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Effect of Tiger Nut Meal on Some Sex Hormones and Sperm Cells in Androgen-induced Benign Prostate Hyperplasia in Adult Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author DII designed the study, managed the literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JNE and CLE managed the analyses of the study and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: It is generally believed that *Cyperus esculentus* (tiger nut) has some fertility boosting effects. However, scientific validation of some of the fertility boosting potentials as well as the ameliorative effect of tiger nut on Benign Prostate Hyperplasia (BPH) is lacking.
Objective: The aim of the project is to determine the effect of tiger nuts in reproductive function in rats induced with Benign Prostate Hyperplasia (BPH).
Methods: A total of sixty (60) male rats weighing between 160 – 200 g were used in this study. They were divided into six groups of ten rats per group. Benign prostate hyperplasia was induced in three groups of the rats (as stated in methodology) with 30 mg/kg sub-cutaneous injections of hormones containing dihydrotestosterone (DHT) and estradiol valerate dissolved in olive oil in the ratio of 10:1 (three times in a week, one day interval). Administration of tiger nut meal commenced immediately and lasted for two months. At the end of administration, assay of reproductive hormones (FSH, LH and Testosterone) was done. Comprehensive semen analysis was also done including (count, motility & morphology).

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Results: The study showed that the induction of BPH resulted in a very significant reduction of FSH while the administration of the tiger nut meal did not show any significant effect on FSH ($P>0.05$). The same trend was also observed on the effect of the tiger nut meal on LH level. For testosterone, it was observed that after the initial decrease as a result of the induction of infertility, 20% tiger nut meal administration significantly increased the testosterone level to 2.10±0.06 ng/ml from 0.30±0.01 ng/ml in the infertility-induced group. The effect of tiger nut meal on semen analyses namely, normal sperm cells and sperm motility showed a significant increase ($P<0.05$) following the induction of infertility. The effect of the tiger nut meal on sperm morphological toxicities were also examined. Sperm abnormalities like sperm cells with twisted tails were examined.

Conclusion: The administration of tiger nut significantly ameliorated the abnormalities and thus, restored the morphology of the sperm cells such that it can enhance fertility.

Keywords: Tiger nut meal; sex hormones; twisted sperm cells; normal sperm cells.

1. INTRODUCTION

Plants have continued to be used worldwide for the treatment of diseases and novel drug entities continued to be developed through research into natural products [1]. Most of these traditional medicinal plants owe their medical activity to the presence of secondary metabolites like alkaloids, terpernoids, saponins, phenolics and flavonoids present in their leaves, stems, barks, fruits, seeds, roots and flowers [2]. They are usually prepared as concoctions, decoctions, infusions, macerations, gargles, powders, pastes and tinctures. They have found usage in several ailments but, are popular in the management of chronic diseases most of which are difficult to treat successfully using orthodox medicine [3]. Among these herbal remedies, consumption of tiger nut (*Cyperus esculentus*) is relatively popular in some societies especially, in Nigeria as an aphrodisiac agent [4,5].

There is a growing evidence indicating a steady decline in human sperm count and quality [6,7,8]. Increased testicular weight, sperm concentration, sperm motility, sperm viability, progressive sperm motility and also, the reduced percentage in sperm morphology abnormalities had been reported in rats and mice treated with tiger nut [9,10], with potentials of attenuating sperm and reproductive toxicities. However, there has been no sufficient scientific validation of the effect of *C. esculentus* on several other parameters of male reproductive function e.g. follicle stimulating hormone, luteinizing hormone, testosterone, bent midpiece etc. Therefore, this study was aimed at investigating the effect of *C. esculentus* tubers (tiger nuts) on the reproductive hormones and some sperm parameters of adult male rats.

In furtherance of the aphrodisiac effect of *C. esculentus*, Allouh et al. [11] investigated the Influence of *Cyperus esculentus* tubers (Tiger Nut) on male rat copulatory behaviour. This study aimed to investigate the influence of tiger nut on the copulatory behaviour of sexually active male rats.

According to [11], Liquid Chromatography/Mass Spectrometry Liquid Chromatography coupled with tandem Mass Spectrometry (LC-MS/MS) was applied to identify the tiger nut constituents that may be responsible for elevating serum testosterone level. The LC-MS/MS analysis revealed the presence of several compounds in tiger nut that may boost serum testosterone levels and contribute to the improvement of the copulatory behaviour: quercetin, vitamin E, and vitamin C. In addition, atomic absorption spectroscopy revealed the presence of the mineral zinc in tiger nut. No selenium was detected in the tiger nut sample. These constituents, no doubt, could positively contribute to testosterone production and improve the erectile function.

