Effect of Tiger Nut Meal on Food/Water Intake, Body Weight Changes and Semen Abnormality in Androgen-Induced BPH in Adult Male Wistar Rats

Abstract

Introduction: Food and water intake correlate positively. This may be due to their mutual dependence on body size, but an additional mechanism directly linking food and water intakes may also be involved. It is also 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.

Objective: The aim of this project was to study the effects of tiger nut meal on Food and water intake, Total Protein, Body weight changes, Semen consistency and Total abnormality of the sperm cell following induction of BPH and its treatment.

Method: 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, blood sample was collected from the rat via cardiac puncture for the determination of Total Protein. Semen samples were collected for analysis of semen Total Abnormality and Consistency (Thickness). The food/water intake as well as body weight changes were also studied.

Results: Following the induction of BPH, the administration of tiger nut meal showed some positive effects on the rats. On Total Protein (TP), the administration of the tiger nut meal increased the TP levels in both the induced and non-induced ($p<0.05$). On food and water intake, tiger nut meal enhances the intake of food at high dosage($p<0.05$) while at the same time, decreasing water intake in the rats ($p>0.05$). On weight changes, there is a significant decrease in the weight of the rats. ($p>0.05$). Also, the administration of tiger nut meal to the rats significantly decreased the level of total abnormality of the semen recorded following the induction of BPH ($p>0.05$) while at the same time, enhancing the Semen consistency (Thickness of the sperm) ($p<0.05$).

Conclusion: Tiger nut meal decreases the rate of water intake, body weight of the rats and total semen abnormality while at the same time, enhancing food intake, Total Protein and semen consistency.

1. Introduction

Feeding, drinking and body weight are interrelated and influenced by both genetic and environmental factors (1). They are critical variables in many types of experiments, such as those involving metabolism, nutrition, or dosage of pharmacological agents.

Food and water intake also correlated positively. This may be due to their mutual dependence on body size, but an additional mechanism directly linking food and water intakes may also be involved. Rodents on pelleted diet consume most of their water immediately before and after they eat food, which is probably due to both osmotic and volumetric stimulation of thirst (2). Thus, rats that eat more would tend also to drink more. However, inspection of individual values indicates that a component of the variation in water intake is independent from food intake.

Food intake by the laboratory animal meets the energy requirements for maintenance, growth, physical activity, heat production, and other physiological processes. Water intake compensates for the losses by evaporation and by excretion in urine and faeces. Food and water intake show a marked daily variation, as they are strongly coupled to the circadian rhythm of waking–sleeping behaviour. Numerous biochemical and physiological factors are in turn connected with food and water intake and their subsequent daily variation may affect the responsiveness of the animal to experimental stimuli. The availability of food and water thus contributes to the drama type of the experimental animal.

This study was designed to assess the effects of tiger nut on feeding, drinking and body weight changes including some semen parameters after the induction of Benign Prostate Hyperplasia (BPH). The hypotheses tested in this part of the study reported here were that tiger nut would enhance food and water intake moderately, prevent or reduce overweight and obesity and decrease semen toxicities in measured physiological parameters.

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.

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

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 Lorke (3). 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 and plan of the experiments

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 dysfunction + Low dose (10% of meal)

Group 4 dysfunction + 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.

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 DHT and estradiol valerate dissolved in olive oil in the ratio of 10:1 (three times in a week) as described by Izunwanne (4).

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 after the induction and it lasted a period of two months.

2.9 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 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 fascia,

washed in cold normal saline, blotted with filter paper and weighed on a sensitive balance.

2.10 Qualitative Phytochemical Studies on *Cyperus esculentus*

Phytochemical methods of Trease and Evans (5) and Harborne (6) were used in the study.

2.11 Quantitative Phytochemical Analysis of *Cyperus esculentus*

The quantitative phytochemical analysis of *Cyperus esculentus* was determined using standard methods described by (6,7,8 and 9)

2.12 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.

2.12.1 Macroscopic Examination

The semen colour and consistency were evaluated macroscopically and recorded. The consistency scale (1-4), adopted by (10) was used.

2.12.2 Abnormal Sperm Proportion

The abnormal sperm proportion was determined by the method described by (11). 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, mid-piece abnormalities etc.

