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Action of contractile fraction of *Vernonia amygdalina.* Del ethanolic extract on hormonal profile of Estrogen and Progesterone in female albino Wistar rats

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Abstract

AIMS: To determine the action of contractile fraction of *Vernonia amygdalina.*Del ethanol extract on hormonal profile of Estrogen and Progesterone in female albino Winstar rats.

Methodology: Ethanol 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 the best contractile properties for further investigations. Using physiograph uterine 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 uterine tissue in the presence of agonist ACh. F5 was used for further studies on profile of Estrogen and Progesterone. Adult female albino Wistar rats grouped into five (I, II, III, IV, V) were used for the hormonal study. Group I served as negative control and was administered 20% dimethyl sulphoxide (DMSO) while groups II, III, and IV served as test groups and were administered 40mg/kg, 80mg/kg and 120mg/kg body weight of F5 respectively. Group V was oxytocin treated group which served as positive control and was administered 0.1 µg of oxytocin intra-peritopeally.

Results: After 5 days of administration of F5, the serum estrogen and progesterone concentrations in the serum were measured. There was a dose dependent increase in the serum estrogen levels (491.66±0.08 pg/ml, 616.66±2.02 pg/ml and 673.66±2.02 pg/ml) in the test groups which was significantly higher than the negative control (92.66±0.88 pg/ml). The results showed a dose dependent significant ($P<0.05$) decrease in serum progesterone in groups II, III and IV (58.33±0.88 µg/ml, 40±0.59 µg/ml, and 35.3.66±0.52 µg/ml,) when compared to the negative control (61.33±2.40 µg/ml)

Conclusion: The contractile extract fraction (F5) increased the serum concentration of estrogen in the rats but decrease the progesterone concentration significantly in dose dependent fashion. This supports the concomitant rise in estrogen and fall in progesterone occurring just before parturition.

Key Word: *Vernonia amygdalina*, Estrogen , Progesterone, Uterus.

1.0 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 ethno-medicine 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. The research is to determine the action of contractile fraction of *Vernonia amygdalina.*Del ethanol extract on hormonal profile of Estrogen and Progesterone in female albino Winstar rats. Estrogen and Progesterone are steroid hormones secreted by the corpus luteum [11].

2.0 Materials and Methods

2.1 Collection of plant material

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 of 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 and was filtered with Whatmann no 1 filter paper. From the 360 g powdered leaves 24 g crude extract was obtained. 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. The method of Harborne [7] 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 (R*f*). The fractions with similar R*f* 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 (R*f*) values.

Fraction before pooling	R*f* 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 animal preparation

2.5.1 In vitro rat assay for contractile activity using extract fractions. (F1, F2, F3, F4, F5, and F6)

The vitro rat bio assay for contractile activity was carried out as described by [8].Uterine strip 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 (Harward Oscillator, physiograph) attached to the uterine tissue. Recording paper and contraction amplitude were used to make the reading.The best contractile fraction was therefore selected for further study of their effect on serum concentration of estrogen and progesterone.

2.5.2 Determination of serum estrogen and progesterone level in rats administered contractile fraction (F5) of Vernonia amygdalina.

Five groups of 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 intraperitopeally 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.

2.6 Description principle and sources of kits used

The test kit used for hormonal profile of estrogen and progesterone was Accu Bind ELISA microwells monobind Inc (Lake forest CA USA).Delayed Competitive Enzyme Immunoassay (type 9) for estrogen and Competitive Enzyme Immunoassay (Type 7) for progesterone [9].

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

3.0 Results and Discussion

3.1 Results

3.1.1 Result of in vitro contraction of rat uterine 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 uterotonic activity revealed that F5 had the highest amplitude of contraction among the other fractions when compared to the control agonist acetylcholine (Fig.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 38 mm, 40 mm, 45 mm, 48 mm, 50 mm and 54 mm respectively as compared to acetylcholine, 42 mm, 41 mm, 46 mm, 50mm, 53 mm and 58 mm.

