



Effect of *Vernonia amygdalina* Del ethanolic extract on Serum prolactin in lactating and non lactating female albino Wistar rats

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Abstract

Ethanolic crude extract of *Vernonia amygdalina* was fractionated into six (F1, F2, F3, F4, F5, and F6). The different fractions were subjected to in vitro screening to provide preliminary observations required to select the crude plant extract with best contractile properties for further investigations. Using physiograph mammary tissue contractile amplitudes were determined at 0.25 mg/ml, 0.3 mg/ml, 0.7 mg/ml, 1.0mg/ml, 1.25mg/ml and 1.5mg/ml for the different fractions. Fraction F5 had the best contractile response on isolated mammary tissue in the presence of agonist ACh. F5 was used for further studies on prolactin. For the non lactating animals study, the adult female rats were placed in five groups of three rats each with group 1 as control and were given 20% DMSO and group II, III and IV were the test groups. The test groups received 40 mg/kg, 80 mg/kg and 120 mg/kg body weights respectively. Group V was the positive control group and was given 0.1 µg of oxytocin. For the lactation study, the animals were kept in different cages following parturition. They were also placed in five groups of three animals each but in separate cages with their litters. Group I was the control while group II, III and IV were the test groups. Group V was also the positive control. Treatment doses also were the same as in non lactating groups. After 5 days of administration of F5, the serum prolactin concentrations were measured. The non lactating serum prolactin level of group I (2.3 ± 0.05 ng/ml) was significantly ($p < 0.5$) less than in group II (1.6 ± 0.05 ng/ml), group III (1.43 ± 0.16 ng/ml) and group IV (1.13 ± 0.18 ng/ml). Group V, slightly reduced the level of serum prolactin which was not statistically significant (2.06 ± 0.06 ng/ml). The lactating prolactin serum levels of group I (13.50 ± 0.04 ng/ml) was significantly ($P < 0.5$) higher than in group II (14.82 ± 0.12 ng/ml), group III (17.37 ± 0.31 ng/ml) and group IV (19.20 ± 0.49 ng/ml). Group V showed a significant ($P < 0.5$) decrease in serum prolactin concentration (12.62 ± 0.13 ng/ml). The contractile extract fraction (F5) reduced prolactin level in non lactating rats but increase it significantly during lactation in dose dependent fashion. This supports the claims of using the extract to enhance milk production after parturition.

Keyword: *Vernonia amygdalina*, phytocomponents, prolactin, mammary tissue, lactation.

Introduction

Vernonia amygdalina Del is a shrub of 2-5 m tall with petiolate green leaves of about 6mm diameter and it is popularly known as bitter leaf. The leaves are bitter but the bitterness can be abated by boiling or by soaking in several washing using clean water¹. The stem and root divested of the bark are used as chewing sticks in Nigeria. The leaves are used for popular bitter leaf soup and have been reported to be consumed by goats in some part of Nigeria². All parts of the plant are pharmacologically useful³. The roots and the leaves are used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort^{1,4}. The present study is prompted by previous workers^{5,6} that feeding of *Vernonia amygdalina* leaves produced uterine contraction and increased milk flow after parturition. The LD₅₀ of the contractile fraction of *Vernonia amygdalina* was 290mg/kg body weight.

Material and Methods

Collection of plant materials: The leaves of *Vernonia*

amygdalina were harvested from University Farm in Michael Okpara University of Agriculture, Umudike, Nigeria. The plant was identified by Prof M. C. Dike of College of Natural Resource and Environmental Management of the University. Specimen of the leaves was deposited in the Herbarium of Department of Vet Pharmacology and Biochemistry the University.

Extraction and isolation of plant materials: The leaves were air dried on the laboratory bench for 10 days. The dried leaves were milled and grounded into coarse powder using Wiley machine (model 5 USA). The powdered plant sample 360 g was soaked in 2000 ml of ethanol for 24 hours and was filtered with Whatmann no 1 filter paper. The ethanol extract was concentrated using rotary evaporator to obtain a yield of 19.8g which represent 6.6% yield.