GONADOSOMATIC INDEX (RELATIVE ORGAN WEIGHT) = $\frac{\text{Weight of Organ (g)}}{\text{Live Weight(g)}} \times 100$

12.13 Statistical Analysis

Statistical analysis was carried out using windows (SPSS version 15.0). Data were analyzed 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 Phytochemical Composition of *Cyperus esculentus*

The quantitative phytochemical composition of *Cyperus esculentus* shows the presence of phytochemicals in varying quantities. There was a high concentration of flavonoids and alkaloids. There was moderate concentration of tannins and small quantities of cardiac glycosides, steroids, saponins, phenolic compounds and terpenes (Table 1).

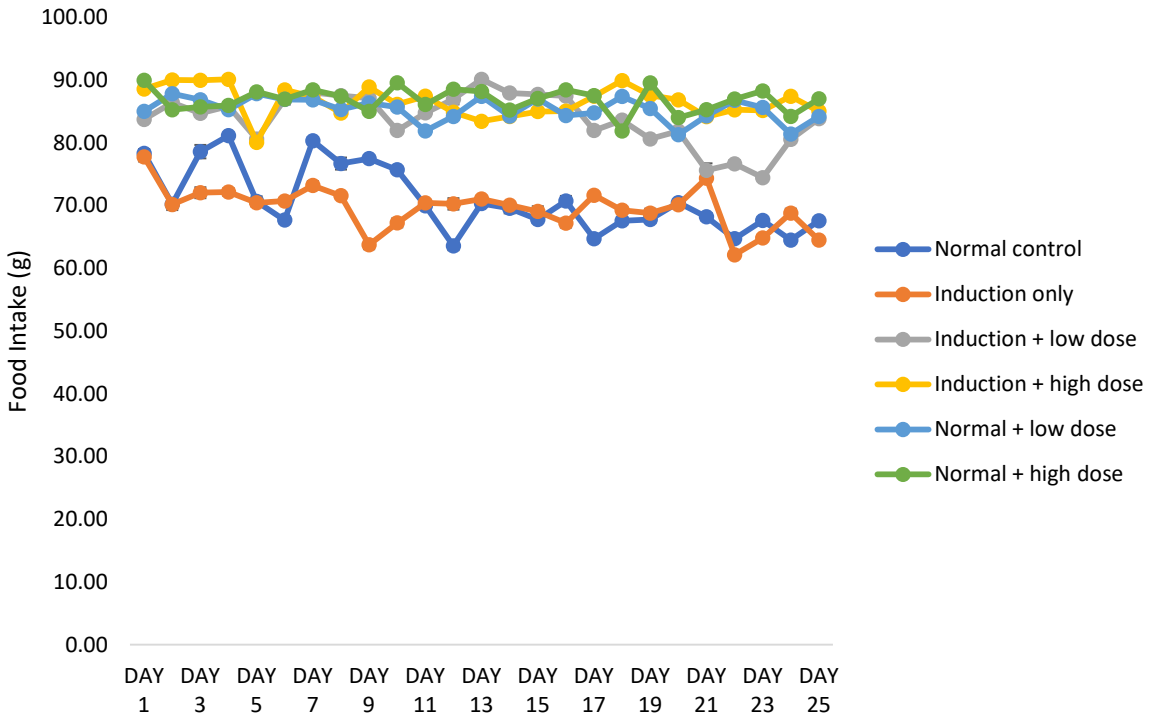
Table 1: Phytochemical analysis of tiger nut extract

Parameters	Qualitative	Quantitative
Alkaloids	+++	8.37 mg/100g
Cardiac glycosides	+	1.15 mg/100g
Steroids	+	1.02 mg/100g
Tanins	++	7.62 mg/100g
Flavonoids	+++	9.82 mg/100g
Saponins	+	1.62 mg/100g
Phenolic compounds	+	3.93 mg/100g
Terpenes	+	0.87 mg/100g

