

## UTEROTONIC EVALUATION OF *CROTON LOBATUS* LINN ON REPRODUCTIVE PARAMETERS OF WISTAR RATS

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**Abstract** - *Croton lobatus* belongs to the crotonoideae family which comprises of 1300 species. The plant possesses some biological properties and its used ethno medicinally. The plant has been active as antimicrobial, anti-parasitic agents, anti-ulcer and anti-cancer agents. The Histological and Uterotonic evaluation of *C. lobatus* on the reproductive parameters of matured female Wistar rats was carried out. Four groups of six rats each received the extracts, at four oral doses 0, 200, 400 and 1000 mg /kg/wt. administered orally for a period of 21 days. The dissected ovaries were weighed and studied histologically. Uterotonic assay was also carried out. The results obtained histologically revealed that the extract of *C. lobatus* was safe at 200, 400 and 1000 mg/kg/wt. The extract acted as fertility booster, since there was increased growth of follicles leading to the production of secondary follicles and oocytes in the ovary on the uterus, the extract was seen to have caused relaxation on the uterine smooth muscles. Therefore, *C. lobatus* leaves favoured reproductive parameters been a tocolytic agent, thus supporting the use of the aqueous leave extract in the prevention of threatened abortion, management of pregnancy and infertility.

**Keywords:** *Croton lobatus*, threatened abortion, fertility booster, uterotonic

### INTRODUCTION

Premature birth is the main cause of neonatal death and illness, meanwhile preterm labour is known as the commencement of labour before the predictable delivery date. This results in the delivery of an under-developed newborn, since premature birth is responsible for neonatal mortality and morbidity in a developed world, there is need for a perfect tocolytic agent that will uniformly affect absolute fetomaternal wellbeing which is non-existent in the real sense. These agents vary in cost, utero-specificity, effectiveness as well as safety. The three major tocolytic agents used all over the world includes calcium channel blockers, beta-agonists and salbutamol an oxytocic receptor antagonist. An assessment of literatures on *S. tragacantha* revealed that there has been no scientific documentation on the effect of the plant on reproductive parameters Lyndrup and Lamant (2007). Infertility is characterized by the failure to attain pregnancy after twelve (12) months of regular unprotected sexual intercourse (Tammy and Kristen, 2015). The main causes of female fertility disorders are numerous. The various possible causes of female infertility may be characterized by the following: Ovulatory disorders, Uterine abnormalities, cervical abnormalities, peritoneal factor, tuba obstruction, infection, injury, toxin exposure, anatomical variances, obesity, smoking and drinking habits (Bown, 1995). These problems

can be managed, with possible management options which includes the use of herbal drugs, with very little or no chances of side effect (Etuck and muhammed, 2009).

The plant, *C. lobatus* was found to possess some biological properties as well as ethno medicinal uses, which were revealed from the works of Kilani *et al.* (2019) and Selowa *et al.* (2010) that the plant was active as antimicrobial and anti-parasitic agents, active as antiulcer and anticancer agents exposed by the works of Salatino *et al.* (2007). The ethno medicinal uses such as anti plasmodial, antibacterial, antifungal and pregnancy problems, eye disease, purgative, the used of the root for treatment of threatened abortion and hiccups was revealed by the works of (Salatino *et al.*, 2007; Schmelzer and Gurib-fakim, 2008).

Euphorbiaceae is a large family of about 300 generals comprising 7,500 species distributed in five sub families which were originally Alcalyphoidae, Crotonoideae, Euphorbiodeae, and old field Diodeae. This research was based on *Croton lobatus* linn. It was revealed that the crotonoideae family comprises of 1300 species (Vanwyk *et al.*, 2008). The biological properties of plants depend on the presence of some chemical constituent, these chemical constituents are often responsible for their physiological actions. Phytochemical are produced by plants to protect itself, but current research demonstrate that they also have the ability to protect humans against diseases (Ajahi and Raji, 2012).

## MATERIALS AND METHODS

### Sample Collection:

Fresh leaves of *C. lobatus* were obtained from Eyean Community and authenticated by the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City; sample of the plant was deposited in the herbarium of the Department and given a voucher number. The leaves were dried at room temperature after collection then put in an oven at 40 °C for 3 days. The dried leaves were then pulverized into fine powder. The dried sample of leaves was stored in an air tight container.

### **Extraction of plant material:**

The 300 g of powdered plant material was extracted with 2000 ml of distilled water using heating mantle with 3000 ml beaker. The resulting crude extract was filtered with a cheese cloth and concentrated in a water bath set at 40 °C for 48 hours.

