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LACTOGENIC EFFECT OF PALM WINE IN ALBINO WISTAR RAT USING OXYTOCIN AND BODY WEIGHT AS BIOMARKER

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ABSTRACT: This study investigated the lactogenic effect of palm wine in albino Wistar rats using oxytocin levels and body weight as biomarkers. Lactation is a hormone-regulated physiological process essential for offspring survival, with oxytocin playing a key role in milk ejection. Ten lactating rats and their sixty pups were divided into control and experimental groups. The experimental group received palm wine (10 ml/kg) daily for 21 days. Maternal and pup body weights were recorded, while serum oxytocin levels were analyzed using ELISA. Statistical analysis was performed using Student's t-test at $p<0.05$. Results showed a significant reduction in serum oxytocin levels ($p<0.05$) and pup body weight ($p<0.05$) in the palm wine group. Maternal body weight and mammary gland number were not significantly affected ($p>0.05$). These findings suggest that palm wine may impair lactation by reducing oxytocin levels and negatively affecting offspring growth. Further research is needed to clarify its physiological mechanisms.

Keywords: Lactogenic Effect. Oxytocin. Mammary gland.

RESUMO: Este estudo investigou o efeito lactogênico do vinho de palma em ratos Wistar albinos utilizando os níveis de oxitocina e o peso corporal como biomarcadores. A lactação é um processo fisiológico regulado por hormônios essencial para a sobrevivência da prole, sendo a oxitocina fundamental no reflexo de ejeção do leite. Dez ratas em lactação e suas sessenta crias foram divididas em grupos controle e experimental. O grupo experimental recebeu vinho de palma (10 ml/kg) diariamente durante 21 dias. Os pesos corporais maternos e dos filhotes foram registrados, enquanto os níveis séricos de oxitocina foram analisados por ELISA. A análise estatística foi realizada utilizando o teste t de Student com $p<0,05$. Os resultados mostraram uma redução significativa nos níveis séricos de oxitocina ($p<0,05$) e no peso corporal dos filhotes ($p<0,05$) no grupo tratado com vinho de palma. O peso corporal materno e o número de glândulas mamárias não foram significativamente afetados ($p>0,05$). Esses achados sugerem que o vinho de palma pode prejudicar a lactação ao reduzir os níveis de oxitocina e afetar negativamente o crescimento da prole. Mais pesquisas são necessárias para esclarecer seus mecanismos fisiológicos.

Palavras-chave: Efeito lactogênico. Oxitocina. Glândula mamária.

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1. INTRODUCTION

Lactation is a vital physiological process in mammals, encompassing the production and secretion of milk from the mammary glands to sustain offspring during the early stages of life. This complex biological mechanism is orchestrated primarily by hormones, with prolactin responsible for milk synthesis and oxytocin facilitating the milk ejection or let-down reflex (Capuco and Akers, 2009). Oxytocin, synthesized in the hypothalamus and released by the pituitary gland, triggers contraction of the myoepithelial cells surrounding the alveoli, enabling milk flow through the ducts to the nipple, while prolactin regulates the quantity and continuity of milk production (Moore, Anderson and Bergman, 2007). The efficiency of these processes can be influenced by physiological, psychological, and environmental factors, including maternal nutrition, stress, and the consumption of bioactive substances (Mennella and Beauchamp, 1991).

Palm wine, a naturally fermented alcoholic beverage derived from the sap of palm trees such as *Elaeis guineensis* (oil palm) and *Raphia* species, has long been used in various cultures not only as a social and ceremonial drink but also for medicinal and nutraceutical purposes (Riffle and Craft, 2003; Ogbonna, Abuajah, Akpan and Udofia, 2013). Traditionally, palm wine is believed to enhance lactation in postpartum women, a practice widely observed in African societies where new mothers are often encouraged to drink it to increase breast milk production (Peter-Ikechukwu et al., 2016). Biochemically, palm wine contains ethanol, sugars, proteins, vitamins A and C, minerals such as magnesium, zinc, and calcium, and live yeasts, which are thought to interact with physiological pathways regulating lactation (Ouoba et al., 2012; Ogbonna et al., 2013).