3.2 Effect of tiger nut meal on Food intake (g)

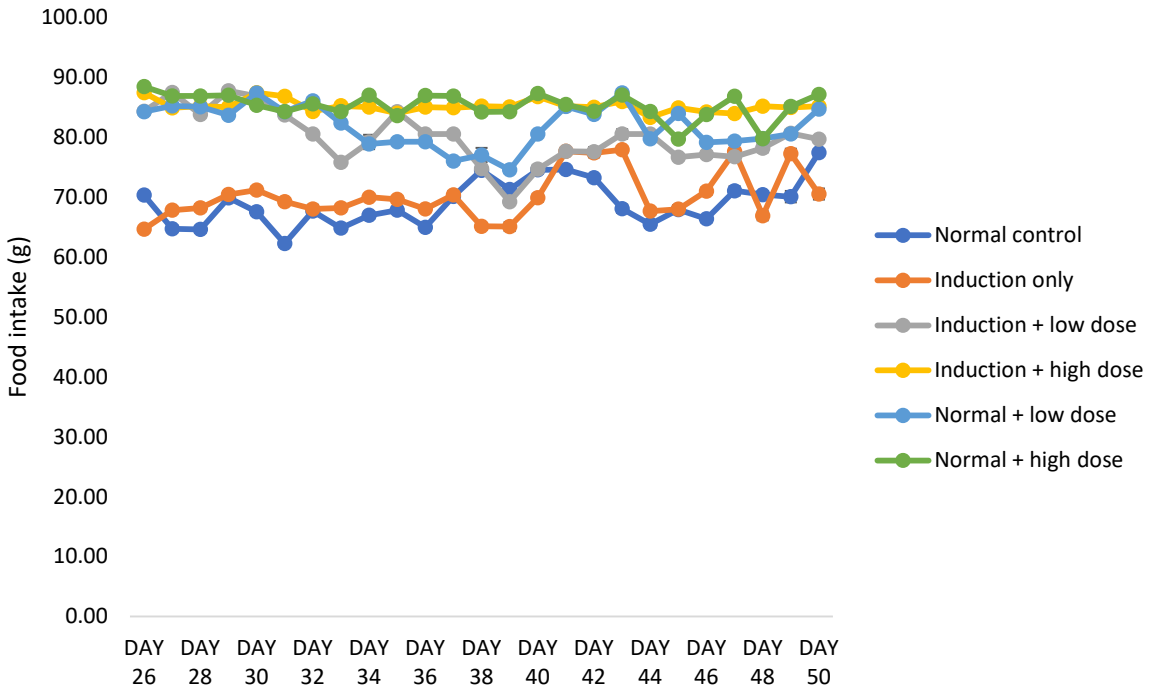
Figure 1 and 2 showed the results of the effect of tiger nut meal on food intake. The control groups were fed with normal animal feed. The food intake of the negative control significantly decreased when compared with the normal control ($p<0.05$).

The administration of both low and high doses of tiger nut meal to the induced and non-induced groups showed a significantly enhanced intake of food by the animals. The higher the percentage of tiger nut in the meal, the more enhanced the food intake. The groups administered with low doses however, showed increase in food intake when compared with controls ($p<0.05$).



