

EFFECTS OF HOT AQUEOUS EXTRACT OF *Telfairia occidentalis* LEAVES (UGU) ON TESTOSTERONE LEVELS IN ADULT MALE WISTAR RATS (*Rattus novergicus*)

ABSRTACT

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.

Commented [u1]: adults

INTRODUCTION

Grown in West Africa as a leaf vegetable and for its edible seeds, *Telfairia occidentalis* a tropical vine [1]. 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 Ikwerre 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 [2]. The leaves have strong antibacterial, hepatoprotective, profertility and antioxidant qualities [3]. The fluted gourd can be used as a blood tonic and is thought to have therapeutic qualities [4].

The main hormone that affects men, testosterone, play key roles in spermatogenesis, male sex characteristics, and sex differentiation regulation [5]. During the first 6 weeks of development, the fetus experiences its initial effects [6]. The **SRY** gene starts the process of developing testicles at week 7, which lead to the eventual formation of seminiferous tubules [7]. 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 [8]. Male external genitalia and the prostate are also formed by testosterone [9].

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 [10]. Testosterone levels can be influenced by several factors [11]. 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 [12]. The Leydig cells produce more testosterone when exposed to LH [13]. By inhibiting GnRH secretion and decreasing the anterior pituitary's sensitivity to GnRH stimulation, testosterone uses negative feedback to control its own secretion [14]. The hypothalamus releases GnRH in pulses every 1 to 3 hours during male reproductive life [15]. However, FSH and LH plasma levels remain constant from puberty to the third decade [16]. Prior to puberty, testosterone levels are low, but changes in neuronal input and brain activity during puberty increase GnRH secretion [14]. LH controls the first stage of the conversion of cholesterol into testosterone by Leydig cells in the testes [17]. 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 [18].

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.

Commented [u2]: Include the full meaning.

METHODOLOGY

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.

Commented [u3]: adults

Eligibility Criteria

Inclusion criteria for selecting research subjects are:

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 for selecting research subjects are:

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.

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.

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

Enzyme linked immunosorbent assay technique ELISA

Enzyme-linked immunosorbent assay (ELISA) is a method of target antigen (or antibody) capture in samples using a specific antibody (or antigen), and of target molecule detection/quantification using an enzyme reaction with its substrate. In ELISA, various antigen-antibody combinations are used, always including an enzyme-labelled antigen or antibody, and

enzyme activity is measured colourimetrically. The enzyme activity is measured using a substrate that changes colour when modified by the enzyme. Light absorption of the product formed after substrate addition is measured and converted to numeric values.

Basic 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 [19].

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 $\text{mean} \pm 3\text{SD}$.

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 $p\text{-value} < 0.05$.

RESULTS

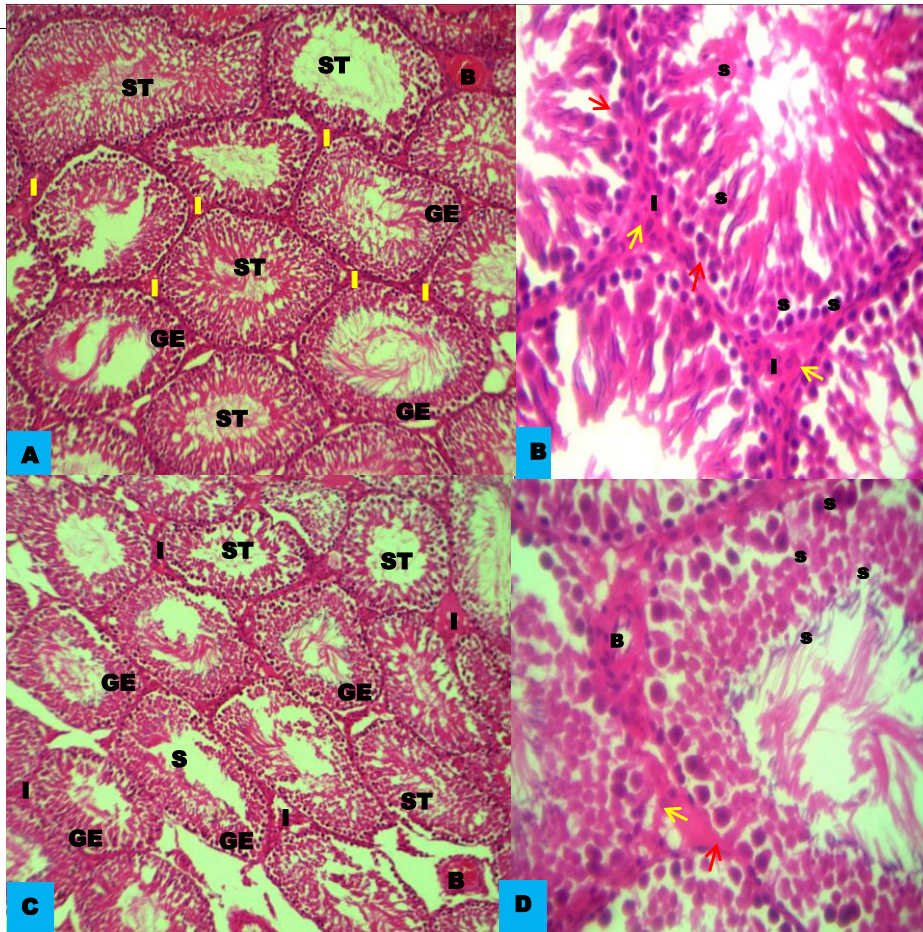
Comparing Testosterone Levels between *Telfairia occidentalis* and Control

