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Effects of Hot Aqueous Extract of *Cyperus Esculentus Lativum* (Tiger Nut) on Testosterone Levels in Adult Male Wistar Rats (*Rattus norvrgicus*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Testosterone plays a crucial role in various aspects of reproductive health and general well-being. Herbal extracts are commonly believed to have potential effects on testosterone levels. This study aimed to explore the impact of hot aqueous extracts of *Cyperus esculentus* latvum (tiger nuts) on testosterone levels and testicular histology in adult male Wistar rats. Ten adult male Wistar rats, each weighing between 100-150g, were divided into two groups (control and treatment), with five rats in each group. Both groups received daily oral administration of 100 mg/kg of hot aqueous extracts according to their assigned treatment for two weeks. After the trial, the rats were sacrificed, and blood and testes were collected for testosterone and histological analysis. T-test was used to compare the levels of each extract group to the control group with statistical significance set at $p < 0.05$. The results showed that there was no significant difference ($p=0.23$) in testosterone levels between the control group (0.85 ng/ml) and the tiger nut treated group (0.77 ng/ml). Histologically, tiger nut extract exhibited very sparse germinal epithelium with small spermatogonia and no recognizable spermatozoa in the tubular lumen. This study has demonstrated that at 100mg/Kg hot aqueous extract of tiger nut daily administration for 14days had no effect on testosterone level but rather had high level of declining testicular histology.

Keywords: Testosterone levels; tiger nuts; testicular histology; wistar rats; herbal extracts.

1. INTRODUCTION

As the global interests in natural remedies and traditional medicines grow, there is a need for rigorous scientific investigation into the potential effects of natural substances, such as aqueous extracts of *Cyperus esculentus* on testosterone. Understanding the impact of the extracts on male reproductive health could have significant implications for both traditional medicine practices and modern health care (Njoku et al. 2018).

Cyperus esculentus, also known as Chufa, Tiger nut, Atadwe, Yellow nutsedge, Earth almond, and in Chishona, Pfende, is a species of plant in the sedge family that is widely distributed throughout most of the world (Zou et al. 2023). It is found in most of the Eastern Hemisphere, which includes Madagascar, Southern Europe, Africa, the Middle East, and the Indian subcontinent (Raju et al. 2023). *C. esculentus* is cultivated for its edible tubers, which are also known as earth almonds or Tiger nuts because of the stripes on them and their hard shell. These tubers are used as a snack food and to make horchata de chufa, a sweet beverage that tastes like milk (Rebezov et al. 2023). In addition, Tiger nut improved sexual performance in treated moderately active rats compared to controls, as evidenced by increased intromission frequency and ratio; serum testosterone levels significantly increased following Tiger nut administration; and phytochemical analyses revealed the presence of quercetin, vitamin C, vitamin E, and mineral zinc in Tiger nut. Tiger nut stimulated sexual motivation in both highly and moderately active rats, as indicated by reduced mount and intromission latencies in these rats compared to controls (Zibaee et al. 2023).

Testosterone is crucial for the development of primary sexual organs, including the testes, spermatogenesis, penis, and testicular growth, as well as for enhancing libido (Omona 2020). Various factors can influence testosterone levels (Amadi et al. 2023). However, it also governs secondary male traits such as deepening of the voice, changes in vocal tone, and male hair patterns. Additionally, testosterone stimulates erythropoiesis, leading to an increase in a man's hematocrit (Warren and Grossmann 2022). The Leydig cells produce more testosterone when stimulated by LH (Chung et al. 2020). Testosterone regulates its own secretion through negative feedback by inhibiting GnRH secretion and reducing the anterior pituitary's responsiveness to GnRH stimulation (Marques et al. 2022). The hypothalamus releases GnRH in pulses every 1 to 3 hours during male reproductive life (Casteel and Singh 2020). FSH and LH plasma levels, however, remain stable from puberty through the third decade of life (Howard 2021). Testosterone levels are low before puberty, but changes in neuronal input and brain activity during puberty increase GnRH secretion (Marques et al. 2022). LH regulates the initial step of converting cholesterol into testosterone by Leydig cells in the testes (Ge et al. 2021). Trace levels of free testosterone in the blood affect tissues such as the seminal vesicles, bone, muscle, and prostate gland. Testosterone

and dihydrotestosterone also bind to cell receptors, regulating protein expression (Narinx et al. 2022).