Solvent fractionation and column chromatography: Silica gel of particle size 0.050 – 0.200 (50 – 200 mesh size) was used as the stationary phase while gradient solvent system of the

combination of petroleum ether, chloroform and methanol was used as the mobile phase. The sample was prepared by adsorbing 12g of the extract to 36g of the silica gel and was dried in a hot air oven. The adsorbed sample was ground into powder using a ceramic mortar and a pestle. The powder was then carefully poured on top of the packed silica gel in the column. It was then covered with glass wool to avoid spattering of the eluant on the extract which may affect the separation process. The solvent system was gently poured on the sample by the side wall of the inside column with the help of glass funnel. The column tap was gently opened to allow the eluant to flow at the rate of 30 drops per minute. The eluted fractions were collected in 100ml test tubes (table-1).

Table-1
Different solvent proportion for the separation of different compounds in Vernonia amygdalina

Fraction before pooling	Petroleum ether (ml)	Chloroform (ml)
F ₁	100	0
F ₂	90	10
F ₃	80	20
F ₄	70	30
F ₅	60	40
F ₆	50	50
F ₇	40	60
F ₈	30	70
F ₉	20	80
F ₁₀	10	90
F ₁₁	0	100
	Methanol (ml)	Chloroform (ml)
F ₁₂	10	90
F ₁₃	20	80
F ₁₄	30	70
F ₁₅	40	60
F ₁₆	50	50
F ₁₇	60	40
F ₁₈	70	30
F ₁₉	80	20
F ₂₀	90	10
F ₂₁	100	0

Thin layer chromatography: Collected fractions were examined by thin layer chromatography (TLC). The method of Harborne⁷ was adopted. The different fractions were spotted on a pre-coated (silica gel 60 F₂₅₄) aluminium plates and eluted with ethyl acetate and chloroform (30: 70) in a small TLC tank.

Each sample was spotted 3 cm from the margin and was slanted into the TLC tank. The distance moved by the sample and the distance moved by the solvent were recorded. The ratio of the distance moved by the sample and the solvent gave the Resolution front (R_f). The fractions with similar R_f values were pooled together as similar compounds (table-2).

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Table-2
Pooling of different solvent fraction of Vernonia amygdalina using their Resolution front (R_f) values

Fraction before pooling	R _f values	Fraction after pooling
F ₁	0.6760	F1
F ₂	0.6665	
F ₃	0.6435	
F ₄	0.6460	
F ₅	0.3320	F2
F ₆	0.3235	
F ₇	0.3165	
F ₈	0.3330	
F ₉	0.7060	F3
F ₁₀	0.7095	
F ₁₁	0.7030	
F ₁₂	0.5060	F4
F ₁₃	0.5030	
F ₁₄	0.5170	
F ₁₅	0.5385	
F ₁₆	0.6165	F5
F ₁₇	0.6115	
F ₁₈	0.6205	
F ₁₉	0.6150	
F ₂₀	0.8260	F6
F ₂₁	0.8720	

Pooled fraction after TLC: F1, F2, F3, F4, F5, and F6

Laboratory animals preparation: In vitro rat assay for contractile activity using extract fractions. (F1, F2, F3, F4, F5, and f6): The *in vitro* rat bio assay for contractile activity was carried out as described by Yeletsehay⁸. Uterus of non pregnant female Winstar albino rats were used for the testing of the different fractions in the plant extract in the presence of against acetylcholine (ACh). Contractile response was translated by physiograph attached to the uterine tissue. Recording paper and contraction amplitude were used to make the reading. The rats were primed with estrogen 24 hours before the experiment by intra-peritoneal administration.

Determination of serum prolactin level in non lactating rats administered contractile fraction (F5) of Vernonia amygdalina: Five groups of 25 matured female rats were employed for the test. Group 1 was the negative control group and groups II, III and IV were experimental groups, Group V was the positive control group. Group 1 was giving 20% Dimethyl sulphoxide (DMSO), groups II, III, IV received 40mg/kg, 50mg/kg, and 120mg/kg body weight respectively, group V received 0.1 µg of oxytocin intra-peritoneally for 5 days. At the end of the dosing period, the rats were sacrificed by cervical dislocation and blood collected by cardiac puncture. Centrifugation of the blood was done immediately using a ultracentrifuge and the supernatant serum was removed with a Pasteur pipette. The serum was kept in the freezer until analysed.