According to [12], there is an increase in the consumption of quercetin. Perhaps, this is as result of the fact that it has been associated with an aphrodisiac property. It is a dietary bioflavonoid. They represent a large class of polyphenolic compounds found in most plants. Many flavonoids, including quercetin, are reported to possess strong antioxidant properties and to have beneficial health effects.

Ma et al. [13] revealed that oral administration of quercetin was associated with a significant increase in serum testosterone level in male rats. This finding by [13] is consistent with the work of [12]. Furthermore, [14] suggested that quercetin

could ameliorate erectile dysfunction in diabetic rats by inhibiting oxidative stress.

Vitamin E is fat soluble organic compound and commonly present in the cell membranes. This vitamin has the strong antioxidant properties and inhibits the lipid peroxidation created by the free hydroxyl and superoxide radicals. This vitamin protects the cell membrane of sperm cell from damages of reactive oxygen species (ROS). According to [15], In vitro studies have proved that the use of vitamin E improves the motility and fertilizing ability of sperm in the egg. In a similar finding by [16], in vivo studies, supplementation of vitamin E was found to be effective in reduced number and motility of sperms caused by ROS.

Ascorbic acid is a sugar acid with antioxidant properties. Its appearance is white to light-yellow crystals or powder, and it is water soluble. Ascorbic acid is a strong antioxidant that facilitates the formation of testosterone, and was found in a considerable concentration in tiger nut in this study. It has been reported that vitamin C increases testosterone content in rat testes in vitro [17]. Vitamin C, also, stimulates vascular nitric oxide production, which consequently improves the erectile function [18].

The element Zinc, is speculated to play a critical role in sexual development. Zinc deficiency was found to disrupt testicular tissue [19], impair spermatogenesis [20], and reduce testosterone levels [21], while zinc supplementation improved the sexual behaviour of adult male rats in a dose-dependent manner by enhancing testosterone secretion [22]. Prasad et al. [23] reported a positive correlation between cellular zinc concentration and serum testosterone level in healthy men.

Zinc deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules. In the male reproductive system, zinc is essential for optimal performance and output. Zinc in seminal fluid helps to stabilize the cell membrane and nuclear chromatin of spermatozoa. Its other roles in male reproduction include: may have a regulatory role in the process of capacitation and acrosome reaction can protect the testis against degenerative changes and it may also be used as an index of prostatic function. Hence, the aim of this work is to ascertain the effect of tiger nut meal in androgen-induced benign prostate hyperplasia in adult male wistar rats.

2. METHODS

2.1 Procurement of Tiger Nut Tubers and Its Authentication

Tiger nut tubers were obtained from the local market at Owerri city, Imo State. The tiger nuts were identified and authenticated at the herbarium of the Department of Plant Science and Biotechnology, Faculty of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State. Its Voucher number is: MOUAU/ZEB/19/004.

For the preparation of tiger nut powder, the tubers were cleaned, washed and dried in a stream of hot air for an hour. The dried tubers were milled using a laboratory electric mill. The research work was carried out at Michael Okpara University of Agriculture, Umudike, Abia State.

2.2 Chemicals and Reagents

All chemicals used were purchased from Sigma Chemicals, St Louis, USA and were of analytical grade. Kits for evaluation of liver and kidney functions, lipid profile and lipid peroxidation were products of QuimicaClinicaApplicada (QCA), Spain.

2.3 Procurement of Experimental Animals

Healthy wistar rats, two months old and weighing 160- 200g were procured from Pharmacology Department, University of Port Harcourt (Rivers state). The rats were housed in wooden netted cages and maintained under environmentally controlled room provided with a 12:12 hours light and dark cycle approximately at 25^0C. They were fed on pellets (Lab Feeds) and tap water. The rats were allowed to acclimatize to laboratory environment for 21 days before experimentation.

2.4 Preparation of Plant Extract

The collected fresh tubers were dried in the shade at 25℃ for two weeks and thereafter, pulverized in a locally fabricated milling machine. Six hundred (600) grams of the pulverized material was packed into the material chamber of the Soxhlet extractor and extracted by ethanol at a specific temperature (60℃) for 48 hr. At the completion of extraction, the solvent in the extract was evaporated at 40℃ in a hot air oven to obtain a crude extract which weighed 49.18 g, representing a yield of 49.18%. The extract was preserved in the refrigerator until needed and is hereafter referred to as *C. esculentus* extract.

