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Cyperus esculentus (Tiger Nut) Improves Fertility and Testicular Histology in Male Sprague Dawley Rats

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ABSTRACT

Male infertility is a public health problem. *Cyperus esculentus* (tiger nut) is an edible nut that is rich in vitamins C and E, and enhances fertility in humans and animals. Tiger nut is widely consumed but there is a paucity of data on its safety profile. This study investigated the effects of administration of different doses of tiger nut crude extract on serum levels of testosterone, vitamins C and E, and histological changes of the testes of Sprague Dawley rats. Four groups of male rats involving: Control (distilled water and animal feed only) and Group 2, 3, and 4 given tiger nut orally at 500 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight respectively for 30 days were used. Testicular weight, serum testosterone, and vitamins C and E levels were determined. Testes were fixed in 10% neutral-buffered formalin, processed by paraffin wax-embedding method, and stained using Haematoxylin and Eosin and Masson's trichrome techniques. There were dose-dependent significant increases in testicular weight ($p < 0.001$), serum testosterone ($p=0.002$), vitamin E ($p < 0.001$), and vitamin C ($p < 0.001$) levels in the test groups. The increase in spermatozoa production and enlargement of interstitial spaces and blood vessels were prominent in the group given 2000 mg/kg. The seminiferous tubule width increased and the collagen fibers were intact and well-preserved in the test groups. Tiger nut improves the production of spermatozoa by increasing serum testosterone, vitamin C, and E levels and enhances erectile functions through vasodilation. Moderate consumption of tiger nuts may help in ameliorating fertility in males.

Keywords: Tiger Nut, Testis, Testosterone, Vitamin C, Vitamin E, Spermatogenesis

Introduction

Cyperus esculentus commonly called tiger nut is a plant that belongs to the Cyperaceae family.[1,2] In Nigeria, it is cultivated mainly in the Northern regions. It is locally called "aya" in Hausa, "akiawusa" in Igbo, "ofio" in Yoruba,[2] and "isipisong" in Annang/Ibibio languages. There is an increase in infertility worldwide.[3] In Nigeria and other developing countries, infertility is a public health problem.[3] It has been shown that male infertility accounts for more than 50% of cases.[3] Due to the increase in infertility among men and the growing need to improve fertility in animals to increase productivity, there has been a rise in research on dietary supplements that can boost fertility in men and animals.[4,5] It has been suggested that the consumption of tiger nut tubers might help to improve the male reproductive system of humans, and may also maintain the normal cytoarchitecture and functions of seminiferous tubules.[4] In the Middle East, it was widely consumed by men due to its ability to improve male sexual activity.[5] Tiger nut is a sexual stimulant and improves libido.[6]

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It has also been shown that tiger nuts can boost male fertility, and spermatogenesis and improve the sexual functions of male animals as well as restore the fertility of male rats after exposure to irradiation.[6,7] The antioxidant properties of tiger nuts have been implicated in these findings.[6,7]

Tiger nuts' potential as a valuable food source and dietary supplement to boost fertility is due to its rich nutrients consisting of fats, carbohydrates, minerals, and vitamins E and C.[8] Studies have shown that its milk is rich in vitamin E, essential for fertility in both men and women.[7,9] Vitamin C also acts as an antioxidant and significantly increases serum testosterone levels in male rats.[10] Due to its numerous health benefits, there has been wide consumption of tiger nuts to boost male fertility.[11] However, there is a paucity of data on the daily recommended quantity safe for consumption as there are concerns that it may disrupt spermatogenesis on prolonged consumption. Thus, the rationale of this study was to investigate the effect of different doses of crude extract of *C. esculentus* (tiger nut) on serum testosterone, vitamin E, vitamin C levels, and histology of the testis of rats.

Materials and Methods

Plant collection

Tiger nut was bought from the Bogobiri area in Calabar on 25th September 2019 and was identified by Mr. Effa Anibeja Effa in the Department of Plant and Ecological Studies University of Calabar, with the number: Bot/Herb/UCC/575. The tiger nuts were washed, air-dried at room temperature for 2 days, and blended to powder using an electric blender. Then 3 kg of the blended nuts were soaked in 1.5 litres of warm distilled water for 48 hours, and the extract was double-

filtered with cheesecloth and Whatman No. 1 filter paper. The crude extract was concentrated to dryness with a rotary evaporator at 30^{O}C under vacuum to yield 300 g of crude extract which was stored in a refrigerator at 4^{O}C until required.

