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Antilactogennic Characteristics Of Methanol Extract Of Aframomum Melegueta Seed In Wistar Rats.

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***Abstract*:** *This study was designed to investigate antilactogenic characteristics of methanol extract of Aframomum melegueta seed by measuring serum concentrations of lactogenic (prolactin, estradiol and progesterone) hormones in lactating and non-lactating wister rats. A total of 18 non-lactating rats were assigned into three (3) treatments of 6 rats each. Treatment 1 and 2 (controls) received 0.11mg/kg/day of reference drug (bromocreptine) and 100mg/kg/day of Dimethylsulfoxide 1ml: 9ml of normal saline (DMSO) which is referred to as normal control in this study. Treatment 3 received 100mg/kg/day of methanol extract of Aframomum melegueta seed administered to experimental rats. Serum samples were collected and analyzed for prolaction, estradiol and progreterone by ELISA techniques. The result obtain showed a significant ($P<0.05$) reduction in serum concentration of prolactin for both non-lactating and lactating rats relative to normal control rats but a significant ($P<0.05$) increase in serum levels of the same hormones with respect to reference control drug (bromocreptine) in both non-lactating and lactating rats. Serum estradiol levels were significantly ($P<0.05$) reduced by methanol extract relative to normal control rats in non- lactating and lactating rats. There was however, no significant ($P>0.05$) changes in serum levels of estradiol relative to reference control drugs in both non-lactating and lactating rats. There was no significant ($P>0.05$) difference in serum levels of progesterone relative to normal control in both non-lactating and lactating rats but there was a significant ($P<0.05$) reduction in serum levels of progesterone in both group of experimental rats. Ability of the extract to significantly lower serum levels of prolactin and estradiol in both non-lactating and lactating rats confers on it anti-lactogenicity despite non-significant ($P>0.05$) changes in serum levels of progesterone.*

Key word: *antilactogenic, methanol extract, Aframomum melegueta, wistar rats.*

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I. Introduction

Breast milk production is a complex physiologic process involving physical and emotional factors and the interaction of multiple hormones, the most important of which is believed to be prolactin (Wolfang *et al*, 2010). With parturition and expulsion of placenta, progesterone level falls and full milk supply is initiated i.e. lactogenesis (Neville *et al*, 2001). In most laboratory species, mammary gland lobuloalveolar growth could be induced after ovariectomy with combination of estrogen and progesterone injection (Falconer, 1970). Extensive proliferations of the mammary gland in response to ovarian hormones occur only if the pituitary gland is intact. In both the rat and the mouse, studies revealed the hormonal requirements for mammary gland proliferation in the absence of endogenous mammogenic hormones i.e. after the removal of the pituitary and the ovaries or the pituitary, ovearies and adrenals (Cowie, 1971). Studies by Talwalker and Meites, 1964 showed that in the hypophysectomized-ovariectomized-adrenalectomized rat, the pituitary hormone by themselves in sufficient doses were capable of inducing tubuloalveolar development (Falconer, 1970). This observation does not imply that the ovarian hormones are of no importance in normal mammary growth but that the pituitary hormones must be regarded the more essential since the ovarian hormones in the absence of pituitary hormones have little or no mammogenic activity in normal mammary growth (Falconer, 1970).

The pituitary hormone of interest in this study is prolactin wich causes milk secretion from the breast after estrogen and progesterone priming. Its effect on the breast involves increased action of messenger ribonucleic acid (mRNA), increased production of casein and lactalbumin (Foltin *et al*, 2003).Increased serum concentration of prolactin during pregnancy causes enlargement of the mammary gland and increase the production of milk (Melmed and Kleinberg, 2008). The high level of progesterone during pregnancy acts directly on the breast to stop ejection of milk (Fleet *et al*.,1975). During pregnancy, high circulating concentration of estrogen promote prolactin production (Daryl, 1996). The secretion is however, inhibited by

hypothalamic and section of pituitary stalk which leads to an increase in circulating prolactin (Stanley, 2005). Prolactin levels peak at rapid eye movement sleep (REM) and in the early hours of the morning (Pagana and Pagana, 2006).

