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Galactagogoue effect of aqueous leaf extract of v*ernonia amygdalina* on serum prolactin secretion in lactating female wistar rats

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Abstract
This study was designed to investigate the effect of consumption of leaf extract of *Vernonia amygdalina* during lactation on serum prolactin level. Twenty-four (24) healthy female wistar rats weighing between 150-180g were used for this study. At day 1 of delivery, the animals were divided into 4 groups labeled A – D with six (6) dams in each group. Group A served as control group and received distilled water only. Groups B – D received 100, 250 and 500mg/kg of aqueous leaf extract of *V. amygdalina* respectively from day 1 of lactation to day 21 via oral route. Blood samples were then collected on day 22 through cardiac puncture and the serum prolactin level was evaluated. Result showed no significant increase ($P>0.05$) in serum prolactin level in test groups B and C when compared with the control group whereas, group D showed significant increase ($P<0.05$) in prolactin level when compared with control group. This study supports the use of *V. amygdalina* in traditional medicine to increase milk production among lactating mothers.

Keywords: *vernonia amygdalina*, lactation, prolactin, maternal, galactagogue

1. Introduction
The number of lactating women who have produced insufficient breast milk is on the rise [1]. It has been claimed that at least 5% of women experience lactation failure (agalactias) while approximately 15% of women experience inadequate supply of their breast milk (hypogalactias) at 3 weeks postpartum [2]. Based on data from the WHO, estimated 130 million babies are born worldwide each year and 4 million babies die within the first 28 days of life due to improper lactation.
Breastfeeding provides numerous benefits for newborn infants and mothers. It reduces the incident of Sudden Infant Death Syndrome (SIDS), allergic/hypersensitivity diseases, and development of Type 1 (insulin dependent) and Type 2 (non-insulin dependent) diabetes mellitus [3] relative to the use of infant formula. Breastfeeding is considered critical for sustaining the health and well-being of newborns and infants [4]. It may also play a role in decreasing post-partum depression, osteoporosis, bleeding, and improving weight control [5]. Prolactin is a polypeptide hormone that is synthesized in and secreted from specialized cells of the anterior pituitary, the lactotrophs. It is in charge of milk production, and also contributes to breast development [6]. Prolactin levels decrease as lactation is established but nursing stimulates prolactin release from the pituitary which promotes continued milk production [7]. Prolactin stabilizes and promotes transcription of casein mRNA; may stimulate synthesis of alpha-lactalbumin, the regulatory protein of the lactose synthetase enzyme system; and increases lipoprotein lipase activity in the mammary gland [7].
The use of medicinal plants to stimulate breast milk production has a long history of use [8] in almost all cultures over the world but has not been extensively studied nor fully exploited for use in lactating mothers [1].
Vernonia amygdalina belongs to the family *Asteraceae.* It is a valuable medicinal plant. It is a common shrub or small tree that grows in tropical Africa and in Asia. Its common name in Africa is bitter leaf [9]. The leaves are green in colour with a characteristic odour and bitter taste [10], but the bitterness can be abated by boiling or by soaking in several washing using clean water. The leaves of *Vernonia amygdalina* are used as soup condiments after washing and boiling to get rid of the bitter taste. Ethno-medical use of *Vernonia amygdalina* in the treatment of ailments such as veneral diseases, gastrointestinal problems, infertility, diabetes and malaria had also been reported [11, 12, 13]. Phytochemical compounds such as saponins, alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides, sesquiterpenes have been extracted and isolated from *Vernonia amygdalina* leaf extract [13, 14]. It has also been reported to possess the following pharmacological properties; antibacterial, hypolipidemic, antihyperlipidemic, antidiabetic, antioxidant and hepatoprotective activities [15, 16].
Native women strongly believe that *Vernonia amygdalina* enhances milk-let-down making this herb important for lactating mothers. This research work was carried out based on this belief as there seems to be paucity of scientific proof to support this claim.

2. Material and Methods
2.1 Plant material and Extract Preparation
Fresh leaves of *V. amygdalina* were harvested from a farm in Okofia, Nnewi Anambra State, Nigeria. The leaves were

identified by a herbarium curator in the Department of Botany, Nnamdi Azikiwe University Awka and a voucher specimen was deposited at the herbarium with voucher number (N.A.U.H #47c). It was classified under the family *Asteraceae.*

The *V. amygdalina* leaves were air-dried for 2 weeks while retaining the greenish coloration. The dried leaves were milled and grounded into coarse powder using Wiley machine. The powder was collected in a clean cellophane bag and then taken to the laboratory where extraction was done using soxhlet extractor. The sample weighed 52g; it was dissolved in 100mls of distilled water for two hours. The mixture was poured into an extractor and heated for eight hours. The mixture was then evaporated and collected through a condenser. The aqueous extract was poured into a tray and put in the oven set at 60 degrees centigrade overnight. 20g of the dried sample was weighed and dissolved in 100mls of distilled water. The derived mixture was shaken continuously and air-tight for complete dissolution.

