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Lactation suppression effect of aqueous seed extract of *Aframomum melengueta* on female albino rats

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Abstract

Background and aims: Alligator pepper, a dietary spice used as food flavor enhancer is a common ingredient in pepper soup, a spicy delight in most parts of West Africa and possesses both medicinal and nutritive properties. Despite the well documented benefits of breastfeeding for mothers and infants, there are a variety of medical situations for which breastfeeding is contraindicated. Therefore, lactation suppression might be indicated for the best interest of the mother and/or infant. This study was undertaken to investigate the Lactation suppression effect of aqueous seed extract of *Aframomum Melengueta* on female albino rats.

Methodology: Thirty (30) adult female albino rats weighing 160 to 200 g were used for the study while eighteen (18) albino mice of body weights (b.w.) range of 25 g to 30 g were used for the preliminary acute toxicity test. Male rats were introduced into the female rat cages of groups II-V within 12h at the expected estrous phase for mating and withdrawn thereafter. Groups I, II and III were orally administered distilled water only, while Groups IV and V received oral doses of 5 µg/kg b.w. Carbagoline and 3,000 mg/kg b.w. aqueous seed extract of *Aframomum melegueta* respectively 24 hours postpartum once daily by oral gavage for 3 days.

Results: LD_{50} determination revealed no death was recorded among the animals up to 5000 mg extract/kg body weight. Study indicates that *Aframomum melengueta* extract elicited significant reduction of prolactin with corresponding increase in estrogen and progesterone in postpartum animals. Histological sections of the mammary gland show extensive fibrosis and congested ducts lobular units within the tissue stroma of the mammary gland of *Aframomum melengueta* treated animals.

Conclusion: The results confirms a positive lactation suppression effect of aqueous seed extract of *Aframomum Melengueta* on postpartum lactating female albino rats

Keywords: Lactation suppression; *Aframomum melegueta*; Carbagoline; Prolactin; Estogen; Progesterone; Postpartum

1. Introduction

Plants or herbs have been found to be of medicinal and therapeutic importance in the prevention, treatment or cure of diseases and ailment. This knowledge is mainly passed down from one generation to another either verbally or in writing (Sofowora, 2008). The universal role of plants in the treatment of diseases is exemplified by their employment in all major systems of medicine (Okeniyi, Adedoyin & Garba, 2013). Since the last decades, researchers have begun to explain these virtues by the ability of plants to limit infection, prevent lipid peroxidation, prevent some cancers, cure

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allergies and many other associated diseases. Among all these virtues, the anti-infectious activity is considered as one of the most important activities (Mohammed *et al.,* 2013). Their role is twofold in the development of new drugs. They may become the base for development of a medicine, a natural blue print for the development of new drugs or a phytomedicine to be used for the treatment of diseases (Prasad, Izam & Maksudur, 2012). Therefore, evaluating their marginal significance can help to understand the worth of plant species in different ecological conditions (Apiamu *et al.,* 2013). Plant remedies (both single plant and multi-herbal preparations) are used since ancient times, even though the mechanisms of action, toxicity and efficacy of very few of them have been evaluated scientifically (Omale & Emmanuel, 2010).

The need to use medicinal plants as alternatives to orthodox medicines in the provision of primary health care cannot be over-emphasized. More so herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and relatively safe for human use and are environment friendly (Apiamu *et al.,* 2013). The World Health Organization (WHO) supports the use of traditional medicine provided they are proven to be efficacious and safe (Omale & Emmanuel, 2010). Herbal products from medicinal plants are preferred because of less testing time, higher safety, efficacy, cultural acceptability and lesser side effects (Prasad *et al.,* 2012). They are also cheap, easily available and affordable and therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medical use and characterization of compounds which will be added to the potential lists of drugs (Apiamu *et al.,* 2013).

*Aframomum melegueta (*Alligator pepper) is a spice that is widely used in many cultures for various purposes such as entertainment, food flavor, religious rites and as part of many herbal medications (Inegbenebor *et al.,* 2009; Omoboyowa *et al.,* 2017). It is served and eaten along with Kola nuts and alcoholic drinks to guests as entertainment (Nwozo & Oyinloye, 2011; Nosiri *et al.,* 2017). Alligator pepper is a very popular spice used mainly as food, in brewing, and in veterinary and traditional medicine (Igwe *et al.,* 1999; Omoboyowa *et al.,* 2017). The seeds are used for culinary reasons (due to the pungency of the seeds) and it is commonly used as seasoning for food products (Van Andel *et al.,* 2012; Obike *et al.,* 2014). Alligator pepper is a dietary spice used as food flavor enhancer in Nigeria and some other parts of the world (Inegbenebor *et al.,* 2014). It is a common ingredient in pepper soup, a spicy delight in most parts of West Africa (Inegbenebor *et al.,* 2009; Nwozo & Oyinloye, 2011). *Aframomum melegueta* (alligator pepper) is a plant that possesses both medicinal and nutritive properties (Nosiri *et al.,* 2017). *Aframomum melegueta* is a popular medicinal plant in Nigeria believed to have many agents acting in different ways to bring about human health benefits (Akpanabiatu *et al.,* 2013). It is popularly used in herbal medicine against a wide range of ailments by many cultures in Africa especially in Nigeria (Nosiri *et al.,* 2017). *Aframomum melegueta fourier-transform infrared spectroscopy* (FTIR) identified twelve functional groups in the seed extracts namely –OH, -NH_2, ≡CH, -NH_3^+, -CH_3, -OH, -N=C=O, -C≡N, -C=C=C, -NH, -CH_3 and -1,3,5-trisubstituted benzenes, while gas chromatography with flame ionization detector (GCFID) determined fifteen bioactive components namely kaemferol, naringenin, sapogernin, flavanones, anthocyanin, flavan-3-ol, cyanogenic glycoside, ribalinidine, rutin, catechin, resveratol, spartein, epicatechin, steroid and phytate. (Olunkwa *et al.,* 2023).

The birth of a baby is an important event in any family and it is very pertinent that for a mother to have a healthy baby, she gives her baby the best nutrition (Mangesi & Dowswell, 2014). For many years, the importance of breastfeeding to both infants and mothers has been emphasised by healthcare providers and various strategies have been employed to promote it globally (Oladapo & Fawole, 2006). Breastfeeding, also called nursing, is the process of feeding human breast milk to an infant, either directly from the breast or by expressing (pumping out) the milk from the breast and bottle-feeding it to the infant (NICHD, 2009). Breastfeeding and human milk are the normative standards for infant feeding and nutrition (AAP, 2019). Breastfeeding is the best source of nutrition for most infants because it reduces the risk for some short- and long-term health conditions for both infants and mothers (CDC, 2019**).** Breastfeeding is particularly advantageous because of the nutritional, immunologic, and psychological benefits (Donovan, 2020).

Given the strong evidence of the benefits of breastfeeding for women and babies, the WHO (2003) recommends that, in all parts of the world, babies should be exclusively breastfed for the first six months to achieve optimal growth, development and health (Mangesi & Dowswell, 2014). The American Academy of Pediatrics recommends breastfeeding as the sole source of nutrition for babies for about 6 months and can be continued until at least one year for optimal health for as long as both mother and baby desire it (AAFP, 2015; Trimeloni & Spencer 2016; AAP, 2019).

