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Full length Research Article

Evaluation of Testicular Function and Structural Changes of Wistar Rats Following Antiretroviral Exposure: Protective Role of *Cyperus esculentus*

***Ogedengbe, O.O.[1,3], Bature, A.[3], Fafure, A.A.[3], Kehinde, S.O.[3], Adekeye, A.O.[3], Akintayo, C.O.[4], Ajiboye, B.O.[2]. and Adeeyo, O.A.[1]**

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Federal University Oye-Ekiti, Ekiti State, Nigeria
[2]Department of Biochemistry, Faculty of Science, Federal University Oye-Ekiti, Oye-Ekiti, Ekiti State, Nigeria
Departments of [3]Anatomy and [4]Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria

Summary: This study examined the ability of *C. esculentus* plants to mitigate testicular dysfunction, which is thought to be a probable side effect of antiretroviral toxicity. Adult Wistar male rats weighing 90–110 g were divided into six groups and administered the prescribed treatments. In addition to testicular histology and stereological parameters, testosterone levels, follicle-stimulating hormone levels, antioxidant markers, malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione levels were also evaluated. The adverse consequences of highly active antiretroviral therapy (HAART) include considerable reduction of germ cells, expansion of the tubular lumen, enlargement of interstitial spaces, and alarmingly low cell counts. Compared to the other treatment groups, MDA levels dramatically increased, whereas GSH and antioxidant enzyme (SOD) levels significantly decreased. Testicular architecture was largely conserved after treatment with C. esculentus, with a notable increase in the cellular densities of germinal and interstitial cells and a notable decrease in the tubular lumen. Vacuolation, architectural malformations, and hypoplastic changes were reduced. Significant improvements were also observed in *C. esculentus* in terms of elevated antioxidant SOD and GSH levels and decreased MDA levels. *C. esculentus* reduced architectural distortions and testicular dysfunction caused by HAART, and improved testicular morphology. Further exploration of these pathways is required.

Keywords*: Cyperus Esculentus, highly active antiretroviral therapy, testicular dysfunction, antioxidants, Testosterone, Follicle-stimulating hormone*

**Author for correspondence: oluwatosin.ogedengbe@fuoye.edu.ng , Tel: +2348163422223*

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INTRODUCTION

The quality of life of those with HIV/AIDS has significantly improved with increased life expectancy, thanks to the discovery and considerable advancement in the use of highly active antiretroviral medication during the last four decades (Achila *et al*., 2022). Highly Active Antiretroviral Therapy (HAART), which has been widely adopted, has altered the treatment and progression of HIV infection, with the consequences of chronic HIV infection and HAART playing an increasingly significant role in morbidity and mortality (Naidu *et al*., 2021). HAART, however, necessitates ongoing administration, which has been linked to negative consequences, such as the emergence of drug resistance, unfavorable drug interactions, and systemic drug toxicity (Olojede *et al*., 2021).

HAART has been proven to be useful in treating retroviral diseases, particularly HIV/AIDS, but it has also been documented to cause infertility, which ultimately leads to a decrease in reproductive ability (Ogedengbe *et al*., 2018c). This is due to the relatively strong permeability of the gonads to many antiretroviral medications that constitute HAART (Trezza and Kashuba, 2014). HAART can have a direct effect on the cells responsible for producing sperm, leading to decreased production or complete infertility in some cases. HAART has been hypothesized to cause topological alterations in sperm cells, resulting in mitochondrial damage, decreased sperm motility, and reduced fertility (Ogedengbe *et al*., 2018a). Such changes in sperm cells may also increase the risk of offspring inheriting genetic mutations from their parents (Haas and Tarr, 2015). Therefore, it is important to monitor the effect of HAART on male fertility. Understanding the effects of HAART on male fertility could provide valuable insights into the implications of this therapy for reproductive health.

Natural medicinal plants are increasingly being used globally for treating illnesses because of their perceived efficacy and low incidence of complications (Sharif *et al*.,

2020). *Cyperus Esculentus* (tiger nut) has been shown to have preventive effects on testicular weight and spermatogenesis (Achoribo and Ong, 2017, Adelakun *et al*., 2021). *C. esculentus* treatment improves testicular weight, sperm concentration, motility, viability, and progression in rats and mice, while reducing abnormal sperm morphology (Atoigwe-Ogeyemhe *et al*., 2018). Its anti-inflammatory, antioxidant, and antimicrobial properties make it a promising herbal remedy for male reproductive issues and for oxidative stress reduction, potentially reducing chronic diseases.