Experimental animals
Twenty male Sprague Dawley rats were purchased from the Animal House in the College of Medical Sciences, University of Calabar. They were kept in standard well-ventilated cages and allowed to acclimatize for 2 weeks during which they were given feed and water *ad libitum*. They were maintained under standard 12 hours a day and night circle. Ethical clearance was obtained from the Faculty Animal Research Ethical Committee of the Faculty of Basic Medical Sciences (FAREC-FBMS, University of Calabar with Ethical approval number: 044MLS2919.

Experimental design and administration
The rats were divided into 4 groups of 5 animals each and the extract was administered orally with a cannula for 30 days. Group 1 was Control given feed and water only. Groups 2, 3, and 4 were given 500 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight of tiger nut crude extract respectively. At the end of the experiment, the rats were sacrificed by anesthesia using chloroform.[12] The blood samples were taken through cardiac puncture. Blood samples were collected into plain containers and centrifuged at 3000 rpm for 10 minutes. Serum samples were separated and stored at 4^{O}C for testosterone, vitamin C, and vitamin E analyses. The testes were excised, weighed, and fixed in 10% neutral buffered formalin for paraffin wax histological tissue processing and staining.[13]

Testosterone measurement
Testosterone levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit manufactured by Krishgen Biosystems, Mumbai. Testosterone was assayed based on the manufacturer's instructions. This assay uses an antibody specific for testosterone pre-coated onto a microplate. Standards and test samples pipetted into their wells react with a biotin-conjugated antibody specific for testosterone. The final reaction with avidin-conjugated Horseradish Peroxidase (HRP) results in a colored end product and the intensity of the color is measured spectrophotometrically at 450 nm.[14]

Vitamin C measurement
Vitamin C level was analyzed using high-performance liquid chromatography (HPLC) based on the manufacturer's instruction from the Eagle Bioscience system. Treatment of the HPLC column was done by equilibrating with a 30 ml mobile phase (ELU). The column was flushed with 15ml deionized water (1 ml/min) before being stored at 50^{O}C methanol in deionized water. Samples were prepared by pipetting 200 µl samples and calibrator into 1.5 ml tubes and the content was vortex and left for 10 minutes at 2-8^{O}C. The content was centrifuged for 10 minutes and 20 µl of the supernatant was injected into the HPLC-System and left in the dark for 24 hours at 2-8^{O}C.[15]

Vitamin E measurement
Vitamin E level was measured using HPLC based on the manufacturer's instruction from the Eagle Bioscience system. The chromatography was performed on HPLC Agilent 1100 series equipped with a pump, degasser, auto-sampler, detector, and a stationary phase. Sample preparation was done using internal standard consisting of 100 ml α–tocopherol acetate mixed with 200 ml of ethanol, 200 ml of water, and 800 ml of hexane after blending for 15 minutes. The mixture was centrifuged for 5 minutes at 4000 rpm before 600 ml of the organic layer was removed. The organic layer was left to dry by evaporation and the deposit was dissolved in 100 ml of solution comprising 88 ml of methanol, 10 ml of ethanol, and 2 ml of hexane. Then, 20 ml of the diluted sample was manually injected into the column and left for 18 minutes with methanol acting as the mobile phase. The flow rate was set at 1.0 ml/min and the detection was monitored at a wavelength of 1 equals 29 nm for tocopherol.[16]

Histological tissue processing/staining
The 10% neutral buffered formalin-fixed testes were processed by paraffin wax-embedding procedure[13]. The embedded tissues were sectioned at 3 micrometers using LEICA RM2125RT rotary microtome (Germany), floated out, and picked on albumenized slides before staining. The tissue sections were stained using Cole's hematoxylin and Masson trichrome staining techniques.[13]

Statistical analysis
Statistical analysis was done with SPSS version 20. All values were expressed as mean±standard deviation. The testicular weight, serum testosterone, and vitamin C and E levels were analyzed using ANOVA and posthoc comparison was done with the Least Square Difference (LSD) test. The seminiferous tubule length and width were measured using the line measurement tool of AmScope digital software (USA) and analyzed with ANOVA. Probability values of ≤ 0.05 were statistically significant.