2.5 Acute Toxicity Test

The oral median lethal dose (LD_{50}) of the extracts was determined in rats according to the method of [24]. The study was carried out in two phases. In the first phase, nine [9] rats were divided into 3 groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight respectively after which they were observed for 24 hours for signs of toxicity and/ or mortality. Based on the results of the first phase, 9 rats were again divided into 3 groups of 3 rats each and were also treated with the extract at doses of 1600, 2900 and 5000 mg/kg body weight respectively in the second phase. The rats were also monitored 24 hours after treatment and for signs of toxicity and/or mortality. The median lethal dose (LD_{50}) of each extract was estimated based on the observations in the second phase.

2.6 Preparation of Tiger-nut Diet

Tiger-nut powder and the animal feed was weighed and calculated to give exactly the ratio of the tiger nut meal needed. For 20% of the tiger nut meal, 20g of tiger-nut powder was added to 80g of the animal feed (high dose) while for 10% of the tiger nut meal, 10g of tiger-nut powder was added to 90g of the animal feed (low dose). The feed was thoroughly mixed before giving it to the animals for consumption.

2.7 Experimental Design

Group 1 Normal Control
Group 2 Negative control (BPH)
Group 3 BPH + Low dose (10% of meal)
Group 4 BPH + high dose (20% of meal)
Group 5 Normal + Low dose (10% of meal)
Group 6 Normal + high dose (20% of meal)

Note: The average weight of the rats is 180 g and the administration of the tiger nut meal lasted for two months.

2.8 Induction of BPH

Rats in the test groups (groups 2, 3 and 4) weighing between 160 - 200g were given 30mg/kg sub-cutaneous injections of hormones containing dihydrotestosterone and estradiol valerate dissolved in olive oil in the ratio of 10:1 three times in a week with one day interval.

The drugs used were purchased from Sigma Chemicals, St Louis, USA and were of analytical grade. The administration of the tiger nut meal commenced immediately the following week.

2.9 Collection of Blood Samples

After two months of administering the meal, the rats were anaesthetized by a brief exposure to chloroform vapour. Blood sample was collected by cardiac puncture and was used in the assay of some sex hormones.

2.10 Semen Collection and Analysis

The sperm cells were harvested from the epididymal reserve. The rats were anaesthetized with chloroform (inhalation), and their epididymis extracted. The caudal portion of each epididymis was incised and a smear made on the preheated glass slides for evaluation.

2.11 Macroscopic Examination

The semen colour and consistency were evaluated macroscopically and recorded. The consistency scale (1-4), adopted by [25] was used.

2.12 Abnormal Sperm Proportion

The abnormal sperm proportion was determined by the method described by [26]. A drop of the semen was stained using E/N stain and the mixture smeared on a glass slide and viewed under a lower magnification of $\times 40$ to check for primary and secondary abnormal sperm cells, percentage of the differential abnormalities such as head abnormalities, tail abnormalities etc.

2.13 Determination of the Hormone Profile of the Rats

- Testosterone assay was done using the method described by [27].
- Luteinizing hormone assay was done using the method described by [28].
- FSH assay was done using the method described by [28].

2.14 Statistical Analysis

Statistical analysis was carried out using windows (SPSS version 15.0). Data were analysed using one-way ANOVA followed by post hoc test-least significant difference (LSD), while charts were done using Microsoft excel. The data was expressed as mean ±SEM and values of $P<0.05$ were considered significant.

3. RESULTS

3.1 Effect of Tiger Nut Meal on FSH (miu/ml)

Table 1 shows the effect of tiger nut meal on FSH level. Following the induction of infertility, there was a significant decrease in the level of FSH in the negative control (induction only) ($P < 0.05$). However, treatment with the tiger nut meal on infertility-induced rats at doses of 10% and 20% showed slight insignificant rises in FSH. However, the administration of tiger nut meal to non-induced rats at high dose of 20% and that of the low dose of 10% daily, showed significant increase ($P < 0.05$).

3.2 Effect of Tiger Nut Meal on LH (miu/ml)

Table 2 shows the effect of tiger nut meal on luteinizing hormone (LH). Following the induction of infertility, the level of LH in the negative control (induction only) significantly decreased. However, treatment of other induced groups with tiger nut meal, did not show any significant increase in the hormone level ($P > 0.05$).

