



Effects of Hot Aqueous Extract of *Telfairia occidentalis* Leaves (Ugu) on Testosterone Levels in Adult Male Wistar Rats (*Rattus novergicus*)

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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 influences various aspects of reproductive health and overall well-being. Herbal extracts are reputed for their potential effects on testosterone levels. This study investigated the testosterone-modulating potentials of hot aqueous extracts of *Telfairia occidentalis* (Ugu), in adult male Wistar rats. Ten adult male Wistar rats weighing between 100-150g were assigned to two groups (control and *Telfairia occidentalis* group) numbering 5 rats per group. Each group received daily oral administration of 100 mg/Kg of hot aqueous extracts based on their treatment assignment for two weeks. The rats were sacrificed; blood and testes were collected after the trial for testosterone and histological analyses. t-Test was used to compare the levels of testosterone in both groups (control and ugu treated) with statistical significance set at $p < 0.05$. The results showed that there was no significant difference ($p=0.19$) in testosterone levels between the control group (0.85 ng/ml) and the Ugu treated group (1.14 ng/ml). Histologically, Ugu leave extract showed sparse germinal epithelium with sparse spermatogonia and moderate spermatozoa. This study has demonstrated that at 100mg/Kg hot aqueous extract of Ugu leaves had no effect on testosterone levels but rather had declining testicular histology.

Keywords: *Telfairia occidentalis*; testosterone; hot aqueous extract; adult male wister rats.

1. INTRODUCTION

Grown in West Africa as a leaf vegetable and for its edible seeds, *Telfairia occidentalis* a tropical vine (Onuguh et al., 2022). The plant is also known by the names fluted gourd, fluted pumpkin, Ikong-ubong (in the languages of Efik and Ibibio), Ugu (in the Igbo language), Okwukwo-wiri (in the Ikwere language), and "Akwukwor ri" (in the Etche language). Southeast Nigeria is home to the Cucurbitaceae species *T. occidentalis*, which is utilized in soups and natural remedies (Ibironke & Owotomo, 2019). The leaves have strong antibacterial properties and their content can vary depending on factors such as extraction method, plant part used, and the specific antibacterial compounds being measured, hepatoprotective, profertility and antioxidant qualities, (Imoseni, 2018). The fluted gourd can be used as a blood tonic and is thought to have therapeutic qualities (Utobo et al., 2023).

The main hormone that affects men, testosterone, play key roles in spermatogenesis, male sex characteristics, and sex differentiation regulation (Sengupta et al., 2019). During the first 6 weeks of development, the fetus experiences its initial effects (Araji et al., 2020). The Sex-determining Region Y (SRY) gene starts the process of developing testicles at week 7, which lead to the eventual formation of seminiferous tubules (Titi-Lartey & Khan, 2020). In order to facilitate the development of the Wolffian duct into the male urogenital tract, fetal Leydig cells and endothelial cells move into the

gonad and create testosterone (Obukohwo et al., 2021). Male external genitalia and the prostate are also formed by testosterone (Gurung et al., 2022).

The hormone testosterone is essential for the development of primary sexual organs, including the testicles, spermatogenesis, penis, and enlarged testicles, as well as enhanced libido (Omona, 2020). Testosterone levels can be influenced by several factors (Amadi et al., 2023). However, it controls secondary masculine traits like deepening of the voice, changes in vocal tone, and patterns of male hair. Additionally, testosterone promotes erythropoiesis, which raises a man's hematocrit (Warren & Grossmann, 2022). The Leydig cells produce more testosterone when exposed to LH (Chung et al., 2020). By inhibiting GnRH secretion and decreasing the anterior pituitary's sensitivity to GnRH stimulation, testosterone uses negative feedback to control its own secretion (Marques et al., 2022). The hypothalamus releases GnRH in pulses every 1 to 3 hours during male reproductive life (Casteel & Singh, 2020). However, FSH and LH plasma levels remain constant from puberty to the third decade (Howard, 2021). Prior to puberty, testosterone levels are low, but changes in neuronal input and brain activity during puberty increase GnRH secretion (Marques et al., 2022). LH controls the first stage of the conversion of cholesterol into testosterone by Leydig cells in the testes (Ge et al., 2021). Tissues such as seminal vesicles, bone, muscle, and the prostate gland are affected by trace levels of free

testosterone in the blood. In addition to controlling protein expression, testosterone and dihydrotestosterone can bind to cell receptors (Narinx et al., 2022).

This study aims to investigate the effects of the hot aqueous extract of *Telfairia occidentalis*, on male wistar rats, providing valuable insights into the potential influence on testosterone levels. The findings of this research may contribute to a broader understanding of the medicinal properties of *Telfairia occidentalis*, and its possible applications in male reproductive health. By conducting a comprehensive study on this topic, we can bridge the gap between traditional knowledge and contemporary scientific understanding, potentially uncovering new avenues for therapeutic interventions and promoting evidence-based healthcare practices.

