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Effect of Contractile Fraction of *Vernonia amygdalina*.Del Ethanolic Extract on the Profile of LH and FSH in Female Albino Wistar Rats

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ABSTRACT

AIMS: To determine the action of contractile fraction of *Vernonia amygdalina* on serum FSH and LH in female albino Winstar rats.

Methodology: 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 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 FSH and LH. 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: The results indicated a dose dependent significant ($P<0.05$) decrease in serum luteinizing hormone concentration in groups II to IV (1.73±0.18 mIU/ml, 1.46±0.03 mIU/ml and 1.2±0.05 mIU/ml) when compared to the negative control (1.23±0.03 mIU/ml), and in the serum concentration of follicle stimulating hormone (0.4±0.01 mIU/ml, 0.31±0.01 mIU/ml and 0.2±0.01 mIU/ml), when compared to the negative control (0.7±0.05 mIU/ml)

CONCLUSION: The extract decreased the serum concentration of both LH and FSH in a dose dependant manner.

Key Word: *Vernonia amygdalina,* LH, FSH, Uterine tissue.

1. 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.

Luteinizing hormone (LH), also known as lutropin and sometimes lutrophin is a hormone produced by gonadotroph cells in the anterior pituitary gland. In females, an acute rise of LH surge triggers ovulation [7] and development of the corpus luteum. In males, where LH had also been called interstitial cell-stimulating hormone (ICSH) [8], it stimulates Leydig production of testesterone [7]. It acts synergistically with FSH. Follicle-Stimulating Hormone (FSH) is a hormone found in humans and other animals. It is synthesized and secreted by gonadotropins of the anterior pituitary gland [9]. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) act synergistically in reproduction.

The pituitary gonadotropins, LH and FSH are presented together because of their similarities and close functional relationships. Both LH and FSH are glycoproteins consisting of alpha and beta subunits associated by covalent bonds [10]. The alpha subunit is common to both hormones whereas the specific hormone activity is associated with the beta subunit. Combination of both subunits is required for biologic activity [10].Gonadotropes are bihormonal,they synthesize both LH and FSH. Gonadotropes have specific membrane receptor for gonadotropin release hormone (GnRH).The two hormones specifically stimulate the ovary and are present in the anterior pituitary [11].The ovarian follicles in mammals are dependent upon LH and FSH for follicular growth and maturation. LH and FSH are essential for the synthesis of estrogen [12]. Rising levels of estrogen suppresses the pituitary release of FSH and LH. Large quantities of estrogen completely inhibit the secretion of gonadotropic hormones. In the male FSH maintains and stimulate spermatogenesis and LH the secretion of testosterone by the Leydig or interstitial cell of the testes [13]

2. 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 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. 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 represented 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 [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 (R*f*). The fractions with similar R*f* values were pooled together as similar compounds.

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

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 preparations

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 [15].Uterine strip of non pregnant female Winstar albino rats were used for the testing of the different fractions of 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 best contractile fraction was therefore selected for further study of their effect on serum concentration of LH and FSH.

2.52 Determination of serum LH and FSH level in rats administered contractile fraction (F5) of Vernonia amygdalina.

Five groups of 20 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 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 LH and FSH in the 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.

3. RESULTS AND DISCUSSION

3.1 Result

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.