Lactation is the production of milk by the mammary glands, which are contained within the breasts (Donovan, 2020). Milk is a complex mixture whose composition reflects the activities of distinct secretion and transport processes of the mammary gland and mirrors the differing nutritional requirements of mammalian neonates (McManaman & Neville, 2003). Lactation is the process of secretion and yielding of breast milk by females after giving birth (Ayoola, 2018;

Donovan, 2020). Changes early in pregnancy prepare the breast for lactation (Lawrence *et al.,* 2015). Breast milk production is an inborn ability of a mother, and it provides an optimal start to an individual's nutritional life, reducing their lifelong risk of many devastating diseases, including obesity, asthma, diabetes mellitus, and childhood leukemia and lymphoma (Domingue, Devuyst, Alexopoulou, Corvilain & Maiter, 2014; Trimeloni & Spencer, 2016; Lucca & Santhosh, 2017; AAP, 2019).

An excessive milk supply may appear to be a cause for celebration by mothers with insufficient lactation (Eglash, 2014). While human milk provides the most complete form of nutrition for infants, including premature and sick newborns, there are rare exceptions when human milk or breastfeeding is not recommended (CDC, 2019). Suppression of lactation becomes essential when breastfeeding is no longer required (as in the events of perinatal death and infant adoption) or when the mother is too ill to breastfeed, example in cases of severe obstetric morbidity (Oladapo & Fawole, 2006). There is very little information in the medical literature regarding the suppression of lactation for those women who cannot or do not want to breastfeed (Marcolina & Denchfield, 2005). In a 100-year review of the literature, nothing new or helpful was found to induce milk suppression or to treat the pain or discomfort of engorged breasts (Spitz, Lee & Peterson, 1998; Moore & Catlin, 2003). There are a variety of medical situations for both the infant and the mother for which breastfeeding is contraindicated (AAP, 2001; Marcolina & Denchfield, 2005). Despite the well documented benefits of breastfeeding for mothers and infants (Clark & Bungum, 2003; ACOG, 2007; AAP, 2012), under some conditions, lactation suppression might be indicated for the best interest of the lactating mother and/or the infant (AlSaad *et al.,* 2017). In spite of the well-known advantages of breastfeeding (for example, infant protection against diarrhoeal morbidity and mortality), there are instances when the wellbeing of the mother or infant requires suppression of lactation (Oladapo & Fawole, 2006). While much is written on breast milk undersupply, little is written on oversupply, sometimes known as hyperlactation or hypergalactia (Trimeloni & Spencer, 2016). Medical contraindications to breastfeeding are rare (AAP 2019). A mother who has established a full milk supply through breastfeeding or pumping will need counseling on strategies to diminish her milk production, especially under the tragic circumstances of a baby's death; the mother's comfort must not be overlooked (Merewood & Philipp, 2001; Moore and Catlin, 2003).

Lactation suppression refers to the act of suppressing lactation by medication or other non- pharmaceutical means (NHS, 2018). Besides medical indications, some mothers in circumstances where alternatives to breastfeeding exist may seek lactation suppression on personal or social grounds as some women may desire to stop the production of breast milk, for example when the mother decides to bottle feed from birth (Moore & Catlin, 2003). It is estimated that over 30% of women in the United States and United Kingdom do not breastfeed their infants while a larger proportion discontinue breastfeeding within two weeks of childbirth (Hamlyn 2002; Ryan 2002; Oladapo & Fawole, 2006). Women who are breastfeeding may need to stop breastfeeding abruptly, for instance if she is taking medication contradicted for breastfeeding or undergoes surgery. Some of the medical conditions that contraindicate breastfeeding include infants with the metabolic disorder classic galactosemia, mothers who are positive for human T- cell lymphotrophic virus type I or II, and untreated brucellosis, among others (AlSaad *et al.,* 2017). The lactating mother's use of some medication types might necessitate the avoidance of breastfeeding, such as mothers undergoing chemotherapy (ACOG, 2007; AAP, 2012). Unlike in the 1970s, when a social reason was the most common indication for lactation suppression Eastham (1976), the need for complete avoidance of breastfeeding by HIV-positive mothers to reduce the risk of vertical transmission of HIV has offered a more compelling reason in the last decade (Oladapo & Fawole, 2006). Lactation inhibition might also be needed following an infant death or miscarriage (Moore & Catlin, 2003; Cole, 2012). In the case of infant death, breastfeeding is no longer necessary and therefore it will be important to support mothers and families during these times by providing practical suggestions about how to prevent painful engorgement and care for their bodies as their breasts involute and milk production ceases (Marcolina & Denchfield, 2005). Also an abrupt weaning process may lead to severe engorgement, extremely painful breasts, and possibly mastitis (Lawrence *& Lawrence, 2011*). Breast engorgement is a painful and unpleasant condition affecting large numbers of women in the early postpartum during a time when mothers are coping with the demands of a new baby it may be particularly distressing (Mangesi & Dowswell, 2014). Up to two-thirds of non-breastfeeding women experience moderate to severe engorgement and breast pain when no treatment is applied (Spitz, 1998; Oladapo & Fawole, 2006). Breast engorgement involves the overfilling of breast milk that causes discomfort and pain to the mother including non-infectious mastitis involving inflammation of the breast due to milk duct blockage (Clarke 2007; Mangesi & Dowswell, 2014). If the decision to suppress lactation or not breastfeed an infant is made, the prevention of breast engorgement is essential to avoid associated complications, such as breast pain and mastitis (Moore & Catlin, 2003; Cole, 2012; Grueger, 2013; AlSaad *et al.,* 2017). Stopping breast milk supply once it's been established can be an uncomfortable process as the breasts will still become engorged as production tapers off, requiring expression of milk to reduce the risk of developing mastitis (Campbell, 2018). The mother who knows that her baby is likely to die within a day or two after birth and does not stimulate her breasts may or may not produce enough milk to cause engorgement (Moore & Catlin, 2003).

The attendant complications (galactorrhea, hyperprolactinaemia, prolactinoma) associated with exclusive breast feeding and the serious side effect (nausa, fatigue, postural hypotension, nasal congestion, exacerbation, psychosis, seizure) associated with orthodox drugs (bromocreptine, pergolide, cabergoline, quinagolide) have necessitated the search for natural alternative with little or no side effect which can be used in alleviating the situation (Ibekwe, 2018). A few herbs are clinically useful to reduce milk supply (Eglash, 2014). Although not well studied, sage and parsley have been reported to reduce milk supply (TOXNET, 2014; Trimeloni and Spencer 2016). Common substances including herbs, homeopathy, pseudoephedrine, estrogen in the form of the combined birth control pills have been found effective (Eglash, 2014).

Severe pains and breast engorgement experienced by non-breastfeeding mothers especially after loss of a child remains a major intolerable side-effect of lactation, with limited available conventional therapeutic and chemoprophylactic options. In this study, we investigated the lactation suppression properties of aqueous seed extracts of Z*ingiberaceae Aframomum melengueta* on female albino rats.

2. Materials and Methods

2.1. Plants Collection and Preparation

The fruits of *Aframomum melegueta* were purchased from Relief Market, Owerri, Imo State, Nigeria. The plants were authenticated by Mr. Francis Iwueze, a botanist at the Department of Forestry and Wildlife, Federal University of Technology, Owerri (FUTO) and compared with existing collections deposited at the herbarium of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, with voucher specimen catalogue no. MOUAU/CVM/VPP/2013/03. The fruits were air dried at room temperature for two weeks. The outer coats of the seeds were removed, and the seeds dried in an oven at a temperature of 50 ℃ for 4 hours. The dried seeds were ground to powder form with electric blender and preserved in labeled air tight container until required for extraction.