The use of synthetic drugs to improve milk secretion in exclusive breast feeding has more often been challenged with hyperprolactinemia, prolactinoma, lactation amenorrhoea and galactorrhoea (Clive et al, 2002). Lactogogues or lactogenic agents are medication of other substances capable of initiation, maintenance or augmentation of maternal milk production (Lawrence and Lawrence, 1999). The galactogogues in common use include metoclopramide, domperidone and sulpiride which act by blocking dopamine receptors and therefore, the prolactin inhibiting action of dopamine. Domperidone is said to increase milk secretion and is safe (dasilva *et al*, 2001). The continued use of these drugs with their attendant side effects led to the discovery and use of antilactogenic agents. The orthodox antilactogenic drugs (cabergoline, pergolide, bromocreptine, quinagolide) required to handle the emerging side effects are associated with serious contra-indications (nausea, fatigue, postoral hypotension, nasal congestion, exacerbation of psychosis, seizure and cardiovascular injuries that limits completion of dosage regimen. It therefore becomes imperative to seek for natural alternative with little or no adverse effect on the recipients.

Aframomum melegueta is the plant of choice. Aframomum melegueta is an herbaceous perennial plant native to swampy habitats along the West African Coast. Its trumpet-shape purple flowers develop into 5-7cm long pods containing small reddish-brown seeds (Paul, 1961). Essential oils, occur only in traces (Maguelonne and Anthea, 2009). The use of Aframomum melegueta root extract in inhibiting excessive milk secretion by nursing mothers has been reported (John 1984). The dose dependent inhibitory effect of Vincristine on milk secretion in goat was reported with 1mg of this alkaloid decreasing milk yield to approximately 40% of previous yield (Henderson and Faukner, 1985). Phytochemistry revealed the presence of alkaloids, glycosides, tannis, flavonoids, sterols, triterpenes and oils (Okwu, 2005). In addition, seed extract of A. melegueta contains polyphenols and hydrocyanic acid.

This experiment is therefore; set to investigate the antilactogenic properties of seed of Aramomum melegueta methanol extract in Wistar rats using bromocreptine as reference control drugs. Its action on the lactogenic hormones (prolactin, estradiol and progesterone) is the interest of this research.

II. Materials And Methods

Preparation of plant material

Fresh fruits of Aframomum melegueta (A. melegueta) weighing 500g were purchased from Apiapum market in Obubra Local Government Council of Cross River State of Nigeria. The fruits were sundried for two weeks to facilitate removal of the seeds from the pods (capsule). The seeds were further sundried to constant weight within four days and stored in an air-tight flask for extraction.

1.1 Preparation of Aframomum melegueta seed extract:

The seeds were ground to powdered form by the use of motor powered milling machine at Root Crop Research Institude,Umudike in Abia State. A 250g weight of Aframomum melegueta seed powder was first extracted with petroleum ether (60-80^{o}c) to defat the powder. The ether extract was carefully decanted leaving the residue for further extraction in methanol. The soxhlet apparatus was filled with methanol and 200g of the residue was wrapped in a thimble and placed in a soxhlet extractor fitted to a 500ml round bottom flask seated on a hot plate. The reflux condenser was attached to the extraction tube and power was supplied by switch linking the electric hot plate cable. The methanol vapour passes up the side tube of condenser and runs back on to the residue in the thimble. After siphoning over for 24 times, the experiment was stopped just before the next lot of methanol was at the point of siphoning over. The methanol soluble fraction or methanolic extract was then preserved at 4^{0}C ready for administration to experimental rats.

1.2 Preparation of stock solution of crude extrat of aframomum melegueta seeds.

One gram (1g) of methanol extract of Aframomum melegueta seed was dissolved in one (1ml) milliliter of Dimethyl sulfoxide Dmso and suspended in 9ml of normal saline (0.9% NaCl). The stock solution was immediately administered to rats in treatment III of this study.