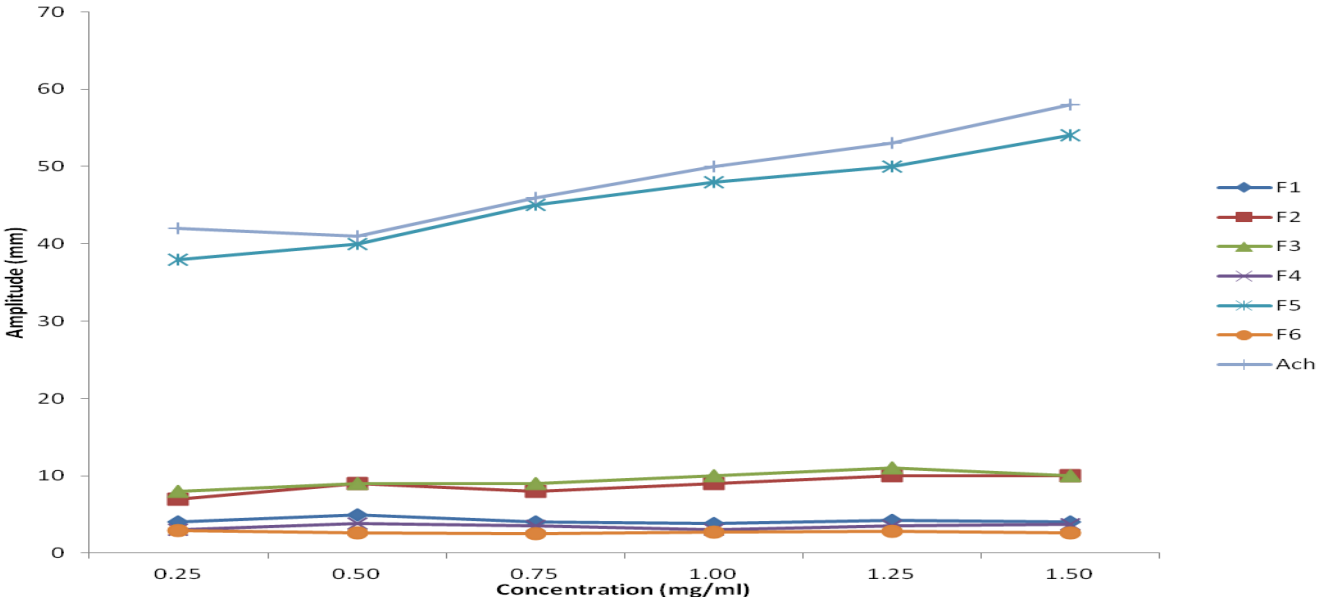

Fig. 1: Shows contractile amplitude of different fractions of *Vernonia amygdalina* on isolated rat uterus at 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 1.25 mg/ml and 1.5 mg/ml compared to the control agonist acetylcholine.

3.12 Effect of F5 Fraction of *Vernonia amygdalina* on Serum Estrogen Concentration

The result of the effect of F5 fraction of *Vernonia amygdalina* on the serum estrogen level of treated rats is presented in Fig. 2. The result showed that *Vernonia amygdalina* caused a dose dependent and significant (P<0.01) increase in the serum level of estrogen of the treated rats (Low 491.66±0.08 pg/ml; Mid 616.66±2.02 pg/ml and High 673.66±202 pg/ml)) when compared to the negative control rats (DMSO 92.66±0.88 pg/ml).

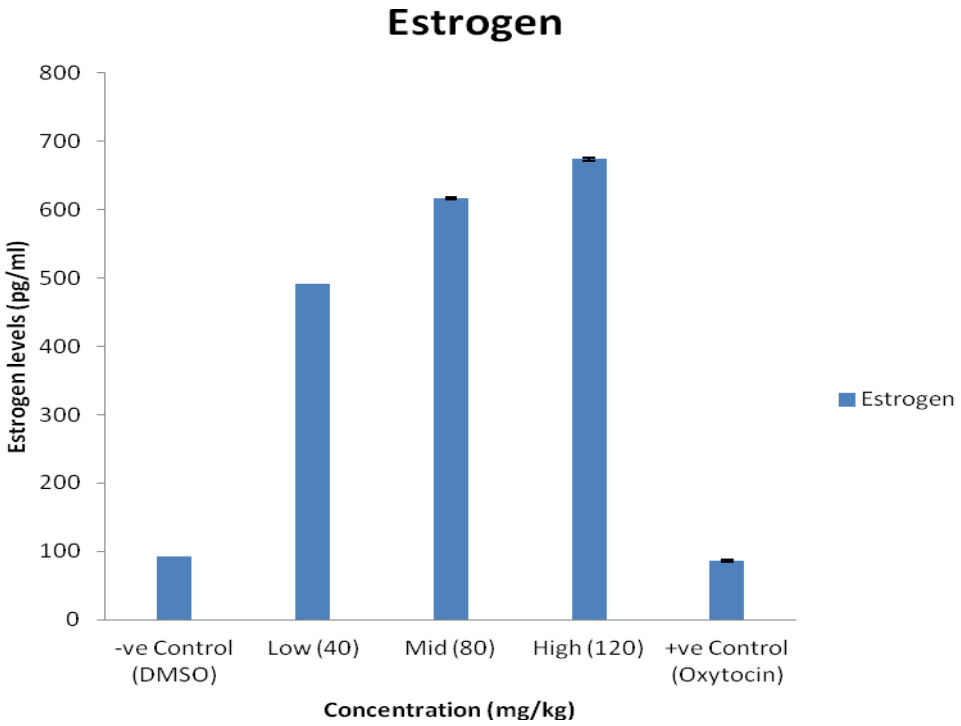

Fig 2: Concentrations of estrogen in the serum of rats administered different doses of F5 of *Vernonia amygdalina* extract.