2.2. Extraction of *Aframomum Melengueta* Seeds

Five hundred milligrams (500 mg) of ground seed were soaked in 500 ml of distilled water. The solution was allowed to stand for 48 hours with intermittent mixing and later filtered with Whatman No. 1 filter paper. Thereafter, the filtrate was then evaporated in a water bath to concentrate the extract. The concentrate was dispensed into airtight sterile container and refrigerated at 4 ℃ until required.

2.2.1. Percentage Yield

The extract obtained was weighed using an electronic weighing balance and the percentage yield calculated as follows:

$$Percentage\ yield = \frac{\text{weight of extract X 100}}{weight\ of\ starting\ material}$$

2.3. Preliminary Acute Toxicity and Lethality LD_{50} Test

2.3.1. Acute Toxicity

Eighteen albino mice of body weights range of 25 g to 30 g were purchased from the Animal Breeding Unit, Zoological Garden, University of Nigeria Nsukka, Enugu state. The animals were kept in a well–ventilated room at of temperature 25 ± 2 ℃ and 55–65% relative humidity with a diurnal 12 h light cycle in stainless–steel cages. The mice had access to water and pelletized standard finisher mesh (Vital finisher, United Africa Company Nigeria Plc., Jos, Nigeria) ad libitum. A period of 1 week was allowed for acclimatization of the mice to the environmental conditions.

2.3.2. LD_{50} Determination

The index of acute toxicity was the LD_{50}, which is the dose of a substance capable of producing death in 50% of the population of animal exposed to the substance. Lorke's (1983) method was used. This method has two different phases.

Phase I

Three groups of three mice each were utilized. The extract was administered at different concentrations of 10, 100 and 1000 mg/kg body weight to group I, II and III mice respectively that formed the LD_{50} phase one groups. The extract was administration orally and the animals monitored for 24 hours for signs of abnormal reactions or death.

Phase II

Phase two consisted of three groups of mice administered with the crude extract at concentrations of 1600, 2900, and 5000 mg/kg body weight in groups 1, 2 and 3 mice respectively. They animals were observed for 24 hours and recorded. Lethal dose (LD_{50}) of the extract was estimated by calculating the geometric mean of the maximum dose with 0 % mortality as well as the minimum dose with mortality.

$$LD_{50} = \sqrt{\frac{maximium\ dose\ with\ 0\%\ mortality\ \times 100}{minimium\ dose\ with\ mortality}}$$

2.4. Standard Drug Preparation

Cabergoline (Dostinex® 0.5 mg, Pfizer Limited) was purchased at Orchad Pharmacy, Owerri and each 0.5 mg tablet was dissolved in 500 ml distilled water, reconstituted and administered as 5 µg/kg body weight of the animals.

2.5. Laboratory Animals

Laboratory animals comprised of 30 female and 15 male adult albino rats (*Rattus novergicus*) weighing between 180 g and 200 g, and aged about five weeks. Animals were procured from Department of Veterinary Medicine, University of Nigeria, Nsukka and housed in cages under standard environmental conditions of temperature (30 ± 1 ℃), humidity (60 ± 0.2%) and a 12 h light/dark cycle in the Animal House Unit of Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria, for 14-day period to acclimatize. The animals were fed standard rodent diet (Growers mash, Vital Feeds Ltd) and water *ad libitum*. Ethical approval was obtained for the study protocol from the University Ethical Committee and Principles of Laboratory Animal Care (DHHS, 1985) was adopted for all the experimental procedures involving the use and handling of the laboratory animals.

2.6. Study Design

Table 1 Animal treatment groups

S/N	Group	Treatment
1	Group 1 (Non-pregnant unmated control)	Orally administered distilled water only
2	Group II (Pregnant control)	Orally administered distilled water only
3	Group III (Post-partum negative control)	Orally administered distilled water only
4	Group IV (Post-partum positive control)	Received single oral dose of 5 µg/kg b.w. Carbagoline (positive control drug) for 3days
5	Group V (Post-partum Test)	Received single oral dose of 3,000 mg/kg b.w. *Aframomum melegueta* for 3 days

The female rats were divided randomly into 5 groups consisting of 6 rats each such that the weight differences within and between groups did not exceed ± 20% of the average weight of the total rats. The animals were grouped and treated as detailed in Table 1. The estrous cycles of the female albino rats was monitored and then 3 male rats thereafter introduced into each of the female rats cages of groups III-V within 12h at the expected estrous phase and left in the cages for three days in order to allow mating to occur and later withdrawn. Subsequently, 14 days thereafter group II female rats were mated as well while group I female rats were not mated at all. The day sperm was observed in the vaginal smear of the female rats or the presence of cornified plug on the floor of the cages was taken as Day 1 of pregnancy (Ojieh *et al.,* 2013; Inegbenebor *et al.,* 2014). The post-partum rats were not allowed to suckle their pups by having their pups removed within 12 hours of birth.

Administration of the drug/extract commenced 24 hours postpartum once daily by oral gavage for total of 3 days. The chosen dosage of aqueous plant extracts used was based on results of preliminary acute toxicity studies on the median lethal dose (LD_{50}).

2.7. Sample Collection

All the animals were weighed on the first and last days of treatment. At the conclusion of the treatment period, animals were fasted overnight, anaesthetized with diethyl ether and sacrificed. Thereafter, 5 ml of whole blood was collected through cardiac puncture and gently dispensed into well labeled bottles of ethylene diamine tetra acetic acid (EDTA) and subsequently utilized for hematological parameters analysis.

2.8. Histological examination

- **Preparation of tissues for histological examination:** After sacrifice by cervical dislocation, the organs (liver, kidney and mammary gland samples) were quickly excised and placed in 10% neutral buffered formalin to prevent putrefaction and autolysis for histological assessment.
- **Tissue Processing:** The tissues were transferred into an automatic processor where they went through a process of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in 10% formaldehyde. The tissues were washed over night in running tap water after four hours in 10% formaldehyde. Dehydration of the fixed tissues was carried out in different percentages of alcohol 50%, 70% and 90% absolute. The tissues were then cleared in xylene and embedded in paraffin wax. Serial sections of 5micron thick were obtained using a rotatory microtome. The tissue sections were deparaffined hydrated and stained using the routine haematoxylin and eosin method. The stained sections were then examined under the light microscope (Obike *et al.,* 2014).
- **Serum Hormonal profile analyses:** Serum Prolactin, estrogen and progesterone were determined using Immunoenzymatic assay method (Wisdom, 1976; Acosta & Wright, 1983) with the aid of ELISA Assay Kit (DiaMetra, Italy).

2.9. Statistical Analysis

Data obtained from experimental groups was expressed as mean ± standard deviation (S.D) and analysed using one-way Analysis of Variance (ANOVA) and Tukey's Multiple Comparism test with the aid of GraphPad Prism Version 5.3 (GraphPad, USA) statistical software. Values for $P<0.05$ were considered statistically significant.

