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# Antifertility Effect of *Costus Lucanuscianus*stem Extract in Male 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, dysmenorrhea, headache and rheumatism. The paucity of information on reproductive effect of Costus lucanuscianus in male animals prompted this study using methanolic stem extract of this plant. Twenty male rats 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 21 days. Thereafter, Animals were anaesthetized and testes collected, homogenized and used for determination of sperm characteristics. Blood was collected for hormonal assay (testosterone) using Enzyme Immunoassay. Histopathological study of the testes and epididymides were conducted. Costus lucanuscianus stem extract (CLSE) had no significant effect ($p>0.05$) on the testicular and epididymal weights as well as the testosterone level relative to the control. CLSE decreased sperm count, sperm viability, sperm motility and normal sperm morphology in a dose dependent manner. However, CLSE at the dose of 300mg/kg only showed a level of significance ($p<0.05$) relative to the control. No abnormality was observed in the testicular and epididymal sections of rats in all the treated groups.The findings suggest that C.lucanuscianus possesses anti-fertility properties evidenced by the reduced sperm count and increased sperm cell defects.*

***Keywords:*** *Costus lucanusianus, antifertility, male, testes, epididymis*

## 1. 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. When these medicinal plants are excessively consumed, they could result in the damage of some body tissues as well as their functions (Soladeye et al., 2010) *Costus lucanuscianus* (Family: Costaceae), locally called 'monkey sugar cane' in the Niger Delta region of Nigeria, is a tall evergreen perennial rhizomatous medicinal plant with thin stems and simple leaves. In the southern Nigeria, *Costus lucanuscianus* and *Costus afer*, which are closely related, produce hybrids (Aweke, 2008). Phytochemical reports indicate that the genus Costus is rich in steroidal saponins, sapogenins, oxalates, furans, furan derivatives and starches (Oliver, 1986). Although *Costus lucanuscianus*leaf extracts have been shown to possess oxytocic (Owolabi et al., 2010), analgesic (Owolabi and Nworgu, 2009), antiglycemic, hepatoprotective and renoprotective (Saliu and Fapohunda, 2016) properties, effect of the stem extract on male fertility is lacking.
This study therefore aims at evaluating the reproductive effect of *Costus lucanuscianus*stem extract in male albino rats.

## 2. Materials and Methods

### *2.1. Plant Material and Authentification*

Fresh stems 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

*2.2. Preparation of Plant Extract*

After collection of the plant, the stems 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*stems were 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.

*2.3. Animals*

Twenty mature male albino rats, *Rattus Norvegicus*, 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.

*2.4. 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 21 days. Animal's weight was taken daily and the dose adjusted accordingly. Body weights of the animals were recorded at the end of the experiment. The animals were anaesthetized under chloroform and blood samples were collected via the retro-orbital plexus into 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 for assessment of Testosterone levels usingAccu-bind ELISA kits (Testosterone Test System Product Code: 3725-300) from Monobind Inc. Lake Forest, CA 92630, USA.

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 epididymides and right testes 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.

*2.5. 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 $p<0.05$.

**3.Result**

Treatment of rats for 21days with 100, 200 and 300 mg/kg doses of *Costus lucanuscianus*stem extract had no significant ($p>0.05$) effect on the weights of left and right testes and epididymides relative to the control as shown in figures 1 and 2.

Figure 3 shows that treatment of rats for 21days with 100, 200 and 300 mg/kg doses of Costus *lucanuscianus*stem extract produced no significant ($p>0.05$) change on the testosterone level relative to the control.

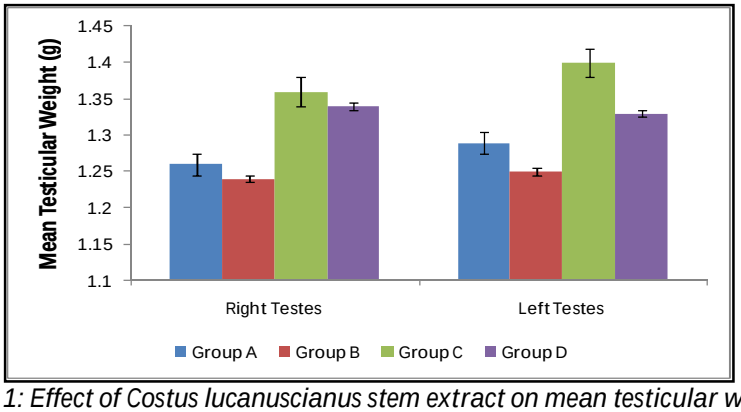


*Figure 1: Effect of Costus lucanuscianus stem extract on mean testicular weights*