1.3 Animal and animal procurement

A total of 18 wistar rats each for non-lactating and lactating groups were assigned on the basis of weight (150-200g) into 3 treatments of 6 rats each. The animals were housed in the Department of Biochemistry, University of Calabar under standard laboratory conditions of ambient temperature of 26^{o}c and adequate ventilation with relative humidity of 50% and a 12 hour day-light cycle. Animal ethic regulations were observed. The administration of Aframomum melegueta seed extract commenced 3 days postpartum and

lasted 3 weeks for both non-lactating and lactating rats. Rats in Treatment Group I: received bromocreptine (reference standard drug) at a dose of 0.11mg/kg/day according to manufacturer's instruction.

Treatment Group II rats received 100mg/kg/day of dimethyl sulfoxide (1ml in 9ml of normal saline) for normal control treatment.

The rats in Treatment III received 100mg/kg/day of methanol seed extract of Aframomum melegueta via oral intubation. The treatment was terminated after a 3 week period of administration of crude extract. The rats were starved of food overnight, weighed and sacrificed by euthanasia with chloroform vapour in an air tight desicator. The blood sample was aseptically collected via cardiac puncture and transferred into sample labeled bottles, while the heart was still beating. The collected blood was allowed to stand for 2 hours to perfect clotting of blood and centrifuged at 1000rpm and serum removed with Pasteur pipette for assay of prolactin, estradiol and progesterone concentrations.

1.4 Estimation of serum prolactin concentration

This was carried out by microwell enzyme immuno-assay using the method of Frantz, 1978. A total of 41 streptavidin located micro-plate was removed from the zip-lock kit being-for a substrate and two each for reference standard and control and 36 micro-plates for the test samples. They were properly labeled and placed in a micro-plate holder. Twenty five (25 µl) micro-liter of standards, controls and samples were dispensed into each micro-well. A total of 100µl of conjugate reagent was dispensed into each well and thoroughly mixed for 30 seconds. These were incubated at room temperature for 60 minutes and the content of the well discarded by decantation and the micro-plate blotted dry with absorbent paper. A 300VI wash was aspirated and repeated twice making a total of three washes. One hundred (100 µl) micro liter of 3,3'5'5'tetramethyl bendine (TMB) was added into each well and gently mixed for 110 seconds. These were incubated at room temperature in the dark for 15 minutes. A 50 µl of stop solution was gently added to each well and mixed after the blue colour of the well has completely changed to yellow. The absorbance of each well was read at 450nm wave length. The average absorbance values were calculated from the duplicate standards, controls and sample and a standard curve was constructed by plotting the average absorbance obtained from each reference standard against its assigned concentration in ng/ml on linear graph paper with the absorbance on the vertical (Y) axis and the concentrations on the horizontal (x) axis. The corresponding concentration of prolactin in the sample was determined from the intersecting point of the curve.

1.5 Estimation of serum estrogen (estradiol) concentration This was determined by microwell enzyme immunoassay according to the method of March *et al*, 1979, and progesterone serum concentration according to the method of Radwanska *et al*, 1978.

1.6 Data analysis: Data collected were subjected to statistical analysis using one way comparison analysis of variance (ANOVA). A one way analysis of variance was used to compare differences among groups of treatment and significant means separated by least significant difference.

III. Result

Table 1.1 – Treatment schedule for experimental rats.

Animal Category	**Treatments or Design of experiment**		
	Bromocreptine	Control	Methanol extract
Non-lactating (NL) rats	6	6	6
Lactating (L) rats	6	6	6

(NL)			
	Treatment Groups		
Lactogenic hormones	Bromocreptine 0.11mg/kg/day	Control 100mg/kg/day	Methanol extract 100mg/kg/day
Prolactin (ng/ml)	12.56 ± 0.89^{b}	33.23 ± 1.82^{c}	26.156 ± 0.15^{m}
Estrogen (estradiolng/ml)	2.81 ± 0.12	3.05 ± 0.06^{c}	2.68 ± 0.08^{m}
Progesterone (ng/ml)	127.96 ± 0.06^{b}	323.43 ± 0.20^{c}	315.72 ± 7.72^{c}

Means are +SD. Mean values on the same row with different superscripts are significantly (P<0.05) different.

b-Bromocreptine
c-Control
m-Methanol extract

Table 1.3 – Serum concentrations of selected lactogenic hormones in lactating (L) rats.