Despite its traditional use, the scientific evidence supporting the lactogenic effects of palm wine remains inconclusive. Alcohol, while capable of temporarily relaxing the mother and aiding milk ejection, can adversely affect mammary gland function, reduce protein content, increase lipid synthesis, and alter the quality of milk produced (Do Carmo, do Nascimento, Martín-Hidalgo and Herrera, 1996). Furthermore, alcohol consumption during lactation may influence maternal and offspring body weight, affect pup growth, and modify oxytocin levels, raising concerns regarding safety and efficacy (Mennella, 1999; Isaac, Obeten and Igiri, 2021). These conflicting findings necessitate

controlled experimental studies to elucidate the precise effects of palm wine on lactation.

Animal models, particularly albino Wistar rats, provide a reliable system for evaluating the impact of bioactive substances on lactation due to their well-characterized physiology, reproducible breeding patterns, and measurable lactation biomarkers such as maternal and pup weights, mammary gland development, and circulating oxytocin levels (Snow and Keiver, 2007). Investigating these parameters allows for an evidence-based assessment of palm wine's influence on both maternal and neonatal health, bridging the gap between ethnomedicinal practices and modern scientific validation.

The lactogenic potential of palm wine may be reflected in changes in oxytocin secretion and body weight of both the lactating mother and her pups. Oxytocin not only facilitates milk ejection but also promotes maternal-infant bonding and physiological homeostasis (Uvnäs-Moberg, 1996), while maternal and pup body weight serve as practical biomarkers for overall health and nutritional status (Abdullahi et al., 2023). Evaluating these biomarkers in response to palm wine intake provides critical insight into its efficacy and potential risks during lactation.

Given the widespread use of palm wine in postpartum care and the limited experimental data on its hormonal and physiological effects, this study aims to investigate the lactogenic effect of palm wine in albino Wistar rats, with a focus on oxytocin levels and body weight as biomarkers. The findings are expected to clarify whether traditional claims are supported by scientific evidence, guide safe consumption practices, and inform healthcare providers and lactating mothers about the potential benefits and risks of palm wine during breastfeeding.

2. MATERIALS AND METHODS

2.1 Location of the Study

The experiment was conducted in the Animal House of the Department of Physiology, Delta State University, Abraka. The rats were housed under standard laboratory conditions with a 12-hour daylight and 12-hour night cycle, an ambient room temperature of 28–30°C, and were fed with standard animal feed and water.

2.2 Ethical Approval

The experimental protocol was reviewed and approved by an accredited Animal Ethics Committee (RBC/FBMC/DELSU/26/1216). All procedures followed established ethical guidelines for the care and use of laboratory animals, and every effort was made to minimize

animal discomfort and reduce the number of animals used.

2.3 Experimental Animals

Ten (10) lactating female albino Wistar rats weighing between 125–200 g, along with their 60 pups, were used. The rats were housed individually with their pups in well-ventilated wooden cages with iron netting and sawdust litter. Rats were allowed to acclimatize prior to the experiment, and their diet consisted of commercial rat feed and water.

2.4 Experimental Design

The rats and their pups were randomly divided into two groups of five (5) rats each: Group 1 (Control): Five lactating rats with their respective pups (n = 25) fed with standard rat chow and water only and Group 2 (Experimental): Five lactating rats with their respective pups (n = 25) fed with standard rat chow, water, and treated with 10 ml/kg of palm wine daily via oral gavage. The treatment was administered daily at 10:00 a.m. for 21 days to ensure maximum absorption. Male rats used for mating did not receive palm wine.

2.5 Apparatus

Apparatus used include: wooden cages with iron netting, sawdust, rat feed, water bottles, laboratory coat, gloves, face mask, weighing balance, electronic weight scale, water bath, centrifuge (Hettich Universal 32, Germany), bucket centrifuge, needles and syringes, sample bottles, cotton wool, Pasteur pipettes, oral gastric cannula, Neubauer hemocytometer, refrigerator, and spectrophotometer.

2.6 Reagents

Fresh undiluted palm wine obtained from oil palm sap was used, along with blood collection tubes containing Ethylenediaminetetraacetic Acid (EDTA) and Enzyme-Linked Immunosorbent Assay (ELISA) kits for the oxytocin assay.

2.7 Test Substance Preparation and Administration

Fresh palm wine was collected from oil palm sap every three days, stored at 4°C, and replaced with fresh samples on the fourth day. The dose of palm wine (10 ml/kg) was calculated based on body weight and administered orally via gavage for 21 days.

