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# Effect of Palm Wine Administration on the Histology of the Mammary Glands of Postpartum Female Albino Wistar Rats.

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#### ABSTRACT

Exclusive breastfeeding has been linked to many positive health outcomes, yet its widespread adoption as the primary mode of providing nutrition to infants remains challenging. The most common reported reason for early breastfeeding cessation is perception of inadequate milk production. Therapeutic approaches to ameliorating insufficient lactation exists in form of pharmaceutical and herbal galactagogues but the activities of herbal galactagogues remain poorly understood. To augment breast milk production, a substantial number of women turn to herbal galactagogues either due to lack of finance for pharmaceutical galactagogues or even following expert recommendations amongst the elite despite lack of information on their efficacy and safety. Worthy of note in this light is palm wine, the paucity of such information as regarding the efficacy and safety of this most commonly used pharmaceutical lactation failure remedy among others remain distressing to the medical field and the society at large. The study was aimed at observinging the histological changes in the mammary glands following administration of fresh palm wine in postpartum female albino wistar rats. Fifteen (15) female albino wister rats and nine (9) male albino wister rats with weight ranging from 180-200g was used for this study. They were acclimatized and vaginal smears were repeatedly collected and analyzed to determine the estrous cycle and fertile males introduced appropriately, at the confirmation of coitus they were randomly divided into three (3) groups of five (5) each. Following parturition, fresh palm wine was administered according to their body weight using the standard value of ten mils per kilogram body weight (10ml/kg BW) for a period of 14 days. On the 15<sup>th</sup> day the animals were then sacrificed, the mammary glands were harvested and fixed in 10% formal saline. Stained with Haematoxylin and Eosin and examined under the microscope. The control group showed a normal lobulo-alveolar system with proliferating ducts and acini lined by vacuolated epithelial cell, stroma present and blood vessels seen. Low dose test group showed very large ducts and lobules with highly branched alveoli lined by large vacuolated cells with irregular larger and empty lumen. The fibrous and fatty stroma is sparsely distributed and blood vessels are hardly visible. Test group 2 showed hyperplasia and deeply stained epithelial cells with closely packed alveoli having irregular lumen containing eosinophillic secretion in the lobules; the ducts were irregular; fatty and fibrous stroma is inconspicuous; blood vessels were also inconspicuous. The progressive increase in hyperplasia of the glandular tissue suggests the efficacy of fresh palm wine as a galactagogue.

KEYWORDS: Mammary glands, Histology, Palm wine, Galactagogue, Breast feeding

## **INTRODUCTION**

Humans and other animals exhibit a significant level of parental care of the offspring, one of which is provision of nourishments in the form of human breast milk which is the primary source of nutrition containing about 88.5% water and 11.5% solids <sup>1,2</sup>. Breastfeeding newborns provide optimal nutrition, immune support and a host of lifelong health benefits to mother and child. Massive tissue remodeling occurs within the mammary gland during pregnancy, resulting in the formation of lobuloalveoli that are capable of milk secretion <sup>3-5</sup>. After parturition, prolactin secreted from the acidophilic cells lactotropes of the anterior pituitary induces lactation by direct stimulation of the synthesis

of milk proteins in the epithelial cells and indirect stimulation of the proliferation of the secretory cells of the breast <sup>6,7</sup>. Nevertheless, lactation failure or deficiency can result in an inability to properly breastfeed the offspring. The most important cause of lactation failure is insufficient secretion of prolactin <sup>8-11</sup>. Furthermore, obesity can result in early lactation failure <sup>12</sup>; Certain drugs such as Delta-2(D<sub>2</sub>) receptor agonist as well as indirect D<sub>2</sub> activators can suppress prolactin secretion from the pituitary gland, traumatic or stressful parturition <sup>13,14</sup>. Pain relievers given during delivery <sup>15</sup>, medical conditions such as gestational diabetes and gestational ovarian theca lutein cysts can cause lactation failure <sup>16-20</sup>. Insufficient glandular tissue of the mammary glands or mammary hypoplasia caused by lack of normal mammary gland growth and maturation during puberty or pregnancy causes lactation failure; a situation that rarely occurs<sup>21,22</sup>.

From a histological point, the mammary gland is a compound tubulo-alveolar gland with lactiferous duct. The smaller ducts are lined by columnar epithelium; in the larger ducts the epithelium has two or three layers of cells; near their openings on the nipple the lining becomes stratified squamous. 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<sup>23,24</sup>.

Insufficient glandular tissue causing lactation failure or deficiency has been ameliorated or treated by using herbal or pharmaceutical galactagogues which are substances that promotes lactation in humans and other animals <sup>9,25</sup>. One of such pharmaceutical galactagogues is Domperidon, while some herbs mentioned as galactagogues include fenugreek (*Trigonella foenumgraecum*), Torbangun (*Coleus amboinicus Lour*), milk thistle (*Silybum marianum*), goat's rue, oats, dandelion, millet, seaweed, anise, basil, blessed thistle, fennel seeds, marshmallow, and a range of others <sup>26</sup>. Reseach suggests that palm wine has also been suggested to have galactogogue properties <sup>27-29</sup>.