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

Figure 1: Effect of tiger nut on Food intake



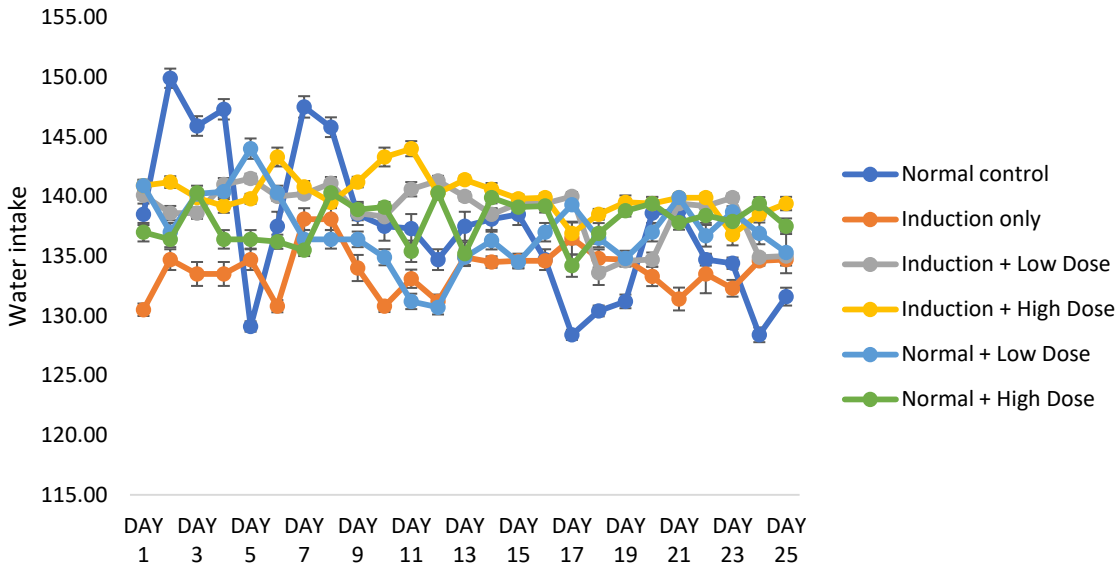
Values are expressed as mean ± SEM, n=10, parameters in the column with the same alphabet are statistically the same ($p>0.05$), parameters with different alphabets are statistically different ($p<0.05$)

Figure 2: Effect of tiger nut on Food intake extension

3.3 Effect of tiger nut meal on water intake (ml)

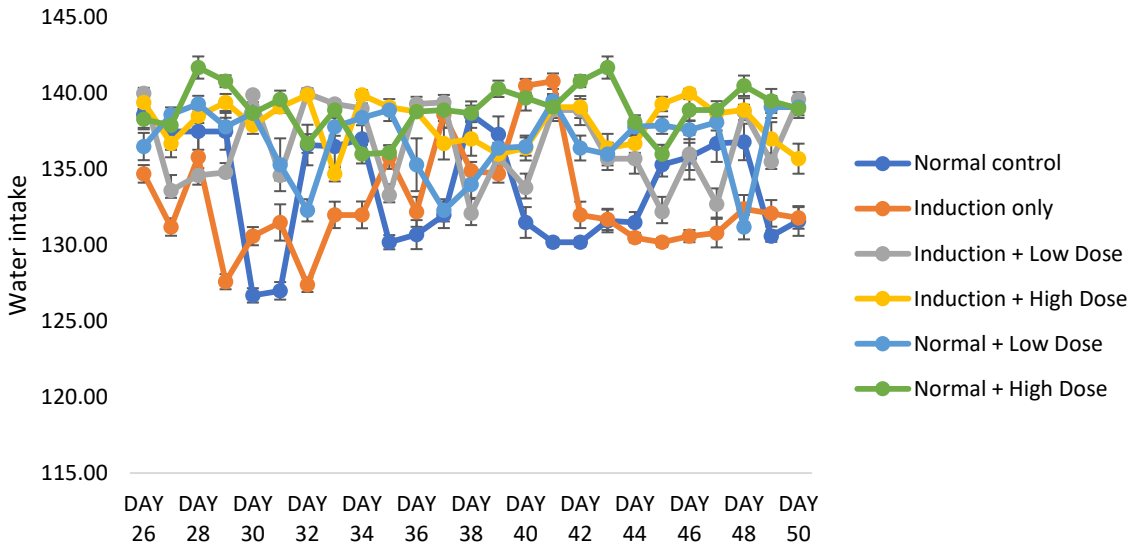
Figure 3 and 4 showed the results of the effect of tiger nut meal on water intake. The control groups and the other experimental groups were given normal water to drink. The water intake of the animals in the negative control significantly decreased when compared with the normal control following the induction of BPH ($p<0.05$).

The results of water intake in post-treatment groups of both low and high doses of tiger nut meal showed that the introduction of tiger nut meal at high doses significantly decreased the intake of water by the animals. This finding is also seen in the apparently normal animals following the administration of tiger nut meal ($p<0.05$).



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

Figure 3: Effect of tiger nut meal on water intake



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

Figure 4: Effect of tiger nut meal on water intake (extension)

3.4 Effect of tiger nut meal on the Total protein (g/dl)

Table 2 showed that after induction of BPH, there was a significant decrease in the level of total protein to 5.69±0.12 in the negative control group ($p < 0.05$).

