

Galactagogue Effect Of Fresh Palmwine In Adult Albino Wistar Rats: Histological Changes In The Hypothalamus And Serum Prolactin Levels

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Abstract

Palm wine is one of the most well-known natural galactagogues used in the treatment of lactation failure in Africa. Traditional healers claim that certain plants are more effective as well as safer than synthetic medications in treating infectious ailments. Despite the descriptions by traditional herbal practitioners on the therapeutic efficacy of herbal products, there is no scientific evidence to support the efficacy of palm wine as a galactagogue. Therefore, the present study was aimed at investigating the effect of fresh palm wine on the histology of the hypothalamus and prolactin levels in postpartum female albino Wistar rats that will be a factor of positive or negative lactation effects. A total of fifteen (15) adult female Wistar rats were procured from the Animal Facility of the College of Medical sciences University of Calabar, and were acclimatized to the conditions of the animal house of the faculty of basic medical sciences, university of Calabar. They were grouped into three (3) of five animal per group. Group A was given water (control); Group B was administered with 1900mg (1.9 mls) of palm wine (low dose); and Group C was given 3,800mg (3.8 mls) of palm wine (high dose). The rats were euthanized using chloroform at day 14 of treatment, and their hypothalami were extracted and prepared for histological examination. Blood samples were also collected for blood serum analysis early hours of the morning from the right ventricle of the heart. The results revealed that the slides from Group A showed several tiny spherical vacuoles and blood arteries, as well as well-distributed neuronal cell bodies. The neuronal cell bodies in Group B were larger and clustered, with larger irregular vacuoles and blood vessels. The slides from Group C showed more hyperplastic and clustered cell bodies, as well as larger irregular vacuoles and blood vessels. In comparison to the control (Group A), blood serum analysis revealed a significant increase in prolactin levels in Group B rats and an even greater increase in Group C. The findings of this study show that a progressive increase in neuronal cell bodies of the hypothalamic cells and serum prolactin levels which suggests the efficacy of fresh palm wine as a galactagogue for treatment of lactation failure.

Index Terms— Blood serum, Galactagogue, Hypothalamus, Lactation failure, Palm wine.

INTRODUCTION

Breast milk is widely regarded as the best source of nutrition for neonates and infants. The properties of human milk facilitate the transmission of life from *in-utero* to *ex-utero*. During critical periods of brain, immune, and gut development, this dynamic fluid provides a diverse array of bioactive substances to the developing infant. Human milk is a unique, species-specific, complex nutritive fluid with immunologic and growth-promoting properties. This unique fluid evolves to meet the changing needs of the baby as it grows and matures. The mammary gland's milk synthesis and secretion involve numerous cellular pathways and processes.⁴

Furthermore, traditional herbal practitioners have described the therapeutic efficacy of many indigenous plants for various diseases, in addition to their ancient historical uses. These traditional healers claim that certain plants are more effective as well as safer than synthetic antibiotics in treating infectious diseases.²¹ However, most users of these medicinal plants do so for its affordability and availability.²¹

The complex physiology of breastfeeding includes a delicate balance of hormones. Oestrogen, progesterone, prolactin, and oxytocin are the four hormones that help the breast to produce milk. To assist the breast in producing milk, the body naturally adjusts the level of these hormones.⁵ Sensory impulses travel from the nipple to the brain when a baby suckles at the breast. In response, the hypothalamus stimulates the anterior lobe of the pituitary gland to secrete prolactin and the posterior lobe to secrete oxytocin. Although estrogen and progesterone are required for physical breast development, oxytocin causes the myoepithelial cells surrounding the alveoli to contract. This causes milk that has accumulated in the alveoli to flow along and fill the ducts. Prolactin is required for the production of milk by alveolar cells. During pregnancy, the level of prolactin in the blood rises dramatically, stimulating the development of mammary tissue. Milk secretion begins shortly after delivery. The amount of milk produced by the alveoli increases in proportion to the amount of prolactin in the blood.⁵ However, not all mothers have the luxury of nursing their newborns after birth. This can be caused by a condition called lactation failure, in which lactation is insufficient or fails due to an inadequacy of breast milk production or a failure of the milk let-down reflex in response to suckling following childbirth, resulting in an inability to properly breastfeed.⁶ Lactation failure is classified into two types: primary and secondary. Secondary failure, which affects 11% of women, means that they were able to produce enough milk at one point, but then their production dwindled, typically due to insufficient nutrition, poor milk supply management, or suckling problems with the child. However, 4% of women experience primary lactation failure. Glandular tissue problems, breast shape, prior breast surgery, or Sheehan's syndrome, in which women who have a massive postpartum hemorrhage experience shock to the pituitary gland, are common causes. Less common causes of primary lactation failure include high or low blood pressure, anemia, and certain medications. This leads to depression, frustration, and anger in women with the condition, making them feel less feminine or capable as mothers because they are unable to breastfeed their babies. It becomes even more tragic when facts from increased awareness campaigns by experts and health professionals show that breast milk is "second-to-none" in terms of nutrition for newborns. The term "lactation failure" has led many women to believe that they have "failed" to provide their child with this basic need.⁷ Women with this condition have tried everything from dieting to changing their habits and posture to using galactagogues (lactation-promoting agents) in the hopes of finding an effective solution.

