# PSA, Sperm Midpiece, Relative Organ Weight, Histological Changes in Adult Male Wistar Rats Treated with Tiger Nut Meal

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**ABSTRACT:** It is generally believed that Cyperus esculentus (tiger nut) has some fertility boosting effects. However, scientific validation of some the fertility boosting belief concerning tiger nut is lacking. Therefore, the aim of the project was to study the effects of tiger nuts on PSA, Sperm midpiece, relative organ weight and histological changes in reproductive function of the rat. BPH was induced in the test groups (groups 2, 3 and 4) of rats weighing between 160 - 200g with 30mg/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). Semen morphological studies was done. Internal organs notably, the prostate and the testes of the rats were removed for histological examination. Results showed that the induction of BPH brought about some adverse effects. On prostate specific antigen (PSA), the administration of the tiger nut meal showed positive trend in the amelioration of benign prostate hyperplasia by significantly reducing the increased level of the PSA which is biomarker for prostate hyperplasia ($P<0.05$). The effect of the tiger nut on sperm morphological toxicities were also examined. Sperm abnormalities like those with bent midpiece was examined. The result showed that the administration of tiger nut meal significantly ameliorated the abnormality and thus, restored the morphology of the sperm cells such that it can enhance fertility. A significant difference was also seen in the relative weight of the prostate. The prostate hyperplasia in the induced group was later observed through histological studies to have reduced significantly following the administration of the tiger nut. In conclusion, these findings indicate that tiger nut meal can ameliorate benign prostate hyperplasia and reproductive dysfunction including sperm cells abnormalities in rats.

**Key Words:** *Exopolysaccharides, food preservation, public health, disease prevention, sensory properties.*

## INTRODUCTION

LBenign prostatic hyperplasia (BPH) is a common age-related disease among the elderly males. BPH is remarkably characterized by histological proliferation of the epithelial cells in the transitional zone of the prostate which leads to lower urinary tract symptoms. The constriction of the urethra can result in increased frequency, urgency and hesitancy of urination, and compromised urine flow, which eventually impacts the quality of life [1][2]

The genesis of the disease is not completely known. However, the development of BPH occurs with an initial hormonal imbalance between testosterone and estradiol [3], ultimately leading to a higher conversion of testosterone into dihydrotestosterone. Such hormonal dysfunction causes an increased proportion of prostatic cell proliferation in relation to parenchymal cell apoptosis [4][5][6].

Furthermore, spermatozoa are particularly susceptible to oxidative stress due to reduced cytoplasm content and, consequently, the limited amount of enzymatic antioxidant [7].Hence, local oxidative stress, in addition to direct effects of BPH, can increase sperm cell damage, resulting in decreased sperm motility, velocity and morphological integrity [8].

Moreover, important sperm functional features are impaired, such as biochemical mechanisms and DNA integrity [9][10]. Sperm DNA fragmentation has a markedly negative impact on reproductive efficiency causing low pregnancy rates and altered foetal formation [11]. Therefore, we hypothesized that BPH can cause important overall sperm damage ultimately leading to decreased reproductive potential in man. The aim of this study was to compare the reproductive potential of healthy rats and those affected by benign prostatic hyperplasia through an overall sperm analyses.

Medicinal therapy remains the first line treatment for most patients. Given the importance of DHT in the development of BPH, inhibitors of 5 alpha-reductase (e.g., finasteride and dutasteride) which prevents the conversion of DHT from testosterone and reduces DHT level and thereby suppresses hyperplastic growth of the prostate are used in the clinical treatment of BPH. Nevertheless, according to Hongcai et al. (2018), finasteride-associated untoward reactions are regularly reported, including gynecomastia, headache, dizziness, chest pain, upper respiratory infections, decrease libido, erectile dysfunction, and male infertility due to a reduced sperm count. Such side effects cause the limitation of conventional drugs used for BPH and, nevertheless, might be prevented by other natural agent such as Cyperus esculentus (Tiger nut).

Tiger nut tubers are edible, with a slightly sweet and nutty flavour. The tubers are used as a foodstuff, particularly in Africa, where it is an important food crop with certain tribes. Tiger nuts have excellent nutritional qualities with a fat composition similar to olives [12]. Moreover, it is the richest food source of flavonoids and also rich in water, fibres, alkaloids, digestible carbohydrates, saponins and fatty oils (glycerides), in addition to some elements, like phosphorus, potassium, calcium, iron, zinc, magnesium and manganese [13][14].

## MATERIALS AND METHODS

### 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.

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### 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.