Treatment of the BPH with the tiger nut meal after induction shows that, at both low and high doses of 10% and 20% respectively, the level of total protein increased significantly ($p < 0.05$).

Furthermore, the administration of tiger nut meal to the rats under normal condition at both low and high doses showed an increase the level of total protein. ($p < 0.05$).

Table 2: Effect of tiger nut meal on Total protein

Parameters	Control	Induction only	Induction + Low dose (10%)	Induction + High dose (20%)	Low dose (10%)	High dose (20%)
TP (g/dl)	**7.05±0.09 ^{c}**	**5.69±0.12 ^{a}**	**6.45±0.11 ^{b}**	**6.58±0.08 ^{b}**	**7.21±0.08 ^{c}**	**7.38±0.07 ^{c}**

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

3.5 Effect of tiger nut meal on the body weight changes (g)

Table 3 showed that after the 50 days of experimentation, there was a statistical increase in the body weight in the treatment groups ($p>0.05$).

The post-induction treatment with low and high doses showed there is a consistent increase in the body weight following the administration of tiger nut meal however, that of low dose tend to increase the weight more than that of high doses ($p>0.05$).

The administration of low and high doses of tiger nut meal to the apparent normal animals showed a consistent increase in the weight of the animals ($p>0.05$).

Table 3: Effect of tiger nut meal on Body weight changes

		Weight changes (g)		
Groups	**Treatments**	**Before induction**	**After induction**	**After experiment**
1	Normal control	135.30 ± 1.71^{b}	170.00 ± 1.60^{d}	211.91 ± 2.44^{a}
2	Induction only	150.59 ± 3.62^{a}	151.53 ± 4.02^{a}	179.37 ± 3.39^{b}
3	Induction + low dose	131.36 ± 2.25^{b}	133.23 ± 2.99^{c}	212.95 ± 3.58^{a}
4	Induction + high dose	133.09 ± 1.78^{b}	134.97 ± 2.26^{bc}	178.86 ± 3.09^{b}
5	Normal + low dose	139.45 ± 5.00^{b}	144.79 ± 6.47^{ab}	224.04 ± 11.36^{a}
6	Normal + high dose	130.81 ± 3.40^{b}	130.96 ± 2.96^{c}	190.65 ± 7.92^{d}

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

3.6 Effect on Semen Consistency (Sperm thickness) (1-4)

Table 4 below shows that the level of the semen consistency in the normal control group was 2.40±0.22. After induction of BPH, there was a significant decrease in the level of consistency of the semen to about 1.30±0.21 in the negative control group. This value is statistically lower compared to the normal control ($p < 0.05$).

Treatment of the BPH with the tiger nut meal after induction showed that, at both low and high doses, the level of semen consistency increased significantly ($p < 0.05$).

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 semen consistency ($p < 0.05$).

Table 4: Effect of tiger nut meal on semen consistency (thickness of sperm)

Groups	Treatment	Semen consistency
1	Normal control	2.40 ± 0.22^{c}
2	Induction only	1.30 ± 0.21^{d}
3	Induction + low dose	2.00 ± 0.26^{c}
4	Induction + high dose	3.10 ± 0.18^{b}
5	Normal + low dose	3.70 ± 0.15^{a}
6	Normal + high dose	3.30 ± 0.15^{ab}