A galactagogue is a substance that promotes lactation in humans and animals. It can be synthetic, plant-derived, or endogenous. Domperidone and metoclopramide are examples of synthetic galactagogues. Herbs and food used as galactagogues include; shatavari (*Asparagus racemosus*), fenugreek (*Trigonella foenumgraecum*), torbangun (*Coleus amboinicus*), chaste berry (*Vitex agnus castus*), oatmeal, marshmallow, moringa, etc.^{8,9} Among many of its nutritional benefits and socio-economic importance, particularly in Nigeria, palm wine is well recognized as an effective galactagogue, not just in rural areas but also among the well-educated. Many nurses and midwives recommend palm wine to postpartum mothers, stating that "it works" from personal experience. The lack of scientific verification or proof of the efficacy of supposed natural galactagogues has left many mothers, among the growing population of women with lactation insufficiency. Also, they are often frustrated and despondent after trying numerous substances rumored to be galactagogues. Many developed countries have created synthetic galactagogue drugs although, many people especially in rural areas of Nigeria lack access to these drugs. Even when available, they are not affordable. As a result, certain foods, herbs, and other natural substances are sought after and used to treat lactation insufficiency. Some of these substances are uncommon and are only known to a few locals. Many of the supposed solutions are culturally specific, giving mothers even more options to experiment with. Due to a lack of scientific evidence of their efficacy, women embark on a research project using themselves as guinea pigs.

MATERIALS AND METHODS

Source of palm wine

Fresh palm wine was acquired on a daily basis from a palm wine tapper at Atimbo Street, Calabar, Cross River State.

Source of animals

Fifteen (15) female and nine (9) male albino Wister rats (weighing 180-200g) were purchased from the Department of Human Anatomy, Cross River State University of Technology (CRUTECH), Okuku, Yala Campus, Calabar, Cross River State. The rats were housed in plastic cages with adequate ventilation, and the bedding (sawdust) was changed every three days to ensure good hygiene.

Maintenance of the experimental animals

The animals were fed a standard pellet diet and water *ad libitum* and kept in good, hygienic, and favourable living conditions. Before the experiment, the animals were acclimatized in laboratory conditions for two weeks at the Animal House, College of Medical Sciences, University of Calabar, Cross River State.

Determination of the animals' estrous phase and experimental grouping

Following the acclimatization period, vaginal smears were collected repeatedly to determine the estrous cycle using a pipette filled with 10% buffered formalin. The pipette tip was gently inserted into the vagina, and normal saline was flushed into the vagina until a cloudy fluid was obtained, indicating the presence of vaginal cells. Following each lavage, a small drop of the

sample was smeared evenly on the glass slide and allowed to dry before repeating the procedure with a different pipette for each of the rats. A light microscope was used to examine the sample slides to determine the animals' estrous phase. The animals that were confirmed to be pre-estrous were separated into individual cages and a male rat was introduced. The following morning, vaginal smears were collected again to examine the possible presence of sperm cells to confirm mating. The animals that were confirmed for successful mating then randomly divided into 3 groups; A, B, and C, each containing 5 rats in a cage based on the administration protocol and weight similarity.