2. METHODOLOGY

2.1 Study Design

This study was an experimental study design. In this study, a total of 10 male rats were obtained. The study lasted for the duration of 14 days and included two (2) groups- group A (control consisting of 5 adult male wistar rats), group B treated with hot aqueous extract of *Telfairia occidentalis* (consisting of 5 adult male wistar rats). The effects of these hot aqueous extracts on testosterone levels were compared with the control group.

2.2 Eligibility Criteria

Inclusion criteria: This study included adult male wistar rats grown in PAMO University of Medical Sciences. This study also included adult male wistar rats of reproductive age, certified healthy by a veterinarian.

Exclusion criteria: This study excluded female rats grown in PAMO University of Medical Sciences. This study also excluded rats weighing below 100g and above 150g. In addition, this study excluded rats that may have been previously used for another research.

2.3 Sample Collection and Analysis

The rats were anaesthetized using chloroform via inhalation in a desiccator. When adequate anaesthetics were given, 2mls of blood samples were collected by cardiac puncture into plain

sample bottles. The collected blood samples were allowed to clot and retract before being centrifuged and the serum was separated into another plain sample bottle, labeled accordingly and stored refrigerated at 2-6°C until needed for analysis. The serum was analyzed for male testosterone levels using the biochemical methods of analysis as stated below.

a. Biochemical analysis

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

ELISA Principle: It employs the principle of immunology (using a known specific antibody or antigen in detection/quantification of antigen/antibody in given sample) and enzymology (using enzyme substrate reaction) in producing readable colour that can be read using ELISA reader (Jurášek et al., 2017).

b. Quality assurance

To ensure the reliability and validity of the data or results obtained from the laboratory analysis, the test was run alongside with control samples such that control samples having values above its known concentration during the analysis subjects the entire batch of analysis to a re-run until the control sample concentration is obtained with $\text{mean} \pm 3\text{SD}$.

2.4 Statistical Analysis

The data gathered from this study were documented in Microsoft Excel and from there the data were exported to SPSS 25.0 for descriptive and inferential statistics. The data were described as $\text{Mean} \pm \text{SD}$ while the hypothesis of the study was tested using t-Test. The study was considered significant at $p\text{-value} < 0.05$.

3. RESULTS

3.1 Comparing Testosterone Levels between *Telfairia occidentalis* and Control

Table 1 shows the comparison of testosterone levels between Ugu treated group and control group. The results showed that the mean level of testosterone in Ugu treated group was 1.14 ± 0.64

4. DISCUSSION

In this study, we investigated the effects of hot aqueous extracts of *Telfairia occidentalis* leaves (Ugu), on testosterone levels in adult male Wistar rats to enable us ascertain whether these extracts impact on testosterone levels in a rat model.

First, the finding from this study as presented in Table 1 revealed that daily administration of hot water Ugu leave extract did not have any effect on testosterone level in adult male wistar rats. This result was supported by the histological analysis that rather showed a declining level of not just Leydig cells but also Sertoli cells, implying that hot water extract of Ugu leave at the studied dose had a negative impact on testicular function by inhibiting steroidogenesis and spermatogenesis respectively in the testes (Chung et al., 2020; Ge et al., 2021). The absence of a significant increase in testosterone levels in the Ugu group suggests that while *Telfairia occidentalis* leaves are nutritionally rich and have antioxidant properties, they do not significantly influence testosterone synthesis.

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 epithelium: spermatogonia (sp), spermatocytes (sc), spermatids (st), spermatozoa (sz); and Sertoli cells (red arrow). The interstitium (I) demonstrates leydig cells (yellow arrow). C, D illustrates photomicrograph sections of the testis of adult male Wistar rats treated with *Telfairia occidentalis* (Ugu leaf). Plate A shows seminiferous tubules (ST) made up of germinal epithelium (GE), surrounded by interstitium (I) containing blood vessels (BV). The sparse germinal epithelium within the seminiferous tubules indicates reduction in steroidogenesis. Plate B shows different cells of the germinal epithelium: spermatogonia (sp), spermatocytes (sc), spermatids (st), spermatozoa (sz); and Sertoli cells (red arrow). The sparse spermatogonia (sp) and moderate spermatozoa (sz) within the tubular lumen; indicates a reduction in spermatogenesis. The interstitial leydig cells (yellow arrow) are also sparse within the interstitium. This aligns with previous study by Okolie et al., 2017, indicating that Ugu primarily benefits overall health and nutritional status rather than directly enhancing androgenic

activity. Phytochemicals in Ugu may interact with endocrine pathways involved in spermatogenesis, however, their specific effects on Leydig cell function or androgen receptor activation might not be potent enough to induce significant changes in testosterone levels (Emmanuel et al., 2018).

5. CONCLUSION

This study found that *Telfairia occidentalis* had no impact on testosterone levels but negatively impacted on testicular morphology. Histological analyses confirmed biochemical results, highlighting decline in spermatogenesis, steroidogenesis, sparse germinal epithelium, liminal cavity and testicular structure induced by this hot aqueous extracts.

6. LIMITATION

The limitations of this study include a small sample size, which may affect the generalizability of the results, and the experimental study design, clinical trials need to be carried out to know the effect of this hot aqueous extract on humans.

ETHICAL APPROVAL

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.

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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