# **Tropical Journal of Natural Product Research**

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**Original Research Article** 



# Aqueous Extract of *Cyperus esculentus* L. (Cyperaceae) Enhances Libido and Spermatogenesis in Male Wistar Rats

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# ARTICLE INFO

Article history: Received 01 November 2018 Revised 23 November 2018 Accepted 24 November 2018 Published online 27 November 2018

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# ABSTRACT

Poor libido is often influenced by social, biological, and psychological factors. Cyperus esculentus (tiger nut), propagated by rhizome, basal bulb and tuber is used in the middle-east to stimulate libido. Hence, we investigated the influence of *C. esculentus* aqueous extract on mount frequency and ejaculatory latency and examined spermatogenic and testicular morphometry in male Wistar rats. Tubers of C. esculentus were prepared and processed, while crushed nuts were soaked in distilled water and filtered. The filtrate was concentrated to dryness using a rotary evaporator and lyophilized. Male rats were selected into groups (I to V; n = 5) and were fed with rat chow and water given ad libitum, while females were introduced much later (one male to a female) in an attenuated room, 10 minutes after treatment with graded doses (250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg) of the extract once daily for 30 days. Animals were monitored by two trained expert observers to determine their sex drive. Spermatogenic (sperm count, motility, morphology, viability, abnormality and pH) and morphometric (Seminiferous tubular diameter, size of interstices and epididymal epithelial thickness) parameters were determined. Mount frequency and ejaculatory latency were significantly reduced in treated rats (P < 0.001). Significant increase in richness of spermatozoa and morphometric parameters of testis and epididymis in treated rats were observed (P < 0.05). Histopathology was consistent with normal tissues. This study provides a rationale for the traditional use of tiger nut tubers against low sex drive in men.

*Keywords: Cyperus esculentus*, tiger nut, libido, sexual arousal, spermatogenic, morphometric Indices.

# Introduction

Libido is simply used to describe an individual sexual desire or arousal especially in men, which sometimes relate to sex drive, which is often influenced by factors which are biological, psychological or social. A variety of plants have been used to improve libido or sexual arousal in traditional systems of medicine and have been well documented.<sup>1,2</sup> Cyperus esculentus (Cyperaceae family) propagated by rhizome, basal bulb and tuber is usually called chufa, earth almond, and tiger nut in English.<sup>3</sup> Other local names is Aya in Hausa, Ofio in Yoruba, Aki Hausa in Igbo and Hausa's groundnut in Pidgin-English and is considered as one of the earliest food sources known to man.<sup>3</sup> The milk derived from C. esculentus has been reported to prevent arteriosclerosis since its consumption helps to avert heart problems and thrombosis and activates blood circulation as the unsaturated fatty acid content is similar to that of olive oil.<sup>4</sup> The tuber has been an excellent dietary food, which is often used in the middle-east to stimulate libido but without scientific backings for it assumed aphrodisiac properties.<sup>3</sup>

Previous research showed a rise in the incidence of male sexual incompetence correlating with sexual dysfunction.<sup>1,2</sup> Orthodox interventions were embraced and have provided succor via psycho-

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therapy, sex enhancing drugs and surgery.<sup>5</sup> However, affordability, inherent adverse effects sensitivity. social stigma and have confined their functions over time.<sup>2,5</sup> Cyperus esculentus was used traditionally to stimulate sexual arousal, treatment of weak erection, premature ejaculation in men, and boosting of breast milk supply in breastfeeding mothers.<sup>6</sup> In view of the numerous benefits of C. esculentus, efforts are made to encourage the consumption in order to solve problems relating to weak erection, potency, libido as well as quick ejaculation in men.7 However, prolong usage has been reported to cause a gradual decline in human spermatogenic parameters.8 Other abnormalities in sperm morphology, viability, motility, concentration as well as reproductive effects have been reported.<sup>7,8</sup> The mechanism by which C. esculentus exerts aphrodisiac action and increase sexual health is yet to be fully understood. Lack of scientific knowledge of the health implication is worrisome as majority of men are turning away from orthodox medicines due to cost implication, stigmatization and side effects of medications. Hence, we investigated the influence of C. esculentus aqueous extract on mount frequency and ejaculatory latency, which are indicative of a good sex drive. Another objective was to examine the spermatogenic effects and morphometric indices in testes and epididymis of male Wistar rats.