Palm wine treatment

Fresh palm wine was administered to the rats according to their body weight using the standard value of 10 ml/kg body weight (BW) from previous research and 20ml/kg/BW for low dose and high dose respectively.¹⁰ After parturition, the animals in Group A (control) were given distilled water, 1.9 ml of palm wine representing a low dose was given to the animals in Group B with an average body weight of 190 g, and 3.8 ml of palm wine representing a high dose was given to the animals in Group C with an average body weight of 190 g. The treatment lasted for 14 days.¹⁰

Sample collection and preparation

At day 14 of palm wine administration, the female Wistar rats were sacrificed by anesthetizing them with chloroform. The brains were harvested and their hypothalami excised with the aid of a sharp surgical blade. The hypothalamus was quickly transferred to a specimen bottle that was appropriately labeled and contained a large quantity of 10% formal saline. Cotton wool was inserted to prevent floating of the hypothalamus to prevent tissue autolysis and putrefaction. The tissues were stained with hematoxylin and eosin and the slides were ready for viewing and microscopic examination. The stained sections of the hypothalami were viewed under a light microscope and photomicrographs were taken. In addition, venous blood samples were collected from the postpartum Wistar rats at 7am from the right ventricle of the heart into plain bottles with a 2 ml syringe, labeled, and sent for hormonal analysis at the medical laboratory at Arubah Specialist Hospital, Calabar, Cross River State. The levels of prolactin were measured in ng/mL of blood.

RESULTS AND DISCUSSION

Histological characteristics of experimental Wistar rats

The hypothalamic sections in Group A (control) rats revealed well-distributed neuronal cell bodies with numerous small rounded vacuoles and blood vessels. The nuclei of the neuronal cells were well-defined, with fine chromatin patterns (Figures 1). In Group B rats that received low dose of palm wine at dose of 1900mg, the hypothalamic sections showed (Figures 2) prominent neuronal cell bodies of various sizes and shapes, as well as abundant neuronal processes, a regular neuronal contour, and a fine chromatin pattern with prominent blood vessels and vacuoles, the cells appear slightly enlarged. As revealed in Figure 3, the hypothalamic sections revealed clusters of densely populated neuronal cell bodies with numerous neuronal processes. The nuclear contour of neuronal cells is regular, and the chromatin pattern is fine. The cells have more hyperplasia, as evidenced by larger irregular vacuoles and blood vessels.

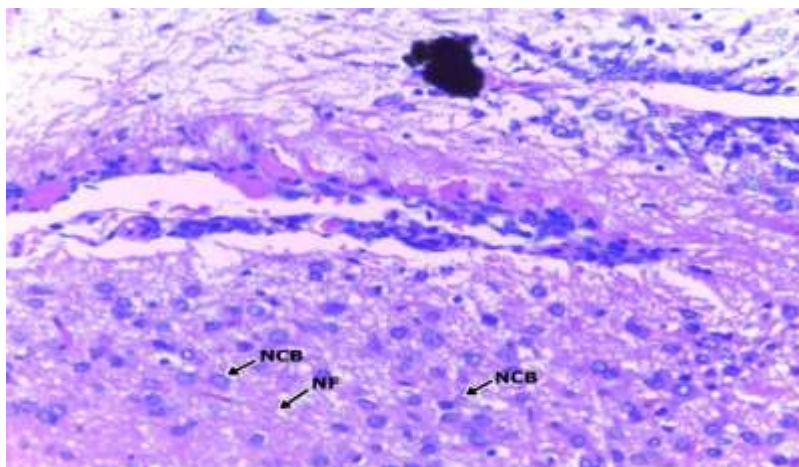


Figure 1: Photomicrograph of the hypothalamus of Group A (control).

NCB: Neuronal cell bodies; NF: Neurofibrils; Magnification = x100

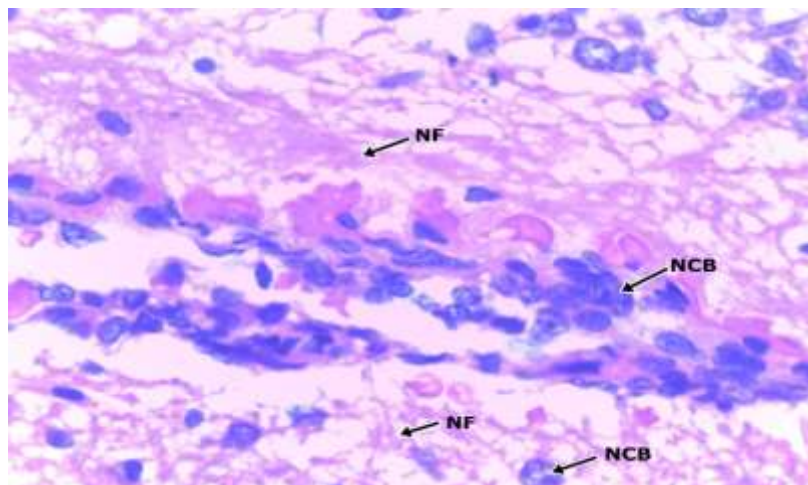


Figure 2: Photomicrograph of the hypothalamus of Group A (control).
NCB: Neuronal cell bodies; NF: Neurofibrils; Magnification = x400