Based on quantitative analysis, *C. esculentus* is known to possess potent phytochemicals that support its strong antioxidant effects, including alkaloids, flavonoids, saponins, tannins, phenols, and glycosides (Nwosu *et al*., 2022). Furthermore, *C. esculentus* increases testosterone and gonadotropin levels in rats (Al-Shaikh *et al*., 2013). This suggests that *C. esculentus* may be useful as a natural supplement for men with low testosterone levels. Additionally, *C. esculentus* has been found to be a potential alternative to sildenafil citrate as a treatment for erectile dysfunction (ED) (Singh *et al*., 2018, Olabiyi *et al*., 2018). Studies suggest that *C. esculentus* help improve libido and sexual performance in men (Malviya *et al*., 2016, Masuku *et al*., 2020). *C. esculentus* are also high in vitamin E, which is important for maintaining hormonal balance and may therefore be beneficial for male reproductive health (Bazine and Arslanoğlu, 2020). Unfortunately, HAART delivery to anatomical sanctuary spots and HIV latent storage sites has not yet exploited the unique properties of C. esculentus. Therefore, this study aimed to assess the therapeutic role of *C. esculentus* against HAART-induced testicular dysfunction.

MATERIALS AND METHODS

Animals and treatments: The present study involved 30 male Wistar rats weighing 90-110 g, housed at the University of Afe Babalola's Animal House in Ado-Ekiti, Nigeria. All animal treatments were performed in accordance with the standards for handling and treating laboratory animals (Albus, 2012). The Animal Ethics Board approved the study protocol, which was assigned the protocol number AB/EC/15/03/007. The animals were subjected to a 12-hour cycle of light and darkness, fed and watered continuously, and allowed to acclimate for a period of two weeks. Zidovex LN, a HAART medication combining zidovudine, lamivudine, and nevirapine (Huff, 2003), was purchased from the Federal Teaching Hospital in Ido-Ekiti, Nigeria. The dosage for animals was estimated using the therapeutic equivalent dose for humans in a rat model (Ogedengbe *et al*., 2018b).

Plant Material: Dried tiger nuts (C. esculentus) were obtained from the Ado-Ekiti market in Southwest Nigeria and validated by the Plant Science Department of Afe Babalola University. Following drying, the samples were finely milled into a powder.The powder were extracted over three days in water. Chemical studies were conducted using aqueous extraction techniques to identify active plant components (Udefa *et al*., 2020). Crude aqueous extraction was performed on air-dried plant powder before evaporation at a low pressure. Constant weight was achieved by evaporating the extracted fraction in a vacuum oven at 40°C. Paste extracts (500 and 1000 mg/kg) were administered after reconstitution with distilled water.

Qualitative phytochemical screening qualitative phytochemical screening: Using the techniques of Sofowora (1993) and Evans (2002), dried extracts were evaluated for the amounts of steroids, flavonoids, saponins, alkaloids, tannins, reducing sugar, terpenoids, glycosides, as well as cardiac glycosides.

Quantitative phytochemical analyses quantitative phytochemical analyses: The dried extracts were quantified for steroids, alkaloids, phenols, tannins, and glycosides content. The Harborne method was used to determine the alkaloids, whereas other phytochemicals were measured spectrophotometrically (Harbone, 1973).

Experimental design: Following an arbitrary distribution of six groups (A–F) of five rats each, animals received the following treatments:
Group A-Control was orally administered distilled water
Group B-Treatment involved administering a HAART cocktail containing zidovex LN, which was diluted in 100 milliliters of distilled water to equal animal doses of 1.35, 0.68, and 0.92 mg/kg body weight, respectively. The equivalent doses of zidovudine, lamivudine, and nevirapine were 600 mg/day, 300 mg/day, and 400 mg/day, respectively(Ogedengbe *et al*., 2018a, Kehinde *et al*., 2021).
Group C- treated with HAART+ 500mg/kg/bw C. esculentus,
Group D- treated with HAART+1000mg/kg/bw C. esculentus,
Group E- treated with 500mg/kg/bw *C. esculentus* (Udefa *et al*., 2020)
Group F- treated with 1000mg/kg/bw *C. esculentus* (Udefa *et al*., 2020)
Daily oral delivery was used for all treatments. The animals were euthanized via cervical dislocation a day after the final dosage following eight weeks of treatment.

