Antilactogenic 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 (bromocrepitine) and 100mg/kg/day of Dimethylsulfoxide (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 prolactin, estradiol and progesterone by ELISA techniques. The result obtained 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 (bromocrepitine) 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 both 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.

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, ovaries 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 which 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 sulphuride 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, bromocriptine, quinagolide) required to handle the emerging side effects are associated with serious contra-indications (nausea, fatigue, postural 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 (Mauguelonne 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 Fautkner, 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 Aframomum melegueta methanol extract in Wistar rats using bromocriptine 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 powder form by the use of motor powered milling machine at Root Crop Research Institute, Umudike in Abia State. A 250g weight of Aframomum melegueta seed powder was first extracted with petroleum ether (60-80°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°C ready for administration to experimental rats.

1.2 Preparation of stock solution of crude extract 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°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
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lasted 3 weeks for both non-lactating and lactating rats. Rats in Treatment Group I: received bromocriptine (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 desiccator. 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.

<table>
<thead>
<tr>
<th>Animal Category</th>
<th>Treatments or Design of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bromocriptine</td>
</tr>
<tr>
<td>Non-lactating (NL) rats</td>
<td>6</td>
</tr>
<tr>
<td>Lactating (L) rats</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Lactogenic hormones</th>
<th>Bromocriptine</th>
<th>Control 100mg/kg/day</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin (ng/ml)</td>
<td>12.56±0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.23±1.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.15±0.15&lt;sup&gt;m&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Estrogen (estradiol ng/ml)</td>
<td>2.81±0.12</td>
<td>3.05±0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.68±0.08&lt;sup&gt;n&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>127.96±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>323.43±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>315.72±7.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>
Means are +SD. Mean values on the same row with different superscripts are significantly (P<0.05) different.

b - Bromocrepine
c - Control
m - Methanol extract

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

<table>
<thead>
<tr>
<th>Lactogenic hormones</th>
<th>Bromocrepine</th>
<th>Control</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin (ng/ml)</td>
<td>10.56±0.29b</td>
<td>19.90±1.16c</td>
<td>14.90±1.16m</td>
</tr>
<tr>
<td>Estrogen (estradiol)ng/ml</td>
<td>2.41±0.80b</td>
<td>3.17±0.09c</td>
<td>2.40±0.03b</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>110.21±1.16b</td>
<td>320.60±0.19c</td>
<td>318.00±2.26c</td>
</tr>
</tbody>
</table>

Means are +SD. Mean 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 bromocrepine (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 (bromocrepine) 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) reduced (127.96±6.06ng/ml) by bromocreptine in comparison with control (323.43±20.20ng/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±20.20mg/ml) non-lactating rats. For the lactating rats, in table 1.3, administration of bromocrepine 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, bromocrepine as well as methanol extract produced significant (P<0.05) reduction (2.41±0.80, 2.40±0.03ng/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 (318.00±2.26ng/ml) relative to control (320.60±0.19ng/ml) rats but bromocrepine 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 bromocrepine may not be unconnected to its action as dopamine agonist which inhibits the secretion of prolactin (Clive et al., 2002). The drug did not onl 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 bromocrepine. This probably may be due to presence of alkaloid in Aframomum melegueta seed as is the case with bromocrepine [Okwu,
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The decreased serum concentration of prolactin observed in this study is in agreement with the report of Bandyopadhyay et al., 2001. The hormone prolactin and estrogen, both play a significant role in the development of the mammary gland. They initiate the process of lactation and regulate the production of milk. In the mammary gland, prolactin and estrogen work in tandem to promote lactation. Estrogen is known to increase the size and number of alveoli, while prolactin stimulates milk production.

Prolactin is the major hormone that stimulates lactation during pregnancy and is produced in response to estrogen and the luteinizing hormone (LH) produced by the pituitary gland. After birth, prolactin levels increase further in response to the decrease in progesterone levels. In this study, the methanol seed extract of Aframomum melegueta did not produce significant reduction in serum prolactin 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 in mind that progesterone withdrawal is the trigger that initiates lactation. Unlike bromocriptine, 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 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 function is generally 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 antilactogenesis 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 bromocriptine and could be a good alternative in managing cases of hyperprolactinemia, galactorrhea and prolactinoma.

References.