### **Experimental animals:**

Female wistar rats weighing approximately 180 g to 200 g were obtained from pharmacology animal house University of Benin, Benin City. These animals were also adapted and maintained in the animal house with standard laboratory conditions met and animals fed with pelletized feed and tap water *ad libitum*.

### **Used drugs and chemicals:**

Drugs/chemicals procured in the course of experiment include: Sabultamol (GlaxoSmith Kline), oxytocin injection (laborate pharmaceutical Indian), acetylcholine (sigma chemicals USA), aqueous extract of *C. lobatus*.

### **Histological study:**

The female wistar rats on attainment of three consecutive oestrus cycles were divided randomly into four groups of 6 six rats each and treated as follows:

Control- received distilled water

Group A received 200 mg/kg/day aqueous leaves extract of *C. lobatus*

Group B received 400 mg/kg/day aqueous leaves extract of *C. lobatus*

Group C received 1000 mg/kg/day aqueous leaves extract of *C. lobatus*

At the end of the 21 day periods the animals were anesthetized and ovaries collected and studied histologically. the following parameters were evaluated:

Ovaries:

- Appearance of ovary after 21 days of treatment,
- Nature of oocytes,
- Production of secondary follicle

Statistical analysis:

The outcome from the studies was articulated as the mean  $\pm$  SEM (standard error of mean). Statistical analysis was carried out using graph pad prism 6 version software (UK). One-way analysis of variance (ANOVA), and comparisons between the control and treated groups were analyzed using Dunnett's multiple comparisons test. Differences at  $P < 0.05$  were considered significant.

## RESULTS AND DISCUSSION

Histological findings:

Rat ovary treated with the extract below (Figure:1), **A, B, C, D** shows normal ovarian architecture composed of mature (secondary follicles) in all treated groups.

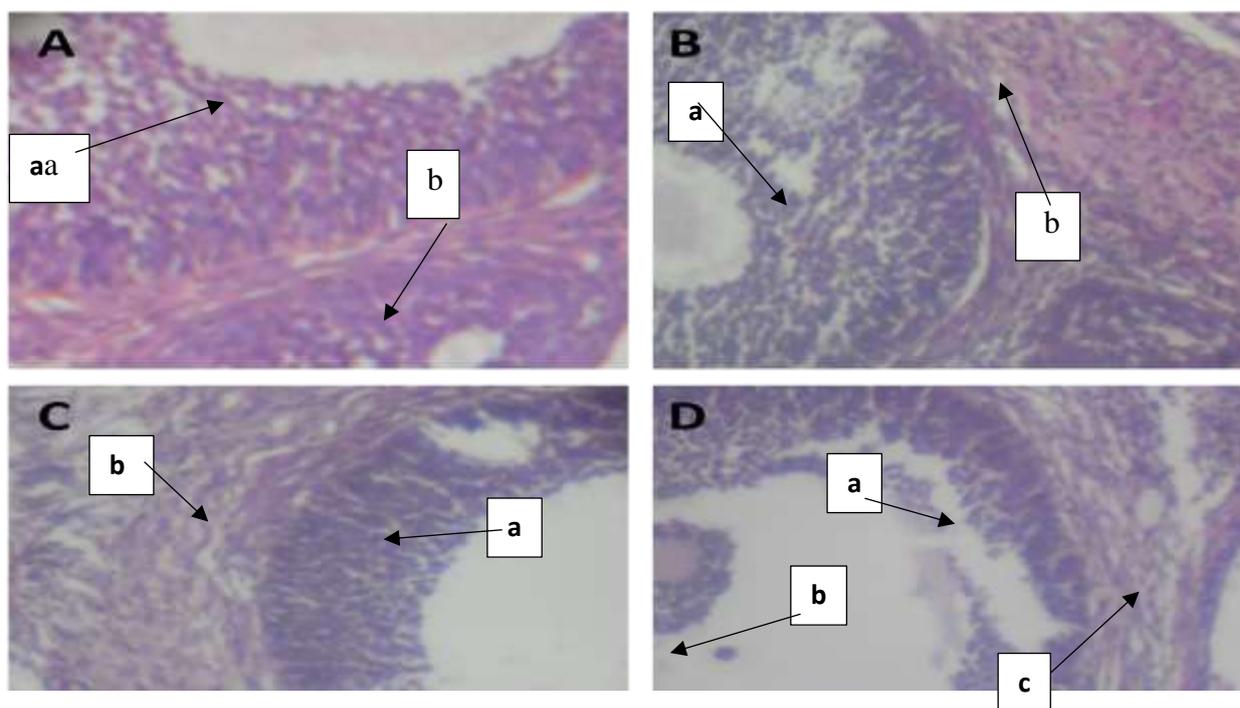
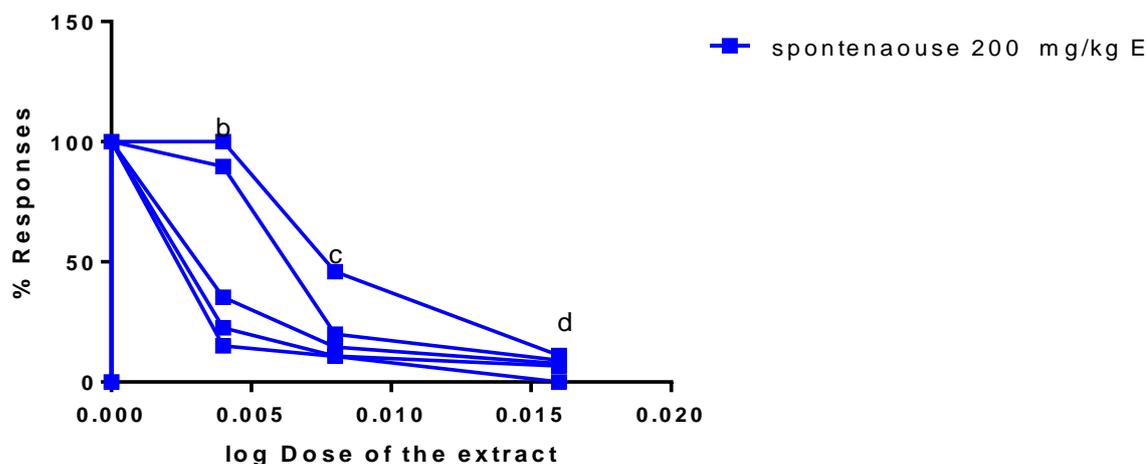


