FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 8, August, 2025, pp 222 – 230
DOI: https://doi.org/10.33003/fjs-2025-0908-3917

ETHYL ACETATE FRACTION OF *HIBISCUS SABDARIFFA* L. (*MALVACEAE*) ENHANCE LACTOGENIC ACTIVITY AND DOWN REGULATE OXYTOCIN RECEPTOR MRNA GENE EXPRESSION IN RAT MAMMARY GLAND

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ABSTRACT

In under developed countries some local people traditionally use herbs to boost milk production or induction of lactation than the antipsychotic drugs that have serious side effects like drowsiness and depression. The objective of this study was to determine molecular pathway for mRNA gene expression of Oxytocin receptor (OTR) in lactogenic effect of Hibiscus sabdariffa L. Fifteen lactating Wistar rats grouped into five (n=5), control group received 1 ml/kg of distilled water, metoclopramide group received 5 mg/kg and ethyl acetate fraction of Hibiscus sabdariffa L group received 800 mg/kg for 14 days. Serum, milk production, milk yield, mammary gland and pup weight (daily) were collected for analysis. Results showed that the ethyl acetate fraction of Hibiscus sabdariffa L. increased significantly ($p<.05$) daily milk yield [(0.72ml) (0.52ml)] and pup weight [(3.3ml) (2.5ml)] compared to the control group. It also increased the level of oxytocin [(19.26±1.3ng/mL) (10.18±1.5ng/mL)], oestradiol [(565.4±28ng/mL) (562.3±38ng/mL)], progesterone [(64.5±19ng/mL) (46.3±12ng/mL)], Thyroxine (T3 & T4) [(1.54±0.1ng/mL) (1.02±0.1ng/mL)] [(73.2±2.8ng/mL) (55.6±2.2ng/mL)] and growth hormone [(54.6±2.1ng/mL) (29.0±1.7ng/mL)] significantly ($p<.05$). Mammary gland of ethyl acetate fraction of Hibiscus sabdariffa L. group showed clear lobuloalveolar development and proliferation of myoepithelial cells, with striking variations observed among the groups. The ethyl acetate fraction of Hibiscus sabdariffa L. down regulate the mRNA expression of OTR receptor [(20.42IU/ml) (21.82IU/ml)]. In conclusion, ethyl acetate fraction of Hibiscus sabdariffa L. enhance lactogenic activity through increasing hormonal level, lobuloalveolar development of the mammary gland and down regulates mRNA gene expression of OTR receptor in rats.

Keywords: Milk, Lactation, Oxytocin, mRNA, Expression, Hibiscus, Sabdariffa

INTRODUCTION

Milk synthesis and secretion by the mammary gland involve numerous cellular pathways and processes (AAP, 2005; Freeman *et al.,* 2000). The processing and packaging of nutrients within human milk changes over time as the recipient infant matures (Cai *et al*., 2015; Sani *et al.,* 2020). Human breast milk has overwhelming advantage as infant's source of nutrition (UNICEF, 2006; Okasha *et al.,*2008). Although it has long been accepted that prolactin is involved in the development of the mammary gland, recent elegant techniques have confirmed such findings (Freeman *et al.,* 2000; Emmanuel *et al*., 2024b).

Apparently, prolactin reaches the milk by first crossing the mammary epithelial cell basement membrane (Ogweje *et al.,* 2019; Emmanuel *et al*., 2024a), attaches to a specific prolactin binding protein within the mammary epithelial cell, and is ultimately transported by exocytosis through the apical membrane into the alveolar lumen (Guyton & Hall, 2006; Freeman *et al*., 2000). The varied effects of prolactin on the mammary gland include growth and development of the mammary gland, synthesis of milk, and maintenance of milk secretion (Ganong, 2007; Sani *et al.,* 2019). It should be noted that none of these actions is solely due to prolactin, but the hormone is merely a player in an orchestra of hormones and growth factors that affect the mammary gland (Sembulingam & Sembulingam, 2012; Freeman *et al*., 2000).

In the process of lactogenesis, prolactin stimulates uptake of some amino acids, the synthesis of the milk proteins casein and α-lactalbumin, uptake of glucose, and synthesis of the milk sugar lactose as well as milk fats (Freeman *et al.,* 2000; Emmanuel *et al*., 2024a). There is evidence that insulin, growth hormone, thyroid hormone, parathyroid hormone, calcitonin, several growth factors (Freeman *et al.,* 2000; Okasha *et al.,*2008; Bako *et al.,* 2013) play a role in galactopoiesis. The study is designed to determine molecular pathway for mRNA gene expression of Oxytocin receptor (OTR) in lactogenic effect of *Hibiscus sabdariffa* L.

