Original Article

Access this article online

Quick Response Code:

Website: jecajournal.com

DOI: 10.4103/jeca.jeca_27_18

Curative effect of aqueous extract of *Cyperus esculentus* on flutamide-induced testicular dysfunction in male Wistar rats

Luqman Adepoju Hassan, Godson Emeka Anyanwu, Nto Johnson Nto, Emmanuel Nebuwa Obikili, Elizabeth Finbarrs-Bello[1], L. Ifeanacho Ezeteonu Abireh

Abstract:

BACKGROUND: The health of the testis is important for a man's sexual functioning and fertility. This study investigated the effect of aqueous extract of *Cyperus esculentus* on flutamide-induced testicular dysfunction in male Wistar rats.

MATERIALS AND METHODS: Sixteen adult male Wistar rats were randomly divided into the following four groups: A–D ($n = 4$). Group A was given distilled water, and Group B was given 5 mg/kg body weight of flutamide daily for the period of the experiment. Group C was given 5 mg/kg body weight of flutamide 1 h before treatment with 150 mg/kg body weight of an extract of *C. esculentus*, and Group D was given 5 mg/kg body weight of flutamide 1 h before treatment with 300 mg/kg body weight of an extract of *C. esculentus* for 21 days.

RESULTS: The hormonal assay showed that the extract produced a significant increase in serum testosterone and luteinizing hormone compared to the controls. The light microscopic study revealed degenerative changes in the germinal epithelium and interstitial tissue of the rats treated with flutamide, whereas the extract produced a marked increase in the number and volume of cells in the germinal epithelium compared to the positive control.

CONCLUSION: The results indicate that *C. esculentus* could enhance testicular activity and ameliorate the adverse effect of flutamide on the testis.

Keywords:

Cyperus esculentus, flutamide, infertility, reproductive biology, testes

Introduction

The use of herbs in the treatment of different diseases is fast becoming revolutionized. It is a well-known fact that most fruits and vegetables are important sources of nourishment. *Cyperus esculentus* (also called chufa sedge, nut grass, yellow nutsedge, tigernut sedge, or earth almond) is locally called "aya," "ofio," and "aki hausa" by the Hausa, Yoruba, and Igbo tribes of Nigeria, respectively (Ibrahim *et al*., 2009). It is a crop of the family; Cyperaceae, native to warm temperate, subtropical regions of the Northern Hemisphere (Imam *et al*., 2013). The biologically active compounds in tiger nut include as follows: alkaloids, saponins, flavonoids, glycosides, phenols, steroids, phlobatannins, and terpenoids (Ibrahim *et al*., 2009). *C. esculentus* has the high contents of Vitamins (A, C, D, and E), minerals (Na, Ca, Fe, Zn, K, Mg, and P), protein, starch, and fat (Simpson and Inglis 2001; Sofowora 1993).

Extracts of *C. esculentus* have been documented to possess antibacterial activity

Department of Anatomy, Faculty of Basic Medical Science, College of Medicine, University of Nigeria Enugu Campus, Enugu, [1]Department of Anatomy, College of Medical Science, Ebonyi State University, Abakaliki, Nigeria

Address for correspondence:
Nto Johnson Nto, Department of Anatomy, Faculty of Basic Medical Science, College of Medicine, University of Nigeria Enugu Campus, Enugu, Nigeria.
E-mail: johnson.nto@unn.edu.ng

How to cite this article: Hassan LA, Anyanwu GE, Nto NJ, Obikili EN, Finbarrs-Bello E, Abireh LI. Curative effect of aqueous extract of *Cyperus esculentus* on flutamide-induced testicular dysfunction in male Wistar rats. J Exp Clin Anat 2018;17(1):13-17.

toward respiratory and urinary tract infections (Ekeanyanwu *et al*. in 2010). Existing body of evidence also suggests that tiger nut is good for cardiovascular, bone, and reproductive health (Prakash and Ragavan in 2009; Hassan *et al*. in 2005). It is widely consumed locally, for its immense nutritional benefits and for the traditional believe that it has aphrodisiac properties. It is important, therefore, to access the effects of *C. esculentus* on the testis.

Flutamide has been reported to affect serum testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, and spermatogenesis resulting in male reproductive dysfunction and infertility (Hassan *et al*. in 2018; Perobelli *et al*. in 2012). Flutamide is a toluidine derivative and a nonsteroidal antiandrogen used primarily to treat prostate cancer and also for hormone therapy (Elks and Ganellin 1990).

