

# The effect of aqueous extract of tiger nut on body weight changes, gonadosomatic index and spermogram of cyclophosphamide treated rats

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**ABSTRACT:** Tiger nut (*Cyperus esculentus*) is consumed by humans all over the world. It has also been found to have many medicinal uses, with notable effect on sperm parameters. Cyclophosphamide is an alkylating agent widely used as an anticancer that has been reported to have some toxic effects on sperm parameters. Hence, this study was designed to evaluate the effect of aqueous extract of tiger nut on reproductive parameters of cyclophosphamide treated male Wistar rats. Thirty-six male Wistar rats weighing between 170 and 200 g, aged between 18 and 20 weeks were randomly divided into six groups (five test groups and a control group) of six rats each. The control group received 1 ml of normal saline per animal, while the remaining five groups were orally administered with 2.5 and 5.0 mg/kg doses of cyclophosphamide alone; 2.5 and 5.0 mg/kg doses of cyclophosphamide with 500 mg/kg aqueous extract of *Cyperus esculentus*, and the aqueous extract (500 mg/kg) alone for 4 weeks. The differences observed for changes in body weight, gonadal volume, sperm motility and sperm livability were not statistically significant ( $p > 0.05$ ), but the sperm count was significantly higher ( $p < 0.05$ ) in the 5 mg/kg cyclophosphamide alone and 2.5 mg cyclophosphamide plus *Cyperus esculentus* treated groups compared to the control and other groups. Total sperm abnormalities were also significantly higher with cyclophosphamide groups and even control group as compared to aqueous extract of *Cyperus esculentus* alone. The result of this study shows that aqueous extract of *Cyperus esculentus* could be used for preserving fertility, as well as to reduce sperm morphological abnormalities that could arise from the use of cyclophosphamide.

**Keywords:** *Cyperus esculentus*, cyclophosphamide, spermogram, body weight, Wistar rats.

## INTRODUCTION

Tiger nut (*Cyperus esculentus*) is an underutilized crop which belongs to the family; Cyperaceae. Tiger nut is one of the earliest domesticated crops and in fact, was found in vases and was used to embalm bodies of the Egyptian Pharaohs (Watt and Breyer-Brandwijk, 1962). In Nigeria, tiger nut is available in fresh, semi-dried and dried form in the markets where it is sold locally and consumed even uncooked (Sanful, 2009). Tiger nuts are under-utilized due to lack of information on their nutritional potential (Sanful, 2009).

Tiger nut can be used to produce drink/milk, which can serve as a substitute for the traditional cow milk (Gambo and Da'u, 2014). Tiger nut milk has never been found to produce allergy (Belewu and Abodurin, 2008). It also finds

uses as a flavouring agent for ice cream, biscuits (Cantalejo, 1997), as well as in making oil, soap, starch and flour (Adejuyitan, 2011). It can also be used to produce a local snack "Dakuwa" (Gambo and Da'u, 2014).

Tiger nut is not a real nut; despite its name, tiger nut is a tuber. However, its chemical composition shares characteristics with tubers and with nuts. The moisture content is lower than the moisture contents reported for true tubers such potato (Lombardo et al., 2012).

The vitamin analysis of raw tubers of *Cyperus esculentus* have been shown to include  $0.12 \pm 0.01$  mg/100g vitamin A,  $7.30 \pm 0.97$  mg/100g vitamin B,  $0.42 \pm 0.02$  mg/100g vitamin D and  $0.74 \pm 0.09$  mg/100g vitamin E. Whereas, the roasted tubers contained

0.20±0.01 mg/100g vitamin A, 4.59±0.09 mg/100g vitamin C, 0.41±0.01 mg/100g vitamin D and 0.57±0.10 mg/100g vitamin E (Ekeanyanwu and Ononogbu, 2010).

Tiger nut has high calcium, sodium and phosphorus and low magnesium, manganese, iron, zinc and copper mineral contents (Arafat et al., 2009). The amino acids profile was dominated by aspartate, which resulted from the conversion of asparagine (Borges et al., 2008 Oladele and Aina, 2007).