MATERIALS AND METHODS

Materials

Hibiscus sabdariffa L. Seed (voucher specimen number1056) were purchased from an agricultural market (Gaya, Adamawa-Nigeria) and were kindly authenticated by Prof. A.Y. Bashir (Department of Botany Ahmadu Bello University, Zaria-Nigeria). The commercial radioimmunoassay kits for Oxytocin (OT), oestradiol (E_2), Progesterone (P), Triiodothyronine (T_3), Tetraiodothyronine (T_4), Growth Hormone (GH), Primers for OTR were purchased from AccuPower® 2X GreenStar™ qPCR MasterMIX, BIONEER Inc. 1301 Marina Village parkway suite 110 Alameda CA, 94501 USA. centrifuged (Hawksley RegNo891481 Ser. No. 07.4.26.®), Homogenizer, Metoclopramide, ketamine, diazepam, ethyl acetate reagents (Sigma Aldrich chemical company Germany) for fractionating the seed extract *Hibiscus sabdariffa* L. All chemical reagent and drugs used were of analytical grade.

Preparation of the Ethyl Acetate Fraction of Hibiscus sabdariffa L. Seed Extract

The extraction of *Hibiscus sabdariffa L.* seed was conducted in Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria. The *Hibiscus sabdariffa* L. seeds were washed thoroughly, shade dried and ground into powder**.** Maceration method was used for aqueous extraction of the seed. Two hundred grams (200 g) of the powdered *Hibiscus sabdariffa* L. seeds were soaked and the mixtures were then shaken for ten hours with mechanical shaker.

The mixtures were macerated and the supernatant liquid (extract) was filtered through a plug of cotton or glass wool (Trease & Evans, 2009). The process was repeated for complete extraction and the extracts were then poured into evaporating dish to evaporate the solvent in the extract over the water bath at the temperature of 40ºC – 45 ºC which yielded (32%) yellowish golden extract weighing 63.5 g. The seed extract was fractionated using the ethyl acetate reagents. Mixtures of basic plants constituents are often separated by fractional liberation. The extract was dissolved in distilled water and the solution was decanted, then ethyl acetate was first used and shaken well for weak constituents to be removed. The mixtures are then gradually basified in increasing order with the addition of an aliquot of ethyl acetate solution at a time and after each addition the mixture is shaken with the organic solvent and fractions are collected separately. The ethyl acetate part was dried and the constituent scribed, weighed (4.6 g) and kept in refrigerator.

Animal Management

Ethical approval was obtained from the Ahmadu Bello University ethical committee on animal use and care with approval number: ABUCAUC/2025/125. The pheromonal induction of estrus was carried out by exposing grouped adult nulliparous females to the bedding materials of the male rats for a period of three days according to the method of Bronson & Whitten (1968). At about 6:00 pm the day 3, the adult female rats were mated with apparently healthy male Wistar rats at the ratio of 1:1. This cohabitation were allowed to continue until obvious signs of pregnancy is observed in the female Wistar rats. By day thirteen (13) of gestation the abdominal enlargement was visible alongside mammary gland development and nipple enlargement according to the method of Baker (1979).

The female Wistar rats were separated from the male Wistar rats and housed separately until parturition. At parturition, the female Wistar rats were randomly grouped into three (3) groups of five (n=5) each and the number of the pups culled to six (6) pups per dam. Administration of ethyl acetate fraction of *Hibiscus sabdariffa* L, metoclopramide and distilled water were from day (2) to day 13 post parturition.

Evaluation of Lactogenic Effect of ethyl acetate fraction of Hibiscus Sabdariffa L. in rats

Milk yield was estimated daily 18 and 23 hours after gavage according to the method described by Sampson & Jansen (1984). Following administration of ethyl acetate fraction of *Hibiscus Sabdariffa* L and metoclopramide at 18:00 pm on day 2 post parturition, the pups from each dam was weighed using an electronic balance at 07:00 am the next morning and the weight recorded as (w1). Afterward the pups are separated from the dams for a period of 4 hours. At 11:00 am, the weight of the pups was taken again and recorded as (w2). The pups were then re-united with their respective dams and allowed to suckle for a period of 1 hour (11:00 to 12:00 pm) after which the pup's weight was taken and recorded as (w3). This brought to the end of the milk yield assessment 18 hours after gavage.

Milk yield 23 hours after gavage was evaluated in a similar manner. At 12:00 pm the pups were separated from their respective dams and allowed to stay for another four hours until 16:00 pm. The pup's weight was again taken and recorded (w4) at 16:00 pm and afterward reunited with their respective dams and allowed to suckle for an hour (17:00 pm). The final weight was taken and recorded (w5), after which administration of drug and ethyl acetate of *Hibiscus Sabdariffa* L was given at 06:00 pm. Daily milk yield were corrected for weight loss due to metabolic processes in the pups (respiration, urination and defecation) during suckling. The values obtained were multiplied by the number of suckling hours per day and then added to the daily sucking gain.

The milk yield (18 and 23) hours after gavage will be calculated using the relationship described below:

Milk yield 18 hours after gavage = $w3 - w2$

w2 = Pre-suckling weight of pups (11:00 am)

Where w3 = Post suckling weight of pups (12:00 pm)

Correction for weight loss due to metabolic processes will be calculated:

Weight loss correction 18 hours after gavage = $\frac{w2 - w1}{4}$

(Morag, 1970; Sampson & Jansen, 1984: Lompo-Ouedraogo *et al*., 2004; Bako *et al*., 2013).