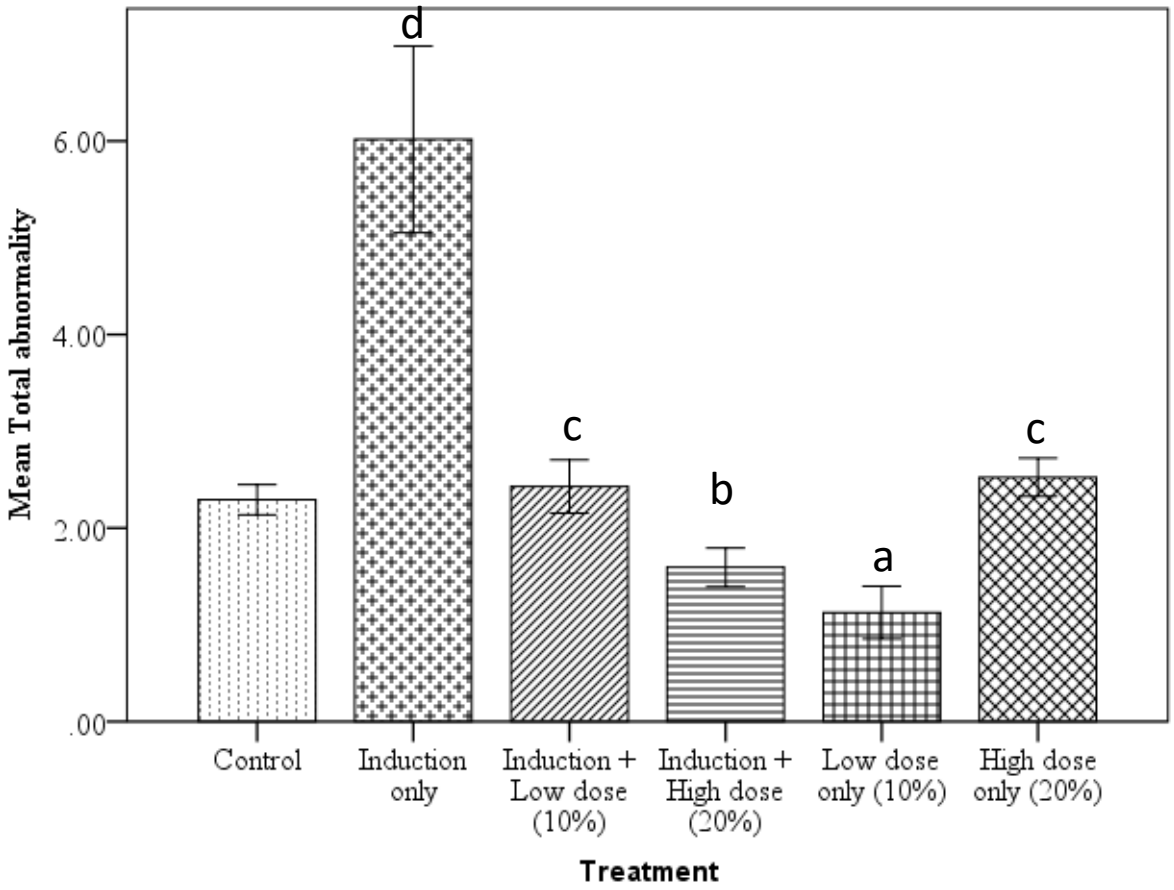
Values are expressed as mean ± SEM, n=10, parameters in the column with the same alphabet are statistically the same ($p>0.05$), parameters with different alphabets are statistically different ($p<0.05$)

3.7 Effect of the tiger nut meal on total abnormality of the sperm cells (%)

Figure 5 shows that the total count of sperm cells with abnormality in the normal control group was 2.29±0.08. After induction of BPH, there was a significant increase in the level of total abnormality in sperm cells to about 6.02±0.48 in the negative control group ($p<0.05$).

However, treatment of the BPH with the tiger nut meal showed that, at both low and high doses of the tiger nut meal, a significant decrease in the percentage of the sperm cells with total abnormality was recorded in the animals when compared with the negative control of 6.02±0.48 ($p<0.05$).

Furthermore, the administration of tiger nut meal to the rats under normal condition at low dose of 10% (1.13±0.14) showed a significant decrease when compared with the normal control ($p<0.05$) however, that of high dose (20%) 2.53±0.10 showed a statistical decrease when compared with the negative control ($p<0.05$) and has no significant decrease when compared with the normal control ($p>0.05$).



Values are expressed as mean ± SEM, n=10.

d = $p < 0.05$ vs control
c = $p < 0.05$ vs induction only
b = $p < 0.05$ vs induction only
a = $p < 0.05$ vs control

Figure 5: Effect of tiger nut meal on the Total abnormality of the sperm cell.

