

Effect of Methanolic Leaf Extract of *Costus Lucanusianus* on Male Reproductive Parameters in Albino Rats

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Abstract: The use of medicinal plants in human and animal health care systems is well documented in ancient literature. However, excessive consumption of these plants could cause damage to some body tissues as well as impair their functions. There is dearth of information on the medicinal use of *Costus lucanusianus* in males hence this study was designed to investigate the effect of methanolic leaf extract of *C. lucanusianus* (MLECL) on male reproductive parameters using albino rats as model. 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 MLECL), Group C (200 mg/kg of MLECL), Group D (300 mg/kg of MLECL) by oral gavage daily for 28 days. Thereafter, Animals were anaesthetized and testes collected, homogenized and used for determination of sperm characteristics. Blood was collected for hormonal assay (testosterone) using ELISA. Histopathological study of the testes and epididymides were conducted. Methanolic leaf extract of *C. lucanusianus* has no significant ($p > 0.05$) effect on sperm cell count and characteristics relative to the control, although the percentage of the sluggishly motile sperm cells increased in a dose dependent manner. No abnormality was observed in the testicular and epididymal sections of rats in the treated groups except the thickened interstitial spaces of testicular sections of rats treated with 200 and 300 mg/kg MLECL. It is therefore concluded that *Costus lucanusianus* methanolic leaf extracts have no deleterious effect on male reproductive parameters and can be considered relatively safe in male fertility.

Keywords: *Costus lucanusianus*, male, reproductive, testis, epididymis, sperm cells, testosterone.

INTRODUCTION

Plants have a long time history in medicine. For centuries, many people have developed different herbal medicines using locally available plants as remedy to their numerous health challenges. Much of the medicinal use of plants seems to have developed by trial and error and or through observations of wild animals [1]. When these medicinal plants are excessively consumed, they could result in the damage of some body tissues as well as their functions [2].

Costus lucanusianus (Family: Costaceae) is a tall evergreen perennial rhizomatous medicinal plant with thin stems and simple leaves [3]. In the southern Nigeria, *Costus lucanusianus* and *Costus afer*, which are closely related, produce hybrids [4]. Although *Costus lucanusianus* leaf extracts have been shown to possess oxytocic [5], analgesic [6] antglycemic, hepatoprotective and renoprotective [7] properties, its effect on male reproductive system is lacking.

This study therefore aims at assessing the effect of methanolic leaf extract of *Costus*

lucanusianus on male reproductive parameters using albino rats as models.

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 [8] 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 28 days. Animal's weight was taken daily and the dose adjusted accordingly. At the end of the experiment, the body weights were recorded

and the animals were anaesthetised under chloroform. 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 micro centrifuge tubes, capped and stored at -20 °C until analysis. The serum was later subjected to hormonal assay by ELISA method for assessment of testosterone levels. The reproductive organs (testes and epididymides) were carefully removed, cleared of adhering tissues and weighed. The left testes were homogenized and used for determination of sperm characteristics. The epididymis and right testes were fixed in Bouin's Solution, and then processed as described by Lillie [9], 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 Tukey post-test. The significance level was set at $p < 0.05$.

RESULT

Treatment of rats for 28days with 100, 200 and 300 mg/kg doses of MLECL had no significant ($p > 0.05$) effect on the mean weights of right and left testes and epididymitis when compared with the control as shown in figures 1 and 2.

Figure 3 shows that treatment of rats for 28days with 100, 200 and 300 mg/kg doses of MLECL produced no significant ($p > 0.05$) change on the testosterone level relative to the control.