Ekaluo et al. (2015) also researched on the effect of aqueous extract of *Cyperus esculentus* on sperm parameters and testosterone level of male albino rats. The study proved that aqueous extract of *Cyperus esculentus* has antioxidant and androgenic properties with the capability of increasing the weights of the testes and epididymes, sperm count, sperm quality and testosterone level. Hence, they posited that aqueous extract of *Cyperus esculentus* could be used as a possible fertility booster and in attenuating sperm and reproductive toxicities.

Cyclophosphamide shows antitumor activities against a broad range of cancers including malignant lymphomas, myeloma, leukemia, neuroblastoma, adenocarcinoma, retinoblastoma, and breast-carcinoma (Moore 1991; Chhipa et al., 2006; Gilbert et al., 1998). Cyclophosphamide given at low doses act either as an anti-angiogenic or an immunostimulatory agent in combination with other immunotherapies in the treatment of cancer (Pietras et al., 2005; Loeffler et al., 2005). It is also a well-known immunosuppressive agent used for graft rejection in case of renal, hepatic, and cardiac transplantation (Anderson et al., 1995).

Cyclophosphamide may be administered either intravenously or orally. To some degree, the side effects of cyclophosphamide differ according to the dose and the way the drug is administered. These side effects include; nausea, vomiting, alopecia, dizziness, infection, bone marrow suppression, hypersensitivity, cancer, bladder problems, infertility, etc (Panigrahy et al., 2011).

Trasler et al. (1986) evaluated the effect of chronic low dose cyclophosphamide treatment of male adult rat's fertility, pregnancy outcome and progeny. The study was carried out for a period of 11 weeks. From the result, a decrease in spermatozoa numbers was observed in all the 3 cyclophosphamide treated groups (at dosage 1.4, 3.4 and 5.1mg/kg), the decrease, however, was significant only for the 5.1 mg/kg group.

Having established previously, the deleterious effect of cyclophosphamide on different body systems including the reproductive organs, as well as the positive influence of tiger nut with regards to its anti-oxidative and protective properties. There is still a dearth of information on the possible effect that consumption of the two will have on reproduction in male animals.

Hence, the objective of this study was to evaluate the effects of aqueous extract of tiger nut on body weight changes, gonadosomatic index and spermiogram of cyclophosphamide treated rats.

## MATERIALS AND METHODS

### Experimental animal and management

Thirty-six sexually matured male Wistar rats were used for this study. Each animal weighed between 180 to 200 g and aged 18 to 20 weeks. These rats were housed in the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Oyo State, Nigeria. University of Ibadan is between latitude 3°54'E and latitude 7°26'N at a mean altitude of 277 m above sea level. The annual rainfall is about 1200 mm, most of which falls between April and October, and a dry season from November to March (Oyeyemi and Fayomi, 2011) during which this work was done. The rats were kept in rectangular plastic cages of about 60 cm by 40 cm, having a height of about 20 cm, and the cover was made of wooden materials and wire mesh.

The bedding was provided with wood shavings, which was replaced every week. They were fed *ad libitum* with commercial rat feed (produced by Premier Feeds Limited, Ibadan), a ration containing 21% crude protein, 3.5% fat, 6% crude fibre, 0.8% phosphorus, 2% calcium, 0.82% Lysine, 0.47% Methionine, and Metabolisable energy-2850kcal/kg. Water was supplied *ad libitum*. The feed and water were given using earthen troughs.

### Preparation of the aqueous extract

The tiger nut (*Cyperus esculentus*) fruit was purchased from Ojoo area of Ibadan, Oyo state, Nigeria. Three kilograms (3 kg) of fresh tiger nuts was dried via a vacuum oven at 40°C for a week (the length of time for drying depends on the moisture content of the item being dried, some take longer time). The dry tiger nuts were then ground and weighed. One kilogram (1 kg) of the pulverised sample was soaked in 4 litres of distilled water for 24 hours and the solution was filtered using Whatman's filter paper. The resulting filtrate was concentrated using Rotary evaporator, and then dried further using vacuum oven at 40°C to yield a 201 g crude extract.

### Extract reconstitution

Five grams (5.0 g) of the extract was weighed using a digital micro-sensitive weighing scale, which was thereafter mixed with normal saline to make a 50 ml solution in a beaker to constitute a 100 mg/ml concentrations preparation. This was gently stirred with a spatula.