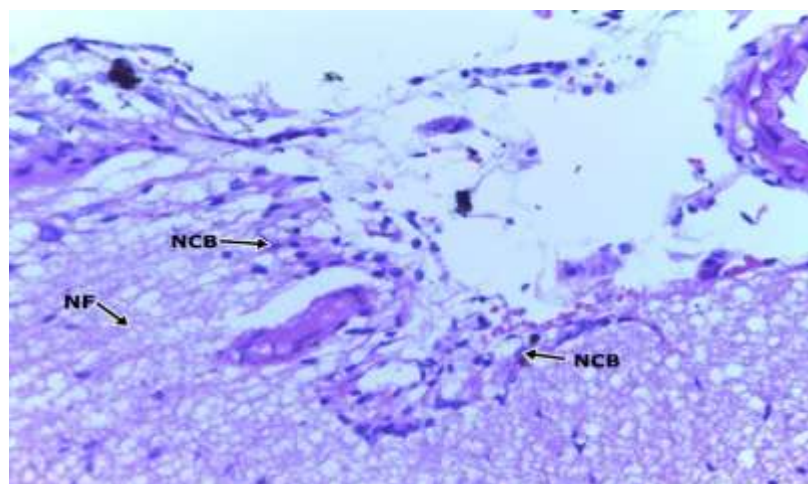


Figure 3: Photomicrograph of the hypothalamus of Group B.
NCB: Neuronal cell bodies; NF: Neurofibrils; Magnification = x100

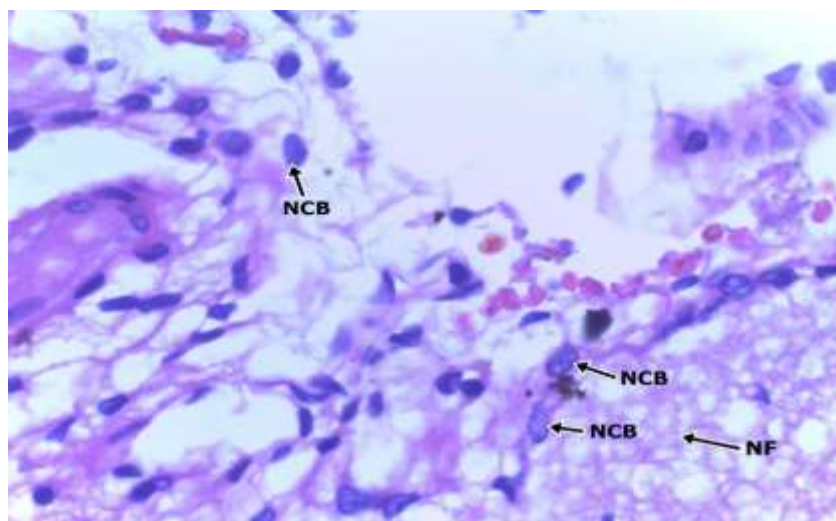


Figure 4: Photomicrograph of the hypothalamus of Group B.
NCB: Neuronal cell bodies; NF: Neurofibrils; Magnification = x400

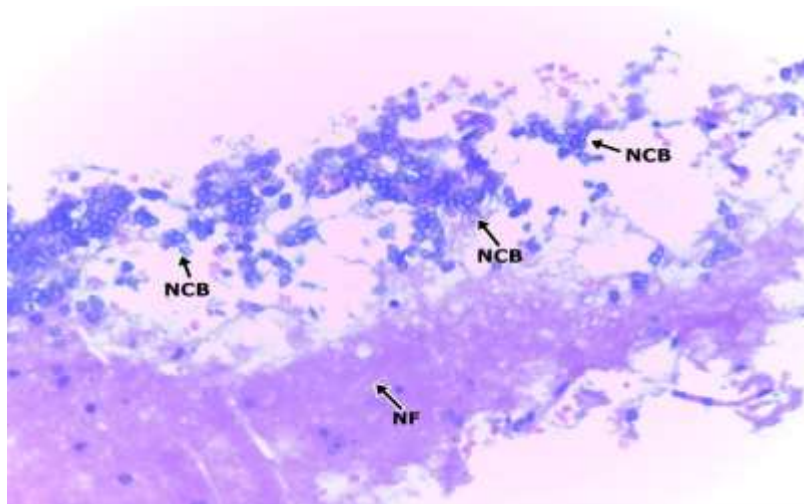


Figure 5: Photomicrograph of the hypothalamus of Group C.
 NCB: Neuronal cell bodies; NF: Neurofibrils; Magnification = x100