Results and Discussion

Testicular weight and serum levels of testosterone, Vitamin C, and Vitamin E levels of the rats were measured. In Table 1, there were significant increases in mean testicular weights of the test groups ($F=44.17$, $p < 0.001$) but the 2000 mg/kg group was significantly higher (1.95±0.23) compared with the control (0.40±0.42). An increase in weight is attributed to the proliferation of Sertoli and Leydig cells.[17,18,19] This suggests an increase in testicular functions due to increased testosterone production from Leydig cells and spermatogenesis. A similar increase in testicular weight was reported to occur due to the production of a high amount of spermatozoa as a result of the increase in testosterone levels in rats.[20] There were dose-dependent significant increases in testosterone levels across the test groups (500 mg/kg, 0.63±0.12, 1000 mg/kg, 0.80±0.03 and 2000 mg/kg, 1.20±1.06) ($F=7.741$, $p=0.002$) compared with the control (0.49±0.12). The study showed an increase in serum testosterone levels and agrees with Abireh *et al.*[21] The mechanism by which tiger nuts enhance testosterone levels may be attributed to its phytochemicals such as quercetin and zinc. Quercetin is a nutritional flavonoid and antioxidant which causes marked increase in serum testosterone levels in male rats.[22] It is also suggested that the increase in testosterone by tiger nut is enabled by zinc which works to facilitate the production of testosterone.[9]
The mean vitamin C levels increased in a dose-dependent manner across the test groups (500 mg/kg =1.02±0.05, 1000 mg/kg =1.17±0.11, and 2000 mg/kg =1.38±0.18) and were statistically significant ($F=164.106$, $p < 0.001$) compared with the control (0.95±0.371). The mean vitamin E levels increased and the 2000 mg/kg (1.62±0.01) was significantly higher among the test groups compared with the control (0.66±0.01) ($F=6166.241$, $p < 0.001$). Vitamins C and E eluted in approximately 4 min (Fig. 1) and 16 min (Figure 2) respectively on the HPLC system. This is within the range of 4 min, 6 min, and 13 min of vitamin C previously reported in a work.[23] The retention time of vitamin E was longer than 2.5 min earlier reported in a study.[24] The use of HPLC for the determination of vitamin C and E levels has been shown to have the advantage of improving specificity and sensitivity.[23,24] The presence of vitamin C and E in tiger nut in this study has some merits. First, vitamins C and E act as antioxidants and cause the proliferation of Sertoli and Leydig cells which can cause an increase in testicular weight.[21,22,25] Also, the increase in vitamin C has been shown to facilitate the formation of testosterone by acting on the hypothalamus-pituitary-testes axis to cause increased testosterone levels which promotes spermatogenesis. It also protects spermatogenesis and maintains fertility both in humans and animals. On the other hand, vitamin E is an essential nutrient that has been found to enhance testosterone synthesis in experimental mice model[25] and improves sperm quality in Boar goats.[5] This vitamin stimulates spermatogenesis by promoting the production of proteins such as FLNA, SPCS3, YBX3, and RARS that are involved in the synthesis of protamine and the plasma membrane of spermatozoa.[26]

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Table 1: Testicular weight and serum levels of testosterone, Vitamin C, and Vitamin E of the rats

Parameter	Mean ± SD	ANOVA	p-value
Testicular weights (g)		F=44.17	<0.001
Group l (Control)	0.40 ± 0.42		
Group 2 (500 mg/kg)	0.45 ± 0.42		
Group 3 (1000 mg/kg)	1.51 ± 0.11		
Group 4 ((2000 mg/kg)	1.95 ± 0.23		
Testosterone (ng/dl)		F=7.741	0.002
Group l	0.49 ± 0.12		
Group 2	0.61 ± 0.12		
Group 3	0.80 ± 0.03		
Group 4	1.20 ± 1.06		
Vitamin C (mg/dl)		F=164.106	< 0.001
Group l	0.96 ± 0.03		
Group 2	1.02 ± 0.05		
Group 3	1.17 ± 0.11		
Group 4	1.38 ± 0.18		
Vitamin E (mg/dl)		F=6166.241	< 0.001
Group l	0.66 ± 0.01		
Group 2	0.77 ± 0.02		
Group 3	0.95 ± 0.01		
Group 4	1.62 ± 0.01		

Key: SD= standard deviation

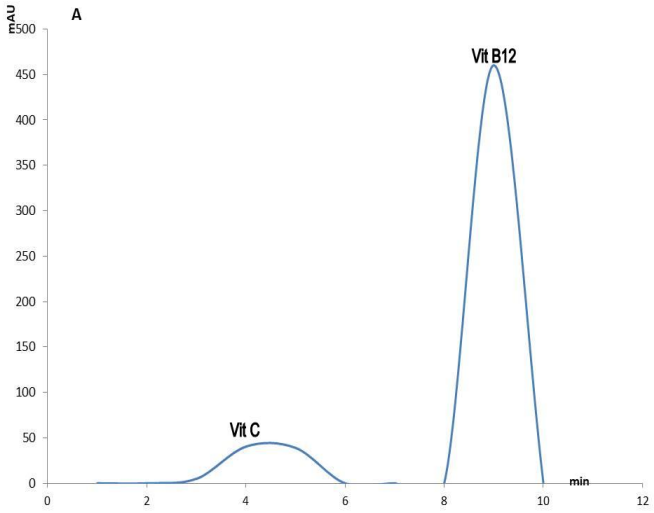

Figure 1: Chromatogram of vitamin C. Retention time 4.3 min.