Palm wine is an alcoholic beverage gotten from the sap of various species of palm tree such as the palmyra, date palms and coconut palms. The unfermented sap is clean, sweet, colourless syrup containing about 10 -12% sugar, which is mainly sucrose <sup>30,31</sup>. Palm wine has been used for several purposes some of which are social and recreational palm wine has also been used in traditional medicine to enhance breast milk production in nursing mothers <sup>27-30</sup> it is also reportedly used for treating eye problems and malaria when combined with other herbs as well as its antimicrobial ability<sup>27</sup>.

This work is therefore aimed at evaluating the histological changes in the mammary glands following administration of fresh palm wine in postpartum female albino wistar rats.

#### **MATERIALS AND METHODS**

**Study Design:** Fifteen (15) female albino wister rats and nine (9) male albino wister rats with weight ranging from 180-200g were used in this study. The rats were housed in plastic cages with sufficient ventilation; saw dust was used for bedding of the animals which was changed every three days in order to maintain good hygiene. The animals were fed with standard pellet diet

and water *ad libitum*. The animals were kept in a good, hygienic and favourable living condition. The animals were acclimatized under laboratory conditions for two weeks at the College of Medical Sciences animal house, University of Calabar before the commencement of the experiment.

After acclimatization period, vaginal smears were repeatedly collected in order to determine the estrous cycle using a pipette filled with 10% buffered formalin. The pipette tip was inserted gently into the vagina and normal saline was flushed into the vagina until a cloudy fluid was obtained which indicated that vaginal cells are present. After each lavage a small drop of the sample was then smeared evenly on the glass slide and allowed to dry, the procedure was repeated for each of the rats using different pipette. Slide evaluation of the sample was carried out using a light microscope to determine the estrous phase of the animals. The reproductive cycle of a female rat is classified into the proestrus phase, estrus phase, metaestrus phase (diestrus I) and diestrus phase (diestrus II)<sup>32,33</sup>. Ovulation occurs from the beginning of the proestrus phase to the end of the estrus phase<sup>34,35</sup>.

Cytology of the cells revealed three basic types of cells, the round/nucleated cells, irregular/non nucleated which are the *epithelial cells*, *cornified cells* and *leucocytes* respectively. The presence of large number of anucleated epithelial cells (cornified cells) indicated that the animal(s) were in their estrus phase. The animals that were confirmed pre-estrous were separated into individual cages and a male rat introduced. The following morning, vaginal smears were again collected to examine possible presence of sperm cells to confirm mating. The animals were then randomly divided into 3 groups, A, B, and C each containing 5 animals in a cage based on the administration protocol and weight similarity.

**Dosage:** Fresh palm wine was acquired on daily basis from a palm wine tapper at Atimbo Street; Calabar, Cross River State. The palm wine was administered to the rats according to their body weight using the standard value of ten mils per kilogram body weight (10ml/kg BW) adopted from the work of Oyedeji *et al.*,<sup>36</sup>.

Administration: After parturition, Group A which represented the control group was administered distilled water, 1.9ml of palm wine representing low dose was administered to the animals in Group B with average body weight of 190g, while 3.8ml of palm wine representing high dose was administered to the animals in group C with average body weight of 190g.

| Group | Quantity   | Duration |
|-------|------------|----------|
| А     | NIL        | 14 days  |
| В     | 10ml/kg BW | 14 days  |
| С     | 20ml/kg BW | 14 days  |

Table 1: Showing groups; quantity of palm wine administered and the number of days.

Animal Sacrifice and Tissue Processing: At the end of 14 days of administration, the animals were then sacrificed by anaesthetizing with chloroform and with the aid of a sharp surgical blade and scissors, the fur was trimmed and the mammary glands were harvested and quickly transferred to a specimen bottle which was appropriately labeled and containing enough quantity of 10% formal saline, cotton wool was inserted to prevent floating of the breast in order to prevent tissue autolysis and putrefaction. The tissues were processed and stained with Hematoxylin and Eosin to produce slides for microscopic examination <sup>22-24,37</sup>.

## RESULTS

#### Weight Observation:



**Figure 1:** (a) showing initial body weight and (b) showing final body weight. No statistically significant difference was observed for initial body weight (a); There was significant difference in final body weight for high dose and low dose when compared to control. Level of significance was set at p<0.05. n=15; 5 Control, 5 Low dose and 5 High dose.

# **Histological Observations**



**Figure A**<sub>1</sub>: Photomicrograph of a lactating mammary gland of albino wistar rat (control group x100). AL- Alveoli; ST-Stroma; BV-Blood vessel. **Figure A**<sub>2</sub>: Photomicrograph of a lactating mammary gland of albino wistar rat (control group x400). L-Lumen; LP-Lipid particle.



**Figure B**<sub>1</sub>: Photomicrograph of a lactating mammary gland of albino wistar rat (test group 1 x100). AL-Alveoli; D-Duct. **Figure B**<sub>2</sub>: Photomicrograph of a lactating mammary gland of albino wistar rat (test group 1 x400). AL-Alveoli; L-Lumen.