2.7.1 Animal Feed

The commercial rat feed (Vital Pelletized Growers Feed, Grand Cereals and Oil Mills Ltd., Jos, Nigeria) contained cereals/grains, animal and vegetable protein, minerals, salts, essential amino acids, antibiotics, antioxidants, and vitamin premix. Nutrient composition per label: crude protein 14.5%, fat 7.0%, crude fiber 7.2%, calcium 0.8%, metabolizable energy 2000 kcal/kg. Feed and water were provided ad libitum to all rats.

2.7.2 Mating, Gestation, and Parturition

Female albino Wistar rats at proestrous (determined by vaginal lavage) were caged overnight with sexually mature males of the same strain for mating. Pregnancy was confirmed around 2 weeks by abdominal palpation and mammary gland development. Delivery occurred on average at 21 days of gestation, which was considered day 0 of lactation (L0).

The litter sizes for each group were as follows: in the control group, the five lactating rats gave birth to 10, 10, 3, 5, and 6 pups, respectively, totaling 34 pups. In the experimental

group, the five lactating rats gave birth to 6, 4, 3, 5, and 8 pups, respectively, totaling 26 pups. Reporting litter size is important because it can influence milk production, pup growth,

and maternal physiology. By providing the exact number of pups per dam, the study ensures transparency in experimental design and allows readers to interpret how differences in litter size may affect the outcomes of lactation and the biomarkers measured

in this study.

2.7.3 Body Weight Measurement

The body weight of each lactating rat and their pups was recorded every three days using a digital weight scale.

2.7.4 Mammary Gland and Physical Observation

Mammary glands were observed and counted. Other physical differences between pups and mothers in the control and experimental groups were recorded.

2.8 Blood Collection

On day 21, blood samples were collected from the rats via the medial canthus using capillary tubes into EDTA sample containers. Samples were centrifuged at 2,000 × g for 10 minutes at room temperature (23°C), and the supernatant was used for hormonal assay.

2.8.1 Hormonal Assay (Oxytocin)

Serum oxytocin levels were measured using an ELISA (Randox Laboratories Ltd., UK). Procedure: 25 µl of serum was added to labeled microtitre wells, followed by 100 µl enzyme-conjugated detection antibody and 50 µl rabbit anti-oxytocin reagent. Samples were incubated at room temperature for 90 minutes, washed three times with deionized water, and 100 µl TMB reagent was added. Wells were incubated for 20 minutes, and 100 µl HCl was added to stop the reaction. Absorbance was read at 450 nm using a spectrophotometer. Concentrations were extrapolated from a standard curve plotted using FSH standards (0, 0.5, 3, 10, 25, 50 ng/ml).

2.9 Statistical Analysis

Data were expressed as mean ± SD. Comparisons between control and experimental groups were performed using Student's t-test with SPSS version 20. Statistical significance was considered at $p < 0.05$.