## **Materials and Methods**

#### Plant Preparation and Extraction

We purchased a good number of *C. esculentus* fresh nuts from New-Benin Market in Benin City, Nigeria and were authenticated by Dr H.A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria, where a specimen was deposited in the herbarium and voucher no. UBHc419 was issued. The method by Innih *et al.*<sup>9</sup> was adopted in extraction of *Cyperus esculentus*. The dried tubers

Citation: 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. doi.org/10.26538/tjnpr/v2i11.2

were crushed into fine powder using a blender (Kenwood 1.6L, BL480 Prestons, Australia) for 10 minutes. The powdered form was soaked in 2 liters of distilled water, stirred vigorously and left to settle for 48 hours at room temperature. The crude aqueous extract was filtered using Buchner funnel and Whatman No.1 filter papers. Filtrate was then concentrated to dryness under reduced temperature and pressure with a rotary evaporator. Extract was stored in an air-tight container and kept in the freezer until use.

#### Ethical Consideration

We conducted this study in compliance with policies outlined in the Guide for the Care and Use of Laboratory Animals (NRC, 2011). Approval was granted by the Animal Care Unit of the Research Ethics Committee at College of Medicine, University of Nigeria, Enugu campus (Protocol Number: COMREC 032/02/2017).

#### **Experimental Animals**

In-bred healthy Wistar rats of different sex with average weight 199  $\pm$ 1.24 g were obtained from the animal facility of University of Nigeria Teaching Hospital (old site) and kept in clean metallic gauze cages with saw-dust as beddings placed in a well-ventilated room with optimum condition (temperature  $25 \pm 5^{\circ}$ C, humidity; 45-50%). and Photoperiod of 12 h light/12 h cycle. The animals adapted to the animal room condition for 7 days, while the cages were sanitized both morning and evening. Male rats were selected into 5 groups (I to V) of 5 rats (n = 5) per cage and were fed with rat chow and water given ad libitum.

#### Acute Toxicity Study

We carried out the acute toxicity study in the initial phase of this study in line with the modified Lorke's method<sup>10</sup>, in which nine rats were divided into three groups of three rats per cage. Rats were treated with graded doses (250, 500 and 750mg/ kg) of the extract orally, while the second part of the experiment had four groups of four rats per cage and were administered (1,000, 1,250, 1,500 and 1,750 mg/kg body weight) orally. The animals were observed closely for the first 4 h for acute behavioural displays and 24 h in all. The median lethal dose (LD50) was calculated using the formula:

 $LD_{50} = \sqrt{(Highest nonlethal dose)} x$  (Lowest lethal dose).

The LD<sub>50</sub> gave an insight for the extrapolated doses that were used for the treatment of animals in the experimental stage of the investigation.

## Experiment on Libido

Allouh<sup>11</sup> experimental design was adopted to determine the mount frequency and ejaculatory latency in male rats. Each rat was placed in a rectangular Plexiglas surveillance chamber measuring 50 x 50 x 40 cm and left to adapt for 10 min prior to each treatment by orogastric tube, which was conducted during the dark phase of the light/dark cycle. Rats were exposed to pre-experimental mating tests to ascertain their sexual experience. After which, graded doses (250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg body weight) of extract were administered to the animals in groups II, III, IV and V once daily for 30 days. Rats in group I served as the control and were not treated but received distilled water in place of extract. A female rat was then introduced into the chamber 10 min after treatment and was monitored by trained expert observers who were blind to the experimental design in a soundattenuated room. Ejaculation was keenly observed following rhythmic contractions of the posterior abdomen, which ended with a slow rising of forelimbs.11

## Histopathology of Testes

After completion of the experiment, the testes were excised, grossed and washed with normal saline. They were fixed in bouin's fluid and processed histologically with Hestion-ATP7000 tissue processor-Germany. Embedding of tissues was conducted with the Hesition- E500 Digital Embedding Machine- Germany. Sections were cut at 3-5 microns with a Hestion ERM 4000 Digital Rotary MicrotomeGermany, and stained using haematoxylin and eosin techniques, and then examined microscopically.