Body weight determination: Prior to starting the therapy, weekly, and finally on the last day of the trial, the animals were weighed. An electric scale was used to measure body weight in the morning from 8:00 to 10:00. (HX-T electronic weighing balance; HX-302 T, China).

Sample collection: Day 57 saw cervical dislocation and slaughter of the animals. Through cardiac puncture, 3 milliliters (mL) of blood was extracted from the heart and placed in simple tubes to clot for two hours. After centrifugation, the supernatant (serum) was collected for biochemical analysis after 15 minutes at 1000 g (Kehinde *et al*., 2021).

Body and Testicular weight: The body weight (BW) of the animals was measured every week until they were sacrificed, and testicular weight (TW) was determined using a digital balance (HX-T electronic weighing balance HX-302 T, China). Testicles were measured separately, and the average value was considered as one observation (TW), which was measured in grams (g).

Semen analysis: The caudal epididymis of each animal was removed from the testis, sliced in 0.5 ml of normal saline, mixed properly, and 20 µl of semen, which was needed to fill the capillary, was drawn out and placed into an automated sperm quality analyzer (SQA IIC-P manufactured by Medical Electronic System Ltd., serial no. 2840) to test sperm quality. Semen parameters were measured, and morphology and motility were measured in percentages. Sperm count was determined in millions/ml.

Organ index: The relative weights of the testes were determined using the following formula:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight}}{\text{Final body weight}} \times 100\%$$

Histological studies: Testicular tissue samples were fixed in 10% neutral-buffered formalin and subjected to histological examination. A rotary microtome (Microm GmbH, serial no. 42861, CAT. no. 02100) was used to produce fine slices with a thickness of 5 µm. Hematoxylin and eosin (H&E) staining is generally used to analyze tissue structures (Schulte *et al*., 2020). The slides were analyzed by a histopathologist who was blinded to the research protocol.

Morphometry: Weibel (2020) point-counting approach was to determine testicular interstitium (I), lumina (L), and germinal epithelium (GE) volumes. Six fields from four testicular sections were randomly selected for analysis using an Olympus microscope (CX 22RFS1, SN 2M82873, with a cameroscope at a magnification of 400 ×). A 10 mm grid with 180 test points per image was overlayed onto 5 m haematoxylin and eosin-stained tissue sections. The number of point crossings on the grid (PN) that covered each tissue component and the total number of points on the grid (PT) were divided to obtain the volume density (Vd) of each tissue component (Bielli *et al*., 2001).

$$Vd = PN/PT$$

One hundred (100) was multiplied by the Vd values for GE, L, and I, and the results are presented as percentages (%). Each testicular component's absolute volume (AV) was calculated using a modified version of Howard and Reed (2004) methodology. This was calculated by dividing the relevant volume density by testicular weight (TW). Values are expressed in mL.

Serum analysis of Testosterone and Follicle Stimulating (FSH) Hormonal levels: Blood was drawn from the heart, coagulated for two hours, and centrifuged for five minutes at 3000 rpm. Hormone levels in the recovered supernatant (serum) were measured. Testosterone and FSH levels were examined using ELISA kits with catalogue numbers TE187S and FS232F (Calbiotech Inc., 1935 Cordell Ct., El Cajon, CA 92020), following the manufacturer's instructions.

Measurement of serum malondialdehyde (MDA) concentration in the testis: The method of Albro *et al*. (1986) was used to determine the malondialdehyde (MDA) concentration in the serum. Serum (0.1 mL of serum was mixed for 15 min in a water bath with 2 mL of a 1:1:1 combination of tert-butyl alcohol (TBA), trichloroacetic acid (TCA), and hydrochloric acid (HCl) (TBA 0.37%, 0.25N HCl, and 15% TCA). After cooling and centrifuging the mixture, the clear supernatant was measured at 535 nm against a reference blank.