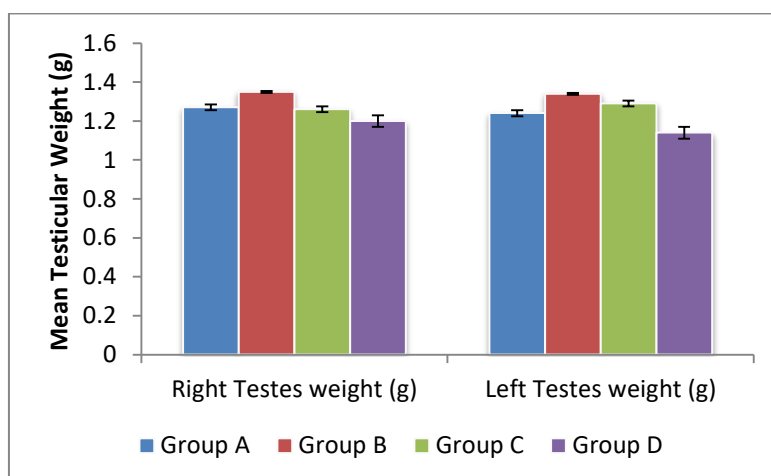


Fig-2: Effect of MLECL on Mean Testicular Weights

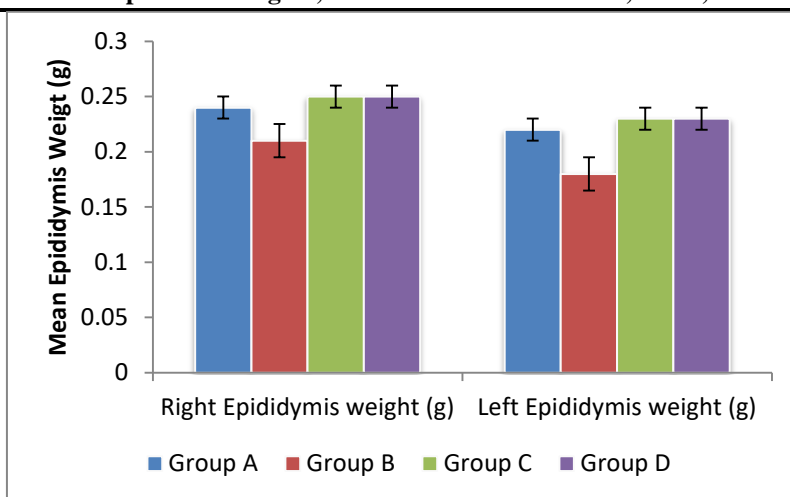


Fig-2: Effect of MLECL on Mean Epididymal Weights

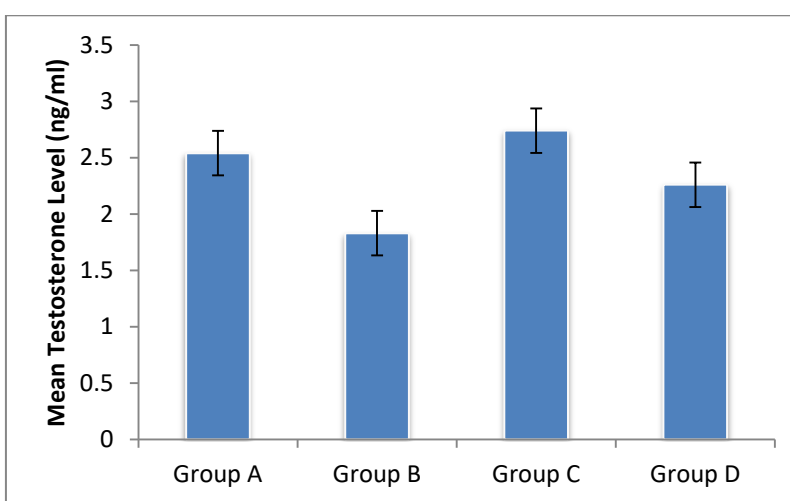


Fig-3: Effect of MLECL on Mean Testosterone Level

MLECL has no significant ($p > 0.05$) effect on sperm cell count and characteristics. However, it has a

dose dependent increase ($p > 0.05$) in sluggishly motile sperm cells when compared with the control (Table 1).

Table-1: Effect of Methanolic leaf extracts of *Costus lucanusianus* on sperm cell count and characteristics

