



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article


May 2019 Vol.:15, Issue:2

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## The Effects of Aqueous Extract of *Cyperus esculentus* on Testis of Male Wistar Rats



IJPPR  
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals



ISSN 2349-7203

**ABIREH Ifeanacho Ezeteonu<sup>1</sup>, ANYANWU Godson Emeka<sup>2</sup>, OBIKILI Emeka<sup>2</sup>, HASSAN Luqman Adepoju\*<sup>2</sup>, Elizabeth Finberris Bello<sup>1</sup>, NTO Johnson Nto<sup>2</sup>**

<sup>1</sup>*Department of Anatomy, Enugu State University of Science and Technology.*

<sup>2</sup>*Department of Anatomy, University of Nigeria Enugu Campus.*

**Submission:** 23 April 2019  
**Accepted:** 28 April 2019  
**Published:** 30 May 2019

**Keywords:** *Cyperus esculentus*; Testis; Spermatogenesis; Leydig cell

### ABSTRACT

**Study background:** *Cyperus esculentus* is presently consumed for various reasons. Its effect on the testis is yet to be defined. This study evaluated the effect of aqueous extract of *Cyperus esculentus* on serum level of testosterone, LH and FSH as well as histological structure of the testes of male Wistar rats. *Cyperus esculentus* was dried and ground and aqueous extract was prepared using distilled water. **Materials and methods:** Twelve adult male Wistar rats were randomly divided into three (3) groups, A-C (n=4). Group A was given distilled water only, Groups B and C were given aqueous extracts of *Cyperus esculentus* at a doses of 150mg/Kg and 300mg/Kg orally respectively for 21 days. The testes of the rats were harvested on day 21 of the experiment and histological studies done using the H&E and the Ki-67 staining. **Results:** Histological examination of the testes revealed increased series of spermatogenesis and Leydig cell proliferation in the rats in groups B and C. Rats in groups B and C had a significant increase in levels of serum testosterone ( $p < 0.05$ ) when compared with control group. Also, there was significant increase in levels of LH in group C when compared with control. There was a significant decrease in levels of FSH in both B and C when compared with control which may be due to feedback mechanism. **Conclusion:** This study demonstrated androgenic effect of the aqueous extract of *Cyperus esculentus*. It also shows that aqueous extract of *Cyperus esculentus* has no deleterious effect on the reproductive organ on the doses used.



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## INTRODUCTION

Many plants have been screened for their medicinal properties [1]. It is a known fact that most fruits and vegetables are important source of balance diet and nourishment [2]. *Cyperus esculentus* (tiger nuts) is an annual or perennial plant, which grows up to 90 cm tall [3]. It has solitary stems that grow from a tuber. The roots are complex system of fine, fibrous roots and scaly rhizomes with small hard, spherical tubers and basal bulbs attached [4]. Tiger nuts contain high percentage of alkaloids, saponins and tannins. Tiger nuts was reported as healthy and helps in preventing heart, thrombosis and activates blood circulation and are responsible for preventing and treating urinary tract infections and other bacterial infections [5]. The tubers are between 0.3 – 1.9 cm in diameter and the colour varies between yellow, brown and black [6]. One plant can produce several hundreds to several thousands of tubers during a single season [7]. When the weather is cold the foliage, roots, rhizomes and basal bulbs die. But the tubers survive and sprout again the following season when soil temperatures remain above 6°C. They can re-sprout up to several years later [4]. When the tubers germinate they initiate many rhizomes that end in a basal bulb near the soil surface. *Cyperus esculentus* is a wind pollinated and requires cross pollination [8]. Miller, [9] reported that tiger nut oil can be used in the cosmetic industry. As it is an antioxidant (because of its high content in vitamin E) it helps to slow down the ageing of the body cells.

Tiger nut also called Chufa sedge, Nutgrass, Yellow nutsedge, Tiger nutsedge, or Earth almond belongs to the sedge family. The Hausas call it Aya, the Yorubas call it Ofio and the Igbos call it Akiawusa [10]. Three varieties are known to be cultivated in Nigeria, namely the yellow, the brown and the black *Cyperus esculentus* [11]. The yellow and brown are more common. The yellow variety is preferred because of its bigger size, attractiveness and fleshy body [10]. As it is antioxidant (because of its high content in vitamin E) it helps slow down the ageing of the body cells. It also favours the elasticity of the skin and reduces skin wrinkles [12].