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 ng/mL and the mean level of the control group was 0.85 ± 0.13 ng/mL. There was no significant difference (T-value = 0.97; $p\text{-value} = 0.19$) in the testosterone levels between both groups.

Table 1: Comparison of Testosterone Levels Between *Telfairia occidentalis* and Control Groups

Groups	Testosterone (ng/ml)	T-value	p-value	Remark
Control (A)	0.85 ± 0.13	0.97	0.19	Non-significant

Ugu Leaf (B) 1.14 ± 0.64



TESTIS X100

TESTIS X400

FIGURE 1 PHOTOMICROGRAPH OF THE TESTIS HISTOLOGY OF THE CONTROL GROUP (A, B) AND *TELFARIA OCCIDENTALIS*LEAF GROUP (C, D)

The testicular sections of adult male Wistar rats show normal seminiferous tubules with healthy germinal epithelium, containing spermatogonia, spermatocytes, spermatids, spermatozoa, and Sertoli cells in the control group. In the treated group, the seminiferous tubules have sparse germinal epithelium, fewer spermatogonia, and reduced spermatogenesis, indicated by a lower number of spermatozoa and sparse Leydig cells in the interstitium.

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 leaf 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 leaf at the studied dose had a negative impact on testicular function by inhibiting steroidogenesis and spermatogenesis respectively in the testes [13] [17]. 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 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 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 [20].

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.

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.

REFERENCES

- 1) Onuguh IC, Ikhuoria EU, Obibuzo JU. Comparative proximate analysis of *T. occidentalis* (fluted pumpkin) leaves sold in three different markets in Benin City, Nigeria. *Int J Agric Anim Prod (IJAAP)*. 2022;2799-0907.
- 2) Ibironke SI, Owotomo I. Haematology and comparative study of fluted pumpkin leave vegetable and seed nutrients (*Telfairia occidentalis*). *Arch Nutr Public Health*. 2019;1(2):1–6.
- 3) Imoseni IO. Review of the toxicity, medicinal benefits, pharmacological actions and morphological effects of *Telfairia occidentalis* Hook. F. *Eur J Pharm Med Res*. 2018;6(7):22–32. Available from: <https://www.researchgate.net/profile/Innocent-Imosemi/publication/344555838>
- 4) Utobo O, Ezeano C, Nwankwo F, Onugu C, Offia E, Nzeocha C. Efficiency use of resources and profitability of “Ugu” (fluted pumpkin) (*Telfairia occidentalis* Hook. F.) production among small-scale farmers in Southeast, Nigeria. *Fudma J Agric Agric Technol*. 2023;9(3):133–42. Available from: <https://jaat.fudutsinma.edu.ng/index.php/jaat/article/view/108>
- 5) Sengupta P, Arafa M, Elbardisi H. Hormonal regulation of spermatogenesis. In: *Molecular signaling in spermatogenesis and male infertility*. CRC Press; 2019. p. 41–9. Available from: <https://www.taylorfrancis.com/chapters/edit/10.1201/9780429244216-5/hormonal-regulation-spermatogenesis-pallav-sengupta-mohamed-arafa-haitham-elbardisi>
- 6) Araj S, Griffin A, Dixon L, Spencer SK, Peavie C, Wallace K. An overview of maternal anxiety during pregnancy and the post-partum period. *J Ment Health Clin Psychol*. 2020;4(4). Available from: <https://www.mentalhealthjournal.org/articles/an-overview-of-maternal-anxiety-during-pregnancy-and-the-post-partum-period.html>
- 7) Titi-Lartey OA, Khan YS. Embryology, testicle. 2020. Available from: <https://europepmc.org/article/nbk/nbk557763>