Determination of serum prolactin level in lactating rats administrated contractile fraction (F5) of Vernonia amygdalin: The matured female rats were grouped into five of three animals but were separated in different cages following parturition. Group I was the negative control group and was given 20% DMSO. Group II, III and IV were the experimental group and were given 40mg/kg, 80mg/kg, and 120mg/kg body weight respectively. Group V was the positive control and was given 0.1 µg of oxytocin intra-peritoneally. The dosing period was 5 days starting from the day of parturition. At the end of the dosing period, the animals were sacrificed by cervical dislocation and blood collected by cardiac puncture. Centrifugation of the blood was done immediately using a ultracentrifuge and supernatant serum was collected and kept in freezer until analysed.

Description principle and sources of kits used: The test kit used for hormonal profile of prolactin in lactating and non lactating rats was Accu Bind ELISA microwells monobind Inc (Lake forest CA USA). Immunoenzymemometric assay (type 3) was used⁹.

Statistical Analysis: Data was analyzed by t-test using SPSS (version 17) software. All values were expressed as the mean value ±standard deviation and the level of significance P<0.05 was considered statistically significant difference between tests and control groups for measured values.

Results and Discussion

Result of *In Vitro* Contraction of Rat Mammary Tissues

Exposed To Different Fractions of Vernonia amygdalina: The result of the screening of the different fractions of *Vernonia amygdalina* F1, F2, F3, F4, F5, and F6 for the peak mammary tissue contractile activity revealed that F5 had the highest amplitude of contraction among the other fractions when compared to the control acetylcholine (figure-1). At 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 1.25 mg/ml and 1.5 mg/ml, the amplitude of contraction was 28 mm, 30 mm, 35 mm, 38 mm, 40 mm and 43 mm respectively, as compared to acetylcholine, 30 mm, 31 mm, 38 mm, 40 mm, 45 mm and 48mm. F5 was therefore selected for further study of their effect on different hormones relevant to mammary tissue contraction

Effect of F5 Fraction of Vernonia Amygdalina on Serum Prolactin Concentration: *Vernonia amygdalina* in all the doses used significantly (P<0.05) decreased the level of prolactin in the blood of treated rats (low 1.6±0.05 mg/ml; mid 1.43±0.16 ng/ml and high 1.13±0.18 ng/ml) compared with control (DMSO 2.3±0.05 ng/ml). This action was dose dependent (figure-2).

Effect of F5 Fraction of Vernonia Amygdalina on Serum Prolactin Concentration During Lactation: Figure-3 shows the result of the effect of F5 of rats. From the results there was a significant (P<0.05) increase in the blood level of prolactin in the lactating rats treated with *Vernonia amygdalina* extract (low 14.82±0.12 ng/ml; mid 17.37±0.31 ng/ml and High 19.20±0.49 ng/ml) when compared to the negative control group (13.50±0.04 ng/ml).

Comparism of Effect of F5 Fraction of Vernonia Amygdalina on Serum Prolactin Concentration in Lactating and Non Lactating Rats: Figure-4 compares the bar graphs of lactating and non lactating rats administered F5 of *Vernonia amygdalina* extract. The result reveals increase in serum prolactin concentration during lactation and decrease when the rats were not lactating. This action was dose dependent.

Discussion: Intra peritoneal administration of contractile fraction (F5) at doses of 40 mg/kg, 80 mg/kg, and 120 mg/kg body weight showed a dose dependant decrease in serum prolactin concentration in non lactating rats of the test groups compared to the control. The result also indicated a dose dependant increase in serum prolactin concentration in lactating rats in test groups compared to the control. *Vernonia amygdalina* may be working to stimulate prolactin secretion from the anterior pituitary that produces and sustains milk production in postpartum lactating mammals¹⁰. This extract could be useful to increase milk flow in mammals just after birth (peurperium). In the non lactating animals, *Vernonia amygdalina* contractile fraction (F5) showed inhibitory effect, probably the extract inhibited the secretion of protactin in non lactating rats. This extract could therefore be useful to check prolactenamia (increased serum level of prolactin) which causes amenorrhea, galactorrhea, loss of libido and infertility, in non lactating mammals, which is in agreement with the work of Ramzi *et al*¹¹.