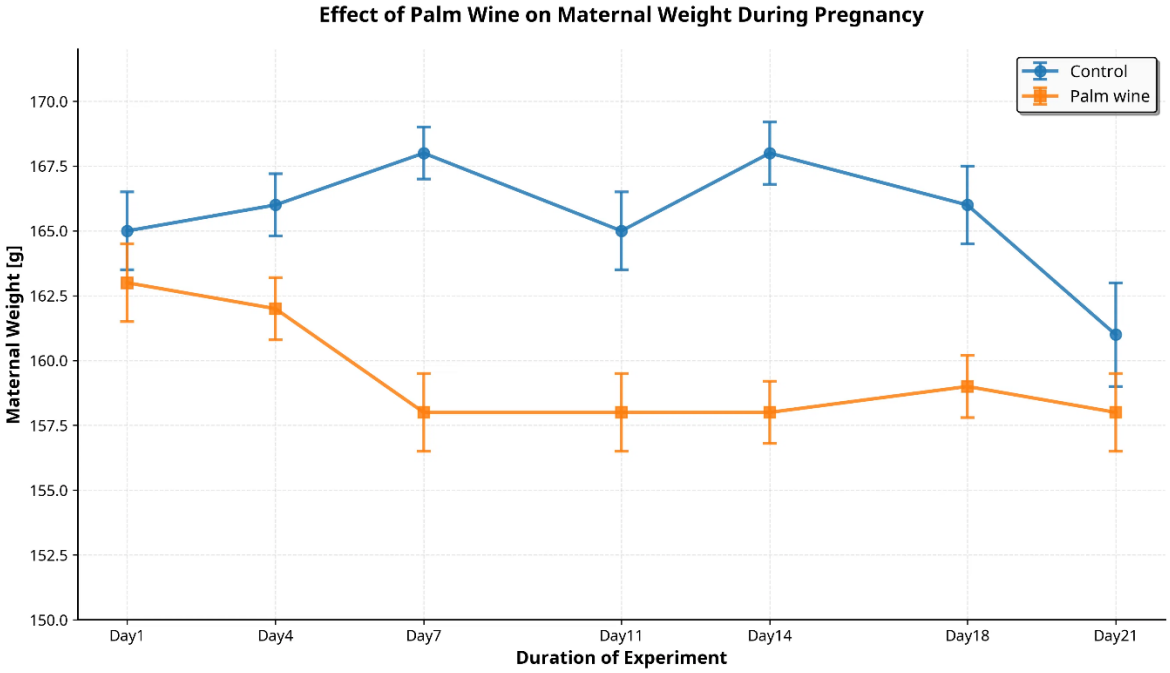
3. RESULTS

The study investigated the effects of Palm wine on lactating albino Wistar rats, focusing on maternal body weight, pup weight, mammary gland number, and serum oxytocin levels. The findings are presented in four parts, each describing the impact of Palm wine on a specific parameter.

3.1 Effect of Palm wine on Maternal Body Weight of lactating albino Wistar rats

The effect of Palm wine on the maternal body weight of albino Wistar rats was evaluated throughout the experimental period. Results indicate that Palm wine administration caused a decrease in maternal body weight when compared to the control group. However, this reduction was not statistically significant ($p>0.05$).

Figure 1: Effect of Palm wine on Maternal Body Weight of albino Wistar rats

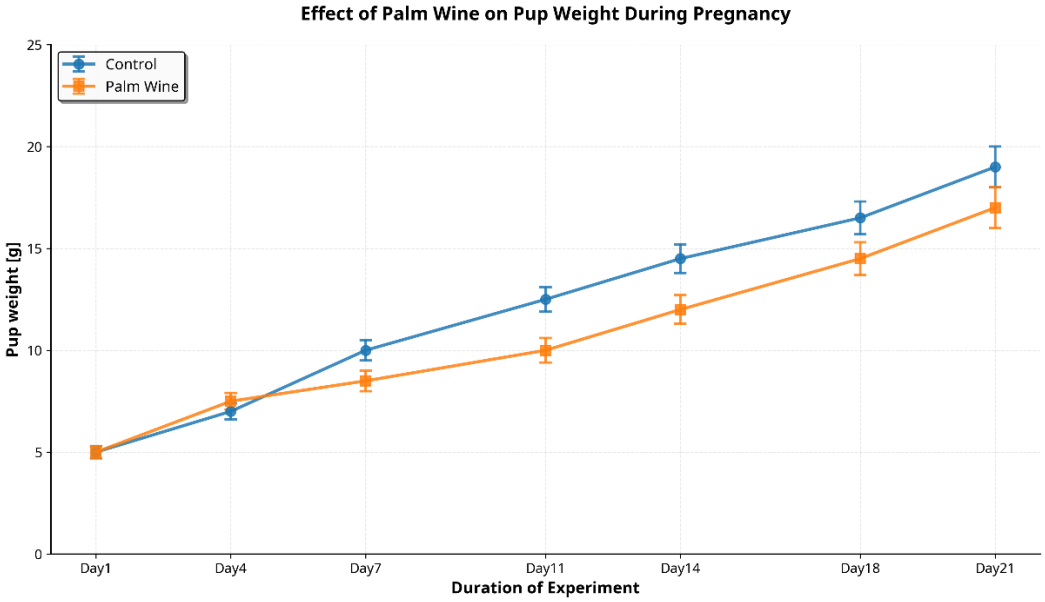


3.2 Impact of Palm wine on weight of Pup weight of albino Wistar rats

The effect of Palm wine on the Pup weight of albino Wistar rats. It was observed that there was consistent increase in pup weight of the litters in both groups, however Palmwine caused decrease in pup weight when compared to control. It was recorded that this decrease in pup weight of Palmwine administered rats was significant ($p<0.05$) after seven (7) days of

treatment.

