EFFECTS OFHOT AQUEOUS EXTRACT OF *Cyperus esculentus lativum* (TIGER NUT) ON TESTOSTERONE LEVELS IN ADULT MALE WISTAR RATS (*Rattus novergicus*)

ABSRTACT

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: *Cyperus esculentus latvum*, testosterone, hot aqueous extract, adult male wister rats.

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 enlarged testicles, as well as for enhancing libido [6]. Various factors can influence testosterone levels [7]. 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 [8]. The Leydig cells produce more testosterone when stimulated by LH [9]. Testosterone regulates its own secretion through negative feedback by inhibiting GnRH secretion and reducing the anterior pituitary's responsiveness to GnRH stimulation [10]. The hypothalamus releases GnRH in pulses every 1 to 3 hours during male reproductive life [11]. FSH and LH plasma levels, however, remain stable from puberty through the third decade of life [12]. Testosterone levels are low before puberty, but changes in neuronal input and brain activity during puberty increase GnRH secretion [10]. LH regulates the initial step of converting cholesterol into testosterone by Leydig cells in the testes [13]. 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 [14].

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.

METHODOLOGY

Study Design

This study utilized an experimental design and involved 10 male rats in total. The research spanned 14 days and included two groups: group A, the control group, which consisted of 5 adult male Wistar rats, and group B, which was treated with hot aqueous extract of Cyperus esculentus and also consisted of 5 adult male Wistar rats. The effects of the hot aqueous extract on testosterone levels were compared with those of the control group.

Eligibility Criteria

Inclusion criteria for selecting research subjects are:

This study included adult male Wistar rats sourced from PAMO University of Medical Sciences. The rats were of reproductive age and certified as healthy by a veterinarian.

Exclusion criteria for selecting research subjects are:

This study excluded female rats from PAMO University of Medical Sciences, as well as rats weighing less than 100g or more than 150g. Additionally, rats that had previously been used in other research were excluded from the study.

Ethical Approval/Informed Consent

This study was approved by the Animal Ethics Committee of PAMO University of Medical Sciences, Port Harcourt, Rivers State, Nigeria with Approval No: PUMS/REC/2024008.

Sample Collection and Analysis

The rats were anesthetized by chloroform inhalation in a desiccator. Once the rats were adequately anesthetized, 2ml of blood was collected via cardiac puncture into plain sample bottles. The blood samples were left to clot and retract before being centrifuged. The serum was then separated into another plain sample bottle, labeled, and stored in a refrigerator at 2-6°C until further analysis. The serum was analyzed for testosterone levels in males using the biochemical methods outlined below.

a. Biochemical analysis

Serum levels of Male Hormone (Testosterone), was estimated using the enzyme linked immunosorbent assay (ELISA) methods of assay

Enzyme linked immunosorbent assay technique ELISA

Enzyme-linked immunosorbent assay (ELISA) is a technique used to capture a target antigen (or antibody) in samples using a specific antibody (or antigen) and detect or quantify the target molecule through an enzyme reaction with its substrate. In ELISA, various antigen-antibody combinations are used, typically involving an enzyme-labeled antigen or antibody. The enzyme's activity is then measured colorimetrically. This is done by using a substrate that changes color when modified by the enzyme. The light absorption of the product formed after substrate addition is measured and converted into numeric values. Depending on the antigen-antibody combination, the assay can be classified as direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA, and so on.

Basic ELISA Principle; It utilizes the principles of immunology, where a known specific antibody or antigen is used to detect or quantify the target antigen or antibody in a given sample, and enzymology, where an enzyme-substrate reaction produces a readable color. This color change can then be measured using an ELISA reader [15].

b. Quality Assurance

To ensure the reliability and validity of the laboratory results, the test was conducted alongside control samples. If the control samples showed values above their known concentration, the entire batch of analyses was re-run until the concentration of the control sample was within the expected range, with a mean±3SD.

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.