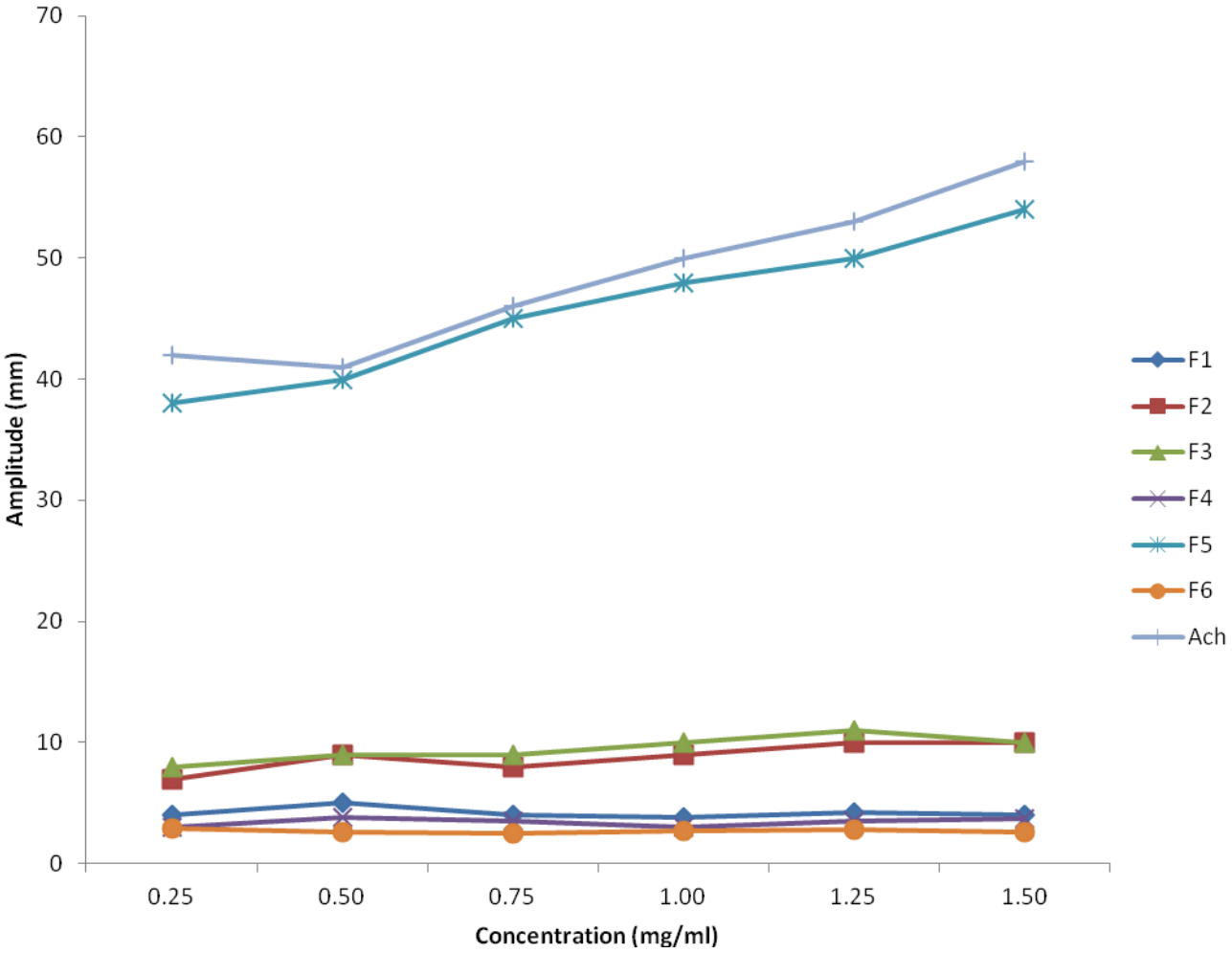

Fig. 1 Shows contractile amplitude of different fractions of *Vernonia amygdalina* on rat uterine tissue at 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1.0 mg/ml, 1.25 mg/ml and 1.5 mg/ml, compared to the control agonist, acetylcholine (ACh).

3.1.2 Effect of F5 fraction of Vernonia amygdalina on serum LH concentration

The result of the effect of F5 of *Vernonia amygdalina* on the serum LH levels of rats is presented in Fig. 2. The result indicates that at doses of 40 mg/kg and 80mg/kg F5 (1.73±0.18 µg/ml; 1.86±0.0 µg/ml) respectively) had no significant effect on the serum levels of the treated rat but at the dose of 120 mg/kg *Vernonia amygdalina* just like oxytocin significantly ($P<0.05$) decreased the level of LH of the treated rats (High 1.2±0.05 µg/ml; negative control 1.23±0.03 µg/ml).