2.3 Extract Administration and Sample collection

Twenty-four female rats weighing between 150g-180g were purchased from the Animal House of College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra state, Nigeria. They were fed with standard rat-pelleted diet (Growers mesh). They had access to feed and water *ad libitum.*

On day1 of delivery the dams and their litters were randomly assigned into four groups of six dams each, labeled groups A, B, C and D. Group A served as the control group that received distilled water only while, groups B – D served as the test groups and received 100, 250 and 500mg/kg of aqueous leaf extract of *V. amygdalina* respectively. Extract administration was done orally using an oral canula from day 1 of delivery to day 21 of lactation. Animals in all the groups received feed and water *ad libitum.* All procedures used in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of Animals [17].

On day 22, blood was collected by cardiac puncture into well labeled plain tubes for hormonal assay. Centrifugation of the blood was done using an ultracentrifuge and supernatant serum was collected. ELISA test kit was used for the serum prolactin hormonal assay.

2.4 Statistical Analysis

All data were tabulated and statistically analyzed using SPSS version 21.0. Results were expressed as Mean ± standard error of mean (SEM). Comparative analysis amongst groups was done using one-way analysis of variance (ANOVA). A post-hoc analysis using Bonferoni multiple comparative tests was performed to identify significant groups. $P<0.05$ was taken as statistically significant.

3. Results

Result showed no significant increase ($P>0.05$) in serum prolactin level in groups B (12.93±0.7ng/mL) and C (14.50±0.3ng/mL) when compared with the control (11.67±0.4ng/mL). However, test group D showed significant increase ($P<0.05$) in serum prolactin level (17.90±1.1ng/mL) when compared with the control group.

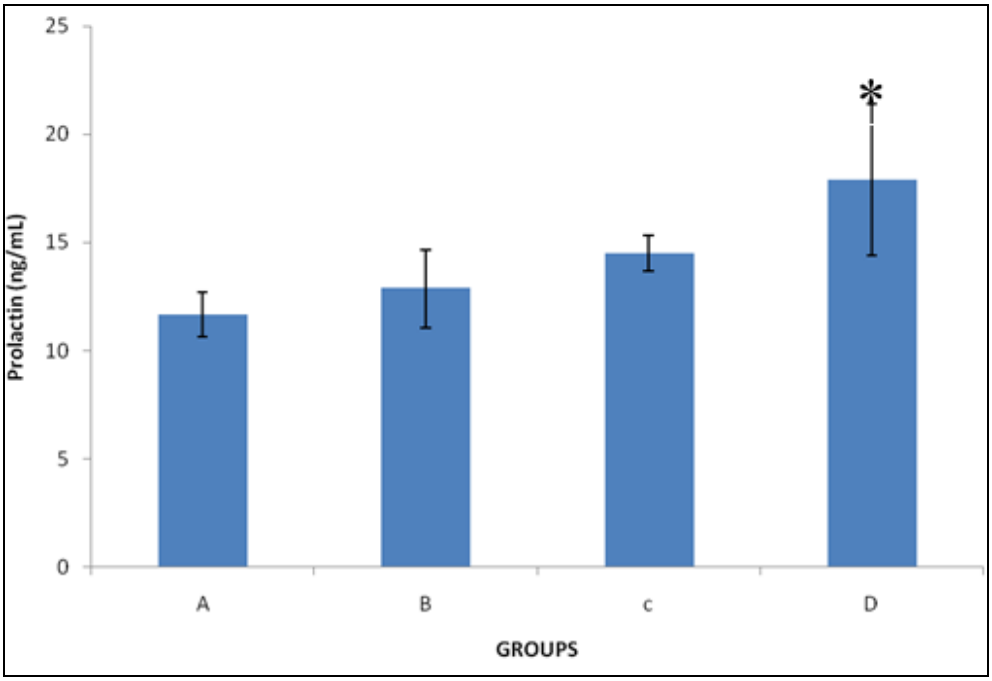

Fig 1: Effect of aqueous leaf extract of V. amygdalina on serum prolactin level of lactating rats

4. Discussion

Proper lactation provides tailored nourishment to the growing needs of infants, offering optimal nutrition, improved cognitive performance and neurological development [18] as well as enhanced immunity [19)].

The result of the present study revealed that, the aqueous leaf extract of *V. amygdalina* produced appreciable increase in serum prolactin level in group D which received the highest dose when compared with the control group. This is in agreement with a similar study done by Igwe *et al* [20] who reported a dose-dependent increase in serum prolactin level in rats administered ethanolic extract fraction of *V.amygdalina* during lactation. The result of the present suggests that the extract may possess the same

phytochemical substances as seen in plants classified as galactogogues. The galactogogue quality exhibited by *V. amygdalina* leaf extract can be explained by their composition of bioactive substances capable of increasing the biosynthesis of milk [21]. These bioactive substances suspected to cause increase in prolactin secretion in this extract are; flavonoids, saponins, and alkaloids which have been reported to show galactogogue quality in some studies [22, 23].

These phytochemicals may either be working to stimulate prolactin secretion from the anterior pituitary that produces and sustains milk production or as dopamine antagonists. Dopamine is a competitive antagonist of prolactin. Thus, an inhibition of dopamine synthesis by bioactive substances would lead to an increase of milk synthesis in postpartum lactating animals [24].

5. Conclusion

This study has shown favorable increase in prolactin level in lactating animals administered aqueous leaf extract of *V. amgydalina.* This therefore supports the use of *V. amygdalina* in traditional medicine to increase milk production among lactating mothers.

6. Acknowledgement

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8. Conflict of interest

None declared

9. References

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