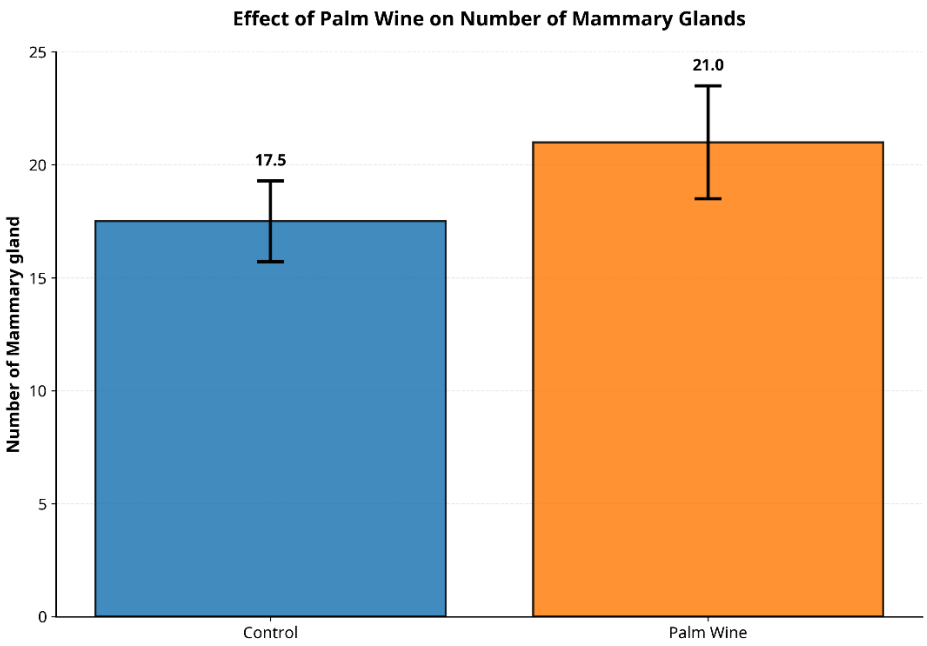
Figure 2: Effect of Palm wine on weight of Pup weight of albino Wistar rats



3.3 Palm wine's effect on the number of mammary gland of lactating albino Wistar rats

The number of mammary glands in Palm wine-treated rats showed a slight, non-significant increase compared to controls ($p>0.05$), indicating no substantial effect on mammary gland development.

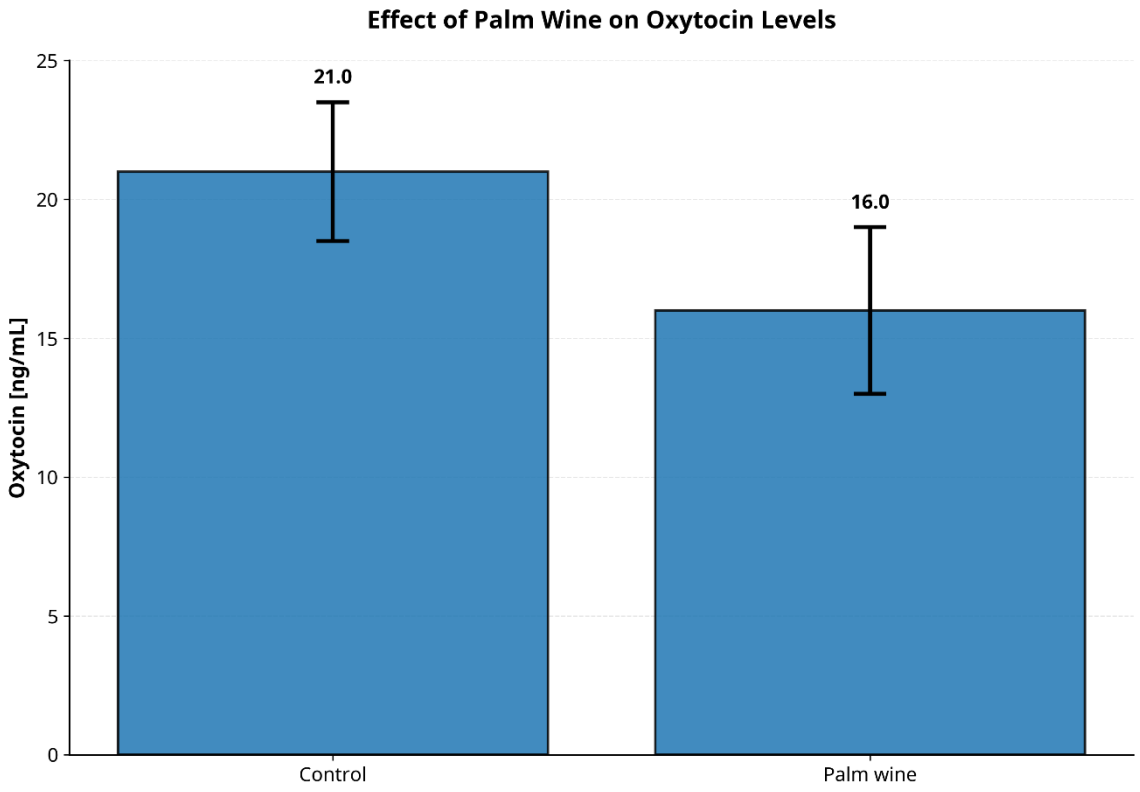
Figure 3: Effect of Palm wine on number of mammary gland of female albino Wistar rats



