



Evaluation of *Costus lucanuscianus* leaf extract for anti-fertility effect in female albino rats

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Abstract

Costus lucanuscianus, a medicinal plant locally called 'monkey sugar cane' in the Niger Delta region of Nigeria, is used in folk medicine for the treatment of diarrhoea, dysmenorrhoea, headache and rheumatism. The dearth of information on effect of *Costus lucanuscianus* in females prompted this study using methanolic leaf extract of this plant. Twenty animals were divided into four groups. Group A (Control) received 0.5ml/kg of 20% Tween 80 (vehicle), Group B (100 mg/kg of extract), Group C (200 mg/kg of extract), Group D (300 mg/kg of extract) by oral gavage daily for 14 days. The estrous cycle was determined daily; and hormonal assays for estrogen, progesterone, LH and FSH were done using Enzyme Immunoassay. Histopathological study of the ovaries was conducted. *C. lucanuscianus* leaf extract caused a significant decrease ($p < 0.05$) in the serum FSH and estrogen levels and non significant effect ($p > 0.05$) on progesterone and LH levels relative to the control. There was also a significant ($p < 0.05$) increase and decrease in the metestrus and diestrus phases of estrous cycle respectively relative to the control. No abnormality was observed in the ovarian sections of rats in the treated groups. The findings suggest that *C. lucanuscianus* leaves may possess anti-fertility effect in female rats.

Keywords: *Costus lucanuscianus*, estrous cycle, female hormones, ovary

Introduction

Plants have a long time history in medicine. The treatment and control of diseases by the use of available medicinal plants in a locality has continued to play significant roles in medical health care implementation in the developing countries of the world (Akharaiyi and Boboye, 2010) and in the management of fertility related problems (Bala *et al.*, 2014). Due to population explosion especially in the developing countries, it has become crucial to source

for biologically active anti-fertility agents from plants which are safe and can interfere with the natural process of reproduction.

The plant *Costus lucanuscianus* commonly known as spiral ginger is a perennial rhizomatous herb growing up to 3 metres tall (Fern, 2017). It is a common species in the forest zone of tropical Africa (Saliu and Fapohuda, 2016). *C. lucanuscianus* is a medicinal

plant locally called 'monkey sugar cane' in the Niger Delta region of Nigeria. Ethnomedicinally, the plant is used in the management of several ailments and conditions including diabetes, eye problems, cough (Burkill, 1985) and impending threatened abortion (Foungbe *et al.*, 1987). The leaf sap of *C. lucanusianus* is used to treat eye troubles and head aches. These usages are however with little information on female reproductive indices.

Experimental studies have reported that plant extracts could adversely affect male and female reproductive indices (Yakubu *et al.*, 2008, Ebeye *et al.*, 2015). Among the few studies carried out on reproductive effect of *Costus lucanusianus*, it was reported that *C. lucanusianus* stem extract possess anti-fertility properties evidenced by reduced sperm count and increased sperm cell defects (Kagbo and Obinna 2017). Also, methanolic leaf extract of *C. lucanusianus* did not cause any obvious abortion in all the treated pregnant rats exposed on gestation days 6-19; neither was any external nor visceral anomaly observed in the foetuses (Kagbo and Obinna, 2018).

This study therefore aims at investigating the anti-fertility effect of methanolic leaf extract of *Costus lucanusianus* in female albino rats.

Materials and Methods

Plant Material and Authentication

Fresh leaves of *Costus lucanusianus* were collected from the forest reserve of University of Port Harcourt, Nigeria. The plant was identified by Dr. I. Agbagwa of the department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, and a sample was deposited at the University of Port Harcourt Herbarium with reference number UPH/V/1212

Preparation of plant Extract

After collection of the plant, the leaves were shade-dried at room temperature (32 – 35°C) to constant weight over a period of seven (7) days. The cold maceration extraction method of Cowan (1999) was used. Fifty grams of dried *Costus lucanusianus* leaves was weighed and grinded to fine powder and dissolved in 1000ml of seventy percent methanol inside a 2-liter conical flask. The flask was shaken vigorously at 30 minute intervals and left to stand for 72 hours at room temperature for effective extraction. The resultant

mixture then was filtered with Watman's No. 1 filter paper and cotton wool to remove particles of plant sample. The clear solution obtained was concentrated with rotary evaporator at 45°C under low pressure and later transferred to evaporating dish over a steam bath. The solid dried powder obtained was stored in sterile pre-weighed screw capped bottles and labelled accordingly. The extract was now stored at room temperature.

Animals

Twenty mature female albino rats weighing an average of 210g, procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria were used for the study. The rats were acclimatized for two (2) weeks before commencing the study. They were fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study.

Experimental Procedure

Following acclimatization, the animals were randomly assigned to four (4) groups of five animals each for treatment as follows:

Group A (Control) received 0.5ml/kg body weight of 20% Tween 80 (vehicle).

Group B received 100 mg/kg body weight of the extract

Group C received 200 mg/kg body weight of the extract

Group D received 300 mg/kg body weight of the extract

Administration of extract and vehicle was by oral gavage daily for 14 days. All administrations were started in the estrus phase. Animal's weight was taken daily and the dose adjusted accordingly.