Consumption of tiger nut tuber is high among men of the Hausa tribe of northern Nigeria. Although there is paucity of research works to support this fact, there is a popular assumption that it has androgenic property. This study was designed to study the androgenic potential of tiger nut tuber using adult male Wistar rats.

## MATERIALS AND METHODS

### Extraction of tiger nuts:

Fresh Tiger nuts were sundried and grounded into fine powder using grinding machine into powder form. The grounded nuts was dissolved in specific quantity of water, stirred and left for 48 hours and boiled for about 20 minutes. The extract was obtained through the filtration. The phytochemical was reported by Adejuyitan *et al.*, [6] to have alkaloid, glycoside, resin, flavonoid, tannins, sterols, saponins, cyanogenic glycoside.

### Experimental animals

Twelve (12) male Wistar rats with mean weight of 170g were used for this experiment. The rats were housed in netted cages and fed with grower's mash. They were kept for a period of two week for acclimatization prior to the experiment.

### Experimental Designs

Twelve male Wistar rats were divided randomly into three groups, A-C (n=4). Group A (control) was fed daily with normal feed and distilled water throughout the twenty-one (21) day period of the experiment. Group B was given aqueous extract of tiger nuts at a dose of 150mg/Kg body weight per day plus water and feed throughout the twenty-one (21) day period of the experiment. Group C was given aqueous extract of tiger nuts at a dose of 300mg/Kg body weight per day plus water and feed throughout the twenty-one (21) day period of the experiment.

### Determination of hormone assay

After 24 hours following the administration of the last dose before the animals were sacrificed, whole blood collected by capillary tube through medial optical plexus and kept in non-heparinised vacutainer which was span at 2500rpm for 10min using a bio-centrifuge (MSE, O-5122A, Germany). The level of free serum testosterone, LH and FSH in serum was measured with ECOBAS-6000 hormone analyzing machine as described by Atlas *et al.*, [13].

## Histological study

Animals in all groups were sacrificed under the anesthetic agent called thiopental at dose 50mg/kg. The abdominal cavities were opened up through the midline incision to expose the reproductive organs (testes). These samples were taken for histological study and these were kept in MDF fluid (modified Davidson's fluid) until the time of sections [14]. The sections were worked in the department of Anatomy College of Medicine, University of Nigeria Enugu Campus.

## Statistical analysis

The data obtained were analyzed with one way ANOVA at 5% level of significance.

## RESULTS

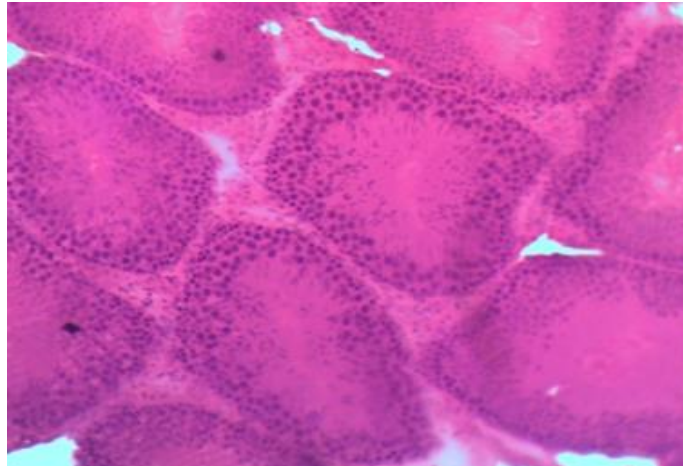
**Table-1: The result of hormonal assay in experimental models**

Hormone	Group A	Group B	Group C
Testosterone	0.3 ±0.01	0.5±0.02	0.7±0.02
Follicle stimulating hormone	11.5±0.02	4.3±0.16	4.0±0.03
Luteinizing hormone	6.6±0.2	5.8±0.01	8.2±0.24

