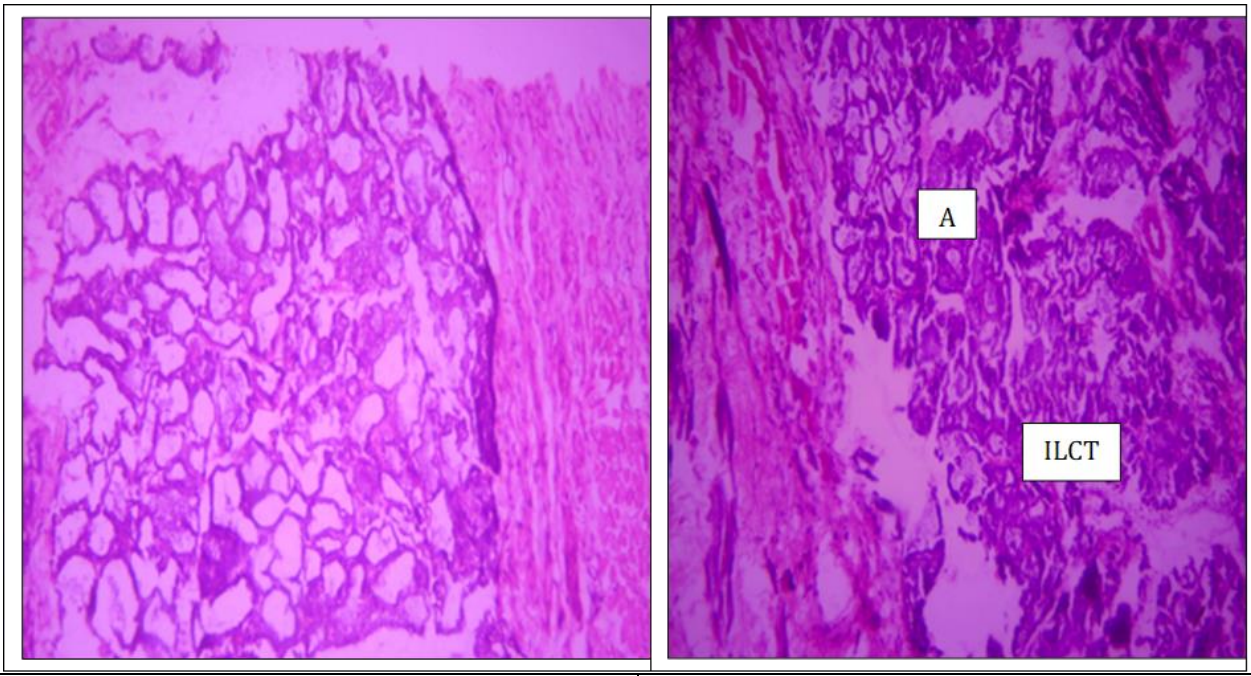
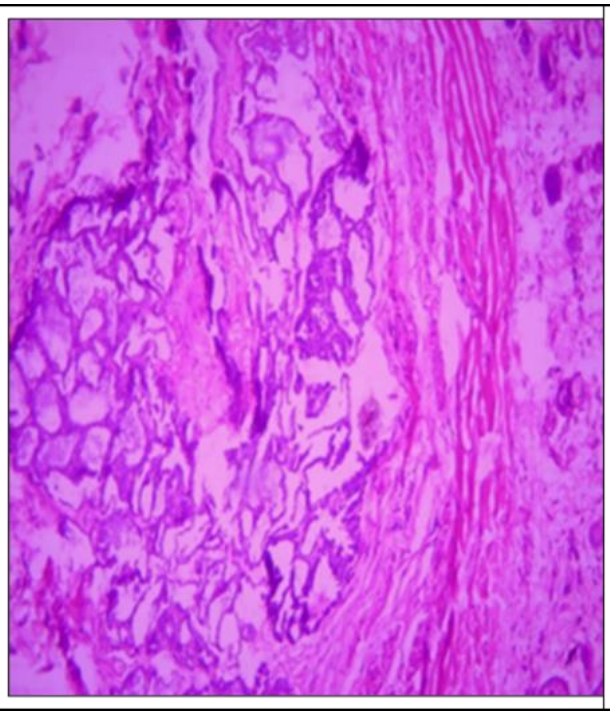


Figure B1	Figure B2

Figure B Photomicrograph section of the mammary glands from the group of lactating rats that received metoclopramide (5mg/kg) showed well defined alveolar development surrounded by interlobular connective tissue (ILCT) with abundance of acini (A) within the numerous lobules H & E × 400.



