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Effcet of Vernonia Amygdalina.Del Ethanolic Extract Fraction on Serum Prolactin in Lactating and Non Lactating Female Albino Wistar Rats

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Abstract: *The effect of contractile fraction of Vernonia amygdalina.Del on serum prolactin in lactating and non lactating albino Winstar rats was investigated using standard protocols 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 serum prolactin concentration in lactating and non lactating rats.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 while 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 1 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 1 (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.*

I. 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 water [1].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 Nigeria [2]. All parts of the plant are pharmacologically useful [3]. 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 LD_{50} of the contractile fraction of Vernonia amygdalina was 290mg/kg body weight.

Prolactin has a wide range of biochemical roles. During lactation it stimulates mammary gland to produce milk. Increased serum concentrations of prolactin therefore causes enlargement of the breasts for the production of milk.

Prolactin provides the body with sexual gratifiction after sexual act. The hormone counteracts the effect of dopamine, which is responsible for sexual arousal. This is thought to cause the sexual refractory period. The

amount of prolactin can be an indicator for the amount of sexual satisfaction and relaxation. Unusually high amounts are suspected to be responsible for impotence and loss of libido (a hyperprolactinemia symptom).

Highly elevated levels of prolactin decrease the levels of sex hormones, estrogen in women and testesterone in men. The effects of mildly elevated levels of prolactin are much more variable, in women both substantial increase and decrease of estrogen levels may result.

Prolactin is sometimes classified as a gonadotropin because within the normal reference ranges can act as a weak gonadotropin[7].Physiologic levels of prolactin in males enhance luteinizing hormone-receptors in Leydig cells, resulting in testosterone secretion, which leads to spermatogenesis[8].

Prolactin also stimulates proliferation of oligodendrocyte precursor cells. These cells differentiate into oligodendrocytes, the cells responsible for the formation of myelin coatings on axons in the central nervous system [9]. Prolactin delays hair regrowth in mice [10]. Prolactin promotes neurogenesis in maternal and fetal brains [11].Progesterone has been observed to upregulate prolactin synthesis in the endometrium but decreases it in myometrium and breast glandular tissue [12].Prolactin levels can rise after exercise, meals, sexual intercourse, minor surgical procedures and early morning [13]. Hypersecretion of prolactin may manifest as amenorrhea and infertility in females as well as impotence in males. Inappropriate lactation is another important clinical sign of hormonal changes manifestation.

II. Materials And Methods

2.1 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.

2.2 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.

2.3 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

2.4 Thin layer chromatography

Collected fractions were examined by thin layer chromatography (TLC). The method of Harborne [14] was adopted. The different fractions were spotted on a pre-coated (silica gel 60 F_{254}) 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 (Rf). The fractions with similar Rf values were pooled together as similar compounds, table-2.

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

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

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

Fraction before pooling	Rf values	Fraction after pooling
f_1	0.6760	
f_2	0.6665	F1
f_3	0.6435	
f_4	0.6460	
f_5	0.3320	
f_6	0.3235	F2
f_7	0.3165	
f_8	0.3330	
f_9	0.7060	
f_{10}	0.7095	F3
f_{11}	0.7030	
f_{12}	0.5060	
f_{13}	0.5030	F4
f_{14}	0.5170	
f_{15}	0.5385	
f_{16}	0.6165	
f_{17}	0.6115	F5
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

2.5 Laboratory animals preparation

2.51 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 Yeletsehay [15].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.

2.52 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-peritopeally 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.

2.53 Determination of serum prolactin level in lactating rats administrated contractile fraction (F5) of Vernonia amygdalina

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-peritopeally. 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 ultracentrifuge and supernatant serum was collected and kept in freezer until analysed.

2.6 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 used [16].

2.7 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.

III. Results And Discussion

3.11 Result of in vitro concentration of rats mammary tissue 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 and1.5 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.