Unfortunately, the administration of tiger nut meal to non-induced rats at high dose of 20% and that of the low dose of 10% daily, showed significant decrease in the level of the LH ($P < 0.05$).

3.3 Effect of Tiger Nut Meal on Testosterone (ng/ml)

Table 3 shows the effect of tiger nut meal on testosterone. Following the induction of infertility in the negative control, there was a significant decrease in the level of testosterone in the negative control (induction only) ($P < 0.05$). However, treatment of the other induced -infertility experimental groups with low and high doses of the tiger nut, the levels of testosterone hormone increased significantly compared to the negative control group ($P < 0.05$).

3.4 Effect of Tiger Nut Meal on Normal Sperm Cells

Table 4 shows the effect of tiger nut meal on the percentage normal sperm cell. Following the induction of infertility in the rats of negative control group (induction only), there was a significant decrease in the percentage of normal sperm cells ($P < 0.05$). However, treatment of the other groups induced with infertility with the tiger nut meal of a low and high doses, a significant increase in the percentage of normal sperm cell was recorded when compared with the negative control ($P< 0.05$).

Table 1. Effect of tiger nut meal on FSH (miu/ml)

Parameters	Control	Induction only	Induction + Low dose (10%)	Induction + High dose (20%)	Low dose (10%)	High dose (20%)
Follicle Stimulating Hormone (miu/ml)	3.7±0.11 [d]	0±0 [a]	0.02±0 [a]	0.09±0 [ab]	2±0.06 [c]	0.3±0.01 [b]

Values are expressed as mean ± SEM, n=10, parameters in the row with the same alphabet are statistically the same (p>0.05), parameters with different alphabets are statistically difference (p<0.05)

Table 2. Effect of tiger nut meal on LH (miu/ml)

Parameters	Control	Induction only	Induction + Low dose (10%)	Induction + High dose (20%)	Low dose (10%)	High dose (20%)
Luteinizing Hormone (miu/ml)	2±0.06 [b]	0.01±0 [a]	0.04±0 [a]	0.03±0 [a]	0.06±0 [a]	0.01±0 [a]

Values are expressed as mean ± SEM, n=10, parameters in the row with the same alphabet are statistically the same (p>0.05), parameters with different alphabets are statistically difference (p<0.05)

Table 3. Effect of tiger nut meal on Testosterone (ng/ml)

Parameters	Control	Induction only	Induction + Low dose (10%)	Induction + High dose (20%)	Low dose (10%)	High dose (20%)
Testosterone (ng/ml)	4.01±0.12 [e]	0.3±0.01 [a]	0.62±0.02 [b]	2.1±0.06 [c]	2.61±0.08 [d]	0.2±0.01 [a]

Values are expressed as mean ± SEM, n=10, parameters in the row with the same alphabet are statistically the same (p>0.05), parameters with different alphabets are statistically difference (p<0.05)

Table 4. Effect of tiger nut meal on normal sperm cells

Parameters	Control	Induction only	Induction + Low dose (10%)	Induction + High dose (20%)	Low dose (10%)	High dose (20%)
Normal Sperm cell (%)	97.71±0.08[bc]	93.98±0.48[a]	97.57±0.14[bc]	98.41±0.1[cd]	98.87±0.14[d]	97.48±0.1[b]

Values are expressed as mean ± SEM, n=10, parameters in the row with the same alphabet are statistically the same (p>0.05), parameters with different alphabets are statistically difference (p<0.05)

3.5 Effect of Tiger Nut Meal on Sperm Motility

Table 5 shows the effect of tiger nut on sperm motility. Following the induction of infertility on the negative control group (induction only), there was a significant decrease in the motility of the sperm cells ($P < 0.05$). However, treatment of the other groups induced with infertility with the tiger nut meal of a low and high doses, recorded a significant increase in the motility of the sperm cells when compared with the negative control ($P< 0.05$)

Finally, the administration of tiger nut meal to the rats under normal condition at low and high doses showed a further positive increase in the level of the Mass Motility of the sperm cells when compared with the positive control ($P< 0.05$).

3.6 Effect of Tiger Nut Meal on Sperm Cells with Twisted Tail

Table 6 shows the effect of tiger nut meal on sperm cells with twisted tail. Following the induction of infertility on the negative control group (induction only), there was a significant increase in the number of the sperm cells with twisted tail ($P < 0.05$). However, treatment of the other groups induced with infertility with the tiger nut meal of a low and high doses, recorded a significant decrease in the number of sperm cells with twisted tail when compared with the negative control ($P< 0.05$). Furthermore, the administration of tiger nut meal to the rats under normal condition at low and high doses, showed no statistical difference when compared with the normal control ($P>0.05$).