### Administration of extract

The rats were randomly divided into six groups (A to F) of 6 rats each. The group A served as the control and

received normal saline, while group B animals were given 500 mg/kg of the aqueous extract of tiger nut (AET). Groups C and D were given cyclophosphamide (CYP) at the dose 2.5 and 5.0 mg/kg, respectively, whereas groups E and F had same treatment as C and D respectively, plus the treatment given to Groups B. i.e. Groups A: normal saline; Groups B: 500 mg/kg AET; Groups C: 2.5 mg/kg CYP; Groups D: 5.0 mg/kg CYP; Groups E: 2.5 mg/kg CYP + 500 mg/kg AET; Groups F: 5.0 mg/kg CYP + 500 mg/kg AET. These dosing were done orally in the morning (07:00 to 09:00 h) using oral cannula and syringe for 28 days.

### Sample collection

At the end of the experiment, the animals were weighed and blood samples collected from the retro-orbital venous complex of the eyes into EDTA bottles and plain tubes (Fried et al., 2015), and all the animals were thereafter euthanized by decapitation. A mid-caudoventral abdominal incision was done with the aid of a sterile scalpel blade to immediately exteriorize the genital tracts. The testes were immediately severed and weighed using a sensitive electronic weighing scale.

### Gonado-somatic index

The gonado-somatic index (GI) was calculated [as  $GI = \text{Gonad weight} / \text{Body weight} \times 100\%$ ], and recorded as mean percentages and standard error of means (Oyeyemi and Fayomi, 2011).

### Spermiogram

#### *Sperm motility*

A small drop of semen was placed on a clean warm glass slide and mixed with one drop of warm 2.9% sodium citrate which was then covered with a coverslip and viewed under a light microscope at a magnification of X40. Only the sperm cells moving in a unidirectional motion were included in the motility count, while sperm cells moving in circles, those in a backward direction or those showing pendulating movement were excluded (Logue and Alstair, 1987).

#### *Sperm livability*

Sperm livability was tested using the eosin-nigrosin stain. This was carried out by staining one drop of semen with one drop of eosin-nigrosin stain on a clean warm slide. A thin smear was then made of the mixture of semen and stain. The smear was air dried and viewed under a light microscope. The ratio of the *in vitro* dead sperm cells was

observed, which is based upon the principle of the eosin penetrating and staining the dead sperm cells whereas viable sperm cells repel the staining (Zemjanis, 1970).

### *Sperm count*

Semen was collected from the epididymal secretion of the right epididymis cauda. The secretion was placed in a 0.3 mL drop of physiological serum extract and later diluted in distilled water. From this homogenate, a sample was taken and a sperm count was obtained using a hemocytometer with improved double Neubauer ruling under the light microscope at X400 magnification. The count was expressed as million/ml of suspension (Moraes, 1994).

### *Sperm morphology*

This is used to determine the presence and incidence of morphologically defective spermatozoa. The sperm cells were observed for morphological defect using Wells and Awa stain. A drop of the semen was placed on a clean warm glass slide, and with another slide a smear was made and then stained with Wells and Awa stain. The stained smear was air dried and observed under a light microscope; OLYMPUS MODEL CX21FS1. The spermatozoa abnormalities noted were classified into head, mid-piece and tail abnormalities according to Zemjanis (1970).

### Data analysis

Data collected were presented as Mean $\pm$ SEM, and were analyzed using the descriptive and inferential statistical method. One-way Analysis of Variance (ANOVA) was used to compare mean values across the experimental groups. Tukey's Multiple Comparison Test was used for post-hoc pair comparison. All inferential statistics were tested at the 0.05 significance level ( $p < 0.05$ ). Data management and analysis were carried out using Microsoft Office Excel 2013 and GraphPad Prism Version 7.00.

## RESULTS AND DISCUSSION

The results in Table 1 show the weekly body weight of the control and treated groups of rats dosed with cyclophosphamide and aqueous extract of tiger nut while the gonadosomatic index of the control and treated groups of rats are presented in Table 2. The results in Table 3 show the spermiogram and in Table 4 present sperm morphological abnormalities of the control and treated groups of rats dosed with cyclophosphamide and aqueous extract of tiger nut.