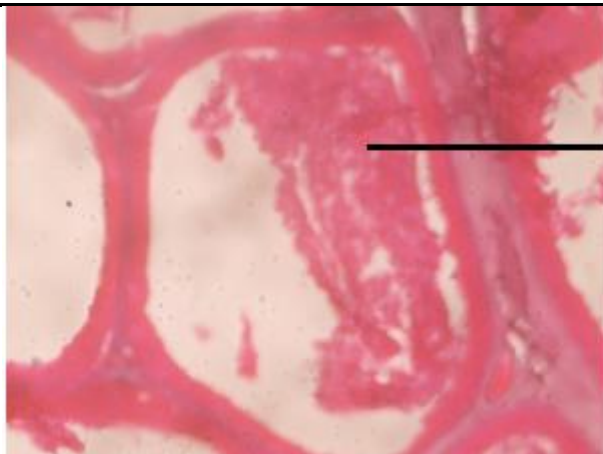
Groups	Viability (%)	Spermatozoa Morphology (%)		Sperm Cell Parameters (%)				Sperm Cell Count ($\times 10^6$)
		Normal	Abnormal	Active	Sluggish	Motile	Dead	
B	82.00±4.64	76.00±4.85	24.00±4.85	78.00±4.36	11.00±2.45	89.00±2.45	11.00±2.45	820.00±25.50
C	69.00±3.67	64.00±3.67	36.00±3.67	59.00±5.34	14.00±1.87	73.00±4.36	27.00±4.36	560.00±99.25
D	74.00±3.67	71.00±3.67	29.00±3.67	54.00±4.30	16.00±1.87	80.00±3.16	20.00±3.16	700.00±35.36

a. Values are Expressed as Mean ± SEM;

b. No significant variation exists across the table at 95% confidence interval ($P > 0.05$).

Epididymides of rats treated with 100, 200 and 300 mg/kg doses of MLECL 28 days showed no obvious abnormality in comparison with the control (Plate 1). However, at doses 200 and 300 mg/kg of

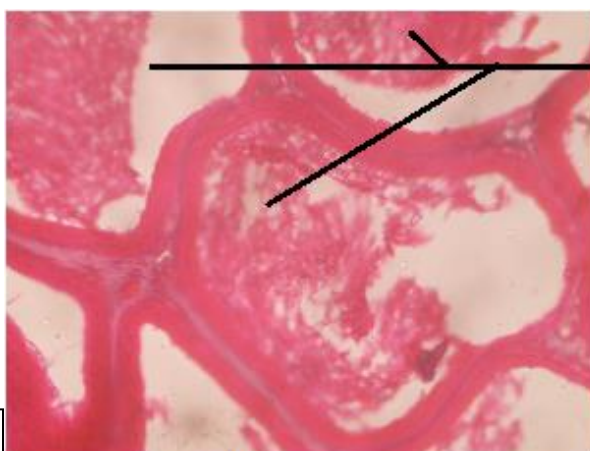
MLECL, testicular photomicrograph shows thickening of interstitial spaces with no obvious abnormality in the seminiferous tubules (Plate 2).