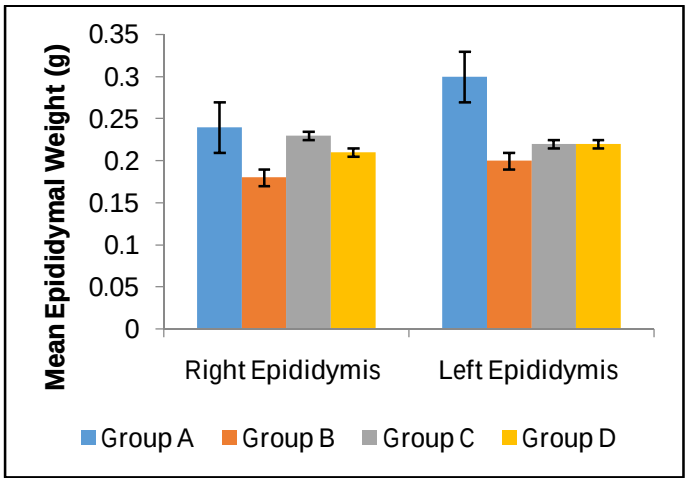


*Figure 2: Effect of Costus lucanuscianus stem extract on mean epididymal weights*

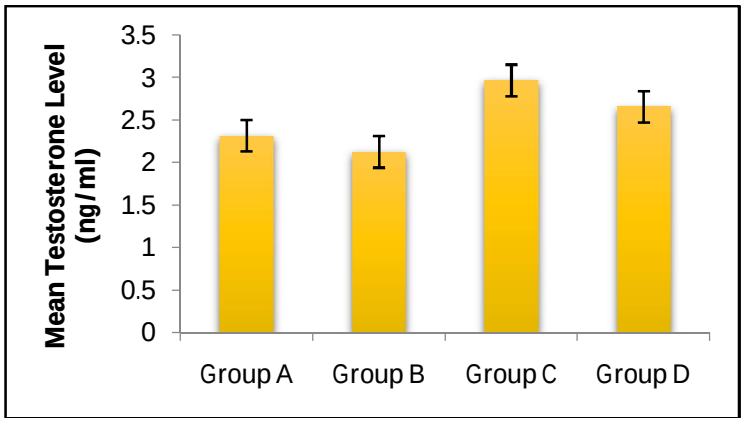


*Figure 3: Effect of Costus lucanuscianus stem extract on mean Testosterone level*

Table 1 shows that generally, there is a dose dependent decrease in sperm viability, normal sperm morphology, motile sperm and active sperm cells across the C*ostus lucanuscianus* stem extract treated groups when compared with control. However, only treated group D(300 mg/kg extract)produced a significant effect ($p<0.05$) on the three indices whereas treated group C (200mg/kg extract) had a significant effect ($p<0.05$) on the sperm viability alone. C*ostus lucanuscianus* stem extract at the doses of 200 and 300 mg/kg caused a significant ($p<0.05$) increase in the abnormal sperm morphology. The high dose (300mg/kg) of C*ostus lucanuscianus* stem extract resulted in significant ($p<0.05$) decrease in sperm cell count and motile sperm as well as significant ($p<0.05$) increase in sluggish and dead sperm cells relative to the control.

| GROUPS | VIABILITY (%) | SPERMATOZOA MORPHOLOGY (%) | | SPERM CELL PARAMETERS (%) | | | | SPERM CELL COUNT (x10⁶) |
|---|---|---|---|---|---|---|---|---|
| | | NORMAL | ABNORMAL | ACTIVE | SLUGGISH | MOTILE | DEAD | |
| A | 79.00±5.79 | 79.00±4.00 | 21.00±4.00 | 78.00±6.44 | 8.00±2.00 | 86.00±4.85 | 14.00±4.85 | 730.00±75.17 |
| B | 72.50±2.50 | 73.75±5.15 | 26.25±5.15 | 71.25±6.57 | 10.00±2.04 | 81.25±5.15 | 18.75±5.15 | 812.50±31.46 |
| C | 70.00±5.24 | 69.00±2.92* | 31.00±2.92* | 66.00±7.31 | 13.00±2.55 | 79.00±5.57 | 21.00±5.57 | 670.00±84.56 |
| D | 59.00±1.00* | 62.00±2.00* | 38.00±2.00* | 42.00±6.25* | 12.00±1.22 | 54.00±6.96* | 46.00±6.96* | 350.00±96.18* |

*Table 1: Effect of Costus lucanuscianus stem extract on sperm cell count and characteristics*

a. Values are Expressed as Mean ± SEM
b. * Indicates a Significant Variation when Compared with the Group A ($P<0.05$)

Testes and Epididymides of rats treated with 100, 200 and 300 mg/kg doses of Costus *lucanuscianus* stem extract for 21 days showed no obvious abnormality in comparison with the control (Figures 4 and 5)