Where w2 = pre-suckling pups' weight, w1 = pre-isolation pups' weight

Milk yield 23 hours = $w5 - w4$

W4 = Pre-suckling weight of pups (16:00 pm)

Where w5 = Post suckling weight of pups (17:00 pm)

Weight loss correction 23 hours after gavage = $\frac{[(w2-w1)]+(w4-w3)]}{8}$

Oxytocin, Oestradiol, progesterone, Triiodothyronine, Tetraiodothyronine and Growth Hormone Assay

After the litter weights were recorded on day 16 of lactation, all dams were anesthetized with intraperitoneal injection of ketamine and diazepam at 75 and 5 mg/kg respectively (Molina *et al.,* 2015). Then, blood samples were collected by venipuncture and placed in a plane test tube. The clotted blood samples were centrifuged (Hawksley RegNo891481 Ser. No. 07.4.26.®) at 3000 rpm for 15 minutes and serum was stored at -20°C until use. Radioimmunoassay techniques were performed to quantify hormone concentrations in serum (Ann and Linzell, 1975; Reiter *et al*., 1995). All assays were performed in triplicate. The inter- and intra-assay coefficients of variation were less than 10% for all hormones.

Determination of Oxytocin Receptor (OTR) mRNA Expression in Mammary Gland Tissue Using Real Time Polymerase Chain Reaction (RT-qPCR)

The mammary gland tissue was dissected out from the inguinal region and snap frozen in liquid nitrogen stored at -80°C for further analysis in ACENTDFB Ahmadu Bello University Zaria. Mammary glands tissues were homogenized at 4°C, washed in an ice-cold phosphate-buffered saline buffer (pH 7.4). The homogenized tissue was optimized for appropriate annealing temperature for the mRNA expression. Homogenized sample was placed in a sterile 1.5 ml Eppendorf tube, after which 500 µL of RB buffer added and centrifuge the lysate for 3 minutes at full speed and the supernatant transferred to a new 1.5 ml tube. 200 µL of ethanol was added and mixed immediate using pipette and 400 µL of RB buffer was added to cell pellet and mixed with vortex mixer. 300 µL of ethanol (80%) was added and mixed using pipette. The

sample transferred to a binding column and centrifuged at 14000 rpm for 20 sec. This set up was incubated for ten minutes at room temperature. 100 µL of isopropanol was added then mixed by lightly vortexing for 5 seconds and spined down for 10 seconds to allow the liquid clinging on the walls and lid to go down. The binding column was then fixed into 2 ml collection tube and the liquid emptied into the binding column while avoiding getting the lid wet. The lid was closed carefully and centrifuged for 1 minutes at 8000 rpm. Subsequently, the binding column was transferred to a new collection tube and 500 µL of W1 buffer was added to the column and lid closed and centrifuged for 1 minute at 8000 rpm. The binding column was transferred to another new 2 ml collection tube and 500 µL of W2 buffer added and lid closed and centrifuged again at 8000 rpm. The set up was spined down once more at 13,000 rpm for 2 minutes to remove the ethanol completely. It was ensured that no droplet was hanging from the bottom of the binding column. The binding column was then transferred to a 1.5 ml collection tube and 50 µL of elution buffer was added and allowed to stand for 5 minutes allowing the buffer to permeate the column. The eluted RNA solution was obtained by spinning down at 8000 rpm for 2 minutes and used for this study together with the primers table 1. First-strand cDNA synthesis and amplification of cDNA were performed using reverse transcriptase polymerase chain reaction (PCR) was performed on the samples which were subjected to the following mix and condition for the PCR cocktail mix in table 2. and temperature profile in table 3.

Table 1: Oligonucleotide Commercial Primers used for RT-PCR

Oligonucleotides	Sequence	Location of Gene
Rat OXTR	Primer (forward) 5'CGATTGCTGGGCGGTCTT3'	325 – 345
	Primer (reverse) 5'CCGCCGCTGCCGTCTTGA3'	668 – 649
Rat β-actin	Primer (forward) 5' TTGTAACCAACTGGGACGATATGG 3'	1552 – 1575
	Primer (reverse) 3' GATCTTGATCTTGATGGTGCTGCTAGG 5'	2991 – 2844

Table 2: PCR Cocktail Mix

PCR Cocktail Mix	Quantity
10XPCR buffer	2.5
25 mM MgCl2	1.0
5p Mol forward primer	1.0
5p Mol reverse primer	1.0
25mM DNTPs	2.0
Taq 5µ/µl	0.2
50ng/µl	3.0
H2O	13.3
Reaction Mix	25 µl

Table 3: Temperature Profile

Pre-denaturation	Denaturation	Primer Annealing	Temp	Extension	Final Extension
Temperature	95℃	95℃	50℃	72℃	72℃
Duration (s)	900	10	15	30	60

Histological Examination of Mammary Gland

The two inguinal mammary glands were excised using a pair of sterile scissors and forceps from experimental animals. A portion of the mammary gland tissue were fixed in 10% formalin. Afterwards, the tissues were then dehydrated by passing it through alcohol solutions of increasing concentrations for 15 minutes each. Subsequently, the tissues were cleared using xylene for 20 minutes and embedded in paraffin wax into blocks then clamped into microtome and cut into sections between 5-8 microns. Then, sections were stained with Harris's hematoxylin and eosin according to standard procedures. Tissue preparation was observed and microphotographed under a light microscope.