This study aims to explore the effects of hot aqueous extract of *Cyperus esculentus* on male Wistar rats, offering valuable insights into its potential impact on testosterone levels. The results may enhance the understanding of the medicinal properties of *Cyperus esculentus* and its potential applications in male reproductive health. Through a thorough investigation of this topic, the study seeks to bridge the gap between traditional knowledge and modern scientific understanding, potentially revealing new opportunities for therapeutic interventions and supporting evidence-based healthcare practices. The use of hot aqueous extracts in this study was specifically intended to mimic a tea preparation scenario, as tiger nut tea is consumed in many cultures. However, heating may degrade vital bioactive compounds, such as flavonoids and and antioxidants, potentially reducing their potency and impact on testosterone levels.

2. METHODOLOGY

2.1 Study Design and Period

This study utilized an experimental design to assess the effects of hot aqueous extract of *Cyperus esculentus* on testosterone levels in male rats. The research was conducted over a 14-day period, the rats were divided into two groups: Group A (control group), consisting of 5 rats that did not receive the extract, and Group B (experimental group), consisting of 5 rats that were treated with 100mg/Kg of hot aqueous extract of *Cyperus esculentus* daily for 14 days. The effect on testosterone levels and their testicular histology were compared between the two groups.

2.2 Study Area

The study was conducted at PAMO University of Medical sciences, located in Port Harcourt, Rivers State, Nigeria. The study included 10 adult male Wistar rats, which were sourced from the university's animal breeding facility. Theses rats were of reproductive age, certified as healthy by a veterinarian, and housed under standard laboratory conditions throughout the study.

2.3 Ethical Consideration

This study was approved by the Animal Ethics Committee of PAMO University of Medical sciences (Approval No: PUMS/REC/2024008). All procedures followed the guidelines for the ethical use of animals in research, ensuring that the welfare of the animals was maintained throughout the study. Informed consent for the study was not required, as the research involved animal subjects rather than human participant.

2.4 Inclusion Criteria

The study included adult male Wistar rats sourced from PAMO University of Medical Sciences. The rats were selected based on their reproductive age and health status, as confirmed by a veterinarian. Only rats that weighed between 100g and 150g were eligible for inclusion in the study.

2.5 Exclusion Criteria

Rats were excluded from the study if they were female, weighed less than 100g or more than 150g, or had been previously used in other research studies. Additionally, any rats showing signs of illness or abnormalities were excluded from participation.

2.6 Sample Collection and Analysis

Blood samples were collected from the rats after they were anesthetized using chloroform inhalation in a desiccator. Once adequately anesthetized, 2ml of blood was drawn via cardiac puncture into plain sample bottles. The blood samples were allowed to clot and retract before being centrifuged. The serum was then separated and stored at 2-6°C until further analysis.

a. Hormonal analysis

Testosterone levels were measured in the serum using the Enzyme-Linked Immunosorbent Assay (ELISA) method. ELISA is widely used technique for detecting and quantifying specific molecules, such as hormones, in biological samples. In this assay, an enzyme-labelled antigen or antibody is used to bind to the target molecule. A substrate is then added, which produces a color change that can be measured using an ELISA reader. The testosterone concentration is determined by measuring the light absorption of the reaction product (Jurášek et al. 2017).

b. Histological Analysis

At the end of the study, the rats were sacrificed, and the testes were harvested for histological

examination. The testes were fixed in 10% formalin, embedded in paraffin, and sectioned into 5µm slices. The sections were then stained with Hematoxylin and Eosin (H&E) for general tissue examination. The histological features of the testes, including cell morphology, structure, and any signs of tissue damage or alterations due to the extract treatment, were observed under a light microscope. The histological analysis aimed to provide additional insight into the effects of *Cyperus esculentus* on testicular tissue.

2.7 Quality Assurance

To ensure the accuracy of the results, control samples with known concentrations of testosterone were included in each batch of analysis. If the control samples did not fall within the expected range (mean±3SD), the analysis was repeated until the control values were consistent with the expected results.

2.8 Statistical Analysis

The data collected from this study were recorded in Microsoft Excel and then exported to SPSS 25.0 for both descriptive and inferential statistical analysis. The data were presented as mean ± SD, and the hypothesis was tested using a t-test. A p-value of less than 0.05 was considered statistically significant for the study (Bazine and Arslanoğlu 2020).