3. Results

Table 2 LD_{50} Acute toxicity test result of albino mice administered aqueous seed extract of *Aframomum melengueta*

Phase I		
Groups	**No. of mice**	**Mortality**
I 10mg/kg body weight	3	0
II 100mg/kg body weight	3	0
III 1000mg/kg body weight	3	0
Phase II		
IV 1600mg/kg body weight	3	0
V 2900mg/kg body weight	3	0
VI 5000mg/kg body weight	3	0

Acute toxicity test using LD_{50} determination revealed no death was recorded among the animals up to 5000mg extsract/kg body weight administration.

Table 3 Hormonal profile of animals treated with aqueous seed extracts of *Aframomum melengueta*

Parameter	Non Pregnant CTRL	Pregnant CTRL	Postpartum CTRL	Postpartum + STD Drug	Postpartum + Extract
Prolactin	0.29 ± 0.04[a]	0.30 ± 0.06[a]	0.52 ± 0.02[b]	0.28 ± 0.03[a]	0.27 ± 0.05[a]
Progesterone	29.02 ± 3.77[a]	41.78 ± 2.45[b]	15.24 ± 2.60[c]	44.96 ± 4.68[b]	44.10 ± 3.28[b]
Estrogen	36.80 ± 2.39[a]	34.25 ± 2.99[ab]	24.60 ± 2.07[c]	32.40 ± 2.07[b]	35.00 ± 1.23[ab]

Values are mean ± standard deviation. The values having different alphabet letters per row are statistically significant (p<0.05)

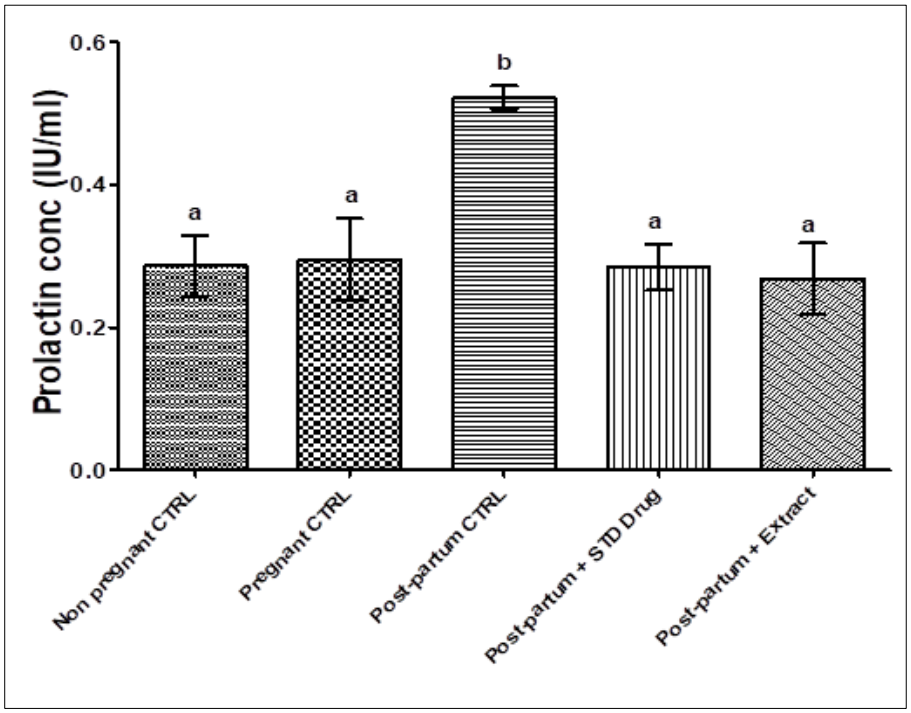

Figure 1 Serum prolactin concentration (ng/dl) of pregnant and post-partum albino rats administered aqueous seed extract of *Aframomum melengueta.* Bars are mean ± standard deviation. Bars with different alphabet letters are statistically significant (p<0.05)

The results show that prolactin concentration (ng/dl) of Non- pregnant control (0.29 ± 0.04), Pregnant CTRL (0.30 ± 0.06), Post-partum + STD drug (0.28 ± 0.03) and Post-partum + Extract (0.27 ± 0.05) groups were not significantly (p<0.05) different from each other. However, prolactin concentration (ng/dl) of the Post-partum CTRL (0.52 ± 0.02) was significantly (p<0.05) elevated when compared to the Non- pregnant control (0.29 ± 0.04), Pregnant CTRL (0.30 ± 0.06), Post-partum + STD drug (0.28 ± 0.03) and Post-partum + Extract (0.27 ± 0.05) groups.

The progesterone concentration (ng/dl) results show that progesterone concentration (ng/dl) of Pregnant CTRL (41.78 ± 2.45), Post-partum + STD drug (44.96 ± 4.68) and Post-partum + Extract (44.10 ± 3.28) groups were not significantly (p<0.05) different from each other. Consequently, the progesterone concentration (ng/dl) of Post-partum CTRL (15.24 ± 2.60) was significantly (p<0.05) reduced when compared to Non- pregnant control (29.02 ± 3.77), Pregnant CTRL (41.78 ± 2.45), Post-partum + STD drug (44.96 ± 4.68) and Post-partum + Extract (44.10 ± 3.28) groups.

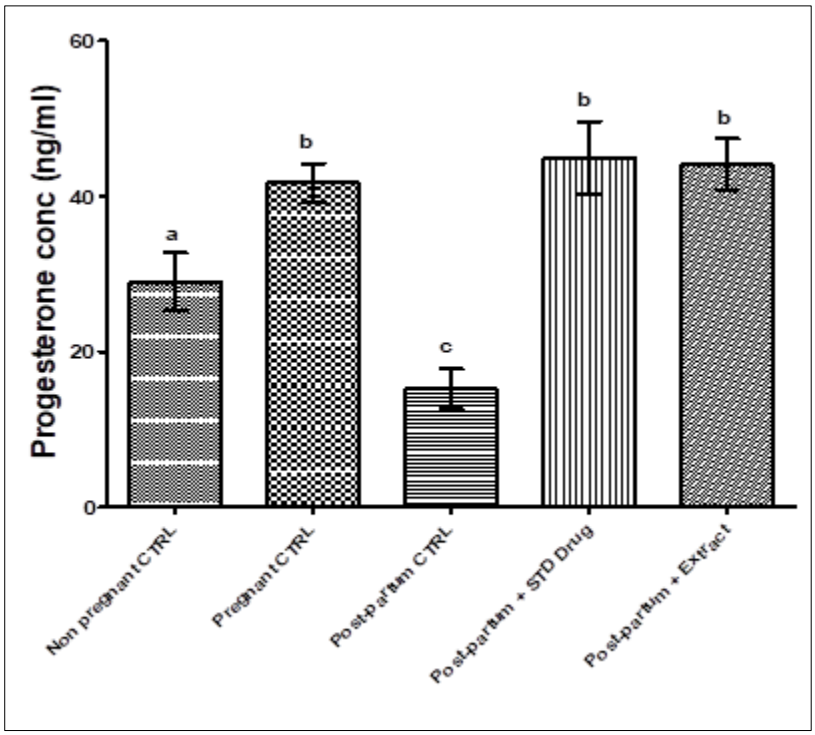

Figure 2 Serum progesterone concentration (ng/dl) of pregnant and post-partum albino rats administered aqueous seed extract of *Aframomum melengueta.* Bars are mean ± standard deviation. Bars with different alphabet letters are statistically significant ($p<0.05$)