#### Analysis of Spermatogenic Indices Sperm Count

By teasing the right epididymis, sperm cells were released into a sterile container to which 5 mL of 9.5% normal saline has been added to make

a suspension. Two drops of semen suspension were applied onto an improved Neubauer haemocytometer (Hawksley Christalite) fitted with a cover slip and mounted beneath the microscope and counted.

## Morphology

A cresyl violet-stained sperm was also made on a glass slide to investigate sperm morphology while counting from varying fields.

#### Motility

One to two drops of sperm suspension were dropped on a microscope slide and cover slipped. Actively moving sperm cells were then counted and divided by the overall number of spermatozoa (viewed with the X40 lenses) and expressed as a percentage.

#### Viability

The method adopted from Bjorndahl et al.12 was used to conduct the viability testing with Eosin-Nigrosin one-step staining technique. Briefly, sperm suspension was mixed with Eosin-Nigrosin stain in equal proportion, while five air-dried smears were prepared on a grease free glass slide. Slides were examined in the first 15 min for percentage viability. Active sperm cells avoided the stain by appearing whitish, but dead sperm cells retained the stain and appeared pinkish. Percentage viability was calculated with the number of active sperm cells out of the entire spermatozoa under observation.12

#### Sperm Abnormality

Sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min. Smears were air-dried and prepared on a glass slide ready for observation. Five air-dried smears were prepared on glass slides for each sample. Percentage sperm abnormalities (head, tail, mid-piece and multiple) were examined in every 200 spermatozoa observed on each slide. The method adopted by Ekaluo et al.13 was used to calculate the percentage of sperm abnormality.

#### Semen pH

Using a sterile pin, a puncture was made in epididymis after dissecting the animal. Semen smear from the pin was rubbed on a pH paper of pH range 4.0-10.0. Thereafter a colour change was monitored, which corresponded to a particular pH that was read from the paper.

# Analysis of Morphometric Parameters

Seminiferous tubular diameter, size of interstices and epididymal epithelial thickness were measured with a standard ocular micrometer (Graticules Ltd. Ton Bridge Kent England). Five slides were studied for each rat and five different measurements were taken on each slide.8 The average of measurements from five slides was taken as the value for each rat. Seminiferous tubular diameter was measured at X100 while size of interstices and epididymal epithelial thickness were measured at x400 respectively.12

#### Statistics and Photomicrography

Group data were expressed as mean ± standard error of the mean. Statistical analysis of data was determined using the Standard student's t-test,  $P \le 0.05$  were regarded as significant. The resulting slides were then viewed under the light microscope and photomicrography was conducted at a total magnification of x400.

# **Results and Discussion**

Ability to perform sexually entails composite interactions within the nervous, endocrine, and vascular systems and their usual co-ordinated activities, which are said to be in relations to sexual rejuvenation, performance and potency.14 In adult males, response to sexuality when agitated should yield to a physiological consistent sequence beginning with an uninterrupted flow of libido to erection, then orgasm to ejaculation. Thus, disruption in any of the named sequence often brings about sexual dysfunction.<sup>14</sup> By assessing libido in rats, we try to unravel reasons behind the widespread consumption of tiger nut in the northern part of Nigeria with a wide speculation that the crop is capable of stimulating sexual arousal and restoration of sexual desire in men. Mount frequency and ejaculatory latency in this study both reduced significantly compared to the control (Table 1), which is suggestive of an increased libido. Mount frequency of 4.46 ± 0.54 and ejaculatory