Sample collection

The vaginal smear was collected daily in the morning using the pipette smear technique. The tip of a Pasteur pipette containing few drops of normal saline (0.9% NaCl) was inserted into each rat's vagina. The fluid was used to flush cells from the vaginal lining after which the resulting suspension was placed on a clean glass slide and examined under light microscope. The phases of estrous cycle were confirmed depending on the different characteristic cells.

The animals were anaesthetized under chloroform at the end of the experiment. Blood samples were collected from the retro orbital plexuses into the sterile plain bottles. The Collected blood was allowed to stand for 30-45 min in order to coagulate and then centrifuged for 15 min at 3000 rev/min to obtain the serum for hormone analysis. The serum was then tipped into a separate vial, placed in microcentrifuge tubes, capped and stored at -20 °C until analysis. The serum was later subjected to hormonal assay by ELISA method for assessment of estrogen, progesterone, LH and FSH levels.

The reproductive organs (ovaries) were carefully removed and were fixed in Bouin’s Solution, and then processed as described by Lillie (1965), embedded in paraffin, sectioned at 4-5 µm and stained by Haematoxylin and Eosin blue.

Statistical Analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean ± SEM and data were assessed by one-way ANOVA followed by the LSD post-test. The significance level was set at p<0.05.

Results

Table 1 shows that treatment of rats methanolic leaf extracts of *Costus lucanusianus* for 14 days had no significant effect (p>0.05) on progesterone and Lutenizing Hormone (LH) concentrations relative to the control. However, significant decrease (p<0.05) was recorded in the concentrations of estrogen and Follicle Stimulating Hormone (FSH) when compared with the control.

Table 1: Effect of Methanolic leaf extracts of *Costus lucanusianus* on Female Sex Hormones

Groups	Estrogen (pg/ml)	Progesterone (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)
A	96.63 ± 5.12	22.80 ± 0.53	0.59 ± 0.09	0.96 ± 0.02
B	67.10 ± 2.41*	22.83 ± 2.68	0.48 ± 0.04	0.78 ± 0.04*
C	81.83 ± 1.15*	25.98 ± 2.71	0.65 ± 0.08	0.70 ± 0.05*
D	78.70 ± 0.53*	26.31 ± 0.76	0.62 ± 0.14	0.80 ± 0.01*

a. Values are Expressed as Mean ± SEM;

b* Indicates a Significant Variation when Compared with Group A (P<0.05)

Methanolic leaf extracts of *Costus lucanusianus* caused a significant (P<0.05) increase and decrease in the metestrus and diestrus phases of estrous cycle

respectively but had no significant effect (p>0.05) on proestrus and estrus phases of the cycle when compared with the control (Table 2).

Table 2: Effect of Methanolic leaf extracts of *Costus lucanusianus* on Estrous cycle

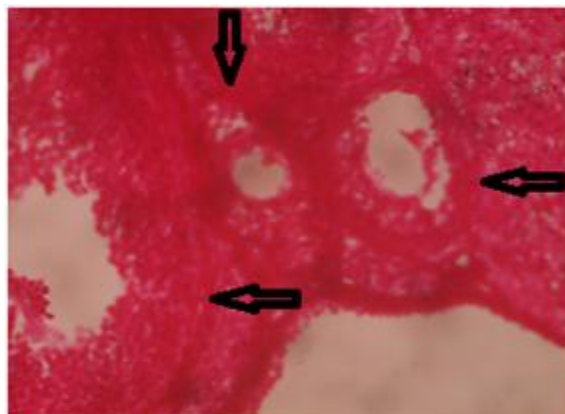
Groups	Proestrus	Estrus	Metestrus	Diestrus
A	2.80 ± 0.49	3.00 ± 0.32	2.80 ± 0.20	5.20 ± 0.58
B	3.00 ± 0.55	4.00 ± 0.63	4.00 ± 0.45	3.00 ± 0.32*
C	2.80 ± 0.37	3.80 ± 0.37	4.20 ± 0.37*	3.20 ± 0.37*
D	2.40 ± 0.51	3.80 ± 0.58	4.40 ± 0.40*	3.40 ± 0.75*

a. Values are Expressed as Mean ± SEM;

b* Indicates a Significant Variation when Compared with the Group A (P<0.05)

Plates 1-4 shows that Methanolic leaf extracts of *Costus lucanuscianus* caused no observable pathology

in the histology of the ovaries of treated rats relative to the control.



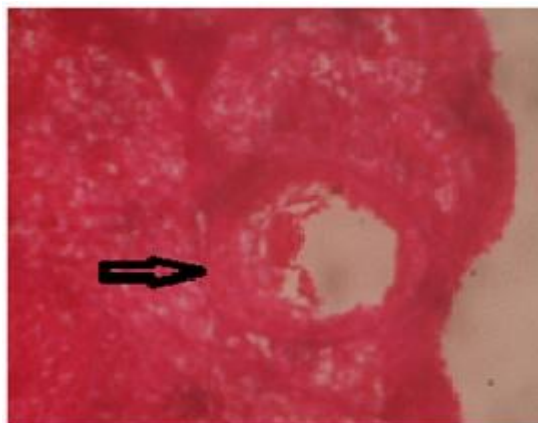
Group A - Control

Plate 1: Photomicrograph of ovarian section from control rats stained with H & E ($\times 400$) showing normal histoarchitecture of a cycling ovary characterised by ovarian follicles (arrows) at different stages of development.