Figure 1: Effects of plant extract on rat ovary (A) Control rat ovary composed of , secondary follicles and, (stromal) graphian follicle (H and E x 4), (B) Rat ovary administered with 200 mg/kg Extract showing , secondary follicle and, (stroma) graphian follicle (H and E x 40), (C) Rat ovary administered with 400mg/kg Extract showing, secondary follicles and, (stroma) graphian follicle (H and E x 40), (D) Rat ovary administered with 1000 mg/kg Extract showing, secondary follicle, oocyte and stroma (H and E x 40).

The photomicrographs of rats in figure 1: B, C, D was dose dependent revealing increased growth of ovarian cells by the production of graphian follicle, secondary follicular cells resulting in increase in follicular number at extract dose of 200, 400 and 1000 mg/kg correspondingly resulting in changes in primordial and primary follicles giving rise to the mentioned production of secondary follicles by

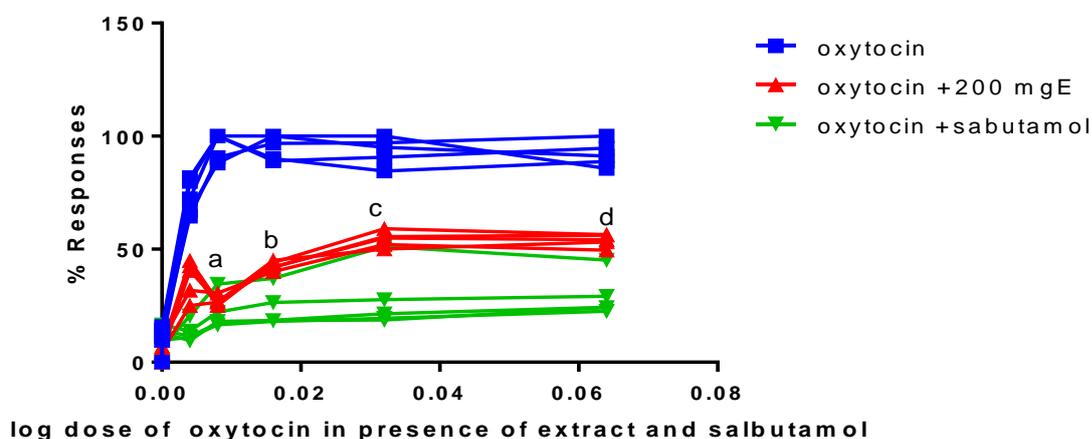
increase in follicular growth when compared to control. It is referred that *C. lobatus* had positive effect on the ovary and was the factor causing increased female fertility by its multipurpose application in the management of other medical conditions by alternative practitioners. The result was not constant with the findings of Eweka (2009) rather the result suggests increased fertility level. This result is consistent with the findings of (Adaay *et al.*, 2013). As result was investigative of increased fertility level.