In this study, there was no significant difference ( $p > 0.05$ )

**Table 1.** Weekly Body weight of the control and treated groups of rats dosed with cyclophosphamide and aqueous extract of tiger nut.

Weeks	A	B	C	D	E	F
Week 0 (g)	195.8±6.208	196±2.236	196±4.806	196.4±7.593	196.4±6.242	195.8±9.047
Week 1 (g)	207.4±8.858	191.2±6.224	206.2±6.461	203.6±5.240	201.6±3.970	196.4±7.652
Week 2 (g)	206.4±9.811	188.2±6.844	200.6±6.742	207.8±2.332	200.6±3.487	197±8.044
Week 3 (g)	209.2±11.218	201.8±5.267	196.8±9.942	208.8±1.800	204.6±5.066	183±12.239
Week 4 (g)	187.2±15.392	175±11.756	187.4±13.732	208.2±1.112	188±11.683	191±8.905

Mean ± SEM with similar superscripts are statistically significant at  $p < 0.05$ .

**Table 2.** Gonadosomatic index of the control and treated groups of rats dosed with cyclophosphamide and aqueous extract of tiger nut.

Parameter %	A	B	C	D	E	F
Testis to body weight	0.599±0.0356	0.615±0.0478	0.720±0.0826	0.548±0.0486	0.643±0.0441	0.646±0.0205

Mean ± SEM with similar superscripts are statistically significant at  $p < 0.05$ .

**Table 3.** Spermogram of the control and treated groups of rats dosed with cyclophosphamide and aqueous extract of tiger nut.

Parameters	A	B	C	D	E	F
Sperm motility (%)	78±7.517	82.6±5.316	83.2±3.917	82.2±8.80	97.4±0.980	85.6±5.980
Sperm liveability (%)	83±5.385	84.2±4.152	89.2±2.577	88.6±3.750	95.4±0.600	88.4±3.696
Sperm count	140.2±9.947	136.8±3.597	157.2±9.058	179.6±3.356 <sup>ab</sup>	184±5.450 <sup>ab</sup>	154.6±5.741 <sup>e</sup>

Mean ± SEM with superscripts **a** are statistically significant to group A at  $p < 0.05$ ; Mean ± SEM with superscripts **b** are statistically significant to group B at  $p < 0.05$ ; Mean ± SEM with superscripts **e** are statistically significant to group E at  $p < 0.05$ .

**Table 4.** Sperm morphological abnormalities of the control and treated groups of rats dosed with cyclophosphamide and aqueous extract of tiger nut.

Parameters	A	B	C	D	E	F
Bent Tail	12.4±3.076	5±1.140	15.2±2.083 <sup>b</sup>	11.6±0.812	6±0.775 <sup>c</sup>	8.8±1.281
Coiled Tail	13±2.811	11.6±1.503	18.6±2.542	15.6±2.293	13.2±2.396	20.2±3.056
Abnormal Mid-piece	0.6±0.4	5.8±1.356 <sup>a</sup>	3.2±0.860	3±0.316	2.4±0.927	7.2±1.497 <sup>ae</sup>
Tailless Head	1.4±0.678	1.8±0.663	6.8±1.114 <sup>ab</sup>	4.6±0.510	6.4±1.364 <sup>ab</sup>	5.8±1.281 <sup>a</sup>
Headless Tail	1.4±0.4	1.8±0.2	10.2±2.354 <sup>ab</sup>	5.6±0.927	3.4±0.510 <sup>c</sup>	3.4±0.927 <sup>c</sup>
Abnormal Head	0±0	0.25±0.193	0±0	0±0	0.4±0.245	0.25±0.194
Curved Tail	73±3.0	44±2.846 <sup>a</sup>	74.8±9.030 <sup>b</sup>	79.6±4.007 <sup>b</sup>	62.4±6.524	66.2±4.852
Rudimentary Tail	0.6±0.245	0±0	2±0.949 <sup>b</sup>	0.68±0.365	0±0 <sup>c</sup>	0±0 <sup>c</sup>
Total Cell Counted	429.8±13.407	437±16.817	409±5.206	414.6±1.600	416.6±3.156	419.8±5.054
Total Abnormality	104.2±6.560	69.2±3.707	130.8±14.105 <sup>b</sup>	120.6±1.806 <sup>b</sup>	94.2±10.190	113±8.729 <sup>b</sup>
% Abnormality	24.42±1.937	16.08±1.292	32.08±3.614 <sup>b</sup>	29.1±0.549 <sup>b</sup>	22.58±2.298	26.96±2.156 <sup>b</sup>