Data Analysis

The values obtained are expressed as the mean – standard error of mean (SEM) for five animals in each group. The data were analysed using SPSS 22.0 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Values with $p<0.05$ were considered statistically significant

RESULTS AND DISCUSSION

Some Lactogenic Hormones Content of Serum

Hormone concentrations were measured in the serum and the results are shown in Table 4. Oxytocin (OT) serum levels in the ethyl acetate of *Hibiscus sabdariffa* L. group showed remarkable variations among the studied groups ($P<0.01$). The other hormone concentrations, including E_2, P, T_3, T_4 and GH, from the ethyl acetate of *Hibiscus sabdariffa* L. group were significantly higher than the control group. Moreover, the hormone levels of ethyl acetate of *Hibiscus sabdariffa* L. groups were all higher than those of positive groups, especially the P ($P<0.05$), E_2 ($P<0.05$), T_3 ($p<0.05$), T_4 ($p<0.05$). These indicated that the ethyl acetate of *Hibiscus sabdariffa* L. was effective for increasing hormone concentrations in the circulation.

Table 4 Serum Lactogenic Hormones in Lactating Rats

Groups	OTR ng/mL	E2 ng/mL	P ng/mL	GH ng/mL	T3 ng/mL	T4 ng/mL
Control	10.18±1.5	562.3±38	46.3±12	29.0±1.7	1.02±0.1	55.6±2.2
Metoclopramide	14.98±2.6a	560.4±28	54.7±23a	31.2±2.2a	1.28±0.1a	65.4±4.1a
Ethyl acetate	19.26±1.3b	565.4±28a	64.5±19b	54.6±2.1b	1.54±0.1a	73.2±2.8b

a = ($p<0.05$); b = ($p<0.01$)

Effect of ethyl Acetate Fraction of *Hibiscus sabdariffa* L. on Milk Yield at 18 and 23 Hours

Milk production of both the groups receiving 5 mg/kg metoclopramide and 800 mg/kg of ethyl acetate fraction of *Hibiscus sabdariffa* L. was significantly higher than that of the control group as shown figure 1a. Milk production increased from 1.56±0.2, 1.92±0.3 and 1.82±0.2 g/pup/per day to about 3.67±0.1, 4.1±0.3 and 5.78±0.2 g/pup per day for the control, metoclopramide and ethyl acetate group respectively. The significant differences observed started from day 4 until the end of treatment day 13 ($P<0.01$). The mean milk yield was 2.5±0.4, 2.6±0.3 and 3.36±0.4 g/pup per day throughout the experimental period respectively ($P<0.05$) as shown in figure 1b. Milk yield data at 18 and 23 hours after gavage showed that milk yield was significant in group receiving ethyl acetate fraction. The mean milk yield for the metoclopramide group was 0.42±0.02 and 0.52±0.03 g/pup at 18 hours after gavage with normal saline respectively. For the group receiving the ethyl acetate fraction, the milk yield was 0.64±0.04 and 0.72±0.03 g/pup at 23 hours after treatment respectively figure 1c. The mRNA expression for OTR receptor of real time polymerase chain reaction RT – PCR showed no statistical significance when ethyl acetate fraction of *Hibiscus sabdariffa* L. [(21.82 IU/ml)] and metoclopramide group [(21.52 IU/ml)] compared with the control group [(20.42 IU/ml)] figure 2.