Measurement of serum reduced glutathione (GSH) concentration in the testis: With a few minor modifications, the measurement was performed using serum samples in accordance with the procedure outlined by Sedlak and Lindsay (1968). The concept was based on the precipitation of proteins using a tungstate/sulfuric acid solution and the formation of a yellow color following the reaction with 5, 5′-dith-iobis-2-nitrobenzoic acid (DTNB). In comparison to the control, the absorbance was measured at 412 nm for 30–60 s. Using a standard GSH curve, the concentration of glutathione (GSH) were calculated.

Measurement of serum superoxide dismutase (SOD) concentration in the testis: The method of Berwal and Ram (2018) was used to measure the superoxide dismutase (SOD). Microsome dilution was achieved by diluting 0.1 mL serum (1:10) in 0.9 mL of distilled water. To make an aliquot, 2.5 ml of 0.05M carbonate buffer were mixed with 0.2 ml of diluted microsome. The procedure was initiated by the addition of 0.3 ml of 0.3 mM adrenaline. The reference mixtures used were 2.5 ml of 0.05 M carbonate buffer, 0.3 ml of 0.3 mM adrenaline, and 0.2 ml distilled water. The absorbance was measured at a wavelength of 480 nm from 30 to 150 s.

Statistical analysis: GraphPad Prism version 5.00 for Windows was used to analyze morphometric data using conventional parametric tests. The findings are shown as mean ± standard error of the mean (GraphPad Software, San Diego, CA, USA) after one-way analysis of variance (ANOVA) and Tukey's multiple comparison test was conducted. Statistical significance was set at $p < 0.05$.

RESULTS

Phytochemical Analysis: A qualitative analysis of the aqueous extraction of *Cyperus Esculentus* showed the presence of saponins, steroids, cardiac glycosides, and terpenoids. Quantitative analysis also showed the presence of phenols, tannins, cardiac glycosides, steroids, and alkaloids, and their respective amounts, with steroids and alkaloids having higher values than the other phytochemicals present (Table 1).

Table 1:
Quantitative and qualitative phytochemical analyses of aqueous extraction of *Cyperus Esculentus*.

Phytochemical	Status	Phytochemical	Quantity (%)
Alkaloid	+	Phenol (%)	0.542
Tannin	+	Tannin (mg/100g)	2.762
Flavonoid	-	Cardiac glycosides (%	0.945
Glycoside	-	Steroid (%)	28.76
Reducing sugars	-	Alkaloid (%)	10.00
Saponin	+		
Steroid	+		
Cardiac Glycosid	+		
Terpenoids	+		

(Key: + = present; − = not present).

Organ-body weight changes: The following groups were compared: B, C, D, E, and F versus A; C and D vs. B; E vs. C; and F vs. D. All groups showed insignificant increases in body weight, with Group D presenting the greatest weight difference, while Group A showed the smallest. For both testicular weight (TW) and relative organ weight (TW/BW X100), there were no significant differences ($p > 0.05$) observed (Table 2).

Changes in sperm counts: Compared to the control group A, group B (HAART) showed a significant ($p < 0.05$) decrease in sperm counts (Table 3).

Changes in sperm motility

Progressive motility: Compared to control group A, group B showed a significant reduction ($p < 0.001$) in progressive motility. Adjuvant treatment with *C. esculentus* significantly increased this parameter in groups C ($p < 0.05$) and D ($p < 0.001$) compared with that in group B (Table 3).

Non-progressive motility: Significant increases in non-progressive motility were recorded in groups B ($p < 0.001$) and C ($p < 0.05$), in contrast to group A (the control group). Additionally, co-administration of *C. esculentus* significantly lowered non-progressive sperm motility compared to HAART group B in groups C ($p < 0.01$) and D ($p < 0.001$) (Table 3).

Immotile sperms: HAART-treated group B had the highest number of immotile sperms, whereas group A had the lowest, with no significant difference ($p < 0.05$) observed between the groups (Table 3).

Changes in germinal epithelium (GE)

Volume density: Group B had a significantly reduced GE volume density ($p < 0.05$) compared with the control group. Group D also showed a significant increase ($p < 0.05$) in this parameter following adjuvant therapy with C. esculentus, in contrast to Group B (Table 4).

Absolute volume: Adjuvant co-treatment with *C. esculentus* substantially enhanced ($p < 0.05$) the absolute volume in group D, as opposed to HAART group B.