Mean ± SEM with superscripts **a** are statistically significant to group A at  $p < 0.05$ ; Mean ± SEM with superscripts **b** are statistically significant to group B at  $p < 0.05$ ; Mean ± SEM with superscripts **c** are statistically significant to group C at  $p < 0.05$ .

between the mean values for sperm motility across all groups, although some variations were recorded amongst them (Table 1). A dose-dependent decrease in sperm abnormalities between the cyclophosphamide (CYP) treated groups C and D, as well as between the cyclophosphamide + aqueous extract of tiger nut (AET) treated groups E and F are suggestive of a protective

effect of the aqueous extract of tiger nut. In like manner, the sperm motility showed same pattern, and if overwhelming it can result in reduced fertility, since the sperm cells will not be efficiently propelled towards the ovum for fertilization.

The mean values for sperm livability also varied across all groups, with an increase recorded in CYP treated

groups C and D, and CYP + AET treated groups E and F (E and F with the highest values) when compared to that of the control group A and AET treated group B. The increase however was of no statistical significance ( $p > 0.05$ ). With very low livability, infertility or sterility may occur because the available sperm cells are not viable to fertilize the ovum from the female. However, the values recorded are equally not likely to produce any clinical significance. On the other hand, sperm count showed a significant difference ( $p < 0.05$ ) between the CYP treated groups when compared to the control group.

Likewise, there was no statistically significant difference ( $p > 0.05$ ) in the mean values of testis-to-bodyweight (gonado-somatic index) across all the groups, which shows that AET and CYP have no significant effect on testicular weight at the dosages used in this study. Although, there are variations in the values recorded for each of the groups.

The outcomes of this experiment are in contrast to what was reported by Ekaluo et al. (2015) where there was dose-dependent significant increase in sperm motility and sperm count after administration of varying doses of AET for 9 weeks. Likewise, Kim et al. (2013) also reported that sperm headcount and sperm motility in CYP treated groups at 150 mg/kg decreased significantly compared to the control group and that no significant difference was recorded in morphological abnormality of the sperm in any group.

Several morphological sperm abnormalities were observed in the CYP treated groups C and D including the bent tail, tailless head, headless tail, curved tail, rudimentary tail, etc. all of these amounted to substantial increase in percentage abnormality in the CYP treated groups C and D when compared to the control group A, as well as AET treated group B. Whereas, the observed increase was not statistically significant when compared to the CYP + AET treated groups (i.e. groups E and F).

However, the percentage of sperm abnormalities in group F was significantly higher ( $p < 0.05$ ) than the AET treated animals in group B. This may be due to the higher dose (5mg/kg) of CYP given to animals in group F compared to those of group E which received 2.5 mg/kg, and showed no significant difference to group B. A high percentage abnormality will result in infertility because the sperm cells produced will not be of good quality thereby resulting in defective fertilization of the ovum.

Qiu et al. (1995) reported that after 6 weeks of treatment with CYP at 6.1 mg/kg, there was a significant increase in both DNA single strand breaks and cross-links in spermatozoal nuclei. Findings from present study did show that CYP administered at 2.5 and 5.0 mg/kg for 4 weeks resulted in increased sperm abnormalities, which can be likened to the findings of Qiu et al. (1995).

It is therefore concluded that aqueous extract of Tiger nut (AET) has some androgenic properties with the capability to protect cyclophosphamide (CYP)-induced morphological abnormality of sperm, although without extensive effect on sperm motility and sperm count. Also,

it can be said that the consumption of tiger nut does not portend any danger to fertility in male Wistar rats, and may be used to combat sperm morphological abnormalities that could arise from the use of some agents such as cyclophosphamide.

Going forward, it is recommended that further works be done to know the particular constituent of the *Cyperus esculentus* that is responsible for the effects seen.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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