	Treatment groups		
Lactogenic hormones	Bromocreptine	Control	Methanol extract
	0.11mg/kg/day	100mg/kg/day	100mg/kg/day
Prolactin (ng/ml)	10.56 ± 0.29^{b}	19.90 ± 1.16^{c}	14.90 ± 1.16^{m}
Estrogen (estradiol ng/ml)	2.41 ± 0.80^{b}	$3.17+0.09^{c}$	2.40 ± 0.03^{b}
Progesterone (ng/ml)	110.21 ± 1.16^{b}	$320.60\pm0.19c$	318.001 ± 2.26^{c}

Means are + SD. Means values on the same row with different superscripts are significantly (P<0.05) different .

Serum concentrations of selected lactogenic (prolactin, estrogen and progesterone) hormones were presented in table 1.2. The result indicates that bromocreptine (standard reference drug) produced a significant (P<0.05) decrease in serum concentration of prolactin (12.56±0.89ng/ml) relative to (33.23±1.82ng/ml, 26.15+0.15ng/ml) control and methanol extract treated non-lactating rats (approximately 62 and 21%) respectively. The standard reference drug (bromocriptine) did not produce significant (P>0.05) changes (increase or decrease) in serum concentration of estradiol (2.81±0.12ng/ml) with respect to control (3.05±0.06ng/ml) and methanol extract treated in this category of rats. The serum concentration of progesterone was however, significantly (P<0.05) reduce (127.96±6.06ng/ml) by bromocreptine in comparison with control (323.43±0.20ng/ml) and methanol extract treated (315.72±7.72ng/ml) rats of non-lactating category. Methanol extract produced a significant (P<0.05) changes in serum concentration of estradiol (2.68±0.08ng/ml) with respect to control (3.05±0.06ng/ml). Methanol extract did not however, produce significant (P<0.05) reduction in serum concentration of progesterone (315.72±7.72ng/ml) relative to control (323.43±0.20ng/ml) non-lactating rats. For the lactating rats, in table 1.3, administration of bromocreptine brought about significant (P<0.05) lowering (10.56±0.26ng/ml) of prolactin in comparison with the control and methanol extract (19.90±1.16ng/ml) and (14.90±1.16ng/ml) respectively. Methanol extract also caused significant (P<0.05) reduction (14.90±1.16ng/ml) relative to control (19.90±1.16ng/ml) with respect to serum concentration of prolactin. For serum concentration of estradiol, bromocreptine as well as methanol extract produced significant (P<0.05) reduction (2.41±0.80, 2.40±03ng/ml) relative to control (3.17±0.09ng/ml) treatment but there was no significant (P>0.05) changes between them. The serum concentration of progesterone was however, not altered by methanol extract (318.001±2.26ng/ml) relative to control (320.60±0.19ng /ml) rats but bromocreptine still caused a significant (110.21±1.15ng/ml) decrease relative to control and methanol extract (320.60±0.19ng/ ml, 318.00±2.26ng/ml) respectively.

IV. Discussion

The result obtained did not establish a definite pattern of reduction of these selected lactogenic hormones in non-lactating and lactating rats. One would expect a more reduction in serum levels of prolactin in non-lactating rats than in lactating since the volume of prolactin increases in lactating than in non-lactating rats. However, methanolic extract of Aframomum melegueta seed produced significant reduction in serum levels of prolactin and estradiol in both non-lactating and lactating rats. The lowered serum concentration of prolactin in rats treated with standard reference drug bromocreptine may not be unconnected to its action as dopamine agonist which inhibits the secretion of prolactin (Clive *et al*., 2002). The drug did not only reduce the serum concentration of prolactin but went ahead to decrease that of estrogen and progesterone in both lactating and non-lactating rats relative to control treatment. Methanol extract of Aframomum melegueta seed produced a significant decrease in serum concentration of prolactin of wistar rats similar to bromocreptine. This probably may be due to presence of alkaloid in Aframomum melegueta seed as is the case with bromocreptine [Okwu,