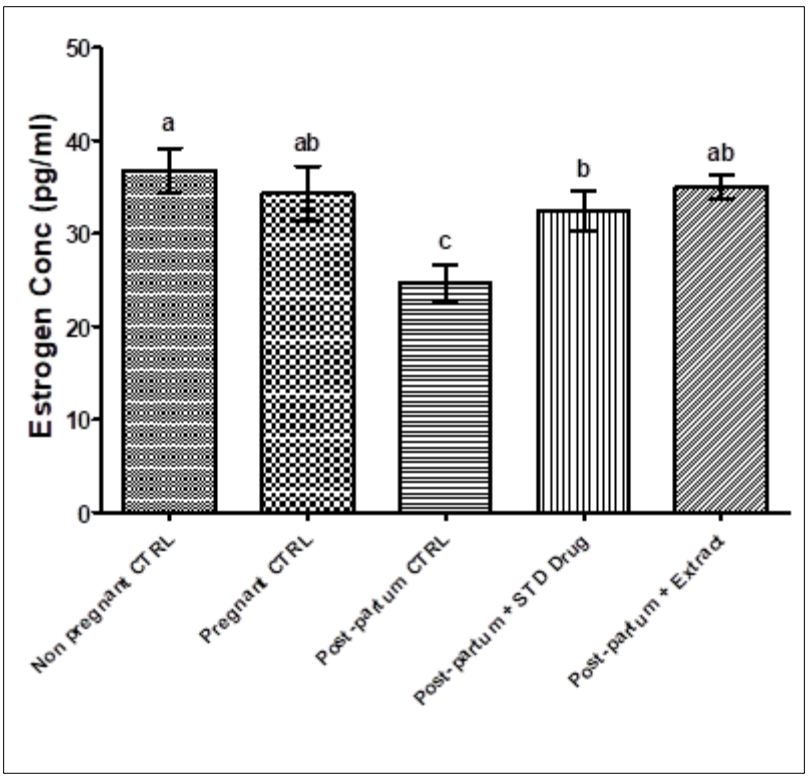

Figure 3 Serum estrogene concentration (pg/ml) of pregnant and post-partum albino rats administered aqueous seed extract of *Aframomum melengueta.* Bars are mean ± standard deviation. Bars with different alphabet letters are statistically significant ($p<0.05$)

The results show that there were no significant ($p<0.05$) difference in the estrogen concentration (pg/ml) of Non-pregnant control (36.80 ± 2.39), Pregnant CTRL (34.25 ± 2.99), Post-partum + STD drug (32.40 ± 2.07) and Post-partum + Extract (35.00 ± 1.23) groups. However, the estrogen concentration (pg/ml) of Post-partum CTRL (24.60 ± 2.07) was significantly ($p<0.05$) reduced when compared to Non- pregnant control (36.80 ± 2.39), Pregnant CTRL (34.25 ± 2.99), Post-partum + STD drug (32.40 ± 2.07) and Post-partum + Extract (35.00 ± 1.23) groups.

3.1. Histology of mammary gland

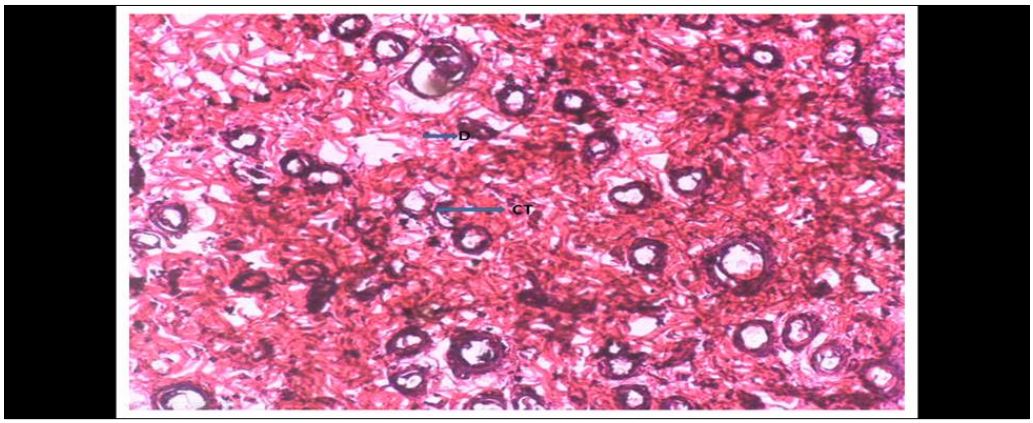

Figure 4 Photomicrograph of Non-pregnant albino rats mammary gland section administered distilled water using Hematoxylin and Eosin Stain

Histological section of the mammary gland shows normal ducts lobular units (D) and connective tissue (CT).

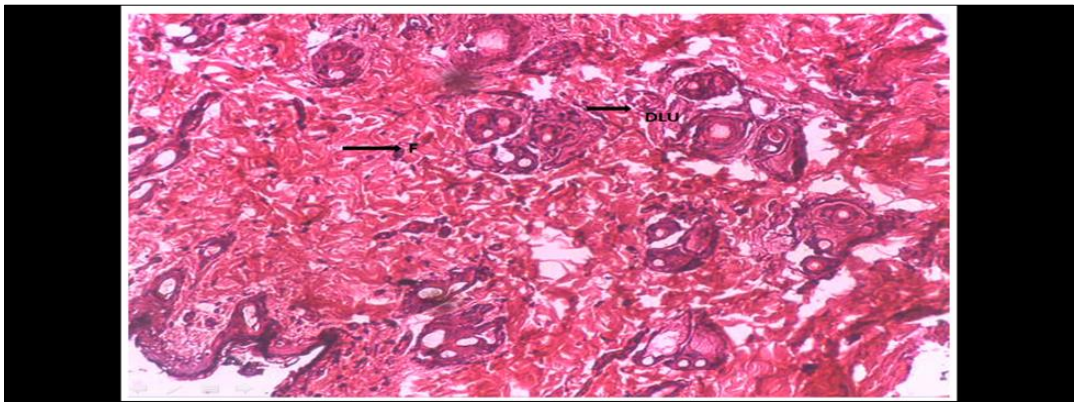

Figure 5 Photomicrograph of Mammary gland section of post-partum + Extract albino rats administered aqueous seed extract of *Aframomum melengueta* using Hematoxylin and Eosin Stain

Histological section of the mammary gland shows extensive fibrosis with congested ducts lobular units (DLU) within the tissue stroma.

4. Discussion

It appears that milk production is held back during pregnancy, however, the mechanism by which this inhibitory effect is brought about, or by which lactation is initiated at delivery, has long been the subject of an argument that revolves around the opposing actions of estrogen, progesterone, and prolactin, as studied in laboratory animals (Donovan, 2020). A number of investigations have been geared towards the influence of pregnancy, lactation and tumor implantation on the metabolic activities of the mammary gland as alterations in metabolic activities in the mammary gland are mostly due to such hormonal factors. Steroid and peptide hormones play very crucial role in the regulation of metabolic activities in mammary gland (Srinivasan *et al.,* 1980).