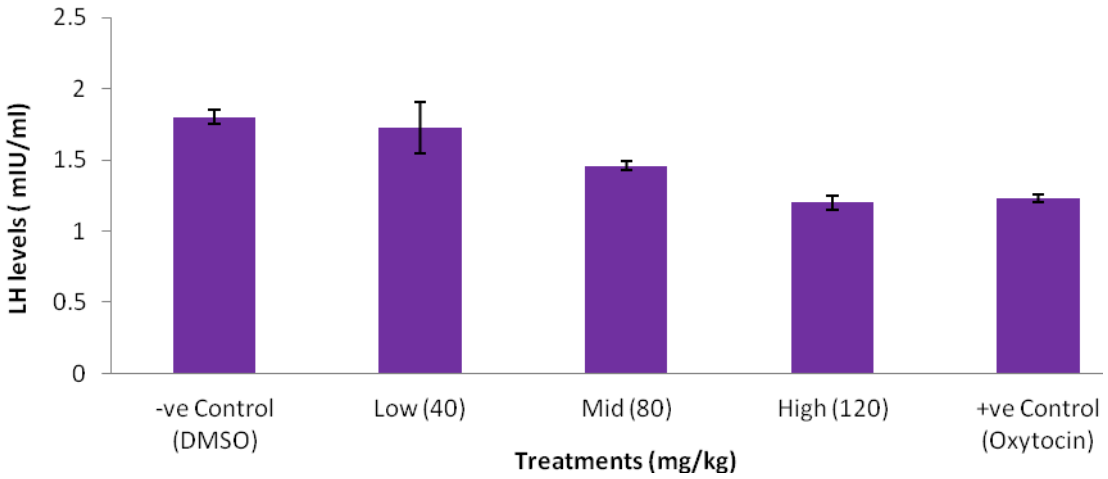

Fig. 2: Concentrations of LH in the serum of rats administered different doses of F5 of *Vernonia amygdalina* extract.

3.1.3 Effect of F5 fraction of Vernonia amygdalina on serum FSH concentration

Fig. 3 shows the result of the effect of *Vernonia amygdalina* on the serum FSH of rats. From the results there was a dose dependent and significant ($P<0.05$) decrease in the serum level of FSH (Low 0.4±0.01 mIU/ml, mid 0.31±0.01 mIU/ml, High 0.2±0.01 mIU/ml) when compared to the negative control rats (DMSO 0.7±0.05 mIU/ml). The positive control drug, oxytocin (0.1µg/ml) increased the level of FSH in the blood of rats (0.8±0.05 mIU/ml) when compared to the negative control through the increase was not statistically significant.

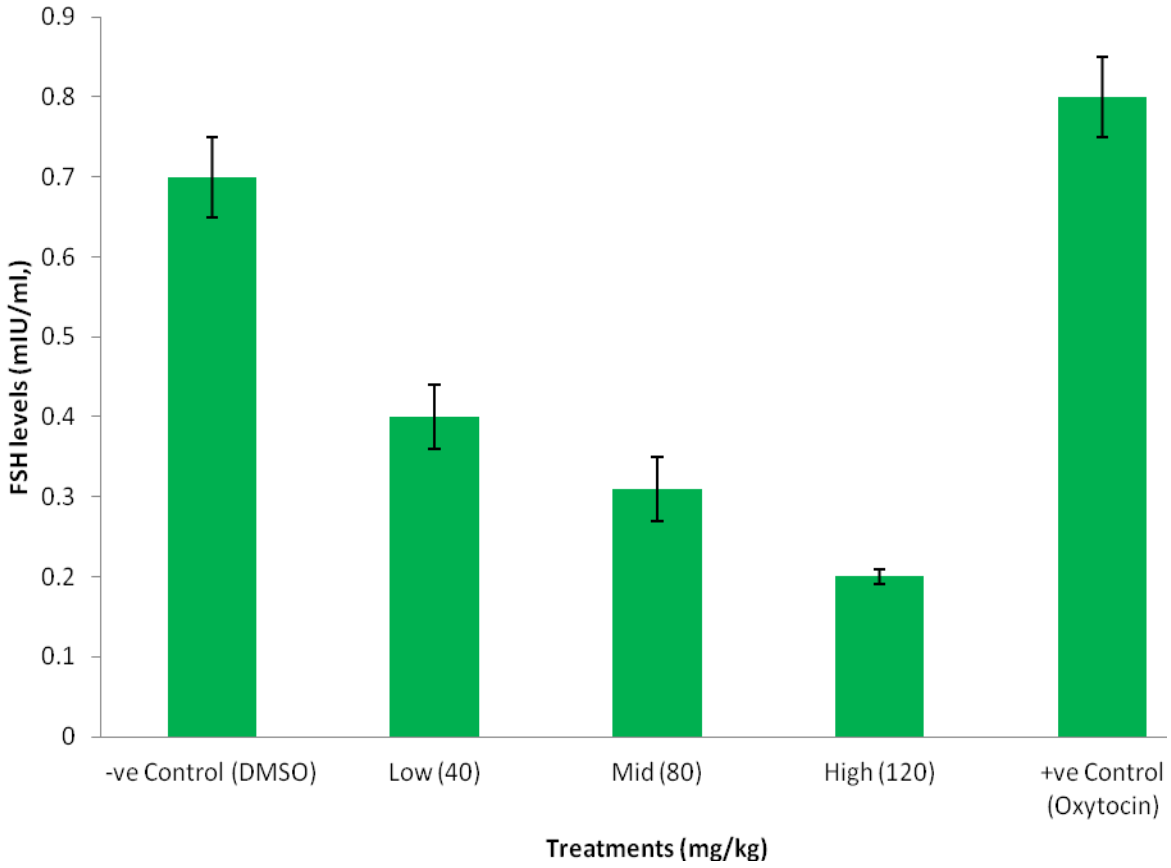

Fig. 3: Concentrations of FSH in the serum rats administered different doses of F5 of *Vernonia amygdalina* extracts

3.1.4 Comparison of effect of F5 fraction of Vernonia amygdalina on serum LH and FSH concentration in rats.

Fig. 4 compares the bar graphs of LH and FSH of rats administered F5 of *Vernonia amygdalina* extract. The result reveals decrease in serum LH and FSH concentration in a dose dependent fashion.