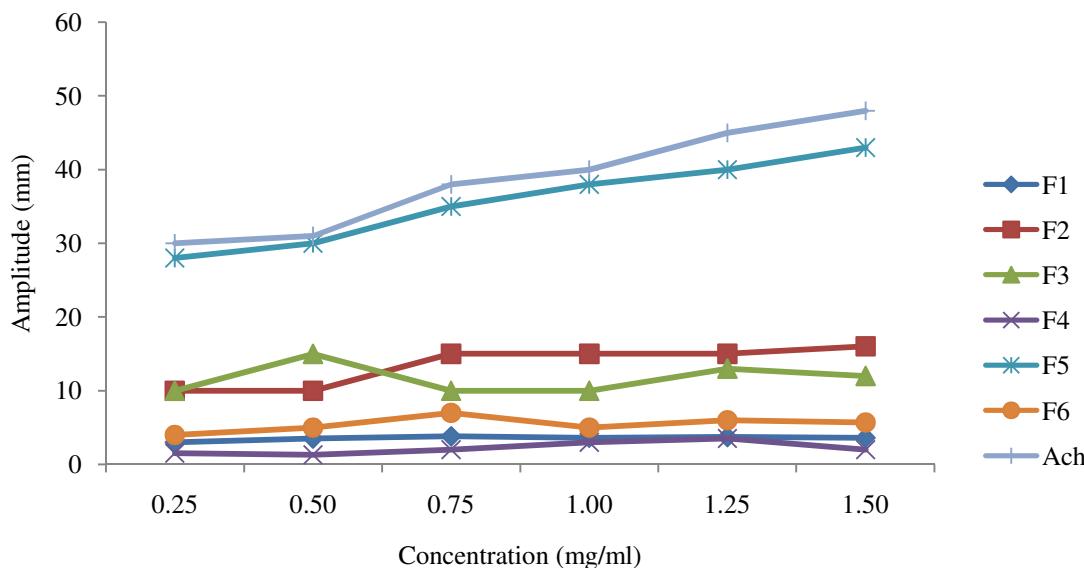


Figure-1

Shows contractile amplitude of different fractions of *Vernonia amygdalina* on rat mammary tissue at 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 1.25 mg/ml and 1.5 mg/ml, compared to the control agonist, acetylcholine