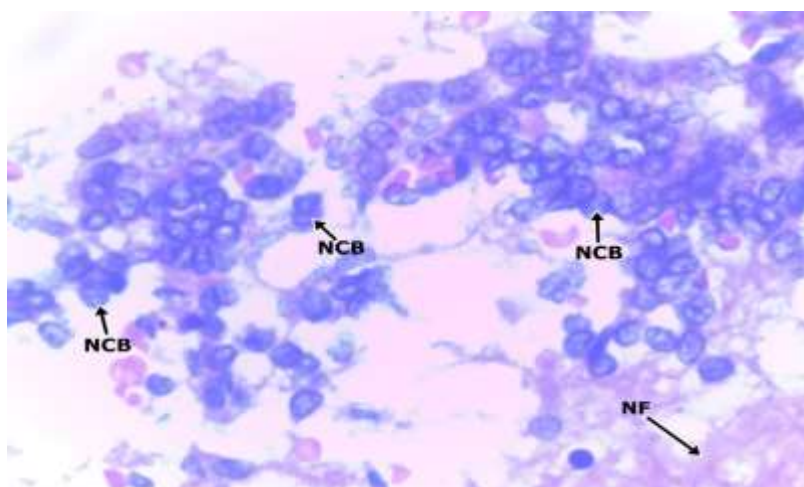


Figure 6: Photomicrograph of the hypothalamus of Group C.
 NCB: Neuronal cell bodies; NF: Neurofibrils; Magnification = x400

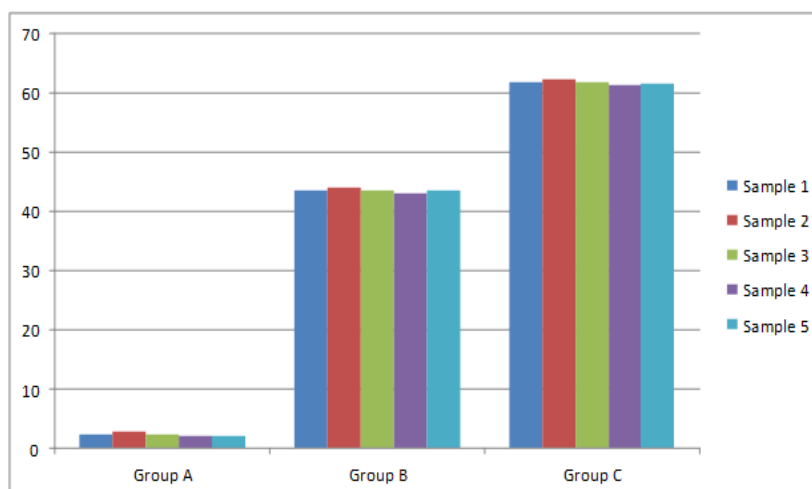


Figure 7: Relationship between Groups A, B, C and prolactin levels in each group.
 Y-axis: Prolactin (ng/mL); X-axis: Different groups.

Table 1: Serum prolactin levels in experimental groups.

Sample	Group A (ng/mL)	Group B (ng/mL)	Group C (ng/mL)
1	2.30	43.60	61.80
2	2.80	44.10	62.30
3	2.40	43.70	61.90
4	2.20	43.20	61.30
5	2.00	43.50	61.70
Mean Value	2.34	43.62	61.80

Group A: Control; Group B: Low dose of palmwine; Group C: High dose of palmwine

Prolactin levels in experimental Wistar rats

The results show that the average prolactin level in the control (Group A) was 2.34 ng/mL. In Group B, which received a low dose of palm wine 1,900mg (1.9mL), prolactin levels averaged 43.62 ng/mL, representing a 93% increase over the control group. The highest level of prolactin (61.80ng/mL) was recorded in Group C, which was administered a high dose of palm wine, 3,800mg (3.8mL). About a 76% increase was observed compared with Group B. The serum prolactin analysis of Group C shows a concurrent increase in prolactin levels among the animals. This represents a 1000% increase over the value obtained for the control group (Figure 7 and Table 1). Therefore, it can be deduced that the cells, which appeared with increasing sizes when viewed under the microscope, were proportional to palm wine dose and dopaminergic.