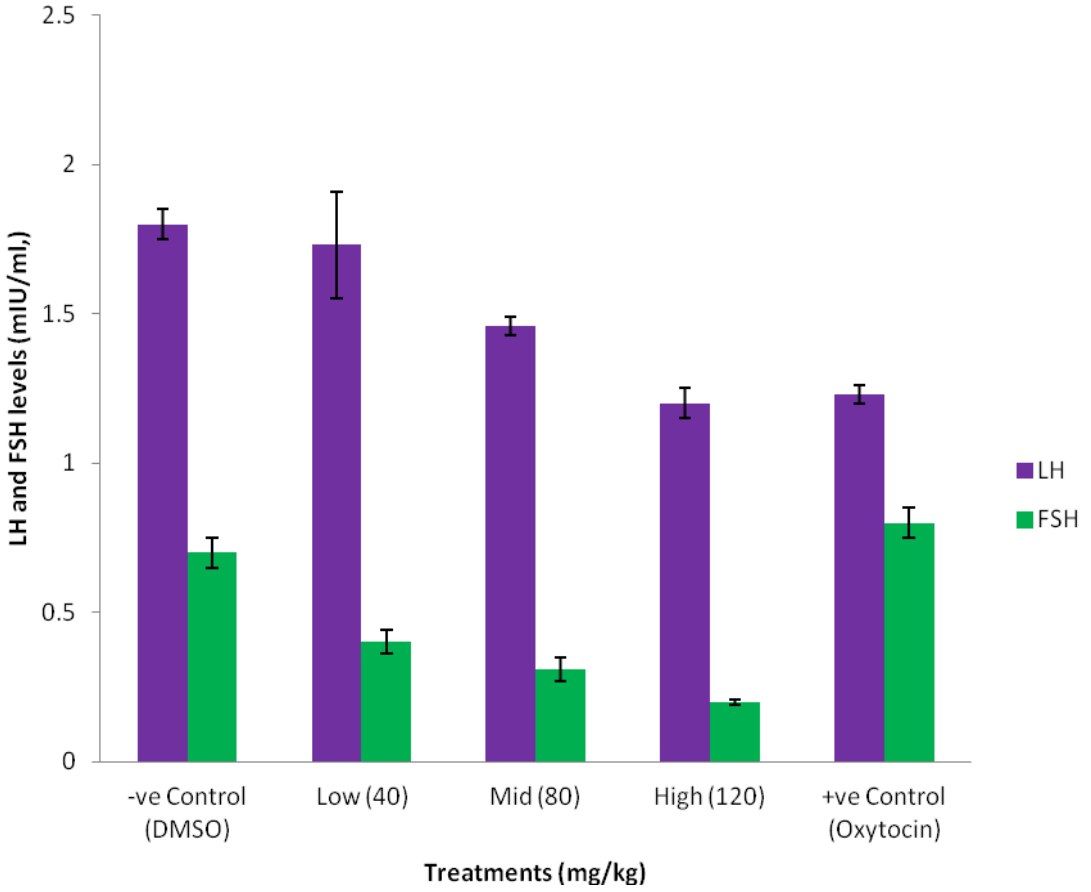

Fig. 4: Comparing the concentrations of LH and FSH in rats administered different doses of F5 of *Vernonia amygdalina* extracts

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 decrease in serum LH and FSH concentration in rats of the test groups compared to the control. The extract could therefore be used to moderate the serum LH and FSH levels because if given at high dose, the LH and FSH serum concentration reduces and vice versa. Although we are not certain if the extract works directly by blocking GnRH receptors or indirectly by elevating estrogen level thus inhibiting LH and FSH secretion, it could be a useful extract for LH and FSH manipulation. In females, an acute rise of LH triggers ovulation [7] and development of the corpus luteum.

4. CONCLUSION

The contractile activity of *Vernonia amygdalina* on uterine tissue was identified in F5 fraction. The compounds synergistically caused the dose dependant decrease in serum LH and FSH concentration.

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