3. RESULTS

3.1 Comparing Testosterone Levels between *Cyperus esculentus lativum* and Control

Table 1 shows the comparison of testosterone levels between Tiger nut treated group and control group. The results showed that the mean level of testosterone in Tiger nut treated group was 0.77±0.17 ng/mL and the mean level of the control group was 0.85±0.13 ng/mL. There was no significant difference (T-value = –0.76; *p*-value = 0.23) in the testosterone levels between both groups.

In the control group, the testis sections show normal seminiferous tubules with healthy germinal epithelium and interstitium containing blood vessels and Leydig cells. The germinal epithelium includes spermatogonia, spermatocytes, spermatids, spermatozoa, and Sertoli cells. In the group treated with *Cyperus esculentus latvum* (tigernut), the seminiferous tubules show sparse germinal epithelium with small-sized spermatogonia. The tubular lumen contains no recognizable spermatozoa, indicating the presence of testicular fluid and some immature or defective sperm. Additionally, the interstitium contains fewer Leydig cells.

4. DISCUSSION

In this study, we investigated the effects of hot aqueous extracts of *Cyperus Esculentus Lativum* on testosterone levels in adult male Wistar rats to enable us ascertain whether these extracts impact on testosterone levels in a rat model.

The histological analysis of testes of rats in *Cyperus Esculentus Lativum* (Tiger Nuts) group revealed the presence of very sparse germinal epithelium within seminiferous tubules indicating a pronounced reduction in germ cell populations. Small-sized spermatogonia and the absence of recognizable spermatozoa in the luminal cavity suggest impaired spermatogenesis. Tiger Nuts contain bioactive compounds such as fatty acids and flavonoids, which may exert antioxidant and anti-inflammatory effects. However, their influence on specific stages of spermatogenesis, including spermatogonia maturation and sperm production, may be insufficient at the concentration used in the study. The lack of significant increase in testosterone may be attributed to the complex interactions of Tiger Nuts' bioactive components with steroidogenic enzymes or androgen receptor signaling pathways (Bazine & Arslanoğlu *et al.,* 2018). The lack of significant impact on testosterone levels could be attributed to the complex pharmacological profile of these compounds, where androgenic effects may not be pronounced. This could explain the observed lack of significant testosterone modulation. Study conducted by Nwakanma *et al.,* 2022 revealed an increase in testosterone level with intraperitonial administration and not oral administration as in this study. Furthermore, tap water was used as a diluent in the study by Nwakanma *et al.,* 2022 and not hot aqueous extract as used in this study. The use of hot aqueous extract was done, to achieve a tea extract scenario to ascertain its efficacy in normal human tea diet. The hot extract may destroy some vital active ingredients that would have been necessary in boosting testosterone levels and enhancing testicular histology. However there are several reasons why the study was still

conducted despite the potential biases, such as to understand the effects of heat on active ingredients, comparing different methods of extraction and replicating real-world scenario.

The histological findings in Fig. 1 A, B illustrates photomicrograph sections of the testis of adult male Wistar rats (Control group). Plate A shows normal seminiferous tubules (ST) made up of germinal epithelium (GE), surrounded by interstitium (I) containing blood vessels (BV). Plate B shows the components of the germinal epithlium: spermatogonia (sp), spermatocytes (sc), spermatids (st), spermatozoa (sz); and Sertoli cells (red arrow). The interstitium (I) demonstrates leydig cells (yellow arrow).

Table 1. Comparison of testosterone levels between *Cyperus esculentus lativum* and control groups

Groups	Testosterone (ng/ml)	T-value	P-value	Remark
Control (A) Tiger Nut (B)	0.85 ± 0.13 0.77 ± 0.17	-0.76	0.23	Non-significant