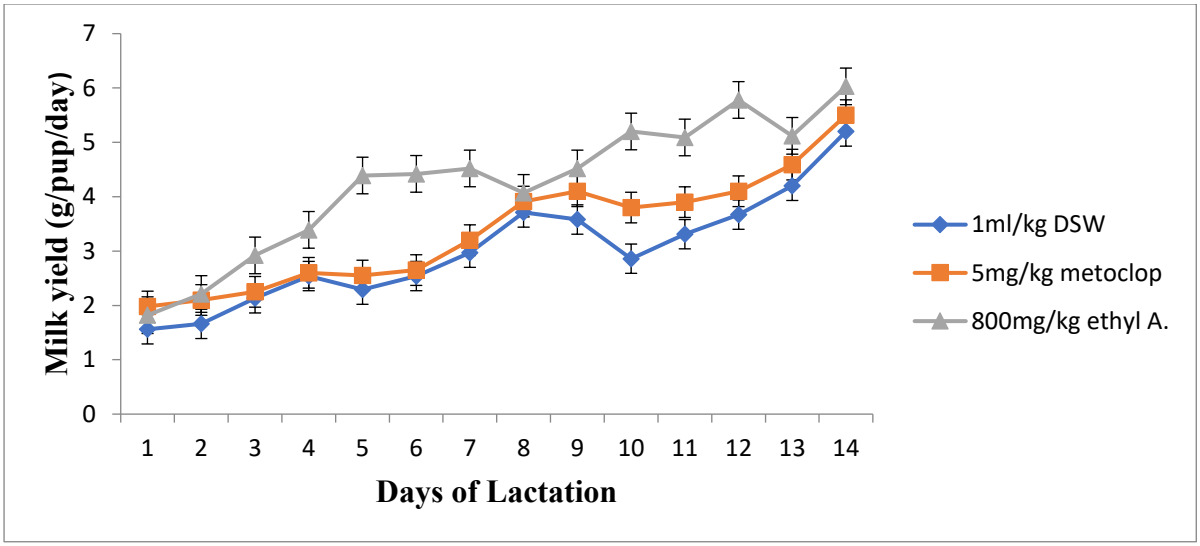


Figure 1a: Effect of Treatment with 800 mg/kg ethyl Acetate Fraction of *Hibiscus Sabdariffa* L. on Daily Milk Production 23 hours after Administration in Rats

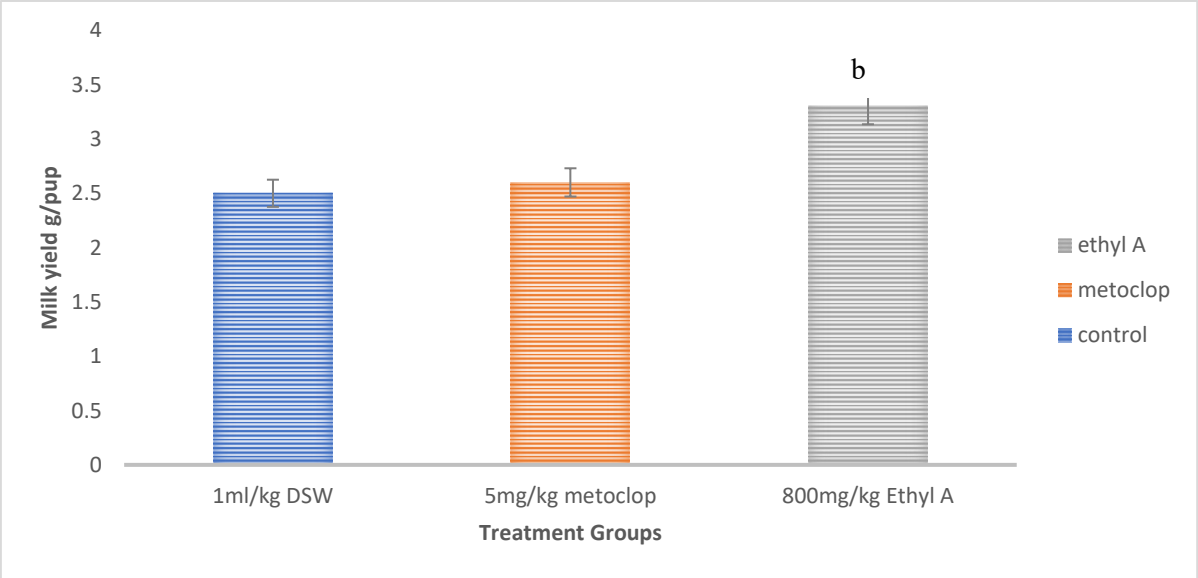


Figure 1b: Average Milk Yield per day in Rats Treated with 800mg/kg ethyl Acetate Fraction of *Hibiscus Sabdariffa* L. for 14 days. b = ($p<0.01$)