### 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 250C. They were fed on pellets (Lab Feeds) and tap water. The rats were allowed to acclimatize to laboratory environment for 21 days before experimentation. All experimental protocols were subjected to the scrutiny and approval of Institutional Animal Ethics Committee.

### 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.

### ACUTE TOXICITY TEST

The oral median lethal dose (LD50) of the extracts was determined in rats according to the method of Lorke, (1983). 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 (LD50) of each extract was estimated based on the observations in the second phase.

### 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.

### 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 180g and the administration of the tiger nut meal lasted for two months.

### INDUCTION OF INFERTILITY AND 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 DHT and estradiol valerate dissolved in olive oil in the ratio of 10:1 (three times in a week) as described by Ejike and Ezeanyika, (2011).

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.

### COLLECTION OF BLOOD SAMPLE

After 2-months of administering the extract, the rats were anaesthetized by a brief exposure to chloroform vapour, and bled exhaustively by cardiac puncture. The sera were carefully separated and used for the prostate specific antigen (PSA) and other biochemical analyses. Each rat's carcass was promptly dissected and the prostates were carefully excised. Two prostates per group were randomly selected out and immediately processed for histology. The other prostates per group were freed of external fascias, washed in cold normal saline, blotted with filter paper and weighed on a sensitive balance.

### SEMEN COLLECTION AND ANALYSIS

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

### MACROSCOPIC EXAMINATION

The semen colour and consistency were evaluated macroscopically and recorded. The consistency scale (1-4), adopted by Chibundu, (2013) was used.

### ABNORMAL SPERM PROPORTION

The abnormal sperm proportion was determined by the method described by El- Sherbiny (1987). 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 ×40 to check for primary and secondary abnormal sperm cells, percentage of the differential abnormalities such as head abnormalities, tail abnormalities, mid-piece abnormalities etc.

### 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.

## RESULTS

### EFFECT OF TIGER NUT MEAL ON THE PSA

After induction, there was a significant increase in the level of PSA in the negative control group. Again, this discovery shows that there was an enlargement of the prostate. Treatment of the BPH with the tiger nut meal after induction showed that, at low dose of 10% and that of 20%, the level of PSA decreased significantly ($P < 0.05$).

Finally, the administration of tiger nut meal to the rats under normal condition at low dose of 10% and 20% showed a positive decrease in the level of the PSA. However, both the low and high doses of treatment groups which were not induced did not show any statistical difference when compared with the normal control ($P > 0.05$).

**TABLE 1:** Effect of tiger nut meal on the PSA of the rats

| Parameter | Control | Induction only | Induction + Low dose (10%) | Induction + High dose (20%) | Low dose (10%) | High dose (20%) |
|---|---|---|---|---|---|---|
| PSA (ng/ml) | 0.19±0.02 [a] | 11.46±0.56 [d] | 5.4±0.4 [c] | 2.05±0.1 [b] | 0.34±0.03 [a] | 0.41±0.05 [a] |

Values are 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 different ($p<0.05$).

### EFFECT OF TIGER NUT ON THE RELATIVE WEIGHT OF THE PROSTATE

Following the induction of BPH, there was a significant increase in the

prostate weight relative to the body weight when compared to the negative control group (P<0.05). However, treatment of the BPH with the tiger nut meal after induction showed that, at low dose of 10% showed a significant decrease in the weight of the prostate when compared with the negative control of 0.14±0.02 (P<0.05).

Furthermore, the administration of tiger nut meal to the rats under normal condition at low dose of 10% also showed a statistical decrease when compared with the negative control (P<0.05).

**TABLE 2:** Effect of tiger nut on the relative weight of the prostate

| Parameters | Control | Induction only | Induction + Low dose (10%) | Induction + High dose (20%) | Low dose (10%) | High dose (20%) |
|---|---|---|---|---|---|---|
| Weight of Prostrate Relative to Body Weight | 0.10±0.01 [a] | 0.19±0.02 [b] | 0.08±0.02 [a] | 0.13±0.01 [a] | 0.1±0.01 [a] | 0.14±0.01 [a] |

Values are 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)

## EFFECT OF TIGER NUT ON THE RELATIVE WEIGHT OF THE TESTES

Following the induction of BPH, there was a significant decrease in the level of testes weight relative to the body weight in the negative control group (P<0.05). This result showed that with the induction of BPH in the rats, the weight of the testes relative to body weight of the rats decreased. However, treatment with the tiger nut meal after induction showed that, at low dose of 10% and a high dose (20%) of the tiger nut meal, a significant increase in the weight of the testes relative to body weight in the animals was recorded when compared with the negative control (P<0.05).