# ISSN 2616-0684 (Print) ISSN 2616-0692 (Electronic)

latency of  $545.20 \pm 97.41$  s was observed in the untreated rats while the treated animals at 1,000 mg/kg b.w after 30 days with an interval of two days showed mount frequency of  $2.94 \pm 0.01$  and ejaculatory latency of  $243.35 \pm 96.54$  s. These reductions were statistically significant by ttest (P < 0.001). We defined mount frequency as the sum total of mounts prior to ejaculation while ejaculatory latency as the time from first intromission until ejaculation was achieved.<sup>11</sup> In this study, tiger nut stimulated sexual arousal in rats, which is evident by the reduction in mount frequencies and ejaculatory latencies that have been keenly surveyed. Our observation however, supports the report by Allouh<sup>11</sup> and Allouh et al.<sup>15</sup> in which factors being investigated have been considered as parameters for determining increased libido in male rats. Improved libido in animals after ingesting tiger nut extract could be due, in part, to increased serum testosterone levels.15 Mount frequency and Ejaculatory latency are important parameters for enrichment of sexual desire. Though, mount frequency points at sexual motivation, improved ejaculatory latency displays the comfort by which ejaculatory reflexes are maintained.16 Hence, the dose-dependent activities of mount frequency and ejaculatory latency after exposure to tiger nut are indicative of increased libido.17 Such increment may result from increased levels of anterior pituitary hormones and testosterone, which subsequently inspired sexual competency by intensive stimulation from dopamine receptors.<sup>16</sup> The mechanism of action has not been fully established but is said to arise primarily from increased amount of blood flow to the erectile muscles thereby increasing the testosterone levels.<sup>15</sup> However, the role of testosterone in the improvement of sexual behavior was not investigated in this study. In a similar report<sup>17</sup> which suggested that increased fertility index of mice exposed to Cyperus esculentus alcoholic extract significantly improved compared to control rats. It has also been documented that C. esculentus plays an important role in resolving fertility problems in human going by results obtained from lower animals.12

Weight loss was prominent in a dose-dependent manner, while physical activities of the animals were marked in high dose treated rats (Table 2). The present study differs from some literatures that reported increased weight gain resulting from improved appetite in rats exposed to *C. esculentus crude extract.* On the contrary, Table 2 showed that

there is an insignificant increase in body weight of animals treated with low dose of *C. esculentus*. This observation was earlier reported by Allouh *et al.*<sup>15</sup> and Ekaluo *et al.*<sup>19</sup> in which body weight of rats did not have a significant improvement after treatment with *C. esculentus* extract but had interesting effects on organ weight. Our study disagrees with weight gain exerted by *C. esculentus* extract in another study suggesting that the extract may not affect metabolism.<sup>20</sup> This study disagree with the report by Ezeh *et al.*<sup>3</sup> where it was reported that regular consumption of tiger nut tubers is capable of improving appetite.

Significant increase in morphometric parameters of the testis and epididymis of treated rats was observed in this study (P < 0.05) compared to the control (Table 3). Rats treated with high dose of C. esculentus extract (500 mg/kg to 1000 mg/kg) were prominently affected and has been published.<sup>21</sup> Elevated weights of the testes and epididymis have been reported to mediate in the production of high amount of spermatozoa in some studies, which was attributed to androgen biosynthesis elevation resulting from spontaneous rise in testosterone level in rats.<sup>7, 22</sup> Consequently, tiger nut extract as it affects testicular weight and epididymis in rats is in agreement with Amaal and Essraa<sup>7</sup> report, which was linked to vitamin C rich content and defensive function against oxidative stress<sup>4</sup> and morphological changes of the testis in particular.<sup>23</sup> Fewer number of sperm abnormalities that were obvious in this study (Table 4) complimented the notion that tiger nut has been a good dietary food supplement for improving sperm quality and health.3 There is an enormous reduction in the head, mid piece and tail abnormality which are quite numerous in the control than in treated rats but significantly varied (P  $\ge$  0.05). Spermatogenic indices also showed that tiger nut extract was capable of improving the quality of sperm as observed in the present study (Table 5). This agrees with Greenspan and Stawler<sup>24</sup> work in which association between dosedependent sperm count and sperm quality was reported with a positive improvement. They then suggested that it may be due to increased level of testosterone's stimulation from spermatogenic cells undergoing successful spermatogenesis as well as sperm maturation in epididymal and secretory actions of the accessory sex glands.24