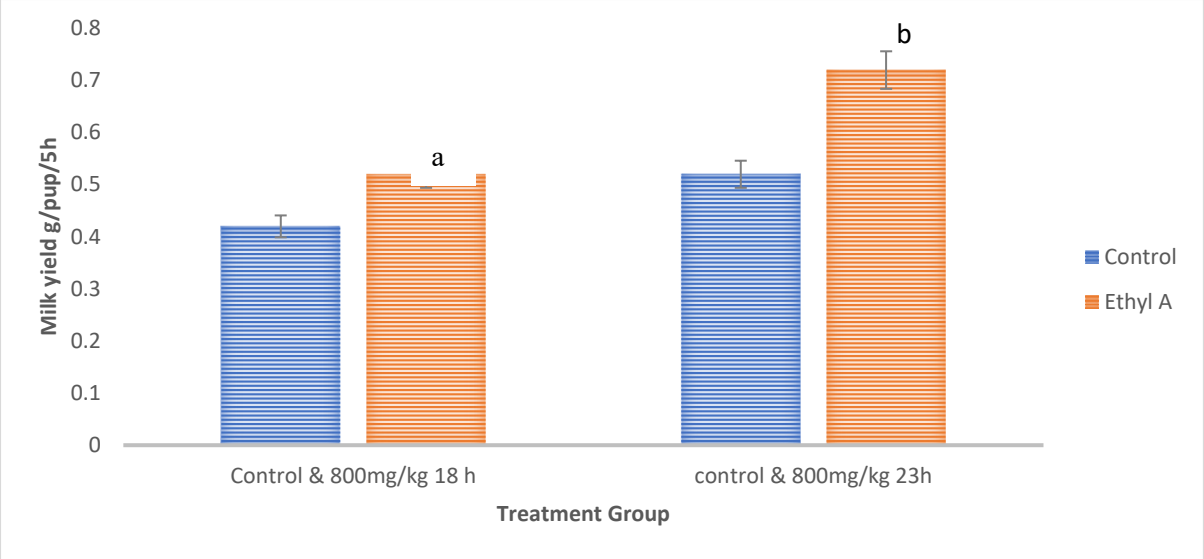


Figure 1c: Milk Yield at 18 hours and 23 hours after Treatment with 800 mg/kg ethyl Acetate Fraction of *Hibiscus Sabdariffa L.* in Rats a = ($p<0.05$); b = ($p<0.01$)

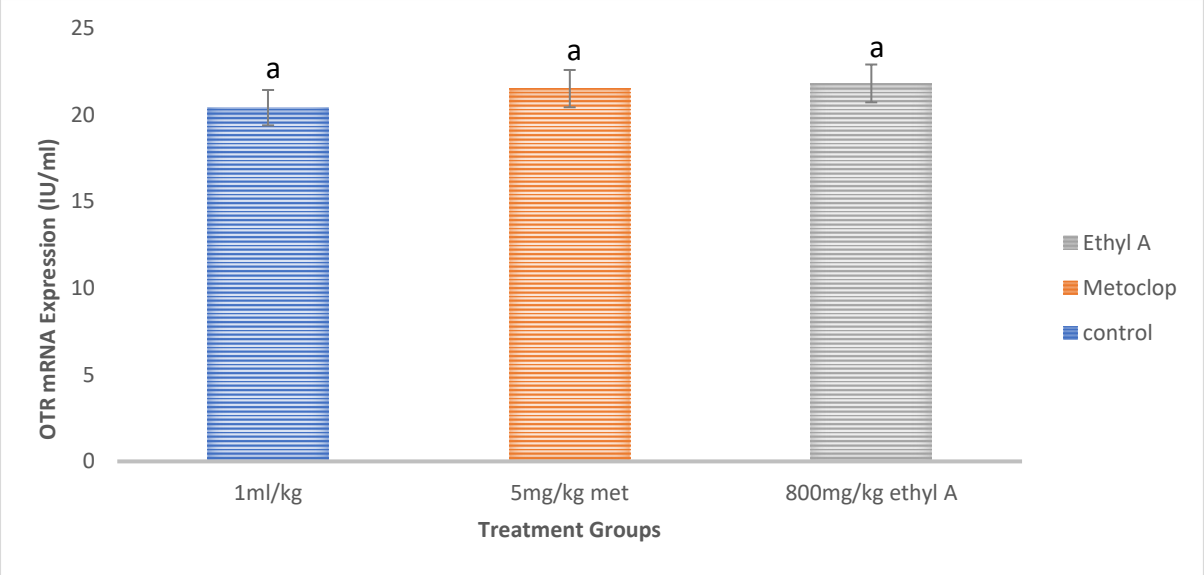
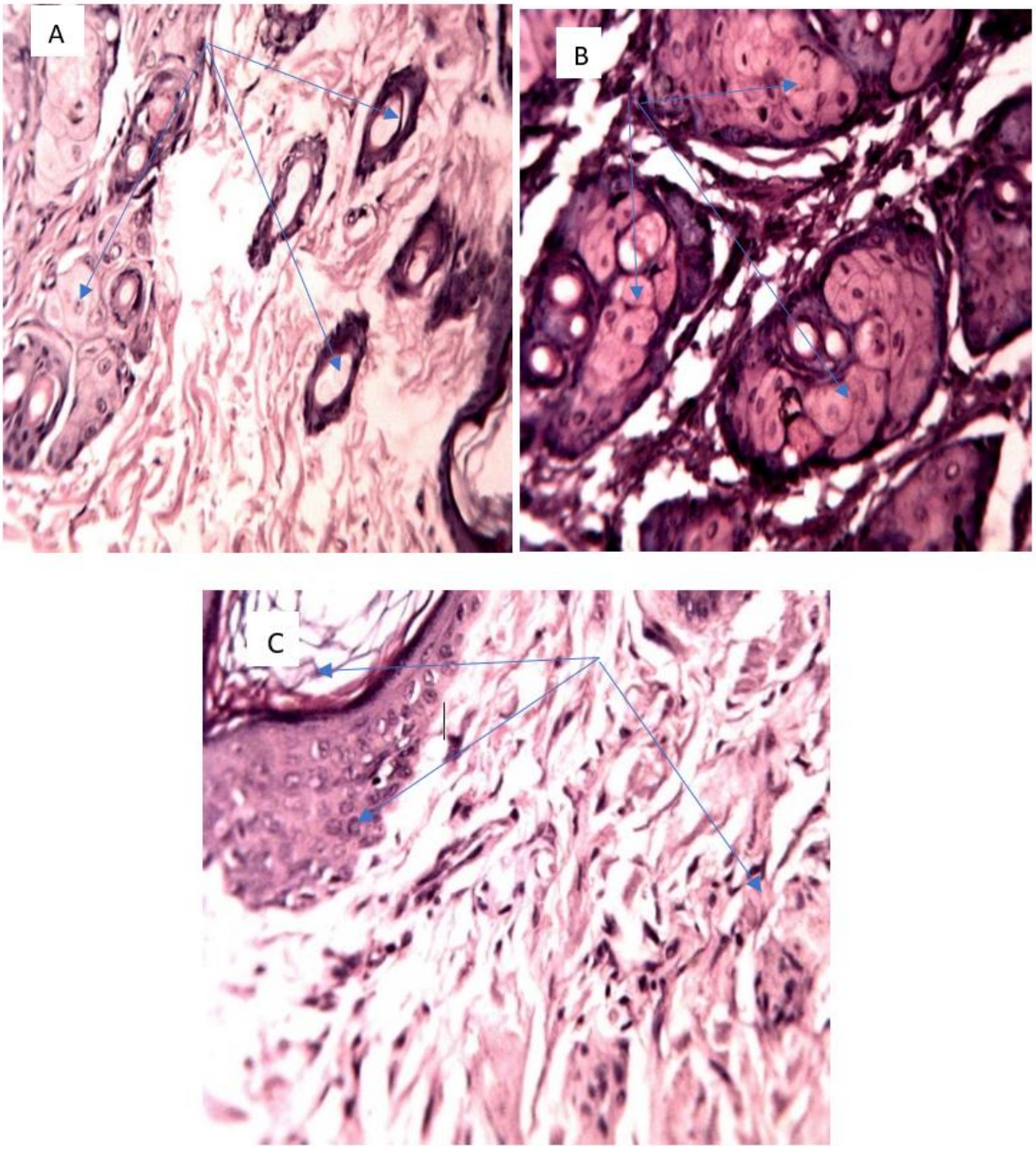