3.1. 3 Effect of F5 Fraction of Vernonia amygdalina on Serum Progesterone Concentration

The result of the effect of *Vernonia amygdalina* on the serum progesterone concentration of rats is presented in Fig. 3. The result showed that there was a significant (P<0.05) decrease in the serum progesterone concentration (Low 58.33±0.88µg/ml, Mid 40.53±0.59 µg/ml, High 35.3±2.52 µg/ml) of rats treated with graded doses of *Vernonia amygdalina* and the reference drug oxytocin when compared to the negative control (DMSO 61.33±2.40 µg/ml).

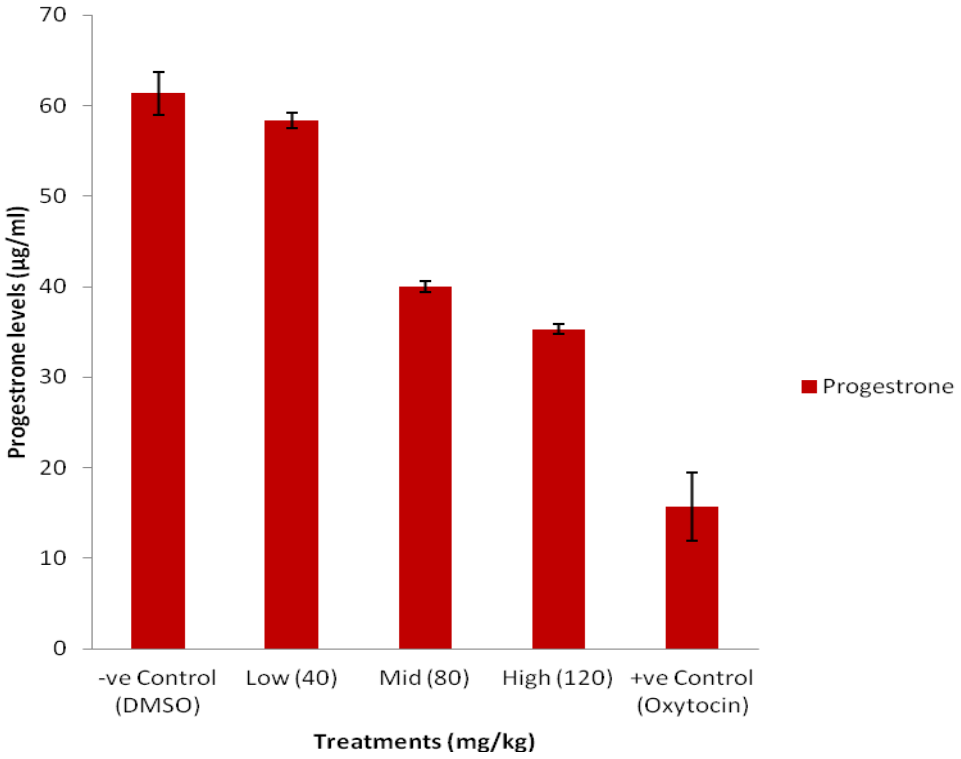

Fig. 3: Concentrations of progesterone in the serum of rats administered different doses of F5 of *Vernonia amygdalina* extract.

3.2 Discussion

Intra peritoneal administration of contractile fraction (F5) at doses of 40mg/kg, 80mg/kg, 120mg/kg body weight showed a dose dependant increase in serum estrogen concentration in rats of the test groups compared to the control. The result also indicated a dose dependant decrease in serum progesterone concentration in rats in test groups compared to the control. The work is in agreement with the work of Gharib *et al*, [10], that membrane receptors for oxytocin are found in both uterine and mammary tissues and these receptors are increased in number by estrogen and decreased by progesterone[10]. This the reason for the concomitant rise in estrogen and fall in progesterone occurring just before parturition [10].Progesterone increase is necessary for the maintenance of pregnancy in all species whether supplied by the corpus luteum,by placenta or both [11] and decrease in the serum concentration occurring immediately before parturition [10].Therefore there is a correlation between estrogen and progesterone in reproductive cycles [12]

4.0 Conclusion

The contractile activity of *Vernonia amygdalina* on uterine tissue was identified in F5 fraction. The compounds synergistically caused the dose dependant increase in serum estrogen concentration and decrease in serum progesterone concentration. The contractile fraction (F5) of *Vernonia amygdalina* caused increase in serum concentration of estrogen in the female rats and decrease in progesterone concentration in a dose dependent manner.

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