4. DISCUSSION

The effect of *Cyperus esculentus* (Tiger nut) on reproductive hormones namely; FSH, LH and Testosterone were studied in BPH induced rats. The results of this study showed that tiger nut did not ameliorate the low levels of FSH and LH in animals with induced infertility. Tiger nut also reduced the levels of FSH and LH in apparently normal (control) animals that were not treated with any drug to induced infertility.

In reproductive studies, the pituitary-testicular axis is immensely important in the regulation of male reproductive function. Androgens especially testosterone which is being regulated by luteinizing hormone (LH) from the anterior pituitary gland, plays a vital role in the final maturation of the spermatozoon while follicle stimulating hormone (FSH) is needed for the maintenance of the gametogenic function of the testis.

The inability of the tiger nut meal to restore the level of FSH and LH may suggest that it does not have any direct effect on the hypothalamo-pituitary-gonadal pathway. Also, it has been suggested that the negative feedback effect of testosterone on the hypothalamus may cause a decrease in the secretion of FSH and LH by the anterior pituitary gland [29]. However, in addition to the suggestion of [29], the author suggests that, the inhibition of FSH and LH may be as a

Table 5. Effect of tiger nut meal on sperm motility

Parameters	Control	Induction only	Induction + Low dose (10%)	Induction + High dose (20%)	Low dose (10%)	High dose (20%)
Mass motility (%)	74±1.54 [b]	35.9±2.8[a]	69.42±1.19 [b]	83.26±0.52 [c]	87.71±0.94 [c]	84.52±1.33 [c]

Values are expressed as mean ± SEM, n=10, parameters in the row with the same alphabet are statistically the same (p>0.05), parameters with different alphabets are statistically difference (p<0.05)

Table 6. Effect of tiger nut meal on Sperm cell with twisted tail

Parameters	Control	Induction only	Induction + Low dose (10%)	Induction + High dose (20%)	Low dose (10%)	High dose (20%)
Sperm cell with twisted tail	0.59±0.07[a]	2.34±0.48[b]	0.32±0.05[a]	0.24±0.02[a]	0.31±0.06[a]	0.22±0.02[a]

Values are expressed as mean ± SEM, n=10, parameters in the row with the same alphabet are statistically the same (p>0.05), parameters with different alphabets are statistically difference (p<0.05)

result of its negative effect on the central nervous system that can inhibit the neural stimulus essential for the release of the anterior pituitary hormones that are essential for initiating and completing spermatogenesis and steroidogenesis in the testis.

The effect of tiger nut on testosterone showed that there was a significant rise in testosterone levels following tiger nut administration to animals that had induced infertility. Furthermore, low dose administration of the tiger nut meal significantly increased the testosterone levels more than that of the high dose therapy. This finding on testosterone hormone is however, consistent with other research findings by [15,27].

The exact mechanism by which tiger nut boosts testosterone levels is not entirely clear. However, we speculate that tiger nut may act directly on testicular cells, and not through the hypothalamus-pituitary axis, since no variations in FSH and LH levels were observed following the tiger nut treatment. This speculated mechanism is possible following the work of [11] on the phytochemistry of tiger nut that showed the presence of several components (quercetin, Vitamins E and C and the mineral Zinc) which may positively contribute to testosterone synthesis and as such, improve the reproductive function.

The effect of *Cyperus esculentus* (tiger nut) on semen parameters namely; sperm motility, percentage of normal sperm cells and sperm cells with twisted tail were also studied. The results of this study showed that tiger nut ameliorated the low levels of sperm motility, sperm cells with twisted tail and also, enhanced the normal sperm cells in the animal groups with induced infertility. There was a significant increase in the levels of these parameters following the administration of Tiger nut meal to animals that had induced infertility. Also, tiger nut enhanced the levels of the mentioned parameters in apparently normal animals that were not induced with infertility. This finding may be associated with the increase in testosterone hormone level.

5. CONCLUSION

1. Tiger nut enhances testosterone hormone while decreases FSH and LH in BPH induced rats.
2. Tiger nut enhances semen profile, ameliorating associated toxicities such as sperm cells with twisted tail in BPH induced rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental protocols were subjected to the scrutiny and approval of Institutional Animal Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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