- 8) Obukohwo OM, Kingsley NE, Rume RA, Victor E. The concept of male reproductive anatomy. In: Male Reproductive Anatomy. 2021. Available from: <https://books.google.com/books>
- 9) Gurung P, Yetiskul E, Jialal I. Physiology, male reproductive system. In: StatPearls [Internet]. StatPearls Publishing; 2022. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK538429/>
- 10) Omona K. Testosterone. IntechOpen. 2020. Available from: <http://ir.umu.ac.ug/handle/20.500.12280/2611>
- 11) Amadi CF, Okolonkwo BN, Jack I, Odiabara KK. Treatment options for erectile dysfunction in diabetics. Asian J Biochem Genet Mol Biol. 2023;15(4):15–25. Available from: <http://journal.article2publish.com/id/eprint/3505/>
- 12) Warren AM, Grossmann M. Haematological actions of androgens. Best Pract Res Clin Endocrinol Metab. 2022;36(5):101653. Available from: <https://www.sciencedirect.com/science/article/pii/S1521690X22000409>
- 13) Chung JY, Brown S, Chen H, Liu J, Papadopoulos V, Zirkin B. Effects of pharmacologically induced Leydig cell testosterone production on intratesticular testosterone and spermatogenesis. Biol Reprod. 2020;102(2):489–98. Available from: <https://academic.oup.com/biolreprod/article-abstract/102/2/489/5556302>
- 14) Marques P, Skorupskaitė K, Rozario KS, Anderson RA, George JT. Physiology of GnRH and gonadotropin secretion. *Endotext* [Internet]. 2022. Available from: <https://www.ncbi.nlm.nih.gov/sites/books/NBK279070/>
- 15) Casteel CO, Singh G. Physiology, gonadotropin-releasing hormone. 2020. Available from: <https://europepmc.org/article/nbk/nbk558992>
- 16) Howard SR. Interpretation of reproductive hormones before, during and after the pubertal transition—identifying health and disordered puberty. Clin Endocrinol (Oxf). 2021;95(5):702–15. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/cen.14578>
- 17) Ge RS, Li X, Wang Y. Leydig cell and spermatogenesis. In: Molecular Mechanisms in Spermatogenesis. 2021;111–29. Available from: https://link.springer.com/chapter/10.1007/978-3-030-77779-1_6
- 18) Narinx N, David K, Walravens J, Vermeersch P, Claessens F, Fiers T, et al. Role of sex hormone-binding globulin in the free hormone hypothesis and the relevance of free testosterone in androgen physiology. Cell Mol Life Sci. 2022;79(11):543. Available from: <https://link.springer.com/article/10.1007/s00018-022-04562-1>

19) Jurášek M, Göselová S, Mikšátková P, Holubová B, Vyšatová E, Kuchař M, et al. Highly sensitive avidin-biotin ELISA for detection of nandrolone and testosterone in dietary supplements. *Drug Test Anal.* 2017;9(4):553–60.

20) Emmanuel OY, Obasi KK, Lawal I. Spermatogenic and spermatotoxic effects of *Telfairia occidentalis* (Ugu) aqueous leaves extract in adult male Wistar rats (*Rattus norvegicus*). *Toxicol Rep.* 2018;5:954–8. Available from: <https://www.sciencedirect.com/science/article/pii/S2214750018302956>

UNDER PEER REVIEW