**Figure C**<sub>1</sub>: Photomicrograph of a lactating mammary gland of albino wistar rat (test group  $2 \times 100$ ). AL-Alveoli; SE-Eosinophillic secretion. **Figure C**<sub>2</sub>: Photomicrograph of a lactating mammary gland of albino wistar rat (test group  $2 \times 100$ ). AL-Alveoli; D-Duct; SE-Eosinophillic secretion.

| Group A (control) slides labeled plate $A_1$ and plate $A_2$ | Group B (test 1) slides labeled plate $B_1$ and $B_2$ | Group C (test 2) slides labeled plate $C_1$ and $C_2$        |
|--|---|--|
| Proliferating lobuloalveolar system                          | Moderately proliferating lobuloalveolar system        | Highly proliferating lobuloalveolar system                   |
| Numerous and closely packed alveoli moderately branched      | Lobules have highly branched alveoli (hyperplasia)    | Hyperplasia is increased and deeply stained epithelial cells |
| Fatty and fibrous stroma is also present                     | Fibrous and fatty stroma is sparsely distributed      | Fat and stroma is sparsely distributed                       |
| Irregular lumen which are empty                              | Irregular larger lumen which are empty                | Closely packed alveoli having<br>irregular lumen             |
| Lined by large vacuolated cells                              | Lined by large vacuolated cells                       | Contains eosinophillic secretion in the lumen                |
| Blood vessels are also seen                                  | Blood vessels are not seen                            | Blood vessels are not seen                                   |
| small and medium ducts                                       | Large ducts   | Large ducts  |

#### **Summary of Histological Findings Table 2:** changes in group A, B and C respectively.

# DISCUSSION

Reports have shown that breastfeeding new-borns provide optimal nutrition, immune support and a host of lifelong health benefits to mother and child <sup>5,38</sup>. Galactogogues or lactogogues are medications or other substances believed to assist initiation, maintenance, or augmentation of the rate of milk production as such palm wine has been suggested to have galactogogue properties by other authors <sup>27-29,39</sup>.

This research revealed that fresh palm wine administration triggered hypertrophy and hyperplasia of the mammary glandular tissue and the rate of proliferation increased simultaneously and proportionately with the increase in the quantity of palm wine administered, this finding corresponds with the reports of Damanik *et al.*,<sup>40</sup> that investigated the galactagogue property of Torbangun (*Coleus amboinicus Lour*) *and found* Proliferation of secretory mammary cells, with increase proliferation in accordance with increase in administration of Torbagun.

There established theory behind pharmaceutical galactogogues is that dopamine antagonists increase prolactin secretion and subsequently increase the overall rate of milk synthesis. This theory could also be adopted for palm wine owing to the fact that the result of this research showed increase mammoplasia with increase administration which suggests increase in prolactin secretion with simultaneous increase in fresh palm wine<sup>41</sup>.

Research has shown that the palm wine collected between the day contains less alcohol (1.4% and 2.82%) than the palm wine which has been accumulated overnight (3.24% and 4.75% and even over 6% in few cases) <sup>42</sup> and the assurance of taking fresh palm wine which hasn't really started fermentation is hindered by access of mothers to the tapping source and adulteration by businessmen. This

basically is the challenge surrounding the pharmacovigilance of herbal medicines in the general population, and more so "freshness" of palm wine in breastfeeding women remains questioned. Alcohol has been reported a known neurotoxin by <sup>43</sup> and could destroy the developing brain including the hypothalamus; It has been observed that prenatal alcohol alters endocrine functions such as hypothalamic-pituitary-gonadal axis in the offspring<sup>44</sup>.

Furthermore, some studies have suggested that consumption of beer and fermented palm wine may be nephrotoxic to the developing kidney and could cause prenatal osteo-inhibitory effects on bones respectively <sup>45,46</sup>. The findings of Oyedeji *et a.l,* <sup>36</sup> indicates that palm wine causes deleterious effects on the reproductive parameters in male albino wistar rats, these findings are not applicable to this work since female is the gender of study.

There is need to acknowledged the fact that herbal remedies are not always "safe" and there is a risk that some herbal medicines may cause side effects or potentially toxic effects in both the mothers and their infants if certain constituents are transferred into the breast <sup>47</sup>; Nevertheless, there appears to be an innate comfort in using herbal medicines with unknown toxicity profiles over conventional medicine shown to have efficacy and low toxicity in breastfeeding women<sup>48</sup>.

Despite the efficacy of palm wine used as a galactagogue as shown in this research, its safety is not guaranteed and currently the "Academy of Breastfeeding Medicine" does not have recommendations for the use of herbal galactagogues<sup>26</sup>.

## CONCLUSION

The results of this research shows that administration of fresh palm wine has a mammoplastic effect on the postpartum female albino wistar rats mammary glands and consequently increases the quantity of breastmilk production proportional to volume administered, and thus proving the galactagogue ability of fresh palm wine.

Hence, the use of palm wine as an herbal galactagogue is justified by its efficacy in increasing mammoplasia, but its safety remains unguaranteed until further advanced studies.

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