The hormonal profile results show a significant ($p<0.05$) increase in estrogen and progesterone with accompanying significant ($p<0.05$) decrease in prolactin during pregnancy. However, after delivery this trend was reversed resulting

in a significant ($p<0.05$) decrease in estrogen and progesterone level with a significant ($p<0.05$) elevation of prolactin post-partum. In pregnancy, the changes in hormones such as progesterone, estradiol and prolactin may have combined influence over the mammary gland. The inhibitory effect of progesterone on lactogenesis during pregnancy is evident (Ibekwe, 2018; Lopez-fontana, *et al.,* 2012). During pregnancy the combination of estrogen and progesterone circulating in the blood appears to inhibit milk secretion by blocking the release of prolactin from the pituitary gland and by making the mammary gland cells unresponsive to this pituitary hormone (Donovan, 2020). In pregrancy, progesterone and estradiol are elevated while during lactation, prolactin is elevated on and off (Srinivasan *et al.,* 1980).

Stage II lactogenesis (secretory activation) starts with copious milk production after delivery as the placenta supplies high levels of progesterone which inhibit further differentiation (Pillay & Davis, 2021). Previously called lactogenesis II, copious milk secretion is induced during the secretory activation phase of differentiation that occurs at the end of pregnancy in rodents and ruminants, and shortly after parturition in humans (McManaman & Neville, 2003). Milk synthesis begins in the second half of pregnancy and it is supported by prolactin and cortisol, which directly act on enzyme activities and processes of differentiation of the alveolar cells and by late pregnancy, some women can express colostrum (Pillay & Davis, 2021). The role of prolactin during mammary gland morphogenesis has been investigated using prolactin receptor knockout mice (Ormandy *et al*, 1997) which revealed normal development in the transplanted tissues, suggesting that defect in knockout glands are due to lack of prolactin/prolactin receptor signaling in other tissues (Ibekwe, 2018). Due to the effectiveness of this hormonal stimulation on the breast, a mother who delivers at 16 weeks of gestation secretes colostrum although she has a nonviable infant (Marcolina & Denchfield, 2005). In primiparous women, the secretory activation is slightly delayed, which is also observed in women who had cesarean births compared with those who delivered vaginally (Pillay & Davis, 2021). Since lactation ensues after a premature birth, it would appear that milk production is held back during pregnancy, however, the mechanism by which this inhibitory effect is brought about, or by which lactation is initiated at delivery, has long been the subject of an argument that revolves around the opposing actions of estrogen, progesterone, and prolactin, as studied in laboratory animals, goats, and cattle (Donovan, 2020). The sudden surge in the secretion of milk after parturition is most likely due to the rapid decline of the serum levels of progesterone. Late onset of milk production has also been seen in women who have had retained placental fragments, diabetes, and stressful vaginal deliveries (Pillay & Davis, 2021). The blockade is removed at the end of pregnancy by the expulsion of the placenta and the loss of its supply of hormones, as well as by the decline in hormone production by the ovaries, while sufficient estrogen remains in circulation to promote the secretion of prolactin by the pituitary gland and so favour lactation (Donovan, 2020). After delivery of the placenta, levels of placental lactogen, estrogen, and progesterone decline abruptly (Marcolina & Denchfield, 2005). The drop in progesterone levels triggers the initiation of full milk secretion and supply (Neville 2001; Ibekwe, 2018). The removal of the placenta at delivery, the rapid drop in progesterone, as well as the presence of elevated levels of prolactin, cortisol, and insulin, results in the stimulation of this stage (Pillay & Davis, 2021). This phase is characterized by homogeneous expression of milk protein genes by alveolar cells (Robinson *et al.,* 1995), induction of additional milk protein gene and biosynthetic enzyme expression, polarization of organelles, expansion of mitochondria and RER, maturation of the Golgi apparatus, and closure of tight-junction complexes (Nguyen *et al.,* 2001; McManaman & Neville, 2003). Lactogenesis stage II could be inhibited by the continued secretion of progesterone with retained placental fragments, and would continue to be inhibited until removal of the remaining placental fragments (Pillay & Davis, 2021).

The breast is one of the most complex endocrine target organs (Marcolina & Denchfield, 2005). A mother's milk supply is under extrinsic endocrine control from prolactin and oxytocin (Eglash, 2014). Several pituitary hormones seem to be involved in the formation of milk, so that it is customary to speak of a lactogenic ("milk-producing") complex of hormones (Donovan, 2020). During lactation, several key hormones are involved in the regulation of mammary gland cell number, secretary activity and consequent milk production potential (Malgwi *et al.*, 2017). Estrogen, a membrane soluble ligand is released from the ovary and activates gene expression through intracellular receptors (Bandyopadhyay, 2013; Ibekwe, 2018).

Prolactin heralds the principal role in lactogenesis (Kim, 2020). Prolactin is the major generator of lactation competence during pregnancy and it functions both indirectly, through its regulation of ovarian progesterone production, and directly via its effect on mammary epithelial cells (Hector & Lindsay, 2012; Ibekwe, 2018). Prolactin production relies on estrogen, progesterone, glucocorticoids, insulin, thyroid hormone and parathyroid hormone (Kim, 2020). Influencing secretion of prolactin has been proven to be a useful tool for regulating lactation which is maintained by regular removal of milk as prolactin is synthesized and secreted from the anterior pituitary gland in response to nipple stimulation and oxytocin from the posterior pituitary gland resulting in milk secretion from lactocytes (Freeman *et al*, 2000; Eglash, 2014). Some prolactin-inhibiting factors (PIFs) such as catecholamine in the hypothalamus have inhibitory effect as a result of dopaminergic impulses, as such drugs that upregulate the catecholamine effect can act as PIF (Lawrence & Lawrence, 2011; Kim, 2020). Prolactin is both positively and

negatively regulated, but its main control comes from hypothalamic inhibitory factors such as dopamine which act on the D2 subclass of dopamine receptors present in lactotrophs (Pillay & Davis, 2021).

Resveratrol, a component of *Aframomum meleguata* is reported to negatively affect pregnancy and breast-feeding, as it might act like estrogen (WebMD, 2005) thereby suppressing prolactin and lactation. The histological sections of the mammary gland of post-partum animals administered with the plant extract showed extensive fibrosis with congested ducts lobular units (DLU) within the tissue stroma in comparison to the rest of the groups which appeared normal. The severe congestion of the duct lobular units could be attributed to suppression of lactation and mastistis. This collaborates with previous studies showing that fennel (*Foeniculum vulgare Mill.*) leaves and seed infusions (used to increase milk secretion in lactating mice) significantly increased the lumen diameter of the lactoferous ducts in the mammary glands, increase in alveoli diameter as well as the number of active mammary gland alveoli (Rifqiyati & Wahyuni, 2019).

5. Conclusion

The hormonal profile results showed significant increase in estrogen and progesterone as well as significant decrease in prolactin during pregnancy. However, this trend was reversed resulting in a significant decrease in estrogen and progesterone level with a significant elevation of prolactin post-partum. In the light of the overall results obtained in this current study, administration of aqueous seed extract of *Aframomum melengueta* resulted in significant reduction of lactating hormone prolactin with corresponding increase in estrogen and progesterone hormonal activities in the lactating animals typical of the effect of the standard drug carbagoline. This confirms a significant lactation suppression property in the treated experimental albino rats likely due to effect of resveratrol, a component of *Aframomum meleguata* reported to negatively affect pregnancy and breast-feeding, which acts like estrogen thereby suppressing prolactin and lactation. *Aframomum meleguata* also exhibited more effective level of lactation suppression, though non-significantly different with the standard drug cabergoline. Histological sections of the mammary gland equally shows extensive fibrosis with congested ducts lobular units within the tissue stroma of the mammary gland as a result of lactation suppression effect of the aqueous seed extracts of *Aframomum melengueta* treated animals.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflicts of interest.