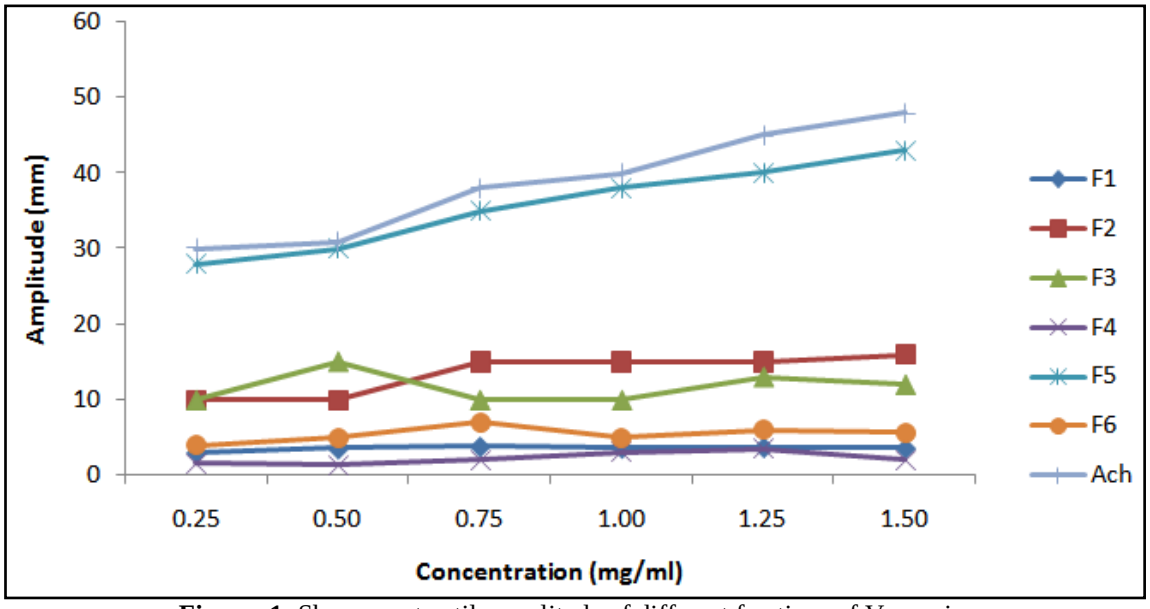
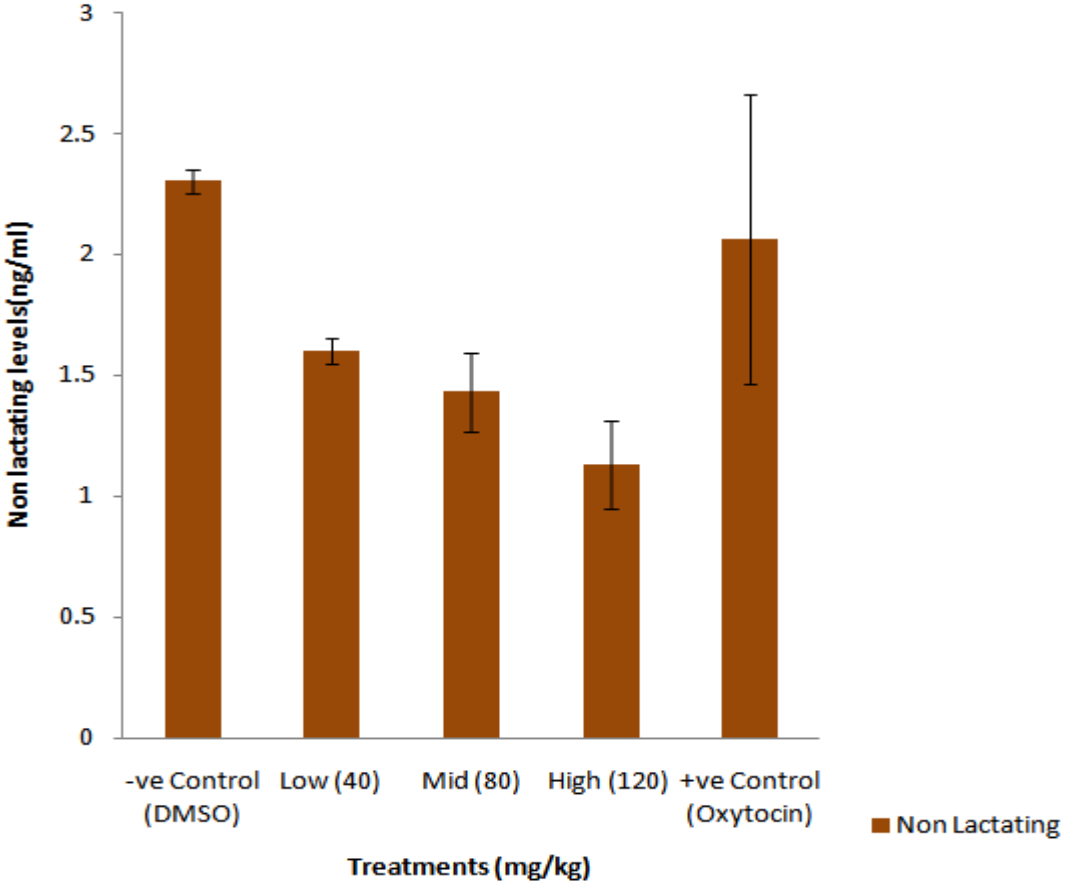


Figure- 1: Shows contractile amplitude of different fractions of Vernonia.

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.

3.12 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



Figur -2. Concentrations of prolactin in the serum of non lactating rats administered F5 of Vernonia amygdalina extract.

3.13 Effect of F5 fraction of Vernonia amygdalina on serum prolactin concentration during lactation

Figure 3, shows the result of the effect of F5 fraction of Vernonia amygdalina on serum prolactin concentration during lactation. 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).