Discussion

The effect of tiger nut meal on total protein showed that there was a significant increase in the total protein level following the administration of tiger nut to animals that had induced BPH. Tiger nut also improved the total protein levels in apparently normal animals that were not induced with BPH. The normal range for total protein levels in blood serum is 6 – 8 grams per decilitre (g/dl). The initial decrease recorded in the animals following the induction of BPH could be as a result of either malabsorption disorder e.g., celiac disease or kidney disease such as nephrotic syndrome or glomerulonephritis which could lower the protein levels. According to (12), this effect of tiger nut to ameliorate the lower levels of the total protein may be as a result of its rich content of essential and non-essential amino acids.

The effect of tiger nut meal on food intake, water intake and body weight changes were also studied. The results of the effect of tiger nut meal on food intake showed that there was an increase in food intake in the groups administered with high doses more than the groups with lower doses. This finding suggests that tiger nut may be stimulating the release of some hormones (such as ghrelin) which increases the appetite of the animals. It has also been discovered that ghrelin reduces energy expenditure of the body. On water intake, the study showed that tiger nut does not stimulate/enhance the intake of water in the animals. On body weight changes, the results showed that the administration of tiger nut over a prolonged period of time, brought about a decrease in the body weight. Irrespective of the fact that tiger nut is rich in fatty acid, previous studies has shown that most of the fats in tiger nut are found in the nut's cell walls which do not easily breakdown during digestion. Most of the fats are passed out through the faeces. Furthermore, tiger nut has been found also to be rich in fibre and calories. These do not add much weight on the body.

The effect of *Cyperus esculentus* (tiger nut) on semen parameters namely; semen consistency (thickness) and total abnormality were also studied. The results of this study showed that tiger nut ameliorated the high level of abnormality in the sperm cells. There was a significant increase in the level of semen consistency following the administration of Tiger nut to animals that had induced BPH. Also, tiger nut enhanced the levels of the semen consistency (thickness) in apparently normal animals that were not induced with BPH. This finding may be associated with the increase in testosterone hormone level and it's consistent with the earlier report of Izunwanne (13).

Tiger nut (*Cyperus esculentus*) was carefully studied and analysis of its phytochemical composition was carried out in order to ascertain the presence of phytochemicals that were qualitatively and quantitatively present. The result as seen in Table 1 showed a relatively high concentration of flavonoids, alkaloids and even tannins while bioactive compounds like saponin, phenolic compounds terpenoids and glycosides were present in small concentrations.

Flavonoid is a potent antioxidant and free radical scavenger and has been shown to protect cell membranes from damage (14). Also, In vitro studies have also shown that flavonoids have anti-allergic, anti-inflammatory and anti-cancer activities (15). Therefore, the *Cyperus esculentus* might be ascribed with these potentials.

Tannins have astringent properties that affect palatability, reduce food intake and consequently body growth. It also hastens the healing of wounds and prevention of decay. Tannin compounds have antimicrobial activities and are responsible for preventing and treating urinary tract infections and other bacterial infections (16). They are known to inhibit the activities of digestive enzymes and nutritional effects of tannins are mainly related to their interaction with protein. Tannin protein complexes are insoluble and protein digestibility is decreased (17).

Saponins are known to reduce certain nutrients like glucose and cholesterols at the gut through intra-lumenal physicochemical interactions (18). Also, when saponins are consumed they may aid in lessening the metabolic burden that would have been placed on the liver. They are known to inhibit the structure dependent biological activities (19). Saponins have been reported to be useful in reducing inflammation of upper respiratory passage and also chiefly as foaming and emulsifying agents and detergents. These compounds serve as natural antibiotics, helping the body to fight infections and microbial invasion (20).

Alkaloids, saponins and tannins are known to have anti-inflammatory activities as well as other physiological activities (21;22). Alkaloids are known for their toxicity but not all alkaloids are toxic. Alkaloids inhibit certain mammalian enzymatic activities such as those of phosphodiesterase, prolonging the action of cAMP.

While some other forms have been reported to be carcinogenic while some have been used either as an analgesic, antispasmodic or bactericidal agents (23). The result obtained from the phytochemical test indicates that the *Cyperus esculentus* possesses some biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine.

Conclusion

From the results analysed, it can be said that tiger nut meal decreases the rate of water intake, body weight of the rats and total semen abnormality while at the same time, enhancing food intake, Total Protein and semen consistency.

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