Table 2:
Body weight, testicular weight, relative testicular weight

Groups	Initial BW (g)	Final BW (g)	Weight diff (g)	Difference %	TW (g)	(TW/BW)X100
A	144.4 ± 4.5	207.7 ± 7.0	63.3	43.8	2.3 ± 0.22	1.10
B	147.2 ± 4.1	216.3 ± 11.4	69.1	46.9	2.7 ± 0.06	1.23
C	146.8 ± 7.6	209.0 ± 4.5	62.2	42.4	2.5 ± 0.07	1.20
D	142.2 ± 7.0	227.5 ± 5.7	85.3	60.0	2.8 ± 0.11	1.22
E	149.0 ± 5.7	226.8 ± 5.6	77.8	52.2	2.9 ± 0.09	1.21
F	145.6 ± 6.7	225.9 ± 8.0	80.3	55.3	2.6 ± 0.18	1.10

The recorded data are shown as mean ± standard error of mean (SEM) (all values compared were $p > 0.05$). A: Control; B (HAART); C (HAART + 500 mg/kg C. esculentus); D (HAART + 1000 mg/kg C. esculentus); E (500 mg/kg C. esculentus); F (1000 mg/kg C. esculentus).

Table 3:
Seminal fluid analysis: epididymal sperm count and motility

Groups	Sperm Count (10^6/ml)	Sperm motility (%)		
		Progressive	Non-Progressive	Immotile
A	44 ± 4.8	47 ± 6.3	14 ± 2.3	14 ± 1.4
B	23 ± 1.3[a]	21 ± 3.6[b]	57 ± 3.4[e]	38 ± 4.8
C	35 ± 2.5	42 ± 2.6[c]	34 ± 2.7[f,g]	30 ± 3.8
D	34 ± 1.5	58 ± 3.1[d]	21 ± 3.8[h]	19 ± 8.3
E	33 ± 1.3	48 ± 3.5	21 ± 2.4	21 ± 2.4
F	35 ± 3.5	55 ± 4.1	23 ± 2.2	18 ± 7.2

The recorded data are displayed as mean ± standard error of mean (SEM) (all values compared were $p > 0.05$). A: Control; B (HAART); C (HAART + 500 mg/kg C. esculentus); D (HAART + 1000 mg/kg C. esculentus); E (500 mg/kg C. esculentus); F (1000 mg/kg C. esculentus).
The following groups were compared: B, C, D, E, and F versus A; C and D vs. B; E vs. C; and F vs. D.
[a]($p<0.05$) B vs A; [b]($p<0.01$) B vs A; [c]($p<0.05$) C vs B; [d]($p<0.001$) D vs B; [e]($p<0.001$) B vs A, [f]($p<0.05$) C vs A, [g]($p<0.01$) C vs B, [h]($p<0.001$) D vs B

Table 4:
Volume density and absolute volume stereological measurements of germinal epithelium, lumen, and interstitium

	Germinal epithelium		Lumen		Interstitium	
Groups	Vd (%)	Av (ml)	Vd (%)	Av (ml)	Vd (%)	Av (ml)
A	66 ± 2.2	1.5 ± 0.05	23 ± 2.2	0.5 ± 0.06	12 ± 0.3	0.3 ± 0.01
B	29 ± 9.2[a]	0.8 ± 0.25	50 ± 2.2[d]	1.4 ± 0.06[f]	15 ± 2.0	0.4 ± 0.05
C	47 ± 1.9	1.2 ± 0.01	27 ± 0.9[e]	0.7 ± .003[g]	18 ± 0.6	0.5 ± 0.01[i]
D	62 ± 2.2[b]	1.7 ± 0.06[c]	27 ± 0.6[e]	0.8 ± 0.02[g]	9.7±1.5[h]	0.3 ± 0.04[j]
E	60 ± 5.0	1.7 ± 0.15	22 ± 4.5	0.6 ± 0.13	10 ± 1.4[h]	0.3 ± 0.04
F	66 ± 5.0	1.7 ± 0.13	19 ± 0.9	0.5 ± 0.02	14 ± 0.9	0.4 ± 0.02