The aim of this study is to evaluate the curative effects of aqueous extract of *C. esculentus* on flutamide-induced testicular dysfunction in adult male Wistar rats.

Materials and Methods

Collection and preparation of plant material

C. esculentus was purchased from a local market in Enugu, Enugu State, South-East of Nigeria. The tubers were screened and cleaned of stones and bad ones, they were then washed and air-dried. The tubers were ground into powder, using wood mortar and pestle. The powdered sample was filtered through a film (2-mm mesh) sieve to remove the residue (Ibrahim *et al*., 2009).

Preparation of aqueous extract

Hundred grams of the dried powdered sample was dissolved in 200 mL of distilled water and allowed to soak for 24 h. The solution was filtered through a Whatman filter (125 mm) paper No. 42 (Adejuyitan *et al*., 2009).

Drugs and chemicals

Flutamide, sold under the brand name Eulexin (Actavis Pharma Inc., USA) a nonsteroidal anti-androgen was used to induce testicular dysfunction (Edoga *et al*., 2006). The drug, routine laboratory reagents, and solvents were purchased from the registered distributors.

Experimental animals and design

Sixteen adult male Wistar rats of the average weight of 150 g were obtained from the animal house of College of Medicine, University of Nigeria Enugu Campus. Animals were allowed to acclimatize for a period of 2 weeks. The rats were weighed and randomly divided into four groups A, B, C, and D ($n = 4$). Group A (normal control) was treated with normal feed and distilled water for the period of the experiment. Testicular dysfunction was induced with flutamide (5 mg/kg body weight) in the rats of Groups B, C, and D. One hour after the induction, Group B (negative control) was treated with placebo, Group C (curative I) was treated with the lower dose of the extract (150 mg/kg body weight), and Group D (curative II) was treated with a higher dose of the extract (300 mg/kg body weight). The flutamide, placebo, and the extract of *C. esculentus were* administered by orogastric intubation once daily for 21 days. At the end of the experimental period, body weights of all the rats were taken and recorded.

Sample collection

All the animals were anesthetized on day 22 by intraperitoneal injection of 50 mg/kg body weight of thiopental sodium (Dorfman 1970). The blood was collected for hormonal assay, and the testes were rapidly dissected for processing and light microscopic study.

Determination of hormonal levels

The blood sample was collected using capillary tube through medial optical plexus and kept in nonheparinized vacutainer which was span at 2500 rpm for 10 min using a bio centrifuge (MSE, O-5122A, Germany). The levels of free serum testosterone, LH, and FSH were measured with ECOBAS-6000 hormone analyzing machine as described by Atlas (Finbarrs-Bello *et al*., 2015).

Light microscopic study

The testes were removed and weighed and then fixed in a modified Davidson's fluid for 24 h (Atlas *et al*., 1995). Standard protocol was followed in processing the tissue for microscopic examination (Latendresse *et al*., 2002). Paraffin sections were cut at 3-µm thick and stained with hematoxylin and eosin (H and E). During the light microscopic examination; testicular architecture, number and volume of germinal epithelium were observed.

Ethical approval

Ethical approval was obtained from the Research and Ethics committee of the Faculty of Basic Medical Science, University of Nigeria, Enugu Campus.

Statistical analysis

Data collected was analysed using Statistical Package for Social Science (SPSS) version 20.0 (IBM Computers, USA). Data were expressed as the mean ± standard deviation. One-way analysis of variance was used to study variations between the groups. Statistical significance was considered at the level of $P < 0.05$.

Results

Body weight

The result in Table 1 showed a significantly higher percentage increase ($P < 0.05$) in the body weight of the rats in Group B. Groups C and D had significantly higher

percentage increase ($P < 0.05$) in body weight compared to the normal control; the increase was however, higher in the Group C.

Observation on hormonal assay

The result of the hormonal assay Table 2 revealed a significant higher ($P < 0.05$) serum testosterone and LH level in Groups C and D than in the controls. The FSH level was significantly higher ($P < 0.05$) in the Group B. The FSH levels in Groups C and D were comparable with the normal control.

Testicular histology

The light microscopic study showed that the testis of the normal control group had an apparently normal architecture and cellular composition (plate I). The histopathology of the negative control group showed degenerative changes in the germinal epithelium and interstitial tissue (plate II). Testicular sections of Groups C and D showed an increase in number and volume of germinal epithelium, which was more pronounced in Group D (plate III and IV).