Figure 2: Oxytocin Receptor mRNA Expression in Lactating Wistar rats Following 14 days Oral Administration of Metoclopramide and Ethyl A Fraction a = ($p>0.05$)

Histology of Mammary Tissues

The histology of mammary gland was to understand the relationship between hormones and mammary tissue growth Photomicrograph 1. Mammary gland morphology showed striking variations among the studied groups. Mammary gland histological sections shown in Photomicrograph 1C were from lactating rats control group, showing little mammary lobules and ductile development and mild hyperplasia of myoepithelial cells. The duct was lined mostly by single-layer cells, some containing secretory material and the amount of surrounding stromal fibroblasts was decreased. The mammary gland of the Ethyl acetate fraction of *Hibiscus sabdariffa* L. Photomicrograph 1B and metoclopramide Photomicrograph 1A groups showed apparent proliferation of myoepithelial cells and more alveolar bud development and ducts branching into ductile. This histology results provided evidence that the *Hibiscus sabdariffa* L. can stimulate lobuloalveolar development, which were the same results demonstrated by other lactation-promoting medicinal plants. These results indicate that the *Hibiscus sabdariffa* L. increases milk production by causing lobuloalveolar development and the increasing of myoepithelial cell amounts leading to milk ejection. There is marked variations in *Hibiscus sabdariffa* L. group than the metoclopramide group when compared to the control group.



Photomicrograph 1: Mammary gland Histology and Morphometric analysis of the dams. (A) Metoclopramide group (B) Ethyl acetate fraction of *Hibiscus Sabdariffa L.* group (C) control group

Discussion

The optimal quantity and volume supply of breast milk has strong impact on the development of the offspring and its health status. This unique fluid actually evolves to meet the changing needs of the baby during growth and maturation. The measurement of milk production rates in rats is difficult (Rhoades & Pflanzer, 2003; Bako *et al*., 2013), however a more feasible direct method is to use the pups to remove the milk from the dam and determine the amount of milk sucked by the litter (Lompo-Ouedraogo *et al.,* 2004; Bako *et al.,* 2014).