3.4 Effect of Palm wine on serum Oxytocin level on lactating albino Wistar rats

Serum oxytocin levels were measured to assess hormonal changes associated with lactation. The administration of Palm wine resulted in a significant decrease in serum oxytocin levels in treated rats compared to the control group ($p<0.05$).

Figure 4: Effect of Palm wine on serum Oxytocin level of albino Wistar rats



4 DISCUSSION

Palm wine is a traditionally fermented beverage obtained from the sap of various palm species and contains ethanol as a result of natural fermentation. In addition to carbohydrates, palm wine may contain trace amounts of proteins, vitamins, and minerals, although its nutritional composition varies depending on fermentation duration and storage conditions (Ogbonna, Abuajah, Akpan and Udofia, 2013; Zongo et al., 2020). It is widely consumed during social and cultural events and is traditionally believed in some communities to possess medicinal properties (Riffle and Craft, 2003).

In the present study, palm wine administration resulted in a reduction in maternal body weight compared to the control group, although this decrease was not statistically significant. This observation is consistent with previous reports that documented a non-significant

reduction in body weight following palm wine administration for twenty-one days (Zongo et al., 2020). The observed reduction in body weight may be related to altered metabolic activity and the ethanol content of palm wine, which has been shown to affect physiological regulation of energy balance and body weight in experimental studies (Isaac, Obeten and Igiri, 2021).

Assessment of mammary gland development revealed a slight increase in the palm wine-treated group compared to the control group; however, this difference was not statistically significant. Since mammary gland number is anatomically fixed in adult rats, the observed variation may reflect minor differences in mammary tissue development rather than an actual increase in gland number. These findings suggest that palm wine does not significantly enhance mammary gland development during lactation.

Pup weight gain was significantly reduced in the palm wine-treated group from day seven of lactation onward. Pup growth is highly dependent on adequate milk intake, and reduced weight gain may indicate impaired lactational performance. Oxytocin plays a central role in milk ejection by stimulating contraction of myoepithelial cells surrounding the mammary alveoli (Uvnäs-Moberg, 1996; Mennella, 2001). In this study, palm wine administration caused a significant decrease in serum oxytocin levels compared to the control group. Reduced oxytocin levels may impair milk let-down, thereby limiting effective milk transfer to the pups and contributing to reduced pup weight gain (Mennella, 2001). Previous studies have shown that alcohol exposure can interfere with neuroendocrine regulation of maternal hormones involved in lactation and mother–infant interaction (Mennella, 2001). Therefore, the observed reduction in oxytocin levels in palm wine-treated rats may be attributed, at least in part, to the ethanol content of palm wine.

5. CONCLUSION

In conclusion, based on the findings of this study, palm wine administration in lactating albino Wistar rats was associated with reduced maternal body weight, significantly decreased pup weight gain, and a significant reduction in serum oxytocin levels. Palm wine did not produce a significant effect on mammary gland development. The reduction in oxytocin levels suggests a potential impairment of milk ejection, which may account for the observed decrease in pup growth. These results indicate that palm wine does not enhance lactational performance and may, in fact, exert adverse effects on lactation, likely due to its alcohol content. Caution is therefore advised regarding the use of palm wine as a lactogenic agent. Further studies are

recommended to investigate the underlying mechanisms and long term effects of palm wine consumption on maternal and offspring health.

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AUTHOR'S CONTRIBUTION

Arawore Aghogho Joy and Okah Miracle Oluwaseun, contributed to the conception and design of the study and drafting of the manuscript Arawore Aghogho Joy and Okah Miracle Oluwaseun conducted the experimental procedures, including animal handling, treatment administration, and performed the statistical analysis. Arawore Aghogho Joy carried out experimental procedures, data collection, data interpretation and laboratory procedures. Both authors contributed immensely to the revision of the manuscript for intellectual content and approved the final draft for publication.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

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