Discussion

The present study showed that flutamide produced a reduced serum testosterone level and increased levels of LH and FSH. The accompanied rise in the levels of LH and FSH following the administration of flutamide may be in response to the feedback from the decreased testosterone level (Azu *et al*., 2010). The reduced level of testosterone following the administration of flutamide may also be responsible for the degenerative changes in the germinal epithelium and interstitial tissue of the testis. This is in agreement with an earlier report by Oremosu *et al*. in 2013 and Azu *et al*. in 2010. Testosterone is essential in maintaining quantitative spermatogenesis (Oremosu *et al*. in 2013), hence decrease in testosterone will be accompanied with disrupted

Table 1: Effect of aqueous extract of Cyperus esculentus on Body Weight after 21 days of treatment

Groups	Day 1 (g)	Day 22 (g)
Normal control (A)	150.0±0.3	168.7±0.5 (12.5%)
Negative control (B)	116.3±0.1	167.5±0.2 (44.1%)
Curative I (C)	132.5±0.1	172.5±0.3 (30.2%)
Curative II (D)	116.3±0.5	143.7±0.1 (23.6%)

Values are expressed as mean±SD for *n*=4 in percentage

Table 2: Effect of aqueous extract of Cyperus esculentus on testosterone, LH and FSH

	A	B	C	D
Testosterone	2.61±0.02	0.31±0.01	4.91±0.01	4.41±0.01
Follicle stimulating hormone	4.30±0.16	11.51±0.02	5.07±0.15	4.30±0.02
Luteinizing hormone	6.60±0.2	9.31±0.01	9.30±0.05	9.20±0.02

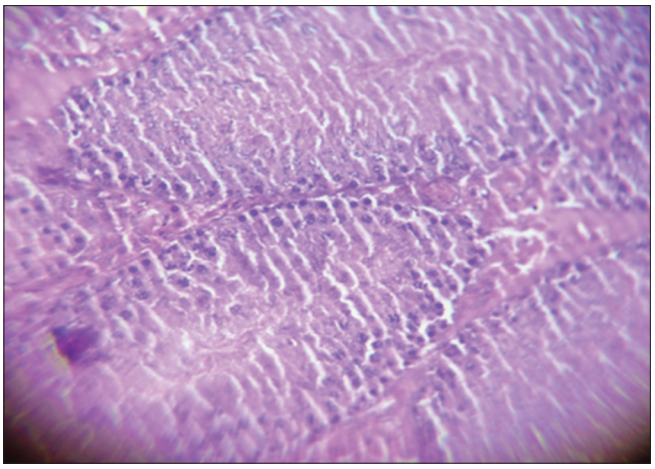

Plate 1: Light Micrograph of the testis of a group A (normal control) rat, showing apparently normal outline of the seminiferous tubules, interstitium and spermatogenic cells (arrows) at different stages of development (H&E, x400)

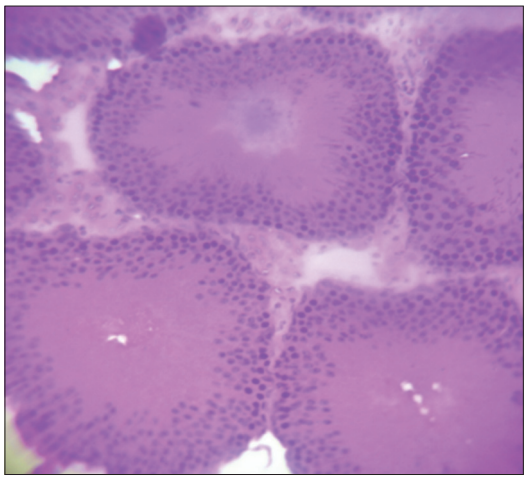

Plate 2: Light micrograph of the testis of a Negative control (group B) rat shows loss of spermatogenic cells, reduced density of mature spermatozoa within the lumens of the seminiferous tubules and some degree of disintegration and degeneration of cells. Leydig cells show reduced size and interstitial spaces are wider when compared with normal control (H%E stain, x400)