In this study the hormonal parameters, milk production, milk yield, mammary gland histology and mRNA gene expression were all evaluated. All the lactogenic hormones significantly increased significantly, which potentiates the lactation process by Ethyl acetate fraction of *Hibiscus sabdariffa* L. Oxytocin is one of the principal lactogenic hormones that act on the myoepithelial cells in the wall of the mammary ducts to contract and squeeze the preformed milk out through the nipples (Freeman *et al*., 2000: Okasha *et al.,*2008; Emmanuel *et al*., 2024b). Estrogen stimulates the growth and proliferation of the duct system of the gland (Freeman *et al*., 2000). It induces fat deposition, increase in blood supply, development of the nipple and darkening of the areolar with permissive factors of other hormones like growth hormones, insulin and Thyroxine (Freeman *et al*., 2000; Simelane *et al.,* 2012). Progesterone stimulates the development of the secretory acini of the gland (Freeman *et al*., 2000; Lompo-Ouedraogo *et al.,* 2004). Milk yield estimation for rats by means of pup weight and weight gains have been used in several studies (Freeman *et al*., 2000; Rhoades & Pflanzer, 2003). Ethyl acetate fraction of *Hibiscus sabdariffa* L.

stimulates milk secretion by release of prolactin (PRL) (Okasha *et al.,*2008; Bako *et al.,* 2014).

For the synthesis and secretion of milk, the mammary gland must receive hormonal signals. PRL acts as a physiological sensor that responds to the demands for milk production by partitioning nutrients away from the adipose tissue in favour of the mammary gland (Ben-Jonathan *et al.,* 2006). Ethyl acetate fraction of *Hibiscus sabdariffa* L. contains bioactive nutrients with high levels of taurine and Aspartate that have been associated with endocrine regulation (Arias *et al*., 1998; Rhoades & Pflanzer, 2003). Taurine, a putative inhibitory amino acid neurotransmitter, has been shown to stimulate PRL release (Rhoades and Pflanzer, 2003; Nzikou *et al.*, 2011). During 14 days of lactation, the increased PRL concentration of serum in the ethyl acetate fraction of *Hibiscus sabdariffa* L group compared with control dams was closely related to high milk yield due to the essential role of PRL for galactopoiesis in rats. Moreover, in the ethyl acetate fraction of *Hibiscus sabdariffa* L-treated group, GH was significantly higher than in the control and metoclopramide group in the mammary gland. Both PRL and GH interact in regulating milk synthesis in the mammary gland through enhancing mammary epithelial cell survival (Tonner *et al.,* 2000) indicating that the ethyl acetate fraction of *Hibiscus sabdariffa* L was beneficial for myoepithelial cell proliferation. It is known that PRL and GH are essential for mammary growth, a combination of E_2 and P is required for lobuloalveolar development of the mammary glands (Tucker, 2000; Horigan *et al.,* 2009). P is essential during lactogenesis for ductal side branching and alveologenesis, acting through receptors on stromal and epithelial cells (Zaidi *et al.,* 2006). Thus, the increased milk production in lactating rats is due to the increase of cell proliferation in their mammary glands, regulated by hormones in ethyl acetate fraction of *Hibiscus sabdariffa* L. The mRNA expression of OTR receptor for ethyl acetate fraction of *Hibiscus sabdariffa* L. and metoclopramide groups compared to control group showed no level of statistical significance.

The regulation of oxytocin production and secretion by the pituitary gland is the result of complex positive and negative feedback mechanisms acting on the pituitary gland itself (Chowanadisai *et al*., 2004). Thus, it is possible that alterations in these secondary negative feedback responses result in an increase in circulating oxytocin. The decrease in oxytocin receptor expression observed in this study could possibly be explained by the high circulating concentrations of oxytocin.

CONCLUSION

In conclusion ethyl acetate fraction of *Hibiscus sabdariffa* L. enhance lactogenic activity through increasing hormonal level, lobuloalveolar development of the mammary gland and down regulate mRNA gene expression of OTR receptor in rats.

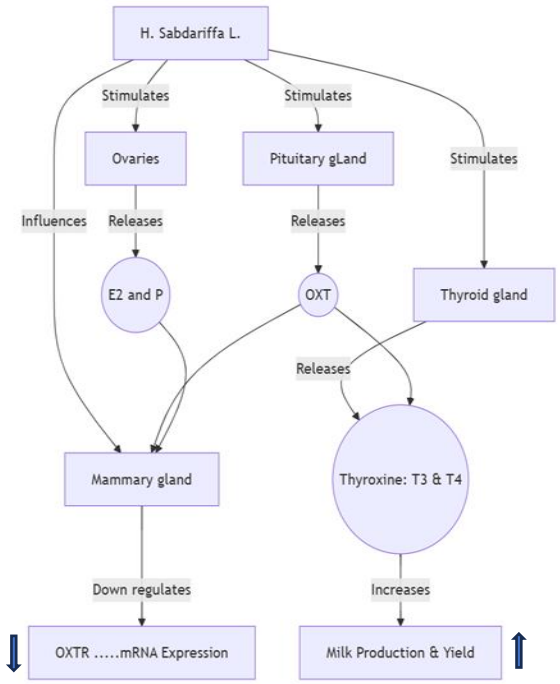


Figure 3: Mechanism of action of milk release and secretion of ethyl acetate fraction of *Hibiscus sabdariffa* L.

ACKNOWLEDGMENTS

This work was sponsored by TETFund through Institution Based Research (IBR) grant Ahmadu Bello University Zaria Nigeria, with grant identification number TETF/DR&D/UNI/ZARIA/IBR/2024/BATCH 8/18.

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