The post hoc test shows the significant differences between the control group and the 2000 mg/kg group for the testicular weight (p=0.001), testosterone (p=0.001), vitamin C (p=0.029), and vitamin E (p < 0.001) in Table 2. This indicates that increased consumption of tiger nuts increases male fertility.[6]

The H&E-stained sections are shown in Plate 1. The testes of Group 1 (control) showed normal morphology with many spermatogonia cells, numerous spermatozoa, normal interstitial space, and intact seminiferous tubules. Group 2 (500 mg/kg body weight) showed few spermatogonia cells, numerous spermatozoa, mildly enlarged interstitial spaces, and mild thinning of walls of seminiferous tubules. Group 3 (1000 mg/kg body weight) testes showed few spermatogonia cells, numerous spermatozoa, moderately enlarged interstitial space and moderate thinning of walls of seminiferous tubules. Group 4 (2000 mg/kg body weight) showed few spermatogonia cells, numerous spermatozoa, marked enlargement of interstitial tissue, marked thinning of walls of seminiferous tubules, and enlargement of blood vessels. Masson trichrome-stained sections are shown in Plate 2.

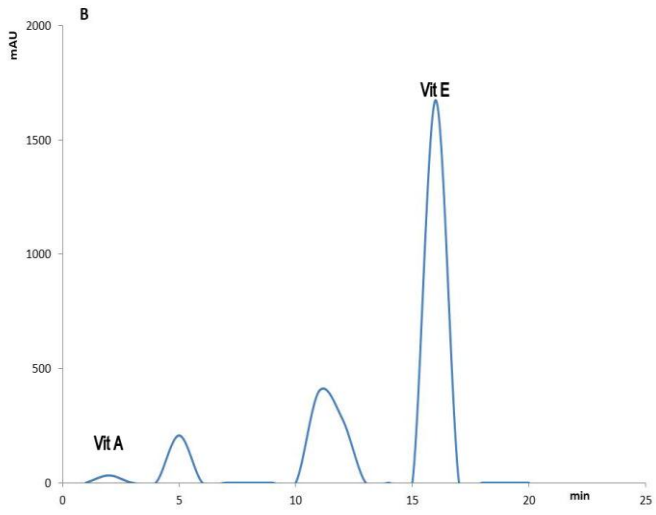

Figure 2: Chromatogram of vitamin E. Retention time 16.8 min.

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Table 2: Post Hoc test of testicular weight and serum levels of testosterone, Vitamin C, and Vitamin E of the rats

Parameter	Groups		Mean difference	Standard error	p-value
Group	Control	LD			
Testicular weight	0.40 ± 0.42	0.45 ± 0.42	1.940	0.682	0.001
Group	Control	MD			
Testicular weight	0.40 ± 0.42	1.51 ± 0.11	6.292	2.250	0.001
Group	Control	HD			
Testicular weight	0.40 ± 0.42	0.45 ± 0.42	6.375	2.465	0.001
Group	Control	HD			
Testosterone	0.49 ± 0.12	1.20 ± 1.06	0.120	0.339	0.001
Group	LD	HD			
Testosterone	0.61 ± 0.12	1.20 ± 1.06	0.312	0.339	0.001
Group	MD	HD			
Testosterone	0.80 ± 0.03	1.20 ± 1.06	1.428	0.339	0.005
Group	Control	LD			
Vitamin C	0.96 ± 0.03	1.02 ± 0.05	0.147	0.021	< 0.001
Group	Control	MD			
Vitamin C	0.96 ± 0.03	1.17 ± 0.11	0.358	0.021	< 0.001
Group	Control	HD			
Vitamin C	0.96 ± 0.03	1.38 ± 0.18	0.065	0.021	0.029
Group	Control	LD			
Vitamin E	0.66 ± 0.01	0.77 ± 0.02	0.178	0.008	< 0.001
Group	Control	MD			
Vitamin E	0.66 ± 0.01	0.95 ± 0.01	0.853	0.008	< 0.001
Group	Control	HD			
Vitamin E	0.66 ± 0.01	1.62 ± 0.01	0.114	0.008	< 0.001

Keys: Control (Group 1), LD=low dose (Group 2, 500 mg/kg body weight), MD=medium dose (Group 3, 1000 mg/kg body weight), HD=high dose (Group 4, 2000 mg/kg body weight)