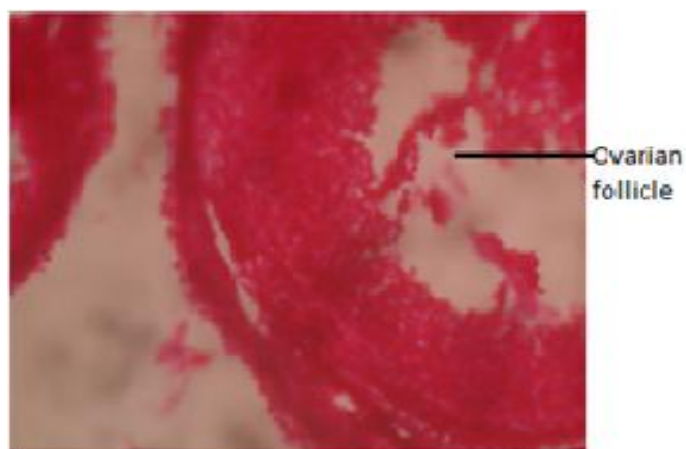
Group B - 100mg/kg

Plate 2: Photomicrograph of ovarian section from 100mg/kg MLECL treated rats stained with H & E ($\times 400$) showing ovarian follicles (arrows) at different stages of development.



Group C - 200mg/kg

Plate 3: Photomicrograph of ovarian section from 200mg/kg MLECL treated rats stained with H & E ($\times 400$) showing Matured ovarian follicle (arrow).



Group D - 300mg/kg

Plate 4: Photomicrograph of ovarian section from 300mg/kg MLECL treated rat stained with H & E ($\times 400$) showing an ovarian follicle.

Discussion

Sex hormones in females are known to regulate the estrous cycle as well as other reproductive functions and characteristics. These sex hormones (estrogen and progesterone) are primarily produced in the ovaries under the influence of FSH and LH from the anterior pituitary through a feedback mechanism (Obinna and Kagbo, 2017). The growth and maturation of ovarian follicles as well as ovulation are regulated by combined activities of these pituitary and ovarian hormones. It has been reported that certain chemical constituents of plants are capable of inducing infertility by suppressing these hormones that are responsible for the regulation of reproductive cycle in humans and animals (Bala *et al.*, 2014).

The result of this study which was undertaken to investigate the antifertility effect of methanolic leaf extract *Costus lucanuscianus* in female rats indicates that the extract reduced the concentrations of estrogen and FSH. *Costus* spp. has been reported to possess estrogen-like activity (<http://www.thesourcenatural.com/ns>). This can cause inhibition of the synthesis and secretion of estrogen from the ovarian follicles as well as reduction in the serum concentration by mimicking the effect of the hormone, estrogen. Estrogen in small amounts has a strong effect to inhibit the production of both LH and FSH (Hall, 2001) hence the reduced serum concentration of FSH observed in the study. Brinker (1997) stated that plants with estrogenic property can directly influence pituitary action by peripheral modulation of LH and FSH, decreasing secretion of these hormones. According to Kumar *et al.*, (1997), reduction in the serum concentration of FSH by the plant extract may affect folliculogenesis as well as cause delay in the pre-ovulatory phase.

This result is in agreement with the study carried out by Asuquo *et al.*, (2013) who reported that ethanolic leaf extract of *Spondias mombin* caused a reduction in the concentration of female hormones in wistar rats. This result is also in line with the findings of Trif *et al.*, (2010) who reported that the decrease in estrogen level could be the consequence of decrease in FSH concentration as a result of chromium exposure which led to decrease of the aromatase in the granulosa cells and androgen transformation into estrogen.

This study also shows that methanolic leaf extract of *C. lucanuscianus* increased the metestrus phase and decreased the diestrus phase of the estrous cycle. Circostal *et al.*, (2001) reported that imbalance in female hormones leads to irregularities in the ovarian functions and duration of the estrous cycle. In line with our findings, Oyedeji and Bolarinwa (2013) documented that post treatment period of methanolic extract of *Portulaca oleracea* showed a significant increase in the metestrus phase relative to pre-treatment period. Our findings contrast that of Monsefi *et al.* (2006) which demonstrated that Ethanolic extract of *Anethum graveolens* increased duration of diestrus phases and total time of estrous cycle in female Wistar rats.

Conclusion

In this study, the findings suggest antifertility potentials of methanolic leaf extract of *C. lucanuscianus* which have important implication for female contraceptive development since it has become necessary to source for biologically active anti-fertility agents from plants which are safe and can interfere with natural procedure of reproduction with minimal side effect.

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