The recorded data are displayed as mean ± standard error of mean (SEM) (all values compared were $p > 0.05$). A: Control; B (HAART); C (HAART + 500 mg/kg C. esculentus); D (HAART + 1000 mg/kg C. esculentus); E (500 mg/kg C. esculentus); F (1000 mg/kg C. esculentus).
The following groups were compared: B, C, D, E, and F versus A; C and D vs. B; E vs. C; and F vs. D
[a]($p<0.05$) B vs A; [b]($p<0.05$) D vs B; [c]($p<0.05$) D vs B; [d]($p<0.01$) B vs A; [e]($p<0.01$) C & D vs B; [f]($p<0.001$) B vs A; [g]($p<0.01$) C & D vs B; [h]($p<0.05$) D & E vs C; [i]($p<0.05$) C vs A; [j]($p<0.05$) D vs C

Changes in Lumen (L)

Volume density: Compared to control group A, there was a significant increase in luminal density in group B ($p < 0.01$). Comparing groups C and D to HAART group B, adjuvant co-treatment with *C. esculentus* considerably ($p < 0.01$) lowered this parameter (Table 4).

Absolute volume: When comparing group B to Control group A, there was a significant ($p < 0.001$) increase in group B's absolute lumen volume. Comparing groups C and D to group B, co-treatment with *C. esculentus* significantly decreased this parameter ($p < 0.01$).

Changes in Insterstitium (I)

Volume density: When compared to group C, the interstitial volume densities of groups D and E decreased significantly ($p < 0.05$) (Table 4).

Absolute volume: In contrast to group A, group C exhibited a statistically significant increase ($p < 0.05$) in this parameter, whereas group D showed a statistically significant decrease ($p < 0.05$) in comparison to group C.

Histopathological examination of testicular tissue:
The testicular cross-sections of groups A, E, and F showed well-preserved cytoarchitecture and minimal **histological** alterations. Seminiferous tubules were occupied by different spermatogenic stages, these groups also have typical basement membranes and interstitial gaps. Cellular infiltration was not observed (Fig. 1A, E, & F).
Seminiferous tubules in HAART-treated groups B and C showed a significant loss of germ cells, enlarged tubular lumen, widening interstitial gaps, and hypocellularity. However, with adjuvant *C. esculentus* co-treatment, groups C and D exhibited notable enhancement with increasing germinal and interstitial cell density and a reduction in the tubular lumen. Architectural deformities, hypoplastic alterations, and vacuolations were also minimized (Fig 1 B, C & D).

Changes in serum testosterone and FSH hormonal levels: Figures 2A and B display the mean and standard error with 95% confidence intervals.

Testosterone: The mean testosterone levels of groups E (61.87 ± 0.82 ng/mL) and F (55.48 ± 7.55 ng/mL) increased significantly ($p < 0.05$) when compared to the Control-group A (15.38 ± 4.2 ng/mL) (Fig. 2A). Meanwhile, when compared to *C. esculentus* alone in Group E (61.87 ± 0.82 ng/mL), HAART co-treatment with *C. esculentus* in Group C (12.44 ± 0.36 ng/mL) considerably ($p < 0.05$) reduced this parameter. For groups B and D, the mean testosterone levels are (40.38 ± 11.45 ng/mL) and (35.57 ± 14.30 ng/mL), respectively (Fig. 2A).