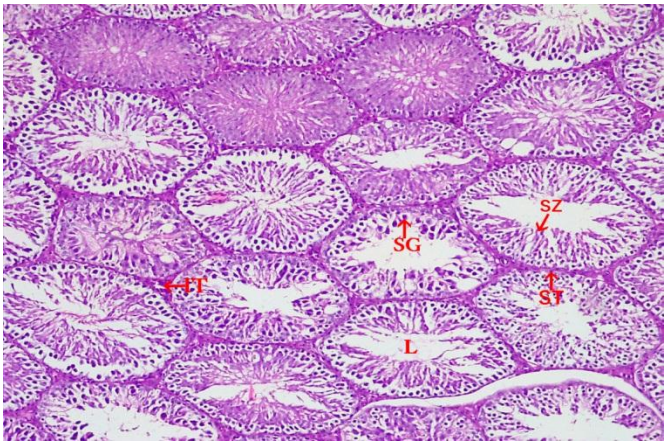

Plate 1: Photomicrograph of the testis of the control group (group 1). The testis shows normal spermatogonia cells (SG), normal size of interstitial space (IT), intact walls of seminiferous tubules (ST), and numerous spermatozoa (SZ) in lumen (L). (H&E X100).

The testis of Group 1 showed normal collagen deposits in interstitial space, in the capsule, and around the blood vessel. Groups 2-4 showed normal collagen deposits in interstitial space and around blood vessels. Histometric measurement of the width and length of the seminiferous tubules is shown in Table 3. There were increases in the mean width values of the test groups (89.40±7.73, 106.24±15.010, 130.14±18.80) when compared with the control (75.9±15.12) but were not statistically significant (F=2.522, p=0.196). The decreases in the mean length values of the test groups (143.80±4.41, 122.36±9.51, 119.88±23.38) compared to the control (153.52±8.80) were not statistically significant (F=1.469, p= 0.350). The histology of the testes revealed an increase in the maturation of germinal cells to spermatozoa, preserved Leydig cells, and intact collagen fibers. This confirms the ability of tiger nuts to improve spermatozoa production. There were marked hypertrophies of the interstitial spaces due to an increase in the functions of Leydig cells responsible for the production of testosterone. These changes were consistent with the observed histometric increase in the width of the seminiferous tubules. These increases in Leydig cell functions and hypertrophies of the interstitial spaces are attributed to the influence of Vitamin E on the testes. A similar increase in the number of epithelial cells, the width of seminiferous tubules, and the number of Sertoli and Leydig cells were observed after vitamin E supplementation in Boar goats.[5]

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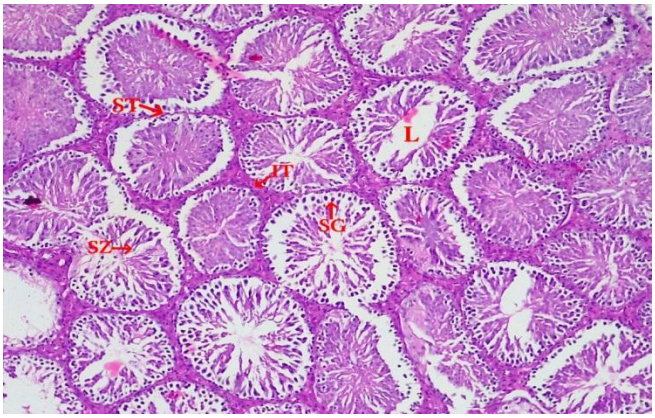

Plate 2: Photomicrograph of the testis of group 2 administered 500 mg/kg of extract. The plate shows few spermatogonia cells (SG), mildly enlarged interstitial space (IT), mild thinning and disruption of walls of seminiferous tubules (ST), and mild occlusion of lumen (L) with numerous spermatozoa (SZ). (H&E X100)

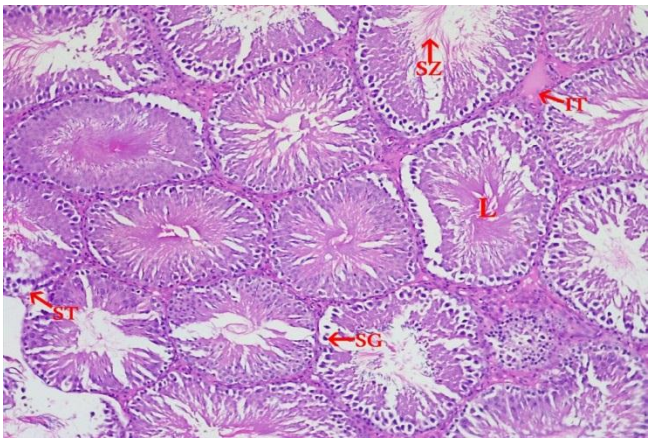

Plate 3: Photomicrograph of the testis of group 3 administered 1000 mg/kg of extract. The plate shows few spermatogonia cells (SG), moderately enlarged interstitial space (IT), moderate thinning and disruption of walls of seminiferous tubules and replacement with interstitial tissue, and moderate occlusion of lumen with numerous spermatozoa. (H&E X100)