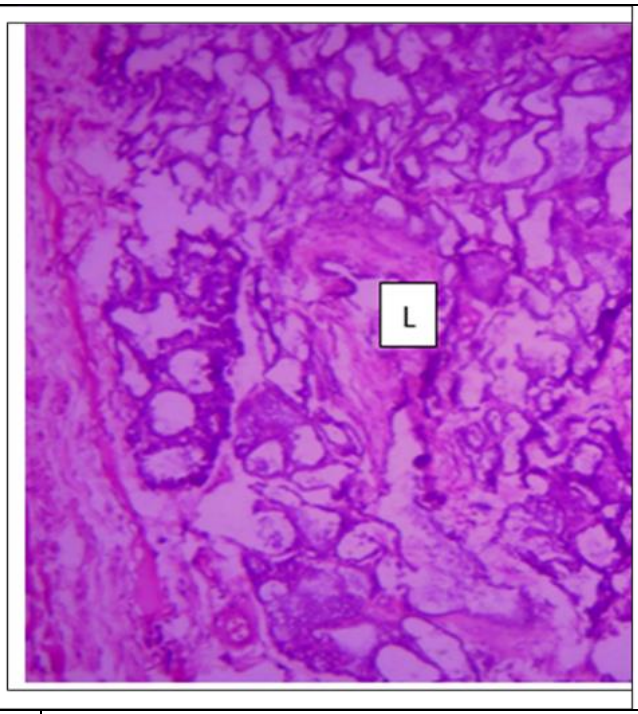
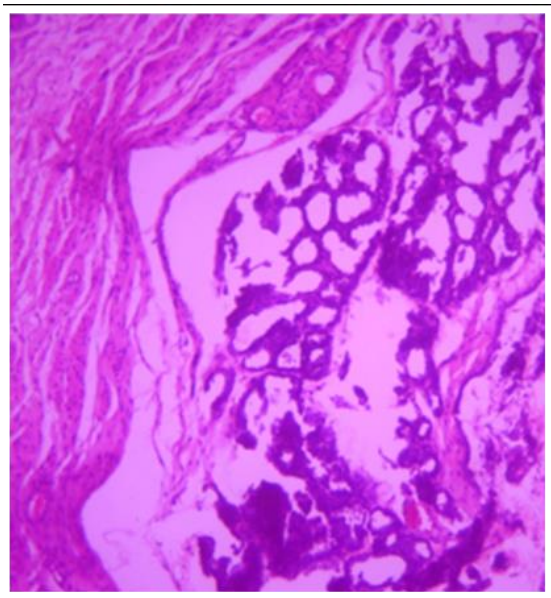


Figure C1	Figure C2

Figure C Photomicrograph of haematoxylin eosin-stained section (×400) of mammary glands of lactating Wistar rats treated with oil palm sap revealed highly proliferating lobuloalveolar system, with increased number of lobules (L) in cross sectional area than surrounding fat connective tissue. Also seen are large acini filled with proteinaceous substances



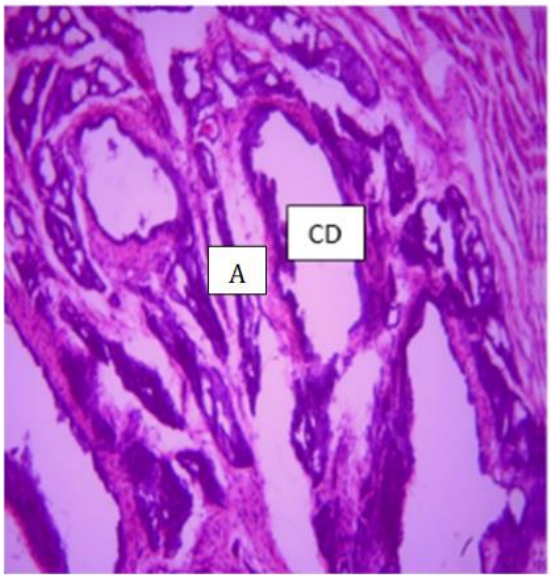


Figure D1	Figure D2

Figure D Photomicrograph of mammary glands of lactating Wistar rats with bromocriptine (2.5mg) showed little alveolar development with moderate to severe degeneration of the breast tissue, cystic dilatation (CD) of the breast duct, atypical lobular necrosis (A)