**Figure 2: Uterotonic Effect of 200 mg/kg aqueous extract on spontaneous contraction on the uterus:**

Key:  $p < 0.01 = b$ ,  $p < 0.001 = c$ ,  $p < 0.001 = d$

Relaxant activity of 200 mg/kg extract on spontaneous contraction on the uterus, from the uterine contractility study it was seen that the extract dose of 200 mg/ml extract inhibited spontaneous contraction on the uterus (Fig. 2). The result showed that the extract relaxed spontaneous contraction from 100 % to 35.96 % and when the percentage difference was calculated it was seen that the extract had inhibited spontaneous contraction by 64.04 % values are the means of percentage responses  $\pm$ SEM,  $n=4$  experiment for each point with significant  $p$ -values at:  $b =$  significant  $p < (0.01)$ ,  $c =$  significant  $p < (0.001)$ ,  $d =$  significant  $p < (0.0001)$ .



**Figure 3: The relaxant Effect of 200 mg/kg Aqueous extract on oxytocin induced contraction on the uterus:**

Values represent mean percentage responses  $\pm$  S.E.M. (n = 5 experiments) for each point.

Key:  $p < 0.05 = a$ ,  $p < 0.01 = b$ ,  $p < 0.001 = c$ ,  $p < 0.001 = d$

The relaxant effect of 200 mg/ml of aqueous leaf extract on oxytocin induced contraction on the uterus, at a concentration of  $6.4 \times 10^{-4}$  I.U /ml alone gave a mean response of 96.00 % of maximum contraction, while the same concentration in the presence of the *C. lobatus* leaf extract relaxant effect gave a mean response of 31.63 % when the difference between oxytocin induced contraction and extract in presence of oxytocin was calculated it was seen that the percentage induced relaxation of the extract was 64.64 % at a dose of 200 mg/kg with significant p-values at: b= significant  $p < 0.01$ , c = significant  $p < 0.001$ , d = significant  $p < 0.0001$  (Fig. 3).

Effect of aqueous extract of *C. lobatus* on acetylcholine induced contraction isolated uterus at  $6.4 \times 10^{-4}$   $\mu$  /ml of acetylcholine alone gave a mean response of 77.5 % of maximum contractile response, while the same concentration in the presence of the plant extract gave a mean response of 37.6 when the difference between ACH induced contraction and the extract, *C. lobatus* was seen to have inhibited contraction on the uterus by 40 % at 200 mg /kg (Fig. 4).

The uses of the extract are recommended for women suffering from persistent miscarriage and premature labour and are at high risk of threatened abortion.

The findings recommend application of *C. Lobatus* to women during pregnancy can be encouraged. This finding is consistent with the belief of traditional practitioners that the plant is useful in the treatment of preterm birth and prevention of abortion with positive enhancement on the ovary and relaxation effect on uterine smooth muscles in accordance with the findings of Etuck and Muhammed (2014).

The uterotonic assay revealed that the aqueous extract was found to have relaxant activity on the uterus, as the extract inhibited spontaneous contraction by 64.04 % acetylcholine and oxytocin contraction on the uterus was also inhibited. *C. lobatus* might have been acting as a physiologic antagonist, such that oxytocin is causing contraction and the extract is causing relaxation due to the presence of phytochemicals as importance was placed on phytochemical constituents by the work of Theuretzbacher (2011). Valuations of the data revealed, that there was a significant relaxation in spontaneous

contraction at 64.04 %, oxytocin and acetylcholine contraction were also inhibited by the aqueous leaf extract at 200 mg/kg concentration by 64.64 % and 40 % tested at a p-value of  $p < 0.01$ ,  $p < 0.001$  and 0.0001 respectively (Figures 2-3). The result in figure 3-4, also showed a reasonable relaxant activity which was created by the plant and sabutamol a clinically used drug in treatment of threatened abortion in pregnant uterus this was backed by the findings of Bafor *et al.* (2013). On contraction induced by both oxytocin and acetylcholine the extract produced a percentage inhibitory effect of about 64.64 %, 40. % at 200 mg/kg of the extract with significant p-value of  $P < 0.01$ ,  $p < 0.001$  and  $p < 0.0001$ . In the acetylcholine induced contraction, since the maximum response was not obtained, it may specify that the extract was acting as non-competitive and nonspecific receptor antagonist. From this observation, it could be suggested that the mechanism of action of the plant might be through a non-specific receptor antagonist path way (Bafor *et al.*, 2010), the plant was capable of causing inhibition of the spontaneous contraction on the uterus by 64.04 %. This result reveals that the aqueous extract of *C. lobatus* is prospective tocolytic agent which could be explored as an alternative management for infertility and treatment of threatened abortion.

## CONCLUSION

The present evaluation has shown that *C. lobatus* has positive effects on fertility parameters.

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