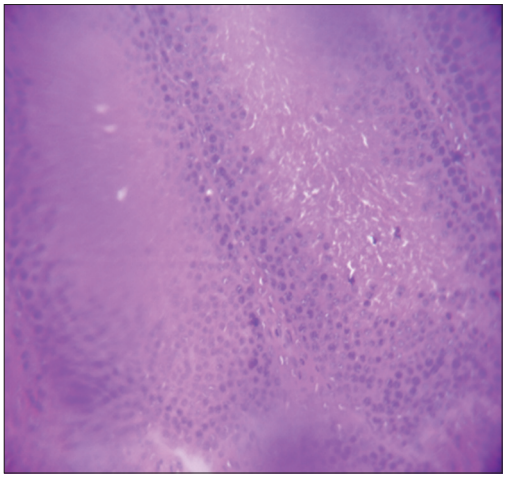

Plate 3: Light micrograph of the testis of a group C rat, showing marked increase in the germinal epithelium and interstitial tissue (H&E stain, x400)

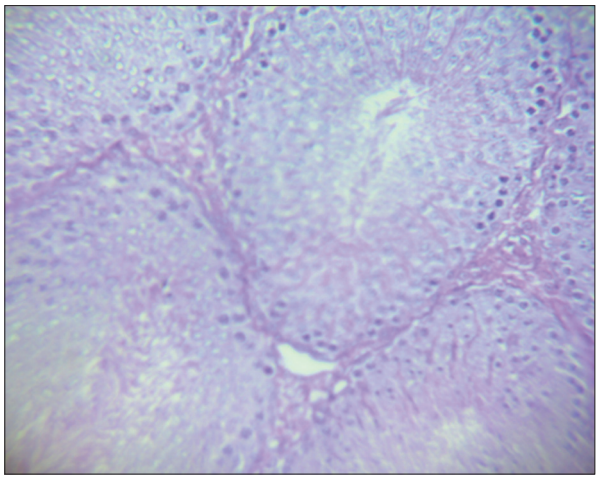

Plate 4: Light micrograph of the testis of a group D rat, showing a more pronounced increase in the number and volume of germinal epithelium, the interstitial cell of leydig and the sizes of the seminiferous tubules. (H&E stain, x400)

spermatogenesis and may result in decreased testicular weight (Azu *et al*. in 2010).

The results of this study suggest that the aqueous extract of *C. esculentus* could ameliorate the adverse effect of flutamide on the testis. This was evident in the observed significant increase in the levels of testosterone and LH, the body weight, the number and volume of germinal epithelium, the interstitial cells as well as the sizes of the seminiferous tubules.

The biologically active compounds in *C. esculentus* include: alkaloids, saponins, flavonoids, glycosides, phenols, steroids, phlobatannins, and terpenoids (Ibrahim *et al*., 2009). The presence of these phytochemicals might have effectively contributed to the ability of *C. esculentus* to ameliorate the adverse effect of flutamide on the testis. Flavonoids in particular, have been documented to have healing effects (Ekeanyanwu *et al*., 2010).

C. esculentus also has high contents of vitamins (A, C, D, and E), minerals (Na, Ca, Fe, Zn, K, Mg, and P), as well as protein, starch, and fat (Simpson and Inglis 2001; Sofowora 1993). The dose-dependent increase in the number and volume of germinal epithelium, the interstitial cells and the sizes of the seminiferous tubules implies an increased secretory activity of nutrients. The high nutrient content of *C. esculentus* may efficiently treat male sexual dysfunction and enhance male fertility; this is evident as seen the result of the hormonal assay, the increased levels of testosterone and LH and the maintenance of normal FSH level. Spermatogenesis is dependent on the action of LH which binds to LH receptor on interstitial cells (of Leydig) and stimulates them to synthesize testosterone, while FSH stimulates Sertoli cells to synthesis and release androgen-binding proteins (ABP) into the seminiferous tubules. ABP combines with testosterone, and in turn, increases its concentration in the seminiferous tubules which then stimulates spermatogenesis (Cheng 2008; Davis and Jane 2001). An increased concentration of testosterone in the seminiferous tubules will result in increased libido (Victor 2013), and it is also essential for quantitative spermatogenesis (Oremosu *et al*., 2013).