References

[1] AAFP (2015) American Academy Family Physicians Breastfeeding Advisory Committee. Breast feeding, family physicians supporting (position paper). Available from: http://www.aafp.org/about/policies/all/breastfeeding-support.html

[2] AAP American Academy of Pediatrics (2001) Committee on Drugs. Transfer of drugs and other chemicals into human milk. *Pediatrics;* 108:776-789

[3] AAP American Academy of Pediatrics (2019) New Mother's Guide to Breastfeeding, 2nd Edition. *American Academy of Pediatrics*

[4] AAP American Academy of Pediatrics (2012) Work Group on Breastfeeding. Policy statement: Breastfeeding and the use of human milk. *Pediatrics;* 129:e827– 41

[5] ACOG (2007) Committee on Health Care for Underserved Women, American College of Obstetricians and Gynecologists. Breastfeeding: maternal and Infant Aspects. *ACOG Clin Rev*.; 12:S1-S16

[6] Acosta, A.A.M.D. and Wright, G.L. (1983) *Journal of Clinical Immunoassays*, 6, 41

[7] AlSaad D., Awaisu A., Elsalem S., Abdulrouf P. V.,Thomas B., AlHail M. (2017) Is pyridoxine effective and safe for post- partum lactation inhibition? A systematic review. *J Clin Pharm Ther.* **42**:373–382

[8] Apiamu, A., Evuen, U. F. & Ajaja U. I. (2013). Biochemical Assessment of the Effect of Aqueous Leaf Extract of *Euphorbia Heterophylla Linn* on Hepatocytes of Rats. *Journal of Environmental Science, Toxicology and Food Technology.* 3(5), 37-41

[9] Akpanabiatu MI, Ekpo ND, Ufot UF, Udoh NM, Akpan EJ, & Etuk EU. (2013). Acute toxicity, biochemical and haematological study of Aframomum melegueta seed oil in male Wistar albino rats. *Journal of Ethnopharmacology*. 150(2):590-4

[10] *Ayoola S. (2018)* Why milk comes out of breast when not breastfeeding. https://www.legit.ng/1181410-why-milk-breast-breastfeeding.html

[11] Bandyopadhyay, S.K., Bhattacharyya, C.R.R. & Basu, S. (2013) Textbook of Veterinary Gynaecology, Artificial Insemination, Obstetrics and Assisted Reproduction. Kalyani Publishers, New Delhi

[12] Campbell M. (2018) The Safest Amount of B-6 to Take When Drying Up Breast Milk. https://www.hellomotherhood.com/article/509418-much-b-6-can-dry-up-breast-milk/

[13] CDC (2019) About Breastfeeding. Centers for Disease Control and Prevention, Division of Nutrition, Physical Activity, and Obesity, National Center for Chronic Disease Prevention and Health Promotion

[14] Clarke, TM. (2007) Is it the flu? Recognised mastitis. http://att.iparenting.com/breastfeed/notflu.htm

[15] Clark S. & Bungum T. (2003) The Benefits of Breastfeeding: an Introduction for Health Educators. *Calif J Health Promot*. **1**:158-163

[16] Cole M. (2012) Lactation after perinatal, neonatal, or infant Loss. *Clin Lact*. **3**:94-100

[17] DHHS (1985). *Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources Commission on Life Sciences*, National Research Council, National Academy Press, Washington, DC, USA

[18] Domingue M.E., Devuyst F., Alexopoulou O., Corvilain B. & Maiter D. (2014) Outcome of prolactinoma after pregnancy and lactation: A study on 73 patients. *Clin Endocrinol (Oxf)*. **80**:642-8

[19] Donovan B.T. (2020) Lactation. *Encyclopaedia Britannica, Inc*. p.763

[20] Eastham, E., Smith, D., Poole, D. & Neligan, G. (1976) Further decline of breast-feeding. *BMJ*; **1**(6005):305–7

[21] Eglash A. (2014) Treatment of maternal hypergalactia. *Breastfeed Med.* **9**:423-5.

[22] Freeman, M.E., Kanyicska, B., Lerant, A. & Nagy, G. (2000) Prolactin: structure, function, and regulation of secretion. *Physiol Rev;* 80:1523-631.

[23] Grueger B. (2013) Weaning from the breast. *Paediatr Child Health*.**18**:210

[24] Hamlyn, B., Brooker, S., Oleinikova, K. & Wands, S. (2002) *Infant feeding 2000.* 6th edn. London: The Stationery Office

[25] Hector, M., Lindsay, H. (2012) Mammary gland development. Wiley interdiscip Rev Dev Biol,. Doi:10.1002/Waev.35

[26] Ibekwe H.A. (2018) Antilactogenic Characteristics of Methanol and Ethyl acetate Crude Extract of *Aframomum melegueta* seeds in Wistar rats. *International Journal of Veterinary Sciences and Animal Husbandry*. **3**(5): 33-39

[27] Igwe S. A., Emeruwa, I. C. & Modie, J. A. (1999) Ocular toxicity of Aframomum melegueta (alligator pepper) on healthy Igbos of Nigeria. *J. Ethnopharmacol*. 65(3): 203-206

[28] Inegbenebor, U. & Ebomoyi, M. (2014) Effect of Saline Extract of Alligator Pepper (*Zingiberaceae Aframomum Melegueta*) On Serum Progesterone in Pregnant Sprague Dawley Rats. *International Journal of Herbs and Pharmacological Research, Ijhpr*, 3(3): 46 – 51

[29] Inegbenebor, U., Ebomoyi, M.I., Onyia, K.A., Amadi, K., Aigbiremolen, A.E. (2009) Effect of alligator pepper (Zingiberaceae aframomum melegueta) on first trimester pregnancy in Sprague Dawley rats. *Niger J Physiol Sci.*

[30] Kim Y. J. (2020) Pivotal roles of prolactin and other hormones in lactogenesis and the composition of human milk. *Clinical and Experimental Pediatrics.* 63(8):312–313

[31] *Lawrence, R.A. & Lawrence, R.M. (2015) Breastfeeding: A Guide for the Medical Professiona* Lawrence, R.A &Lawrence, R.M (2011) Breastfeeding: a guide for medicine profession. 7th eds. Maryland Heights (MI): Mosby, p69, 550–613

[32] Lopez-Fontana, C.M., Masselli, M.E., Salicioni, A.M. & Caron, R.W. (2012) The inhibitory effect of progesterone on lactogenesis during pregnancy already evidenced by mid-to-late gestation in rodent. *Pubmed*. Doi:1071/RD11160

[33] Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of toxicity*. 54: 275-287

[34] Lucca, J.M. & Santhosh, A. (2017) Management of hyperlactation syndrome by full drainage and block feeding methods. *Tropical Journal of Obstetrics and Gynaecology*; 34:3, 250-2

[35] Malgwi, I.S., Tanko, Y., Eze, E.D., Kawu, M.U., Salami, H.A., Emmanuel, N.S. & Mohammed. A. (2017) Lactogenic hormones and histological evaluation in female lactating wistar rats treated with fermented soya bean and vitamin C.; 6(I):14-17