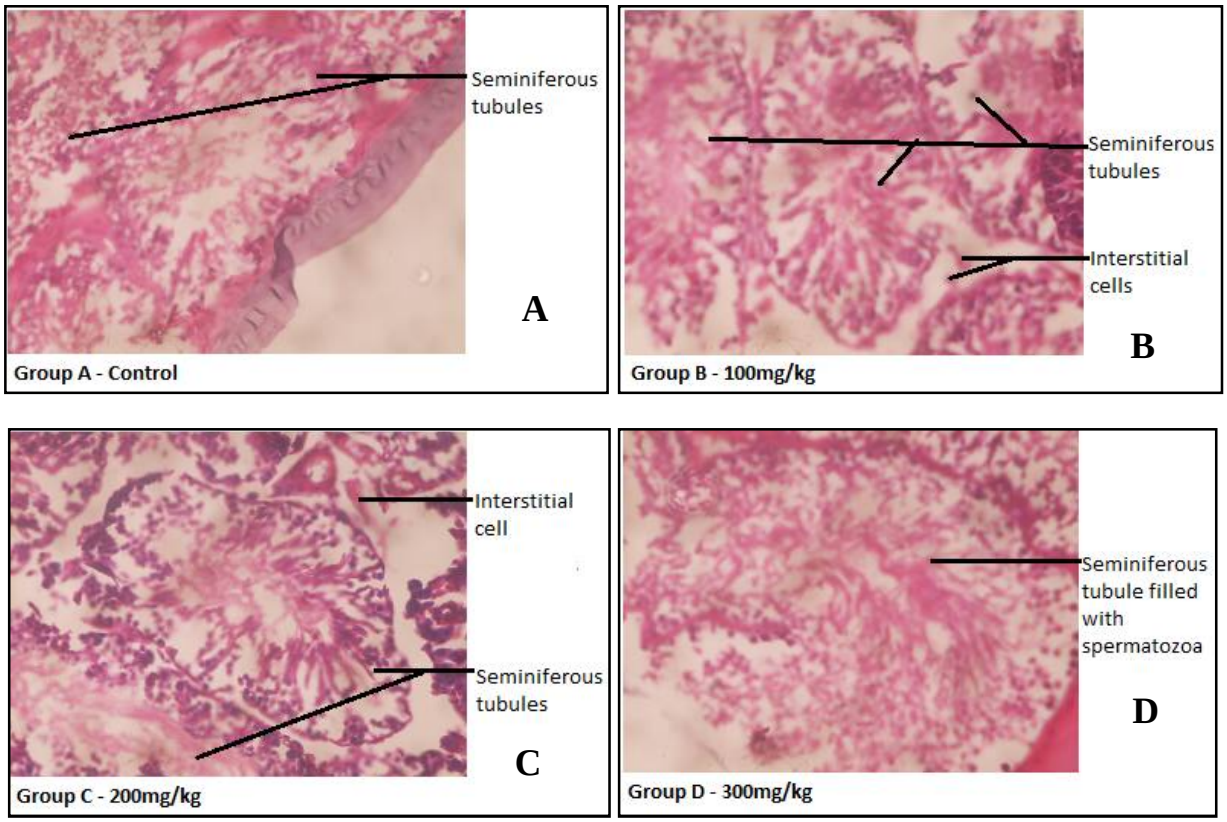


*Figure 4: Photomicrographs of testicular sections of rats from Control (A) and treated groups (B, C and D) stained with H& E (X400). No abnormality in the testes of rats treated with Costus lucanuscianus methanolic stem extract compared to the control*