Profile	0 mg/kg	250 mg/kg	500 mg/kg	750 mg/kg	1000 mg/kg
1-10 days exposure					
Mount frequency	$4.46\pm0.54$	$4.13\pm0.43$	$4.00\pm0.29$	$3.96\pm0.12$	$3.16\pm0.06$
Ejaculation latency (s)	$545.20\pm97.41$	$374.00 \pm 11.23^{**}$	$361.00 \pm 41.05 **$	$360.07 \pm 65.15^{**}$	$344.03 \pm 85.21 **$
11-20 days exposure					
Mount frequency	$4.24\pm0.47$	$4.06\pm0.33$	$4.00\pm0.12$	$3.62\pm0.11$	$3.02\pm0.03$
Ejaculation latency (s)	$551.20\pm11.33$	$352.29 \pm 41.06^{**}$	$324.32 \pm 68.37 **$	$312.11 \pm 57.04 \ast \ast$	301.01 ± 11.06**
21-30 days exposure					
Mount frequency	$4.31\pm0.61$	$4.00\pm0.15$	$3.79\pm0.11$	$3.23\pm0.09$	$2.94\pm0.01$
Ejaculation latency (s)	$542.02\pm47.68$	331.31 ± 42.96**	311.14 ± 11.16**	301.52 ± 67.09**	243.35 ± 96.54**

# Table 1: Mating Profile of Experimental Animals Exposed to Cyperus esculentus Extract for 30 days.

All values are expressed as mean  $\pm$  standard error of the mean. Values are significantly varied (\*\*) at P  $\leq$  0.05 t-test.

Table 2: Empirical Measurements and Physical Activiti	es in Experimental Rats.
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Dose (mg/kg)	Initial Average Weight (g)	Final Average Weight (g)	Difference in Weight	<b>Physical Activities/ Dullness</b>
0	$162.40\pm1.41$	$166.32\pm1.12$	4.12 ± 0.31 ↑	±
250	$191.26\pm1.82$	$193.21\pm4.71$	2.05 ± 3.11 ↑	+
500	$200.12 \pm 1.22$	$201.75\pm2.33$	1.61 ± 1.12 ↑	+
750	$219.20 \pm 1.53$	$219.96\pm3.03$	0.76 ± 1.01 ↓	++
1000	$238.32\pm3.12$	$236.98 \pm 2.21$	2.66 ± 1.13 ↓	++

All values expressed as Mean  $\pm$  SEM. Negligible ( $\pm$ ), present (+), strongly present (++) increase ( $\uparrow$ ), decrease ( $\downarrow$ ).

Group	Seminiferous Tubular Diameter (µm x100)	Size of Interstices (µm x400)	Epididymal Epithelial Thickness (µm x400)
0 mg/ kg	$23.35\pm0.07^{\text{a}}$	$36.50\pm2.18^a$	$10.06\pm0.09^{\text{a}}$
250 mg/ kg	$26.72\pm0.46^{\text{b}}$	$36.59\pm7.11^a$	$10.16\pm0.23^{\mathtt{a}}$
500 mg/ kg	$27.31 \pm 1.33^{b}$	$37.91 \pm 7.02^{\mathtt{a}}$	$11.06\pm2.11^{\mathtt{a}}$
750 mg/ kg	$28.65 \pm 1.68^{\mathrm{b}}$	$39.55\pm2.56^{\text{b}}$	$13.16\pm0.21^{\mathtt{a}}$
1000 mg/ kg	$29.07\pm3.12^{\texttt{b}}$	$42.38\pm2.07^{\text{b}}$	$19.13 \pm 1.01^{\mathrm{b}}$
P-values	0.01	0.03	0.02

Table 3: Morphometric Parameters of Testis and Epididymis in Treated Rats.

All values expressed as mean  $\pm$  standard error of the mean.

Values in super script in same column significantly varied at  $P \le 0.05$ .

Group	Sperm Abnormalities %			
	Head	Mid piece	Tail	Multiple
0 mg/ kg	$2.9\pm0.17$	$0.9\pm0.53$	$5.1\pm0.4$	$0.35\pm0.02$
250 mg/ kg	$2.9\pm0.16$	$0.8\pm0.12\ast$	$5.1\pm0.3$	$0.35\pm0.03$
500 mg/ kg	$2.8\pm0.17\ast$	$0.9\pm0.03$	$4.8\pm0.3$	$0.28\pm0.01*$
750 mg/ kg	$2.8\pm0.11*$	$0.9\pm0.03$	$4.2\pm1.1*$	$0.34\pm0.01$
1000 mg/ kg	$2.8\pm0.66^{\ast}$	$0.8\pm0.02*$	$4.2\pm1.2^{\ast}$	$0.34\pm0.02$
P-values	0.06	0.071	0.67	0.81

# **Table 4:** Sperm Abnormalities in Experimental Rats.

All values are expressed as mean  $\pm$  standard error of the mean.