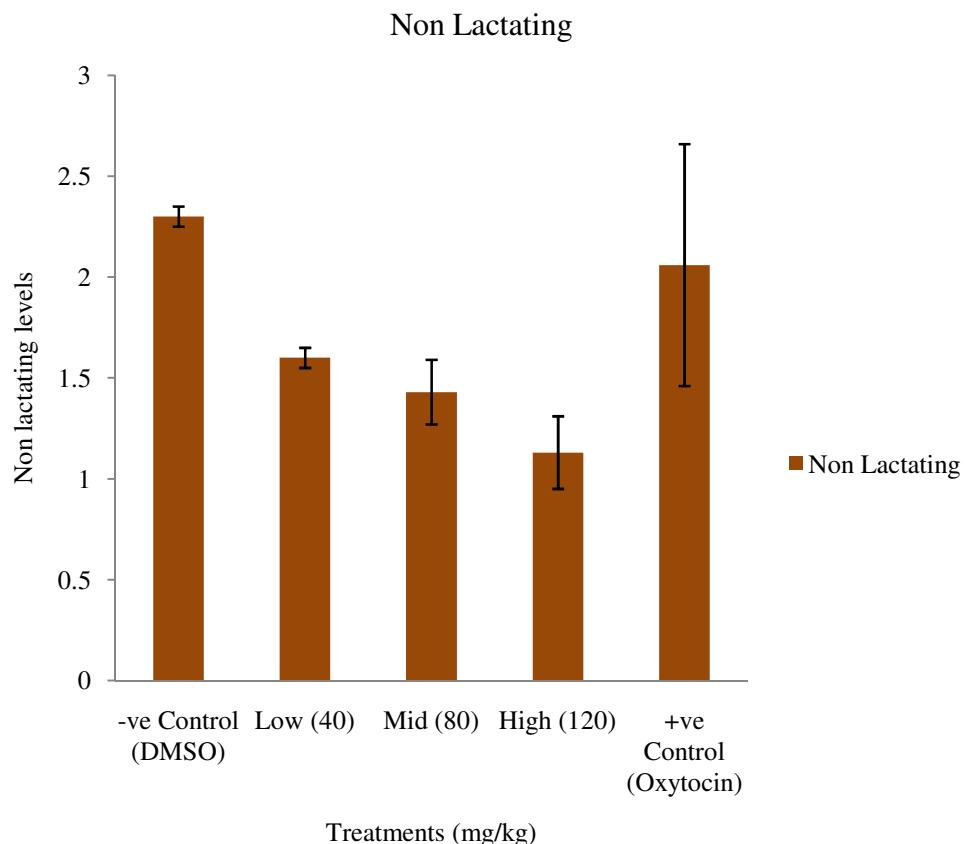


Figure-2

Concentrations of prolactin in the serum of non lactating rats administered F5 of *Vernonia amygdalina* extract

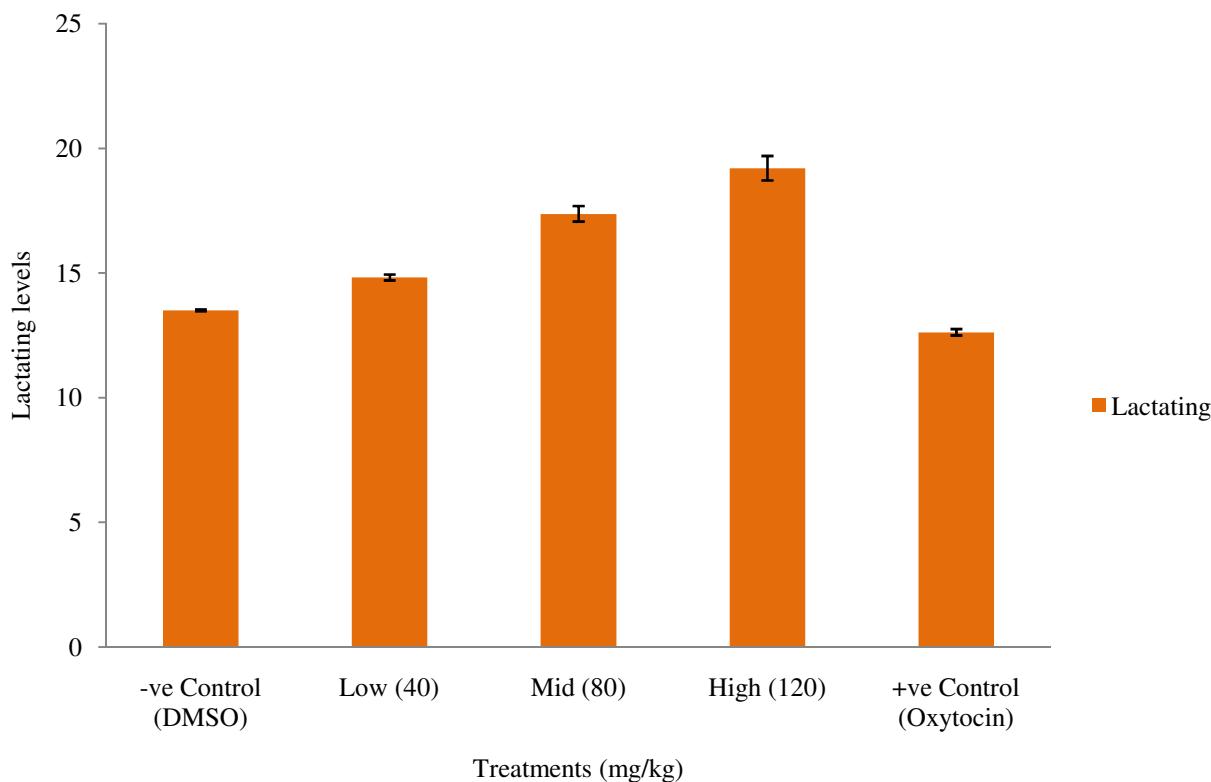


Figure-3
Concentrations of prolactin in the serum of lactating rats administered F5 of *Vernonia amygdalina* extract