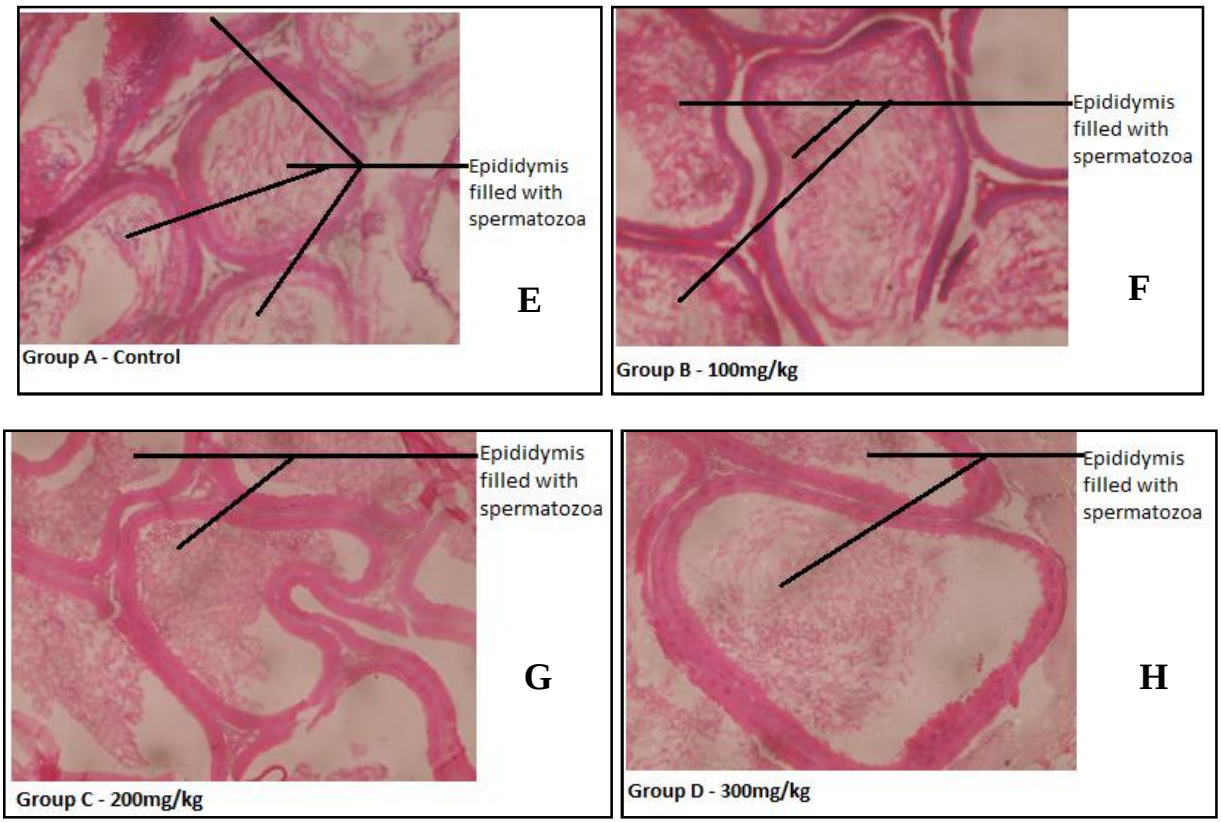


*Figure 5: Photomicrographs of epididymal sections of rats from Control (E) and treated groups (F, G and H) stained with H& E (X400). No abnormality in the epididymides of rats treated with Costus lucanuscianus methanolic stem extract compared to the control*

**4.Discussion**

Indiscriminate use of medicinal plants as a remedy to health problems is becoming worrisome due to the associated adverse effects. The prolonged usage of herbal products without proper monitoring has led to a number of health-related problems like infertility (Leke, 2008). The study investigated the reproductive effect of *Costus lucanuscianus* stem extract in male albino rats.

The study demonstrated that *Costus lucanuscianus* stem extract has a negative effect on the sperm cell count and characteristics especially at the dose of 300mg/kg. It decreased the sperm cell count with an increase in sperm cells that are defective. Sperm characteristics such as sperm motility and morphology as well as sperm cell count are key indices of male fertility, since they are the prime markers in testicular spermatogenesis and epididymal maturation. Sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis (Reddy *et al*., 2006). It is well established that motile spermatozoa in sufficient concentration and free from abnormalities are highly correlated with fertility (Aitken *et al*., 1984) as sluggishly motile or immotile sperms are less likely to penetrate the cervical mucus and fertilize the ova (Abu and Uchendu, 2010). According to Sarkar *et al*., (2000), reduced sperm motility and viability may be an indication of alterations in spermatogenesis. This finding is in line with the studies by Nwaehujor *et al*. (2014) and Reuben *et al*. (2014) in male Albino rats treated with methanolic extract of *Carica papaya* root and seed respectively.

The study also revealed that *Costus lucanuscianus* stem extract had no significant effect on the testicular and epididymal weights as well as the testosterone level relative to the control. This shows that he extracts may not have a direct effect on male hormone – testosterone but on the spermatogenesis. Testosterone and spermatogenesis are stimulated by two different hormones of the anterior pituitary; FSH stimulates the former while LH stimulates the latter. This agrees with the findings by Adeleke *et al.* (2016) in the work carried out in male rats treated with *Cnidoscolous aconitifolius.*

*Costus lucanuscianus* stem extract did not alter the testicular and epididymal histology of treated rats even when the sperm cell characteristics were adversely affected. The exact mechanism for this is not known, but it is suggested that the short duration of exposure (21 days) to the extract mayhave caused immediate harm to mature sperm cells which is yet to be seen at the tissue level.

**5.Conclusion**

The results obtained from this study calls for some level of caution in the consumption of *Costus lucanuscianus* stem (monkey sugarcane) as it has been demonstrated to possess anti-fertility properties evidenced by the reduced sperm count and increased sperm cell defects.

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