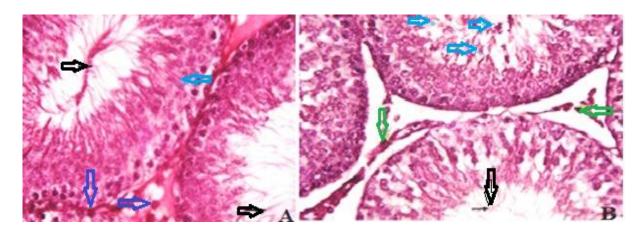
Values are significantly varied at  $P \le 0.05$  using one-way ANOVA.

Table 5: Spermatogenic	Analysis in	Experimental Rats.
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Grouping	Sperm count (×106 mLG1)	Sperm motility (%)	Semen pH	Sperm viability (%)
0 mg/ kg	$7.65\pm2.06^{a}$	$76.11\pm0.34^{a}$	$7.05\pm0.12^{a}$	$86.01\pm1.95^a$
250 mg/ kg	$6.95\pm0.13^{\text{a}}$	$75.65 \pm 1.14^{\text{a}}$	$6.65\pm1.18^{a}$	$88.85\pm1.95^{a}$
500 mg/ kg	$8.15\pm9.67^{\text{a}}$	$79.11\pm0.34^{a}$	$7.15\pm0.88^a$	$89.99\pm3.66^a$
750 mg/ kg	$9.16\pm0.11b$	$87.75\pm0.77^b$	$6.93 \pm 1.07^{\rm a}$	$91.16\pm3.43^a$
1000 mg/ kg	$11.09\pm0.23^{\rm c}$	$98.65\pm2.12^{\rm c}$	$7.39 \pm 1.37^{\rm a}$	$98.01\pm2.02^{b}$
P-values	0.244	0.02	0.686	0.03

All values are expressed as Mean  $\pm$  SEM. (SEM = standard error of the mean).

Values with similar superscripts are not significantly different at (P  $\ge$  0.05).



**Figure 1:** Photomicrograph of control section (A) and highest dose of treated section (B) of testicular tissue showing several normal seminiferous tubules with normal maturation stages (spanned), the spermatogonia (light blue arrow), sertoli and leydig cells (deep blue arrow) are normal, the lumen appears with presence of spermatozoa (black arrows) and normal interstices (green arrows) H and E x250.

Generally, any damage to sperm cells may be linked to cytotoxic induction of cytotoxins in circulating spermatozoa, physiological, and or genetic mechanism.<sup>25</sup> However, exposure to chemicals could lead to damages in the pituitary hypothalamic or sex hormonal impairments, and may affect spermatogenesis.<sup>26</sup> In addition, exposure to cytotoxins or genotoxins in chemicals could result in aberrations in seminal fluid, which may exert functional or structural impairment on viable spermatozoa.<sup>25</sup> Thirdly, natural circumstances may lead to depletion in the level of spermatozoon differentiating process during spermatogenesis.<sup>26</sup> There is a significant rise in richness of spermatozoa seen in the lumen (P < 0.05), which is dose related within each group treated with different doses of tiger nut extract and is evident with the accumulation of spermatozoa making the lumen to appear dense, which is not dissimilar to the report by Ekaluo *et al.*<sup>25</sup>

#### Conclusion

Aqueous extract of *C. esculentus* enhances sexuality and enriches spermatogenic and testicular weight and epididymis in male Wistar rats. This study, however, is in support of regular consumption of tiger nut tubers as local supplement for sex enhancement. Although we recommend that further studies are necessary to substantiate this claim.

# **Conflict of interest**

The authors declare no conflict of interest.

## **Author's 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.

# Acknowledgements

We are thankful to the Pathologist at UBTH and the members of Sexual Health Concerns for Commercial Sex Workers (SHCCSW) Research Group, University of Benin, Benin City, Nigeria for providing technical and expert input for the study.

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