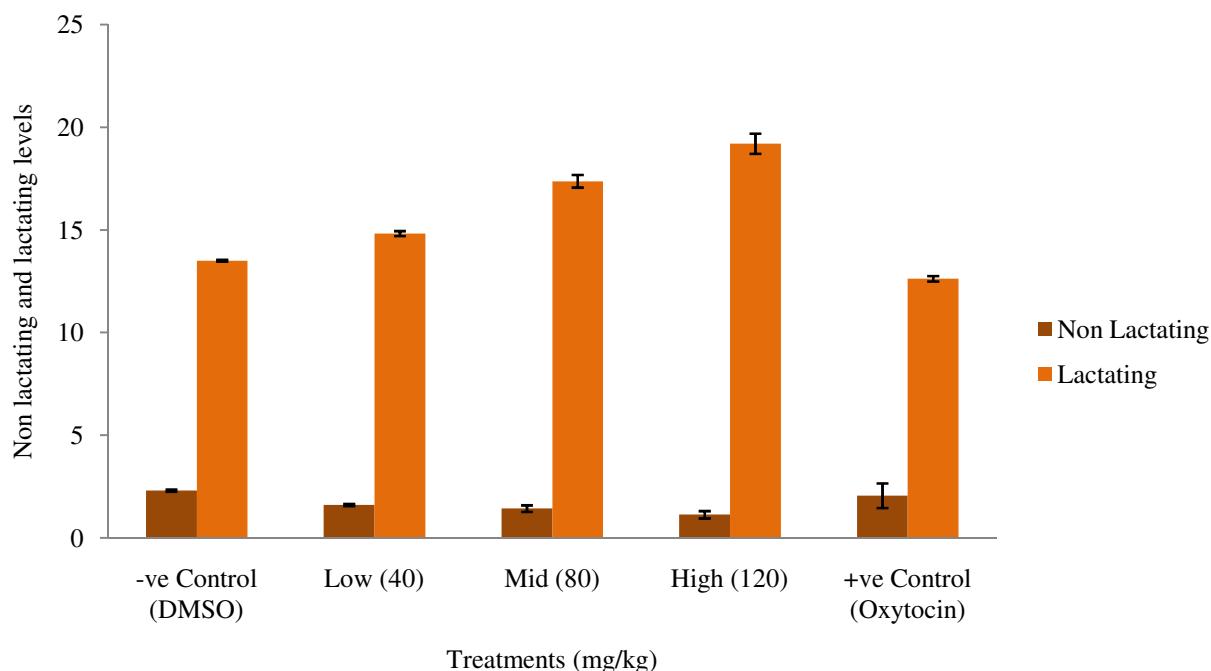


Figure-4
Comparism of concentrations of prolactin in the serum of lactating and non lactating rats administered F5 of *Vernonia amygdalina* extract

Conclusion

The contractile fraction (F5) of *Vernonia amygdalina* caused increase secretion of prolactin in lactating female rats and decrease in secretion in non lactating female rats in a dose dependent manner. Pregnancy, lactating and the administration of oral contraceptives can cause an increase in the level of prolactin¹². Our research finding confirmed increase in prolactin concentration during lactation. Drugs such as morphine, reserpine and the psychotropic drugs increase prolactin secretion^{13,14}. The *Vernonia amygdalina* acted as medicinal plant which caused increase in prolactin concentration during lactation in the experimental rats. Therefore, prolactin hormone concentration in the serum is dependent upon diverse factors other than pituitary homeostasis. *Vernonia amygdalina* could therefore be useful to check prolactenamia (increased serum level of prolactin) which causes amenorrhea, galactorrhea, loss of libido and infertility in non lactating mammals, which is in agreement with the work of Ramzi *et al*¹¹.

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