Finally, the administration of tiger nut meal to the rats under normal condition at low dose of 10% and 20% also showed a statistical increase in the weight of the testes when compared with the negative control and normal control (P<0.05).

**TABLE 3:** Effect of tiger nut meal on the relative weight of the testes

| Parameters | Control | Induction only | Induction + Low dose (10%) | Induction + High dose (20%) | Low dose (10%) | High dose (20%) |
|---|---|---|---|---|---|---|
| Weight of testes Relative to Body Weight (g) | 2.65±0.07 [d] | 1.46±0.35 [a] | 2.25±0.08 [b] | 2.27±0.12 [b] | 3.02±0.37 [e] | 2.55±0.01 [cd] |

Values are 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)

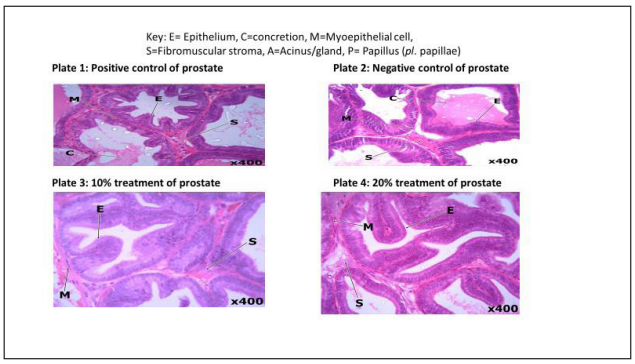
**TABLE 4:** Effect of tiger nut meal on Bent Midpiece of the sperm cells

| Parameters | Control | Induction only | Induction + Low dose (10%) | Induction + High dose (20%) | Low dose (10%) | High dose (20%) |
|---|---|---|---|---|---|---|
| Bent mid piece | 0.18±0.03 [a] | 0.62±0.06 [c] | 0.45±0.02 [b] | 0.39±0.05 [b] | 0.1±0.04 [a] | 0.81±0.02 [d] |

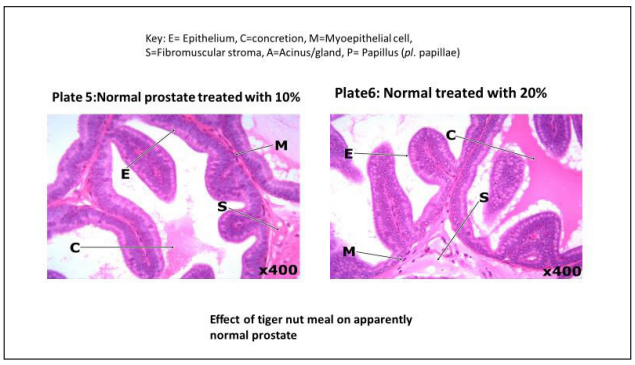
Values are mean ± SEM, n=10, parameters in the row with the same alphabet are statistically the same (p>0.05), parameters with different alphabets are statistically difference (p<0.05)

## EFFECT OF TIGER NUT ON THE HISTOLOGY OF THE PROSTATE

Plate 1 shows a photomicrograph of the prostate gland with well and orderly differentiated prostatic acinar glands lined by luminal columnar cells and basal layer of myoepithelial cells with few papillae with fibrovascular cores. Some of the glands contain prostatic concretions. Plate 2 shows the histology of the prostate gland induced with hyperplasia. It showed a moderate hyperplasia of the acini/glands and stroma. Also, Plate 3 and 4 shows the effect of the induced prostate hyperplasia treated with 10 and 20% of the tiger nut meal. Compared to normal control group of rats and the induced group, there was a visible reduction in hyperplasia of the acini/glands and stroma. Finally, plate 5 and 6 showed the effect of tiger nut meal on non-induced BPH rats. There was no visible pathology seen.