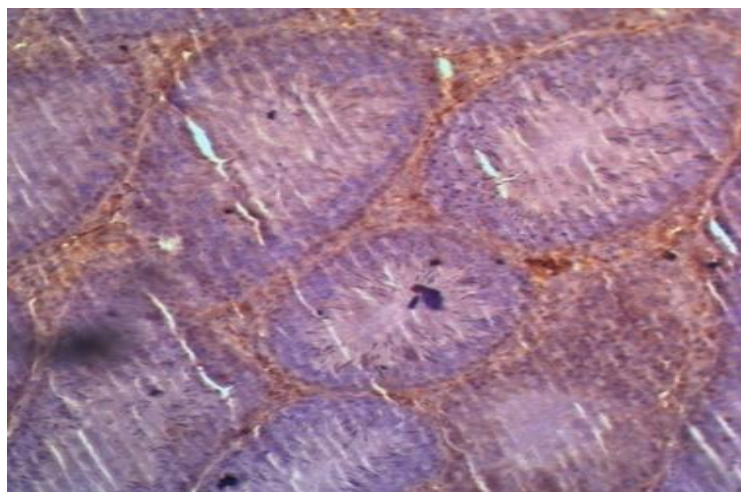
## Observation on hormonal assay

The rats in groups B and C had significant increase in level of serum testosterone with  $p < 0.05$  when compared with control group. There was also significant increase in level of LH in group C with  $p < 0.001$  when compared with control group. FSH was significantly reduced with  $p < 0.01$  in B and C when compared to A (control).

### Histological findings

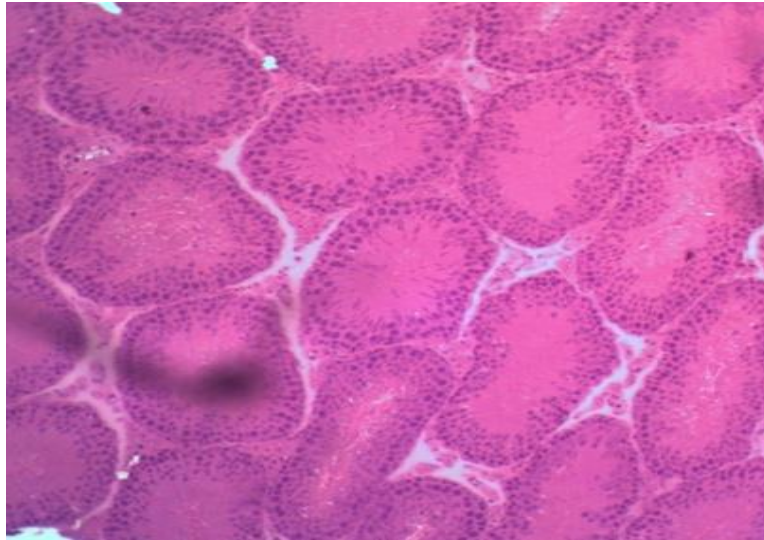


**Fig-1: Histoculture of the testis (H&E, x200) in Group A (control) showing apparently normal outline of the seminiferous tubules, the interstitium and the spermatogenic cells which are at different stages of development.**

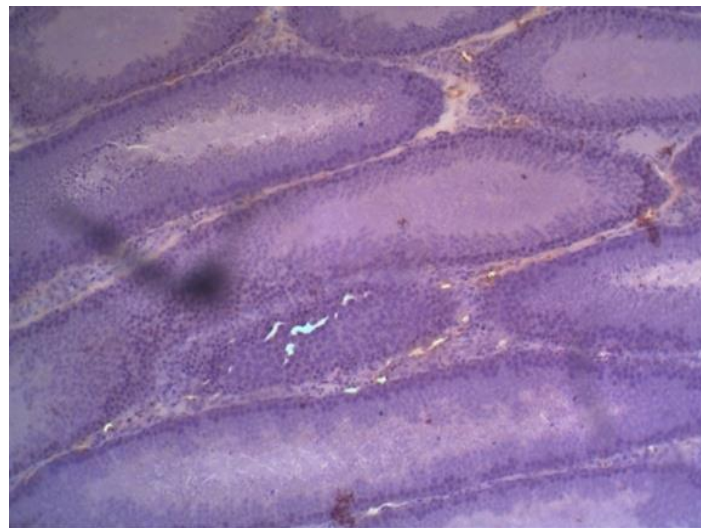


**Fig-2: Histoculture of the testis (Ki 67 stain, x200) in Group A (control) showing apparently normal outline of the seminiferous tubules, interstitium and spermatogenic cells which are at different stages of development.**