Epididymis containing spermatozoa

1

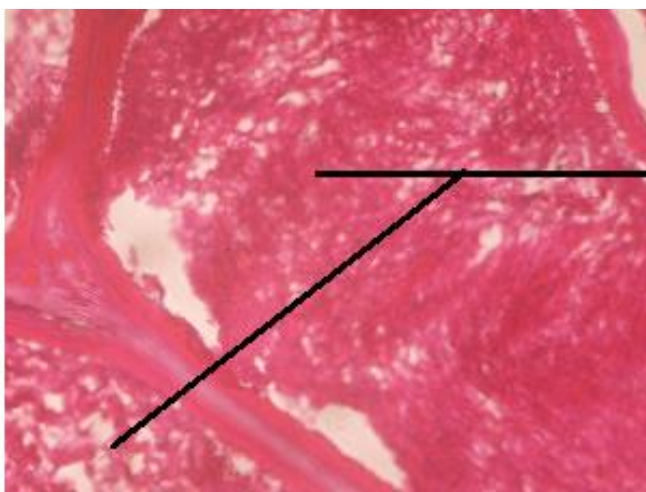
Group A - Control



Epididymis containing spermatozoa

2

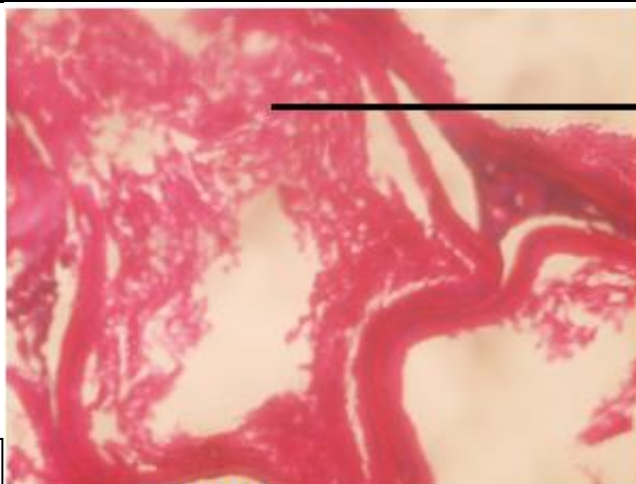
Group B - 100mg/kg



Epididymis filled with spermatozoa

3

Group C - 200mg/kg

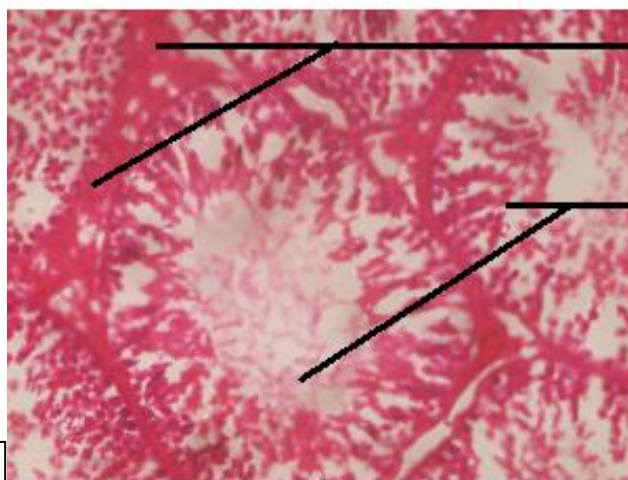


Epididymis
containing
spermatozoa

4

Group D - 300mg/kg

Plate-1: Photomicrographs of epididymal sections of rats from Control (1) and treated groups (2, 3 and 4) stained with H& E (X400). No abnormality in the epididymides of rats treated with MLECL compared to the control.