Conclusion

The results suggest that *C. esculentus* could enhance testicular activity and ameliorate the adverse effect of flutamide on the testis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Adejuyitan J.A., Otunola E.T., Akande E.A., Bolarinwa I.F., Oladukun F.M. (2009). Phytochemical properties of flour obtained from fermentation of tigernut (*Cyperus escullentus*) sourced from a market in Ogbomoso, Nigeria. Afr J Food Sci 3:51-5.
2. Atlas M.R., Alfred E., Lawrence C. (1995). Laboratory Manual Experimental Microbiology. 1st ed. Mosby. USA.
3. Azu O.O., Duru F.I., Osinubi A.A., Noronha C.C., Elesha S.O., Okanlawon A.O. (2010). Prelimnary study on the antioxidant effect of Kigelia Africana fruit extract (*Bignoniacieace*) in male Spraue-Dawley rats. Afr J Biotechnol 9:1374-81.
4. Cheng C.Y. (2008). Molecular mechanisms in spermatogenesis. Adv Exp Med Biol 636:1-289.
5. Davis S.R., Jane T. (2001). Testosterone influences libido and well being of women. Trends Endrocrinol Metab 12 (1):143-50.
6. Dorfman R.I. (1970). Biological activity of anti-androgens. Br J Dematol 82 (6):3-8.
7. Edoga H.O., Okwu D.E., Mbaetie B.O. (2006). Phytochemical constituent of some Nigerian medicinal plants. Afr J Biotechnol 4 (7):685-8.
8. Ekeanyanwu R.C., Njoku O., Ononogbu I.C. (2010). The phytochemical composition and some biochemical effect of *Cyperus escullentus*. Pak J Nutr 9 (7):709-15.
9. Elks, J., Ganellin, C. R (1990). The Dictionary of Drugs: Chemical Data: Chemical Data, Structures and Bibliographies. Chapman and Hall. 1st ed. University of Michigan. USA. p. 573.
10. Finbarrs-Bello E., Eteudo A.N., Nto N.J., Egwu A.O., Ikenna I.E. (2015). Cognitive and immunohistochemical study on the effect of neostigmine on ketamine induced wistar rats. Int J Dev Res 5 (6):4781-4.
11. Hassan M.M., Oyewale A.O., Amupitan A.O., Abdullahi I.O., Abdullahi M.S., Okonkwo E.M. (2005). Prelimnary phytochemical and antibacterial investigation of root back of deterium microcarpum. J Chem Soc Niger 29 (1):26-9.
12. Hassan L.A., Anyanwu G.E., Nto N.J., Abireh I.E., Akunna G.G. (2018). Protective effect of aqueous extract of *Cyperus esculentus* on the flutamide induced testicular defect of male wistar rats. SJAMS 6 (6):2391-5.
13. Ibrahim T.A., Jude B.S., Giwa E.O., Adebote V.T. (2009). Microbial Analysis and effect of selected antibacterial agents on microbial load of fluted pumpkin, cabbage and bitter leaves. Res J Agric & Biol Sci 3 (4):1143-5.

14. Imam T.S., Aliyu F.G., Umar H.F. (2013). Prelimnary Phytochemical screening, elemental and proximate composition of two varieties of *Cyperus esculentus*. Niger J Basic Applied Sci 21 (4):247-51.
15. Latendresse J.R., Warbrittion A.R., Jonassen H., Creassy D.M. (2002). Fixation of testes and eyes using a modified Davidson's fluid: Comparison with Bouin's fluid and conventional Davidson's fluid. Toxicol Pathol 30:524-33.
16. Oremosu A.A., Arowaye V.O., Akang E.N., Bassey R.B. (2013). Effects of *Cissus populnea* and *Panax ginseng* on Flutamide-induced testicular dysfunction in pre-pubertal male rats. Br J Med Med Res 3 (1):173-81.
17. Perobelli J.E., Alves T.R., de Toledo F.C., Fernandez C.D., Anselmo-Franci J.A., Klinefelter G.R., Kempinas W.D. (2012). Impairment on Sperm quality and fertility of adult rats after antiandrogen exposure during prepuberty. Rep Toxicol 33 (3):308-15.
18. Prakash N., Ragavan B. (2009). Phytochemical observation and antibacterial activity of *Cyperus esculentus* L. Anc Sci Life 28 (4):16-20.
19. Simpson D.A., Inglis C.A. (2001). Cyperaceae of Economic, Ethnobotanical and Horticultural Importance. Kew Bulletin, Heidelberg. 56(2) 57-300.
20. Sofowora E.A. (1993). Medicinal plants and Traditional Medicine in Africa. University of Ife Press. 2nd ed. Ile Ife, Nigeria. p. 159-238.
21. Victor P.E. (2013). Male Reproductive System. 12th ed. Atlas of Histology, South Asian. p. 486.