The tissues and organs of the body work in perfect harmony to maintain the homeostasis of the body's internal environment. This is achieved under the control of the nervous system through extensive nervous connections around the body and the endocrine system.¹¹ It connects the nervous system to the endocrine system via the pituitary gland (hypophysis). The pituitary gland is known to be heavily influenced by the hypothalamus. The pituitary and hypothalamic glands form a complex neuro-hormonal circuit that is critical for maintaining normal endocrine status.^{12,13} The hypothalamus is the major neuroendocrine regulatory region of the brain and is restricted by the blood-brain barrier, whereas the hypophysis is not. This could explain why oxytocin, ADH, and inhibitory hormones released by neurons in the hypothalamic region must travel long distances along axons before reaching capillaries in the median eminence, infundibular stalk, and infundibular process.¹⁴ Lactotrophs in the anterior pituitary (adenohypophysis) are responsible for secreting the peptide hormone, prolactin, which is known for its major role in causing milk production in female mammals.¹⁰ This implies that any substance with the ability to influence milk secretion would also act on the hypothalamic-prolactin axis of the endocrine system, which is the usual course of action for synthetic pharmaceutical galactagogues.⁸

When the experimental groups' hypothalami were examined histologically, it was discovered that there was proliferation of neuroendocrine cells proportional to the dose of palm wine administered, when compared to the control group. This implies an increase in the neurosecretory function of these cells. The hypothalamus is made up of numerous, mostly ill-defined nuclei, each with a distinct function and neuroanatomical significance. The hyperplasia in experimental Groups B and C, on the other hand, indicates the presence of cells whose functions are related and have been affected by palm wine.

Neuroanatomical examinations of the hypothalamus show that none of the cell groups found in the hypothalamus directly regulates the secretion of prolactin by the anterior pituitary except dopamine-producing neurons, which produce dopamine as a prolactin-inhibitory factor.^{15,13,11} Thus, histological observation of any or the whole region of the hypothalamus is a valid part of this research. From these histological observations, hyperplasia of the dopaminergic cells increased with an increased dose of palm wine. Dopamine has a tonic inhibitory control of pituitary lactotrophs. The term "tonic inhibitory control" refers to the hypothalamus's ability to secrete dopamine only when serum prolactin levels are too high (hyperprolactinemia). Therefore, the hyperplastic hypothalamic dopaminergic cells are evidence of moderately increased prolactin levels in the postpartum female rat.¹⁶

Palm wine is a popular alcoholic beverage among natives of West African countries.^{17,18} Among its many socio-economic and cultural importance, it is used as a local galactagogue.¹³ When compared to the control, serum prolactin analysis showed a rise in prolactin levels in Group B (low dose group) and a greater rise in Group C (high dose group). These findings suggest that palm wine can help promote lactation and thus act as a natural galactagogue. Although palm wine is usually a medium for a community of bacteria and microorganisms.^{18,19,20} Nonetheless, there have been no reports of serious health risks or deaths associated with the consumption of palm wine.¹⁷ According to previously documented literature, palm wine is a spontaneously

alcoholic beverage with an alcohol content proportional to its length of stay after tapping. Fresh palm wine is palm wine that has recently been tapped and typically contains negligible amounts of alcohol. However, its alcohol concentration rises drastically within two to three hours after tapping. It is difficult for a postpartum woman to find fresh palm wine that is less than two hours old. Most of the palm wine consumed contains significant amounts of alcohol. Alcohol can cause brain damage and various cell-growth abnormalities in the fetus.¹⁷ There is also evidence that palm wine can be teratogenic.¹¹ Serum analysis from this study revealed that fermented and a high dose of palm wine can lead to hyperprolactinemia in postpartum females, hence, the need to regulate its consumption.

CONCLUSION

Galactagogues are substances or action that stimulates the production of milk. The findings of this study show that palm wine is an effective galactagogue based on hypothalamic cellular proliferation and increase in serum prolactin levels and its effect is directly proportional to the dose administered. However, due to the relatively high levels of alcohol in palm wine, its safety and effectiveness in the treatment of lactation failure cannot be guaranteed.

Conflict of Interest

This research has no conflict of interest.

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