[36] Mangesi, L. & Dowswell, T. (2014) Treatments for breast engorgement during lactation. Europe PMC Funders Group. *Cochrane Database Syst Rev*.; **(9)**: CD006946

[37] Marcolina, S.T. & Denchfield, P. (2005) Complementary Therapies and Lactation Suppression. https://www.reliasmedia.com/articles/81994-complementary-therapies-and-lactation suppression

[38] McManaman, J.L. & Neville, M.C. (2003) Advanced Drug Delivery Reviews **55**: 629–641

[39] Merewood, A. & Philipp, B.L. (2001) Implementing change: becoming Baby-Friendly in an inner city hospital. *Birth.* **28**: 36– 40

[40] Mohammed, A., Ngwen, A.L, Umaru, I.J, Ahmed, M.U & Boyi, N.R. (2013). Antimicrobial property of aqueous and petroleum ether leaf extracts of *Jatropha curcas. International Journal of Science Inventions Today*

[41] *Moore, D.B. & Catlin, A. (2003) "Lactation suppression: forgotten aspect of care for the mother of a dying child". Pediatric Nursing. 29 (5): 383–4*

[42] Neville, M.C, Mortan, J., Unemura, S. (2001) Lactgenesis: Transition from pregnancy to lactation. *Paediatrics Clinician of North America*; 48:45-52

[43] Nguyen, D.A., Parlow, A.F. & Neville M.C. (2001) Hormonal regulation of tight junction closure in the mouse mammary epithelium during the transition from pregnancy to lactation, *J. Endocrinol.* 170: 347–356

[44] *NHS, (2018) "What happens if your unborn baby dies". nhs.uk.*

[45] NICHD (2009) National Institute of Child Health & Human Development. Breastfeeding. http://www.nichd.nih.gov/health/topics/Breastfeeding/

[46] Nosiri, C., Anyanwu, C., Agim, C. & Nwaogwugwu, C. (2017) Evaluation of Aframomum melegueta aqueous seed extract on food and water consumption in albino rats. *IOSR Journal Of Pharmacy. Volume 7, Issue 4 Version. 1 , PP. 23-26(e)-ISSN: 2250-3013, (p)-ISSN: 2319- 4219*

[47] Nwozo S. O. & Oyinloye B. E. (2011) Hepatoprotective effect of aqueous extract of Aframomum melegueta on ethanol-induced toxicity in rats. *Acta Biochimica Polonica*. 58(3) 355–358

[48] Obike, H.I., Ezejindu, D.N. & Akingboye, A.J. (2014) The Effects Of Aframomum Melegueta Aqueous Extract On The Adrenal Gland Of Adult Wistar Rats. *International Journal of Research In Medical and Health Sciences* Vol. 3, No.6 ISSN 2307-2083

[49] Ojieh, E.A., Adegor, C.E. & Ewhre, O.L. (2013) Regulated effects of *Capsicum frutescens* supplemented diet (C.F.S.D) on fasting blood glucose level, biochemical parameters and body weight in alloxan induced diabetic wistar rats. *British J. Pharmaceut. Res.;* **3**(3):496-507

[50] Okeniyi, S. O, Adedoyin, B. J. & Garba, S. (2013) Phytochemical Screening, Cytotoxicity, Antioxidant and Antimicrobial Activities of Stem and Leave Extracts of *Euphorbia heterophylla*. *Journal of Biology and Life Science.* **4(**1): 24-31

[51] Oladapo, O.T. & Fawole, B. (2006). Treatments for suppression of lactation (Protocol) *Cochrane Database of Systematic Reviews*, **Issue 2**. Art. No.: CD005937. DOI: 2/14651858.CD005937. Published by John Wiley & Sons, Ltd

[52] Olunkwa U.E, Iheanacho K.M.E., Igwe C.U., Nwaogu L.A., Iheanacho J.N. (2023). Bioactive component analysis of aqueous seed extract of *Aframomum melengueta.* GSC Biological and Pharmaceutical Sciences, 25(02), 249–272

[53] Omale, J. & Emmanuel, T. F. (2010) Phytochemical Composition, Bioactivity and wound healing potential of *Euphorbia heterophylla* (Euphorbiaceae) leaf extract. *International Journal on Pharmaceutical and Biomedical Research* **1**(1): 54-63

[54] Omoboyowa D. A., Aja A. O., Eluu F., & Ngobidi K. C. (2017). Effects of Methanol Seed Extract of *Aframomum melegueta* (Alligator Pepper) on Wistar Rats with 2,4-Dinitrophenylhydrazine-induced Hemolytic Anemia. *Recent Advances in Biology and Medicine,* Vol. 3, Pages 11-17

[55] Ormandy, C.J., Camus, A., Barra, J., Damotte, D., Lucas, B., Buteau, H., Edery, M., Brousse, N., Babinet, C., Binart, N. & Kelly, P.A. (1997). Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. *Genes Dev.*; 11(2):167-78

[56] Pillay J. and Davis T.J. (2021) National Library of Medicine, National Institutes of Health. Publishing LLC

[57] Prasad, D. M. R., Izam, A. & Maksudur, R. K. (2012). *Jatropha curcas*: Plant of medical benefits. *Journal of Medicinal Plants Research.* 6(14): 2691-2699

[58] Rifqiyati, N. & Wahyuni, A. (2019) Fennel (Foeniculum vulgare) leaf infusion effect onmammary gland activity and kidney function of lactating rats. N u s a n t a r a b i o s c i e n c e *11.* (**1**): 101-105

[59] Ryan, A.S., Wenjun, Z. & Acosta, A. (2002). Breastfeeding continues to increase into the new millennium. *Pediatrics*; **110**:1103–9

[60] Sofowora, A. (2008) *Medicinal plants of Traditional Medicine in Africa.* 3rd Edition, Spectrum Books, Ibadan, Nigeria. Pp. 199- 181

[61] Spitz A.M., Lee N.C. & Peterson H.B. (1998) Treatment for lactation suppression: little progress in one hundred years. *Am J Obstet Gynecol*. **179**(6 Pt 1):1485-1490

[62] Srinivasan, N., Govindarajulu, P. & Vanithakumari, G. (1980). Inluence of Sex Steroids and Prolactin On Rat Mammary Gland. *Ind. J. Physiol. Pharmac.* Volume 24 number 4;322-328

[63] TOXNET (2014) Drugs and Lactation Database. Bethesda (MD): National Library of Medicine (US) Sage; *LactMed* Record Number 903, 961

[64] TOXNET (2014). Bromocriptine. TOXNET Database http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/*9MiljI:1

[65] TOXNET (2014) Drugs and lactation database. Bethesda (MD): National Library of Medicine (US) Parsley; *LactMed* Record Number 961. http://toxnet.nlm.nih.gov.

[66] Trimeloni L, Spencer J. (2016) Diagnosis and management of breast milk oversupply. *J Am Board Fam Med.* **29**:139-42

[67] van Andel, T., Myren, B. & van Onselen, S. (2012). Ghana's herbal market. *J Ethnopharmacol.* ; 140(2):368-78

[68] WebMD (2005). https://www.webmd.com/vitamins/ai/ingredientmono-307/resveratrol

[69] WHO (2019). Breastfeeeding. World Health Organisation. www.who.int/topics/breastfeeding/en/

[70] Wisdom, G.B. (1976). Enzyme-immunoassay. *Clin Chem.*; 22(8):1243-55. PMID: 780003