4. Discussion

In most African cultures, breastfeeding implies more than just nutrition. It has varying degrees of cultural, environmental, and psychosocial significance, operating between the mother and the baby within the community (Ibe

et al., 2017). Breast feeding is therefore a cultural practice and young women are often reprimanded for failure to breastfeed their babies. Despite these, some mothers still find it difficult to produce milk post-partum and hence fail to initiate breastfeeding immediately after parturition (Okamkpa *et al*., 2022). It is widely documented that women with milk production deficiencies traditionally use plant extracts to induce milk production or to increase milk yield (Wendy B., 2018). Researchers have reported the age-old traditional practice in Southeastern Nigeria where nursing mothers use oil palm sap from *Elaeis guineensis* tree as galactagogue (Ibegbulam *et al*., 2012); Udiegwe *et al*., 2013; Okamkpa *et al*., 2022). The histological effects of oil palm sap as a galactagogue on the on mammary gland tissue was the subject of this study.

Our results showed clearly that oil palm sap induced well-defined alveolar development with highly proliferating lobuloalveolar system, well defined lobular dilatation (LD), with ducts branching into ductile with abundant mammary adipose cells in the treated group (Figure C2). These features indicate an increased secretory activity and milk synthesis in the mammary epithelial cells with presence of increased accumulation of milky secretions in the alveoli when compared with the control group (Figure A2). This finding is in agreement with the study by Anyiom et al., (2020) which reported that palm wine sap induced hyperplasia of the lobuloalveolar system of the lactating mammary glands when used as a galactogugue. Our result is also consistent with similar studies on herbal galactagogues which showed that aqueous plant extracts induced mammary alveolar glandular development while increasing milk production (Lompo-Ouedraogo *et al.,* 2004; Simelane *et al*., 2012; Bako *et al*., 2013; Al-Bazii *et al*., 2019; and Sani *et al.*, 2019). These histological changes in the mammary glands could be attributed to their effects on lactogenic hormones. Galactagogues have been shown to exert their pharmacological effects through altering the complex hormonal milieu regulating lactation, particularly prolactin and oxytocin (Grzeskowiak et al., 2019).

Metoclopramide is a well-known galactogogue though mostly used as antiemetic, that induces low level hyperprolactinemia to increase milk production while causing mammary gland proliferation (Umur et al., 2022). The alveoli proliferation in the metoclopramide group (Figure B2) compared to the control was clear evidence of the galactogogue effect. However, a recent systematic review and meta-analysis revealed that metoclopramide did not increase the milk volume of the intervention groups when compared with that of the control groups. There was however, a significant increase in the serum concentrations of prolactin when the mothers were administered metoclopramide (Hussain et al., 2021).

5. Conclusion

Oil palm tree (*E. guineensis*) is a common plant in the tropical rainforest belt and very popular in Southeastern Nigeria. The traditional use of the fresh oil palm sap called palm wine as a galactogogue has already been documented (Okamkpa *et al*., 2022; Owoyele and Owolabi, (2014)), and this study has demonstrated further that this galactagogue effect results from the inducement of lobuloalveolar glandular hyperplasia in the mammary glands. The primary mechanism of this action is a subject of further studies but the upregulation of the lactational hormones like prolactin and oxytocin has been suggested.

Compliance with ethical standards

Acknowledgment

We acknowledge the assistance of the staff of the Department of Anatomy and those of the Histopathology laboratory of Enugu State University of Science and Technology for their unalloyed support and cooperation.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical clearance was obtained from the Ethics and Research Committee of University. Permission to conduct the research was also obtained from the Head, Department of Human Anatomy, Enugu State University of Science and Technology.

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