2005]. The decreased serum concentration of prolactin observed in this study is in agreement with the report of Ebong *et al*, 2000, Mark 2004 who observed a significant reduction in serum level of prolactin of wistar non-lactating rats treated with Aligator pepper seed. The hypothesis of a regulatory role of placenta in pituitary prolactin and luteinizing hormone (LH) and release by hypothalamus-pituitary stimulation may have played out here (Tripathi, 2001). Although prolactin under specific condition such as pregnancy, lactation and estrus responds to particular physiological demand (Bray *et al*., 1992) but methanol seed extract of A. melegueta caused significant ($P<0.05$) reduction of serum prolactin in both non-lactating and lactating rats mimicking the actions of bromocreption which is the standard reference drug. Bromocreptine produced a generalized reduction in serum concentrations of the three hormones selected in this research in both non-lactating and lactating rats and this was supported by the report of Ribeiro *et al*, 1997 who reported a significant decrease of serum prolactin by bromocreptine in all estradiol treated rats while researching on the effect of bromocreptine on serum prolactin levels, pituitary weight and immune reaction of prolactin cells in estradiol treated ovariectomize rats. Methanol seed extract of Aframomum melegueta brought about a decrease in serum levels of estradiol in lactating rat. This finding may correlate with the report of Agarwal *et al*, 2014 on stimulating mammary growth development of estradiol just as does prolactin. Conversely the reduced levels of these hormones by the extract manifest the anti-lactogenic effect of the extract. The finding was collaborated by the work of (Hoehn and Marieb, 2007) who reported that estrogen enhances prolactin secretion and that plasma concentration are normally higher in women than men. If estrogen enhances prolactin production and methanol seed extract of A. melegueta reduces the serum levels of estrogen and therefore disrupting the capacity to enhance prolactin production. This may probably contribute to low level of serum prolactin in this study. In other words, decreased concentration of estradiol may probably results in decreased plasma concentration of prolactin as is the case with methanol extract in this study. This is however, slightly different from the report of Ganong, 2003 who stated that prolactin and estradiol synergize in producing mammary growth but estradiol antagonizes the milk producing effect of prolactin on the udder. Unlike bromocreptine methanol seed extract of Aframomum melegueta did not produce significant reduction in serum progesterone concentration of wistar rats used in the study for both non-lactating and lactating rats. The non-significant response of the extract to serum progesterone is advantageous bearing in mind that progesterone withdrawal is the trigger that initiates lactation (Bandyopadhyay *et al*., 2013). These results did not differentiate much the lactating from non-lactating rats since the extracts produced significant reduction in the affected hormones of lactating as well as the non-lactating wistar rats. No reason has been advanced for this. Generally, serum prolactin levels are higher during the early part of lactation and declines toward the end (Miller, 2002)

V. Conclusion.

Prolactin, estrogen and progesterone all play one role or the other in mammary gland development and thereby enhancing lactation. These roles are played by these hormones at different stages of mammary gland development. To create a ductal tree that fills the fat pad, branching morphogenesis is initiated at puberty by growth hormone and estrogen. Upon pregnancy, the action of progesterone and prolactin generate alveoli which secrete milk during lactation. Prolactin is the major generator of lactational competence during pregnancy and this functions both indirectly through its regulation of ovarian progesterone production and directly via its effects on mammary epithelia cells. Agonistic or antagonistic actions to these functions will either elevate or lower concentrations of these hormones thereby increasing or decreasing mammary gland development viza-viz milk secretion. The reduction in serum levels of prolactin and estrogen in both lactating and non-lactating wistar rats treated with methanolic seed extract of Aframomum melegueta demonstrated anti-lactogenesis even though serum progesterone was not significantly affected by the treatment. By the findings of this study, Aframomum melegueta seed extract slightly mimicked the reference drug bromocreptine and could be a good alternative in managing cases of hyperprolactinemia, galactorrhoea and prolactinoma.

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