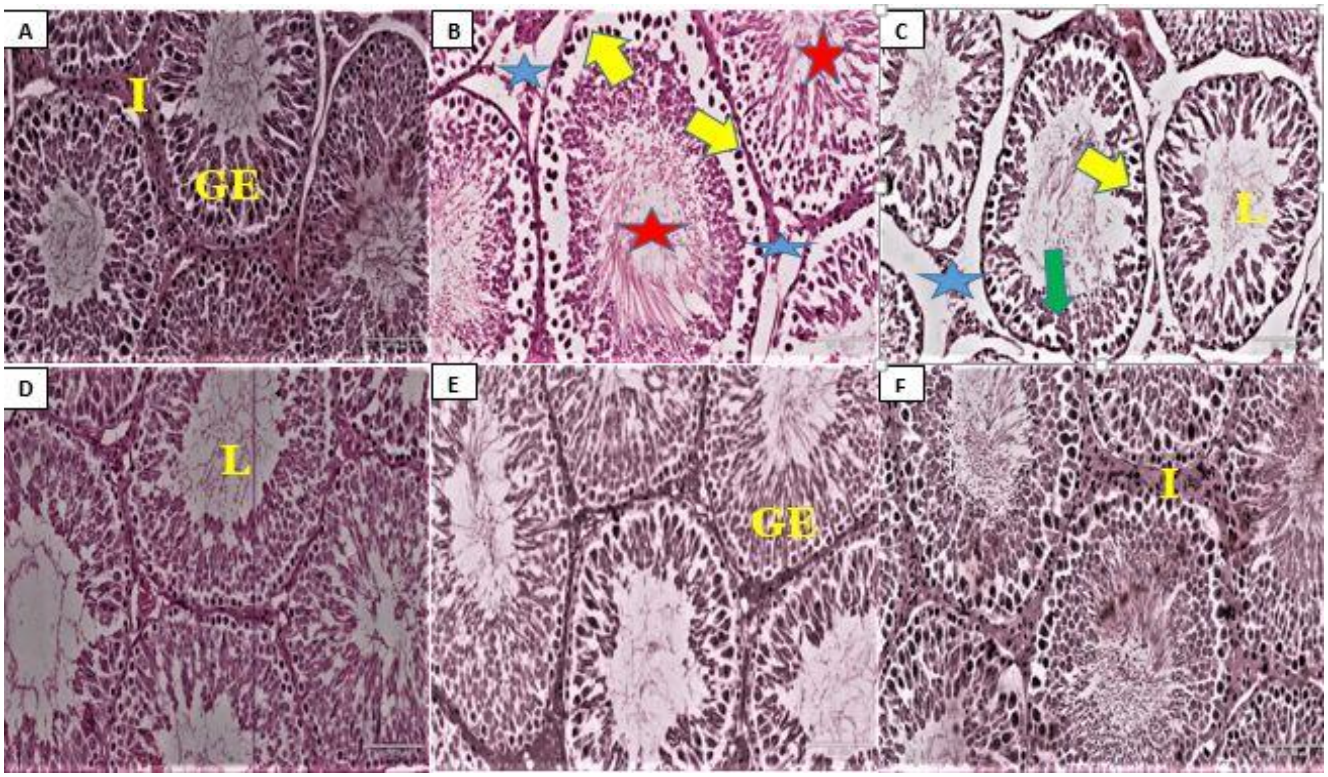

Plate 1:
Photomicrograph of testicular histological sections of groups A-F (H and E). Testicular sections from the control animals 'A revealed a germinal epithelium (GE) with well-preserved cytoarchitecture and normal cellular composition. Leydig cell-filled interstitial (I) spaces were prevalent. Immotile spermatozoa were observed in the lumen (L) (H&E × 100). Group 'B' revealed substantial loss of germ cells (yellow arrow), lumen widening (red star), and an enlarged and broadened hypocellular interstitium (blue star), (H&E X 100). In group C, the cellular densities of the germinal epithelium (green arrow) and interstitium improved significantly. Group D similarly demonstrated increased cellular density of germinal epithelium with an expanded interstitium and some hypocellularity (yellow star). Groups E and F were comparable to the control group (H&E staining, × 100).