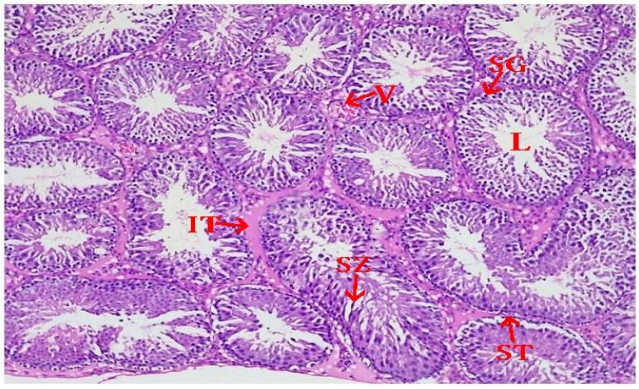

PLATE 4: Photomicrograph of the testis of group 4 administered 2000 mg/kg of extract. The testis shows few spermatogonia cells (SG), marked enlargement of interstitial tissue (IT), marked thinning and disruption of walls of seminiferous tubules (ST) and replacement with interstitial tissue, enlargement of blood vessel (V) in interstitial tissue, and scanty spermatozoa (SZ) in lumen (L). (H&E X100)

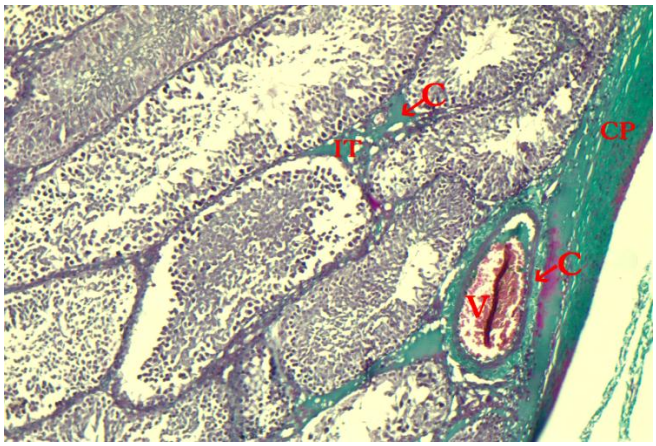

Plate 5: Photomicrograph of the testis of the control group showing normal collagen deposits (C) in interstitial space (IT), in capsule (CP) and around blood vessel (V). (Masson trichrome x100).

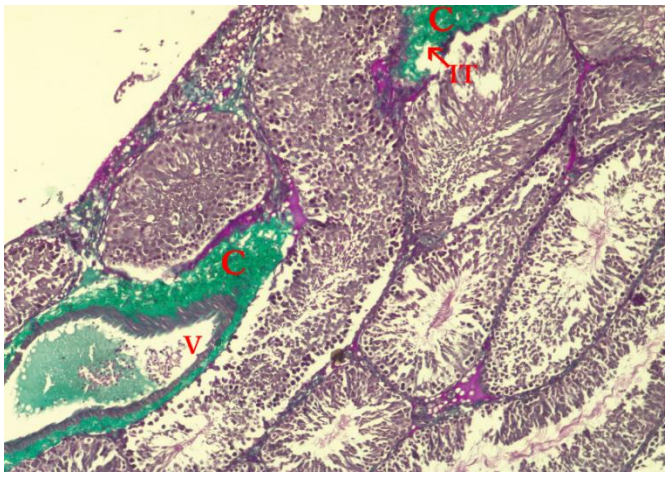

Plate 6: Photomicrograph of the testis of the group 2 administered 500 mg/kg of extract showing normal collagen deposits (C) in interstitial space (IT) and around blood vessel (V). (Masson trichrome x100).

Enlargement of the blood vessels at 2000 mg/kg was also observed in the study. This has been linked to vitamin C that has been reported to stimulate the production of vascular nitric oxide which functions in blood flow regulation and vasodilation leading to improvement in penile erection.[27]

Conclusion

The findings of the study showed that oral administration of crude extract of tiger nut causes an increase in testicular weight, serum levels of testosterone, vitamins C and E, spermatozoa production and interstitial spaces of seminiferous tubules, and dilation of blood vessels. These features are all pointers to an increase in spermatogenesis and improvement in fertility and erectile functions. This study recommends moderate consumption of tiger nuts as a dietary supplement.

Conflict of Interest

The authors declare no conflict of interest.