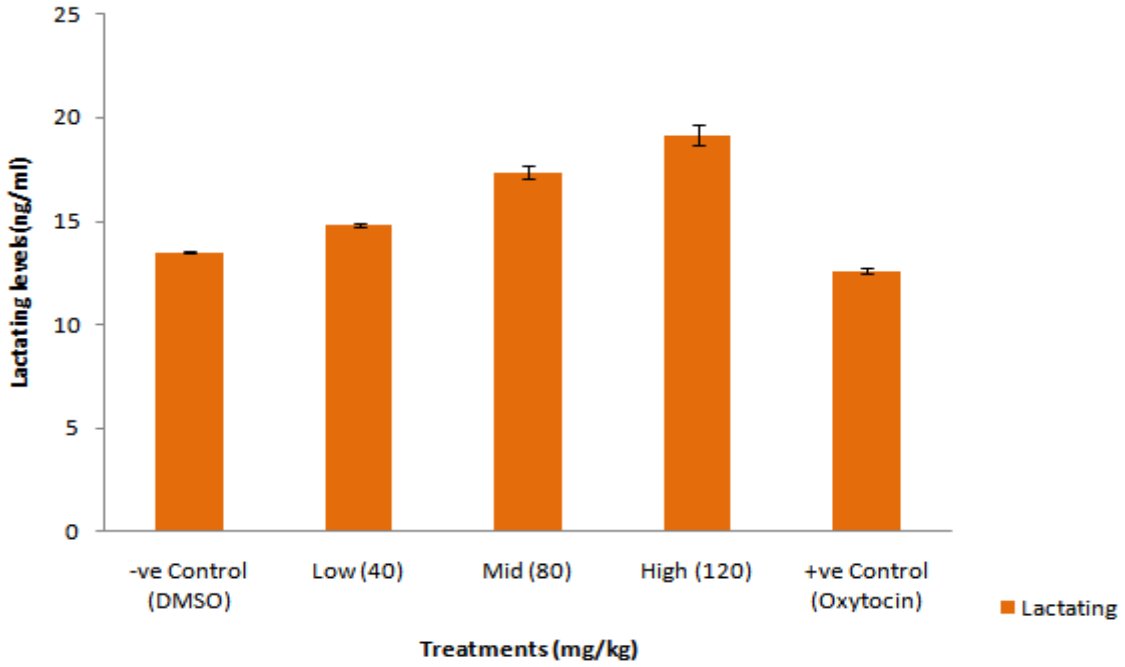


Figure-3 Concentrations of prolactin in the serum of lactating rats administered F5 of Vernonia amygdalina extract.

3.14 Comparison 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 revealed increase in serum prolactin concentration during lactation and decrease when the rats were not lactating. This action was dose dependent.

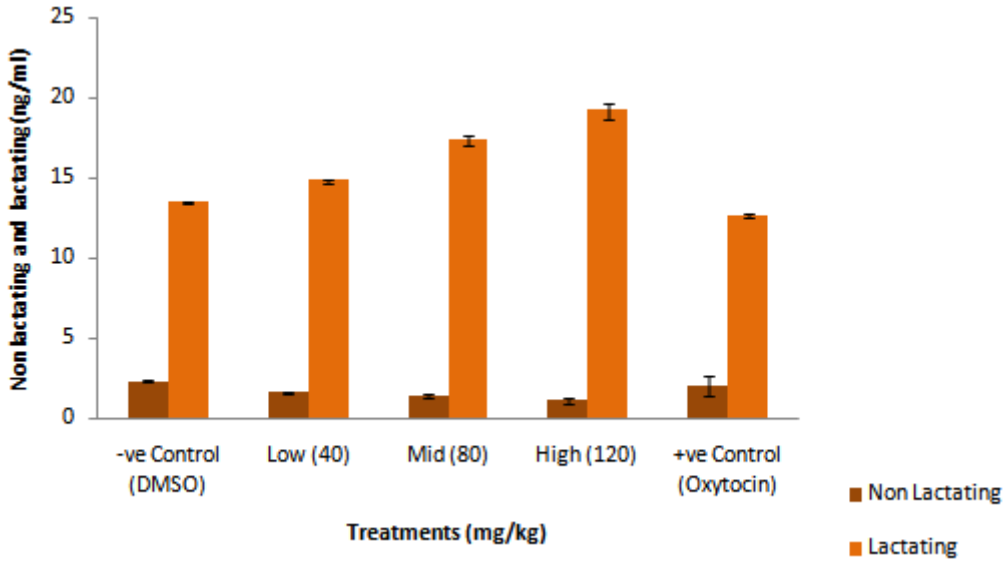


Figure - 4 Comparing the of concentrations of prolactin in the serum of lactating and non lactating rats administered F5 of Vernonia amygdalina extract.

3.2 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 mammals [17]. 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 [18].

IV. Conclussion

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 prolactin [19]. Our research finding confirmed increase in prolactin concentration during lactation. Drugs such as morphine, reserpine and the psychotropic drugs increase prolactin secretion [20, 21]. 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 [18].

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