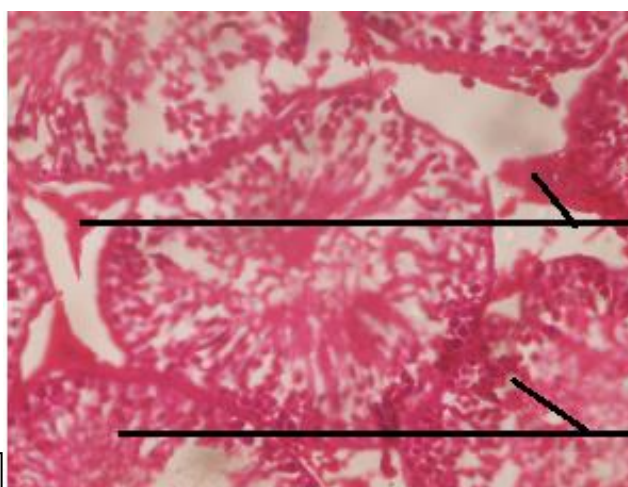


Interstitial
cells

Seminiferous
tubules

5

Group A - Control



Interstitial
cells

Seminiferous
tubules

6

Group B - 100mg/kg

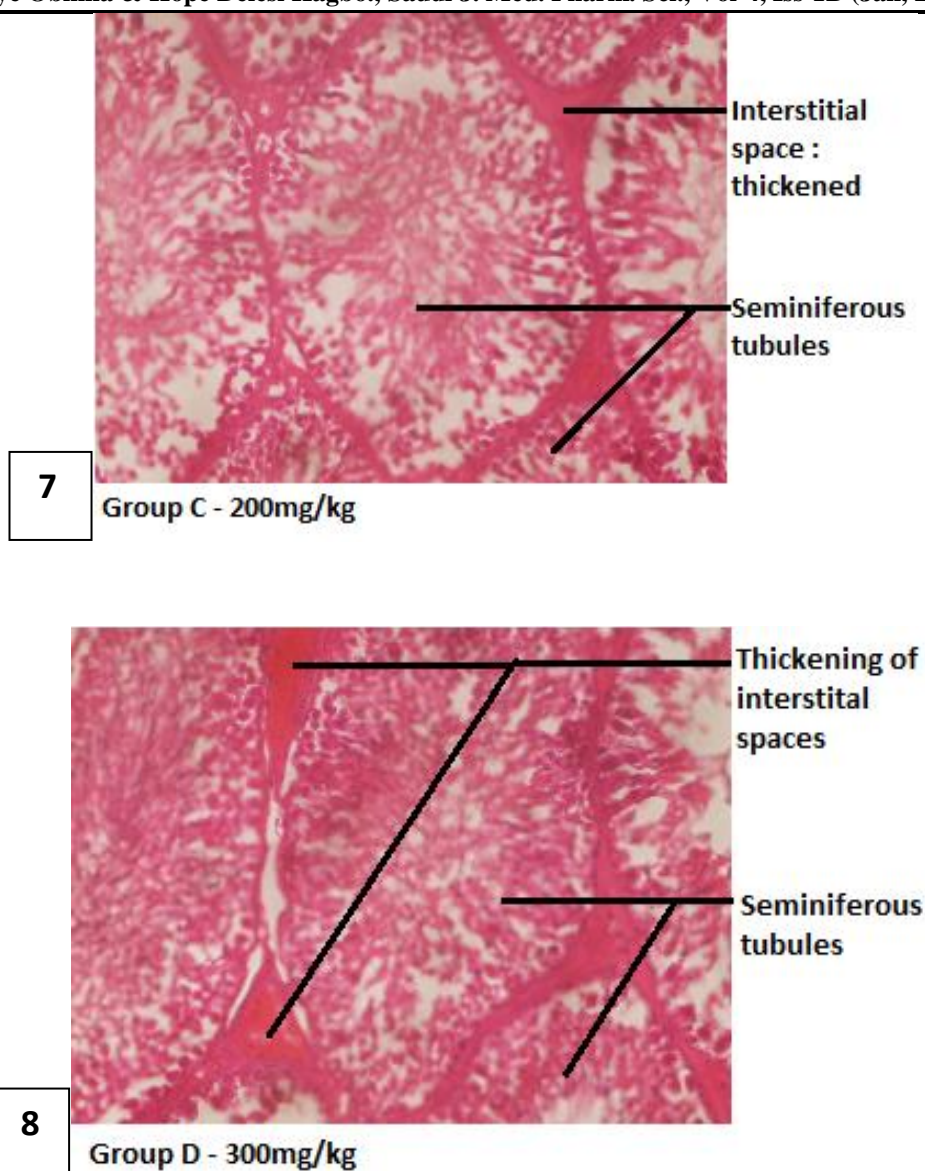


Plate-2: Photomicrographs of testicular sections of rats from Control (5) and treated groups (6, 7 and 8) stained with H& E (X400). No abnormality in the testes of rats treated with 100mg MLECL (6). Testicular interstitial spaces were thickened with no obvious abnormality in the seminiferous tubules of rats treated with 200 and 300 mg/kg MLECL (7 and 8)

DISCUSSION

Previous work done on *Costus lucanuscianus* stem demonstrated that the methanolic stem extract caused reduced sperm count and increased sperm cell defects. However, no abnormality was observed in the testicular and epididymal sections of rats in all the treated groups [10]. The present study showed that methanolic leaf extract of *Costus lucanuscianus* had no significant effect on testosterone levels, testicular and epididymal weights, sperm cell count and characteristics. The testicular and epididymal sections of rats in all the treated groups were not affected.

The reason for the disparity in the results of the previous and present studies could be in the distribution of the phytochemicals in the stem and leaf

parts of the plant. The medicinal values of plants lie in their component phytochemicals, which produce definite physiological actions in a biological system. Generally, phytochemicals and nutrients accumulate in different parts of plants in varying concentrations. Ezeabara *et al.*, [11] demonstrated that the phytochemicals and nutrients found in various parts of *Portulaca oleracea* were in different concentrations.

In agreement with our findings in this study, the methanolic leaf extract of *Costus lucanuscianus* was reported to have no harmful effect on pregnancy as it did not cause abortion in the pregnant rats neither was any external nor visceral anomaly observed in the fetuses [12]. The result of this study is found to be in contrast with the study carried out by Oyedeji and

Bolarinwa [13] who reported that methanolic extracts of *Portulaca oleracea* leaves and stems caused a decrease in testosterone levels, sperm viability and motility as well as increase in the percentage of morphologically abnormal cells.

CONCLUSION

The result of this study revealed that the reproductive parameters determined were similar in both treated and control groups, over the period of treatment, suggesting that the methanolic leaf extract of *Costus lucanusianus* evoked no adverse effects on sperm production and can be considered relatively safe in male fertility.

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