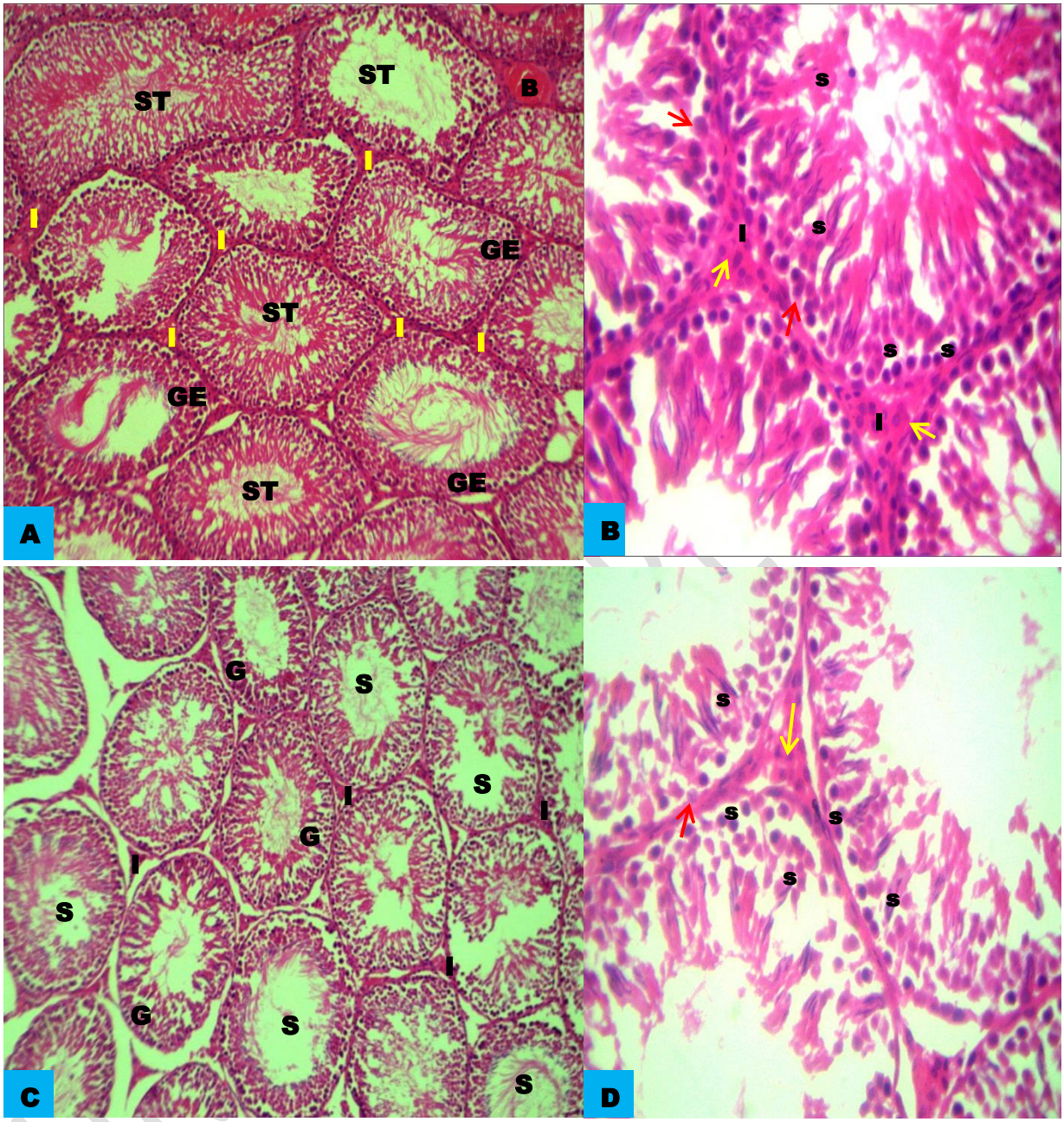
RESULTS

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.

Table 1: Comparison of Testosterone Levels Between *Cyperus esculentus lativum* and Control Groups

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



TESTIS X100 TESTIS X400

FIGURE 1 PHOTOMICROGRAPH OF THE TESTIS HISTOLOGY OF CONTROL GROUP (A, B) AND *CYPERUS ESCULENTUS LATIVUM* GROUP (C, D)

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.

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.

The histological findings in Figure 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).

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).

CONCLUSION

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

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