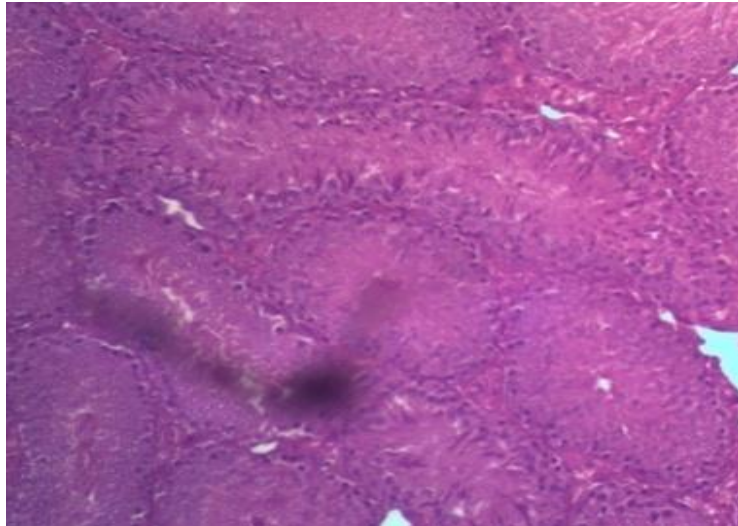




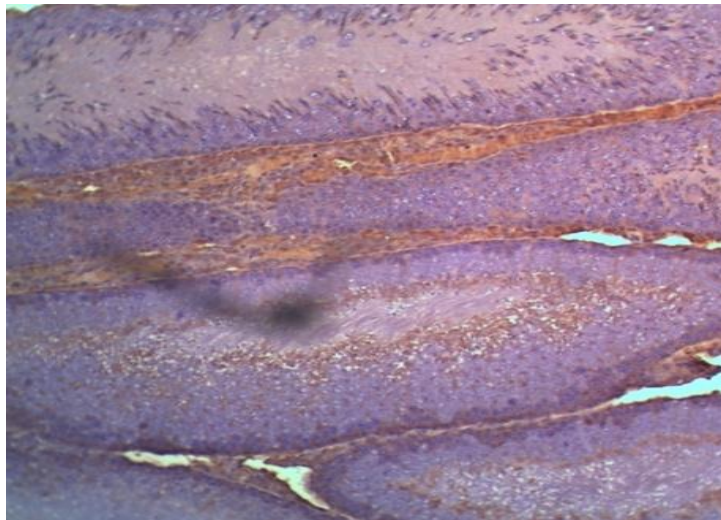
**Fig-3: Histoculture of the testis (H&E stain, x200) in group B treated with 150mg/Kg of aqueous extract of Tiger nut. This shows increased cellular proliferation when compared with the control.**



**Fig-4: Histoculture of the testis (Ki 67 stain, x200) in group B treated with 150mg/Kg of aqueous extract of Tiger nut. This shows increased cellular proliferation when compared with the control.**



**Fig-5: histoculture of the testis (H&E stain, x200) in group C treated with 300mg/Kg of aqueous extract of Tiger nut showing increased spermatogenic cells proliferation with narrowed lumen.**



**Fig-6: Histoculture of the testis (Ki 67 stain, x200) in Group C treated with 300mg/Kg of aqueous extract of Tiger nut showing increased spermatogenic cells proliferation with narrowed lumen.**

## DISCUSSION

Testosterone plays a major role in the growth and development of the male reproductive organs such as the testis and prostate [15]. Testosterone also increases libido, increase frequency of erection, growth of facial, chest, nipple and pubic hair and reduces the fat content beneath the skin, while the muscle mass increases [15].

This study observed increased serum testosterone and luteinizing hormone levels in the groups treated with tiger nut extract when compared with the control. It was observed that tiger nut tuber extract caused a significant increase in serum testosterone level at dose of 300mg/kg. This increase in serum testosterone level observed is dose dependent as no significant increase in the serum testosterone level was observed in the group that was administered with 150mg/Kg of the extract. The feedback control mechanism of testosterone on luteinizing hormone is lost due to active cell multiplication. Lee, *et al.*, 1975, observed that there is parallel increase in LH and testosterone in rapidly dividing interstitial cells.

Decrease in serum follicle stimulating hormone level was observed in the groups treated with the tiger nut tuber extract. Follicle stimulating hormone induces sertoli cells to secrete androgen-binding proteins, inhibin B and activin. The inhibin secreted by the sertoli cells has a negative feedback effect on the anterior pituitary gland [16]. This explains the low level of follicle stimulating hormone observed in this study. The presence of more spermatogenic cells was also observed in group B and C which was dose dependent as more spermatogenic cells were seen in group C when compared to group B. The interstitial cells in group D are more in number and with narrowed seminiferous tubule lumen. The basement membrane of the seminiferous tubules was intact.

## CONCLUSION

From these results, it will be concluded that extract of *Cyperus esculentus* may be positive libido effect because it increases the serum luteinizing hormone and Testosterone levels. This also showed that extract of *Cyperus esculentus* increases the spermatogenic cells within the seminiferous tubules. Therefore this study will validate the use of *Cyperus esculentus* for normal testicular activities.



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