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Table 3: Histometric measurements of the width and length of seminiferous tubules of the testes

Seminiferous tubule	Mean ± SD	ANOVA	p-value
Width (pixels)			
Group 1	75.9 ± 15.12	F=2.522	0.196
Group 2	89.40 ± 7.73		
Group 3	106.24 ± 15.01		
Group 4	130.14 ± 18.80		
Length (pixels)			
Group 1	153.52 ± 8.80	F=1.469	0.350
Group 2	143.80 ± 4.41		
Group 3	122.36 ± 9.51		
Group 4	119.88 ± 23.38		

Keys: Control (Group 1), (Group 2, 500 mg/kg body weight), (Group 3, 1000 mg/kg body weight), (Group 4, 2000 mg/kg body weight

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.\

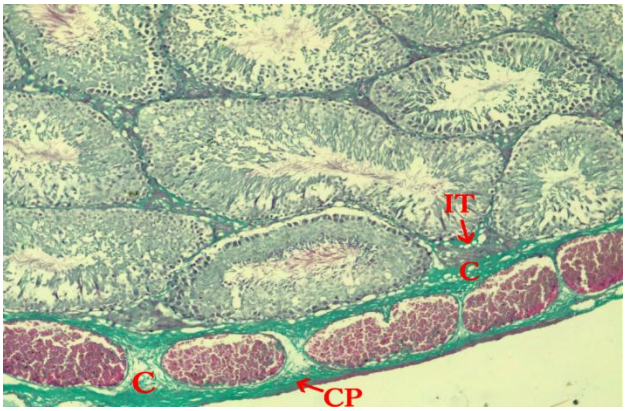

Plate 7: Photomicrograph of the testis of group 3 administered 1000 mg/kg of extract showing normal collagen deposits (C) in interstitial space (IT) and capsule (CP). (Masson trichrome x100).

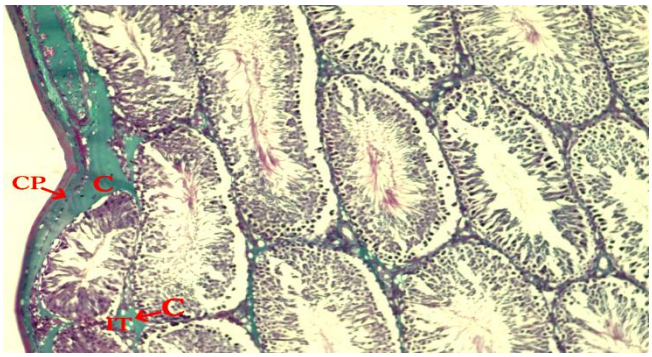

Plate 8: Photomicrograph of the testis of group 4 administered 2000 mg/kg of extract showing normal collagen deposits (C) in interstitial space (IT), and in capsule (CP). (Masson trichrome x100).

References

1. Suleiman MS, Olajide JE, Omale JA, Abbah OC, Ejembi DO. Proximate composition, mineral and some vitamin contents of tigernut. Clin Invest (Lond). 2018; 8(4): 161-165.
2. Oluwakemi, AA, Irewunmi, AF, Sholotan Kazeem Joshua, Nwaemeke David Iweunor. Mineral, Vitamin, and Phytochemical Content of the Tigernut. World J Applied Chem. 2021, 6(3): 36-40.
3. Jeje EA, Alabi TO, Ojewola RW, Ogunjimi MA, Osunkoya SA. Male infertility: An audit of 70 cases in a single center. Afri J Urol. 2016; 22(3): 223-226,
4. Hasan HF, Hamzah AM, Zghair ZR. Study of the comparative effect between *Cyperus esculentus* seeds extracts and gentamicin on induced endometritis in mice. J Physics Conf Series. 2013; 7(7): 40-47.
5. Umesiobi DO. Effect of vitamin E supplementation on the libido and reproductive capacity of large white Boars. South Afri J Animal Sci. 2012; 42: 5.
6. Atoigwe-Ogeyemhe BE, Odigie EB, Achukwu PU. Aqueous Extract of *Cyperus esculentus* L. (Cyperaceae) Enhances Libido and Spermatogenesis in Male Wistar Rats: Trop J Nat Prod Res. 2018; 2(11), 471-475.
7. Udoh BE, Erim AE, Paulinus SO, Eru EM, Ukpong EV, Efanga I, Archibong BE, Egong AE, Udo EE,Egbe NO. Effects of *Cyperus esculentus* (Tiger Nut) Extract on the Irradiated Testes of Wistar Rats. Trop J Nat Prod Res. 2020; 4(11): 966-969.
8. Tunde-Akintunde TY and Oke MO. Thin-layer drying characteristics of tiger nut (*Cyperus esculentus*) seeds. J Food Proc Pres. 2012; 36: 457-464.
9. Innih SO, Eluehike N, Francis B. Effects of aqueous extract of *Cyperus esculentus* (tiger nut) on antioxidant status and hematological indices in the heart of cadmium-induced Wistar rats. Niger J Exp Clin Biosci. 2021; 9: 17-24.
10. Bassey IE, Ikpi DE, Isong IK, Akpan UO, Onyeukwu CC, Nwankwo NP, Udofia IG. Effect of combined calcium, magnesium, vitamin C and E supplementation on seminal parameters and serum oxidative stress markers in fructose-induced diabetic Wistar rats. Arch Physio Biochem. 2022; 128(3): 643-650.
11. Izunwanne DI, Egwurugwu JN, Emegano CL. Effect of Tiger Nut Meal on Some Sex Hormones and Sperm Cells in Androgen-induced Benign Prostate Hyperplasia in Adult Male Wistar Rats. IMSEAR. 2020. Available from https://imsear.searo.who.int/handle/123456789/210250.
12. Udonkang, M. I., Eluwa, M., Enun, B. K., Inyang-Etoh, P., Inyang, I. Studies on antimalarial activity and liver histopathological changes of *Artocarpus altilis* on