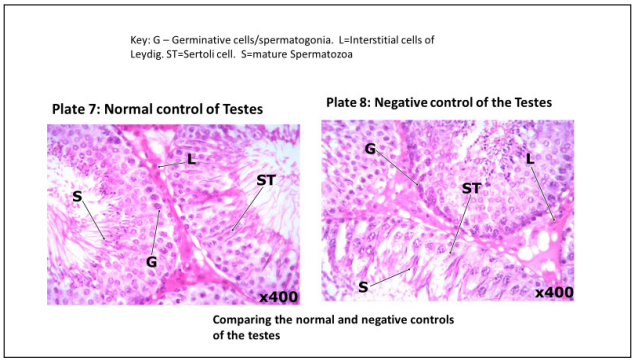


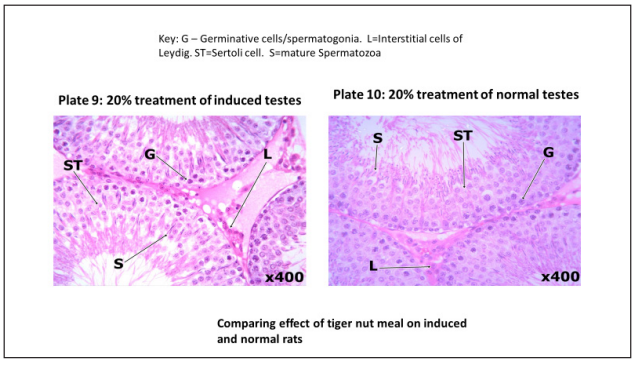

Effect of tiger nut meal on different treatment groups of the rats




## EFFECT OF TIGER NUT MEAL ON THE HISTOLOGY OF THE TESTES

Control photomicrograph of testes shows intact seminiferous tubules of uniform size with orderly germ cell maturation variable around the tubule, supported by the Sertoli cells. The mature spermatid density was variable in tubule and on average were 300 per tubule. The Leydig cells were also orderly differentiated. Testicular cross section of rats induced with BPH revealed significant pathology and distortion of the cytoarchitecture of the testes. Significant azoospermia was however observed in the testicles of rats with an average matured spermatid density of about 30 per tubule. Following the administration of the tiger nut meal, the spermatid density was improved as well as the restoration of normal cytoarchitecture of the Sertoli and Leydig cells of the testes.



## DISCUSSION

The effect of tiger nut meal on the Prostate Specific Antigen (PSA) showed a significant decrease (in both the treated and the apparently normal rats administered with tiger nut) in the levels of the PSA. The PSA is a protein produced mainly in the prostate gland. Its level vary from day to day and it is used in the diagnosis and management of patients with prostatic diseases such as benign prostate hyperplasia (BPH). Serum PSA correlates with prostate volume, and men who have large prostates and high serum PSA are at a higher risk of experiencing more significant symptoms, including progression to acute urinary retention. Thus, following the induction of infertility and BPH in the experimental animals, the levels of the PSA increased in the negative control. This finding is an indication of inflammation of the prostate. Histological studies also indicated the presence of benign prostate hyperplasia in the induced group. The administration of tiger nut meal to the rats (in the treatment groups) was able to significantly reduce the level of the PSA.

The mechanism by which the tiger nut meal was able to protect against BPH in rat model may be due to the level of phenolic compounds present in the plant. A variety of polyphenols are known to have the ability to inhibit testosterone 5alpha-reductase activity and so prevent the development of BPH (Idakwoji et al., 2018). On the other hand, since the administration of tiger nut meal to the rats in normal condition show no variation when compared with the normal control, it shows that the consumption of tiger nut has no adverse effect on the prostate. Again, this report is consistent with the finding of Idakwoji et al. (2018).

The effect of tiger nut meal on the histology of prostate and testes of the rats were studied and the results of this study showed that tiger nut ameliorated the enlargement of the prostate in animals with induced BPH and infertility. Tiger nut also enhanced the prostate of animals that are apparently normal though with visible deposit of adipose tissues. The enlargement of the organ is seen as a confirmation of the diagnosis of the histological pathology characterized by proliferation of the cellular elements of the prostate which involves the stromal cells.

Male fertility requires the cooperation of the different organs of the male urogenital system, each carrying out its assigned function. Male fertility requires the cooperation of the different organs of the male urogenital system, each carrying out its assigned function.

The prostate therefore, is one of the major male reproductive gland involved in male fertility. Indeed, male fertility intrinsically relies upon the content of the prostatic fluid secreted by the prostate epithelium. The key contribution of the prostatic fluid to male fertility is linked to its role as the trigger for each of the molecular pathways involved in ejaculation and, subsequently, in sperm activation and capacitation (Gilany et al., 2015). In this study, tiger nut was seen to have contributed greatly in enhancing this action of the prostate.

The effect of tiger nut meal on the relative weight of prostate was studied and the result showed that there was a significant decrease in relative weight of the prostate following the administration of tiger nut meal to the animals that had induced infertility and benign prostate hyperplasia (BPH). Also, tiger nut meal reduced the relative weight of the prostate in apparently normal animals that were not induced with BPH and infertility. However, it is important to note that lower dosage of the meal has more decrease compared to the high dose. This finding suggests that, ingestion of tiger nut at higher doses may have a negative effect on the weight of the prostate whereas, at a lower dose, it may have a good effect on the prostate.

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