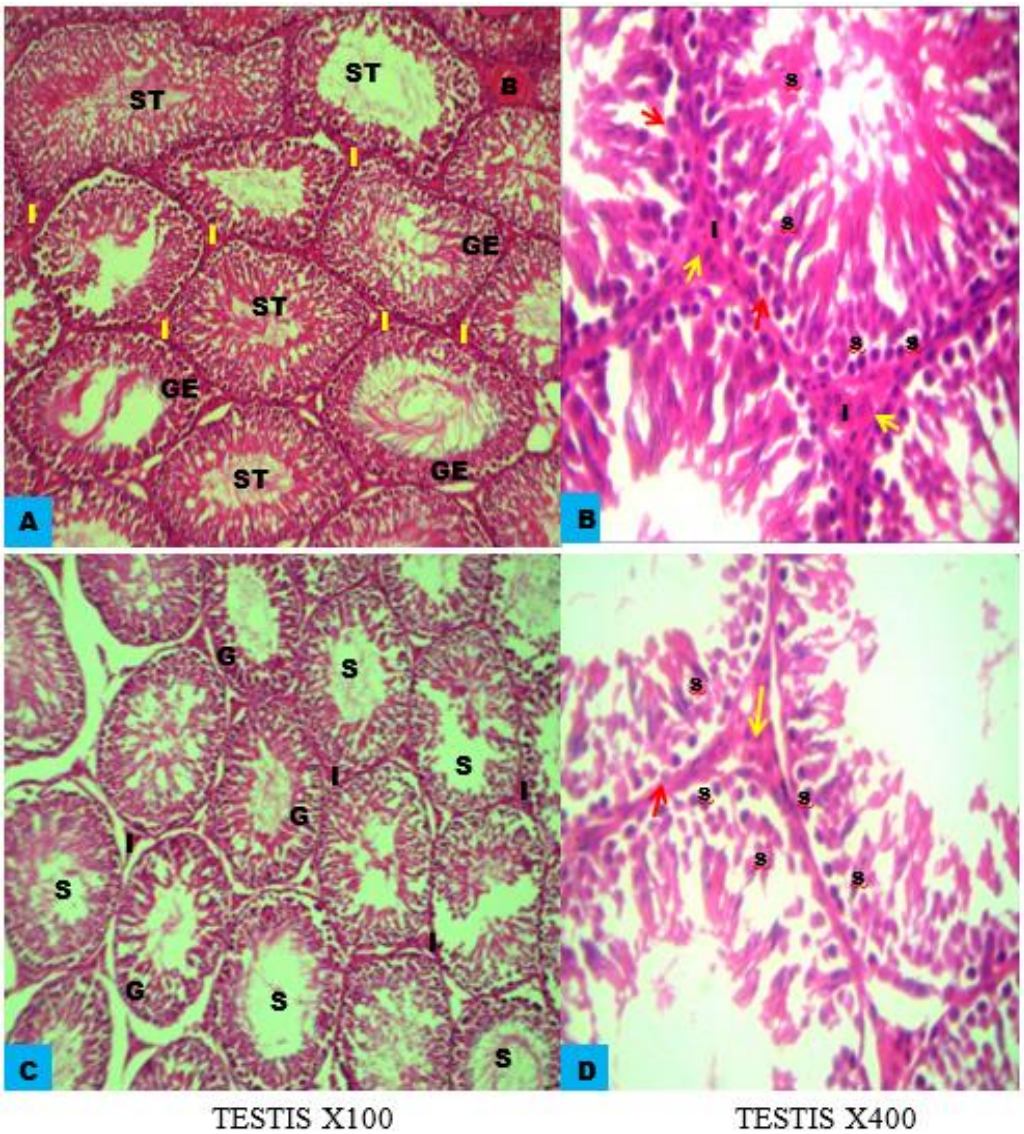

Fig. 1. Photomicrograph of the testis histology of control group (A,B) and *Cyperus esculentus lativum* group (C,D)

Photomicrograph C, D illustrates sections of the testis of adult male Wistar rats treated with *Cuperus esculentus lativum* (Tigernut). Plate A shows seminiferous tubules (ST) made up of germinal epithelium (GE), surrounded by interstitium (I). Note the very sparse germinal epithelium within the seminiferous tubules. Plate B shows different cells of the germinal epithelium: spermatogonia (sp), spermatocytes (sc), spermatids (st); and Sertoli cells (red arrow). Note the small-sized spermatogonia (sp). The luminal cavity has no recognizable spermatozoa (sz), hence the tubular cavity content may only be testicular fluid with a few immature/defective spermatozoa. The interstitium has sparse content of leydig cells (yellow arrow).

5. CONCLUSION

This study found that *Cuperus esculentus lativum* (Tigernut) had no increasing impact on testosterone levels and degraded testicular morphology. Histological analyses confirmed hormonal results, highlighting decline spermatogenesis, steroidogenesis, very sparse germinal epithelium and luminal cavities and testicular structure induced by this hot aqueous extracts.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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