Plasmodium berghi-infected mice. Res J. Life Sci, Bioinfo Pharm Chem Sci. 2018; 4(3): 106-114.
13. Bancroft JD and Gamble M. Theory and Practice of Histochemical Techniques. (6th ed). Elsevier: Churchill Livingstone; 2008. 121-212 p.
14. Konstantinou GN. Enzyme-Linked Immunosorbent Assay (ELISA). Methods Mol Biol. 2017; 1592: 79-94.
15. Eagle Bioscience system. Vitamin C HPLC assay. [Online]. 2020. [cited 2020 Dec 18]. Available from: http:www.eaglebio.com
16. Eagle Bioscience system. Vitamin E HPLC assay. [Online]. 2020. [cited 2020 Dec 18]. Available from: http:www.eaglebio.com
17. Amaal AM and Essraa MA. The effect of *Cyperus esculentus* on sperm function parameters in prepubertal mice as a model for humans. J Baghdad Sci. 2010; 7: 389-393.
18. Chukwuma ER, Obiama N, Christopher OI. The phytochemical composition and some biochemical effects of Nigerian tiger nut (*Cyperus esculentus* L.) tuber. Pakistan J Nut. 2010; 9(7): 709-715.
19. Zhu H, Luo HL, Meng H, Zhang GJ. Effect of vitamin E supplementation on the development of reproductive organs in Boer goat. Animals Reprod Sci. 2009; 113: 93-101.
20. Ekaluo UB, Ikpeme EV, Ibiang YB, Omordia FO. Effect of soursop (*Annona muricata* L.) fruit extract on sperm toxicity induced by caffeine in Albino Rats. J Med Sci. 2013; 13: 67-71.
21. Abireh IE, Anyanwu GE, Obikili E, Hassan LA. Effect of crude extract of (*Cyperus esculentus*) on testis of male Albino Rats. Int J Pharm Pharma Res. 2019; 15(2): 1-9.
22. Allouh MZ, Daradka HM, AlBarbarawi MM, Mustafa AG. Fresh onion juice enhanced copulatory behavior in male rats with and without paroxetine-induced sexual dysfunction. Exp Bio Med. 2014; 239(2): 177-282.
23. Clark ZD and Frank EL. Development and implementation of an HPLC-ECD method for analysis of vitamin C in plasma using single column and automatic alternating dual column regeneration. Pract Lab Med. 2016; 6: 25-37.
24. Abidin SZ, Ibahim MJ, Rajikin MH, Satar NA. Determination of vitamin E isomers in plasma using ultra performance liquid chromatography. The Malaysian J Anal Sci. 2012; 16(1): 71 – 78.
25. Mohammadirad A, Aghamohammadali-Sarraf F, Badiei S, Faraji Z, Hajiaghaee R, Baeeri M. Anti-aging effects of some selected Iranian folk medicinal herbs-biochemical evidence. Iran J Basic Medi Sci. 2013; 16: 1170-1180.
26. Gao Y, Jian L, Lu W, Xue Y, Machaty Z, Luo H. Vitamin E can promote spermatogenesis by regulating the expression of proteins associated with the plasma membranes and protamine biosynthesis. Gene. 2021; 773: 145364.
27. Ahmad A, Dempsey SK, Daneva Z, Azam M, Li N, Li, P-L, Ritter, JK. Role of Nitric Oxide in the Cardiovascular and Renal Systems. Int J Mol Sci. 2018; 19(9): 2605.