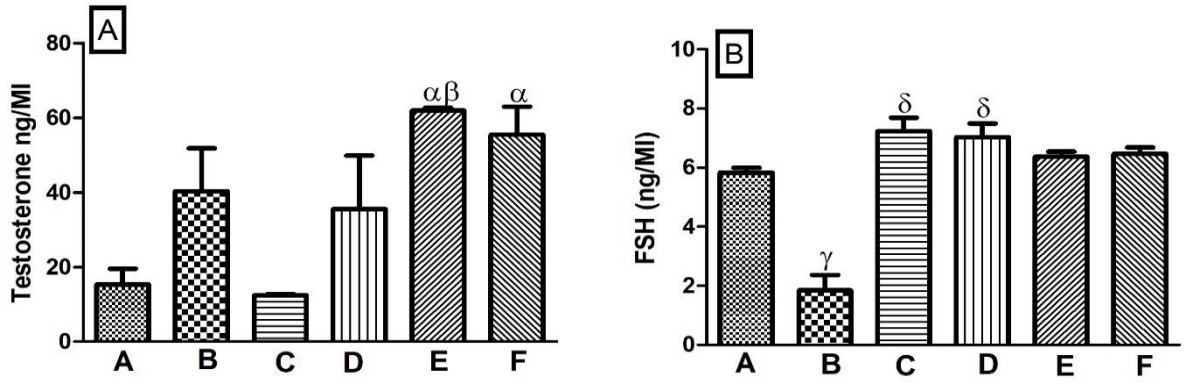

Figure 1:
Effect of *C. esculentus* and HAART treatment on serum testosterone (A) and serum follicle-stimulating hormone (B). The values were all compared at $p < 0.05$, and are shown as the mean ± SEM.; A Control; B (HAART), C (HAART + 500 mg/kg/bw *C. esculentus*), D(HAART + 1000 mg/kg/bw *C. esculentus*), E(500 mg/kg/bw *C. esculentus*), F(1000 mg/kg/bw *C. esculentus*).
The following groups were compared: B, C, D, E, and F versus A; C and D vs. B; E vs. C; and F vs. D.
For serum testosterone: $^{\alpha}(p < 0.05)$ E&F vs A; $^{\beta}(p < 0.05)$ E vs C
For serum FSH: $^{\gamma}(p < 0.001)$ B vs A; $^{\delta}(p < 0.001)$ C & D vs B

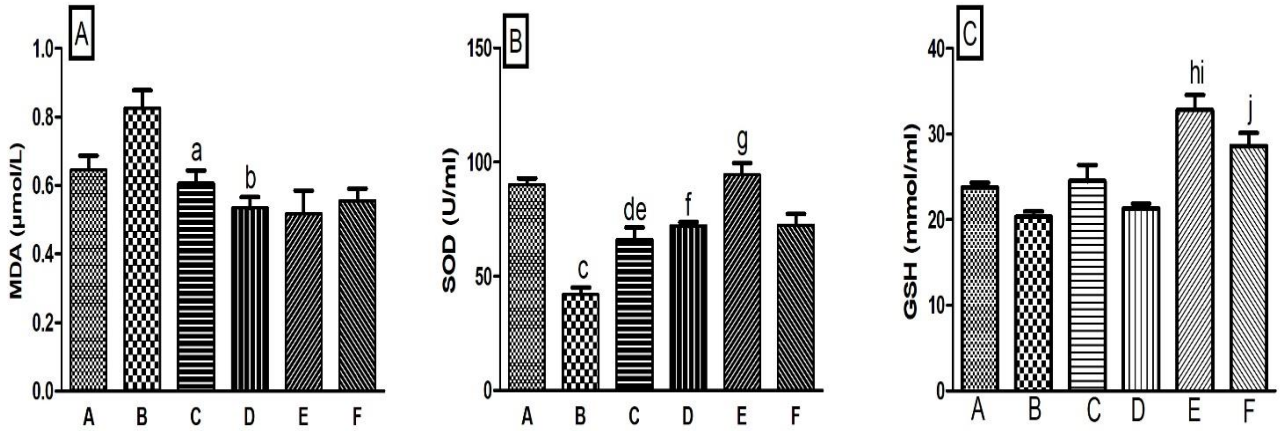

Figure 2:
Effect of *C. esculentus* and HAART treatments on the Serum (A) malondialdehyde (MDA) (B) Superoxide dismutase (SOD) (C) Reduced glutathione (GSH)
The values were all compared at $p < 0.05$, and are shown as the mean ± SEM; A Control; B (HAART), C (HAART + 500 mg/kg/bw *C. esculentus*), D(HAART + 1000 mg/kg/bw *C. esculentus*), E(500 mg/kg/bw *C. esculentus*), F(1000 mg/kg/bw *C. esculentus*).
The following groups were compared: B, C, D, E, and F versus A; C and D vs. B; E vs. C; and F vs. D.
For MDA: $^{a}(p < 0.05)$ C vs B; $^{b}(p < 0.01)$ D vs B
For SOD: $^{c}(p < 0.01)$ B vs A, $^{d}(p < 0.05)$ C vs A; $^{e}(p < 0.05)$ C vs B; $^{f}(p < 0.01)$ D vs B; $^{g}(p < 0.01)$ E vs C
For GSH: $^{h}(p < 0.01)$ E vs A; $^{i}(p < 0.01)$ E vs C; $^{j}(p < 0.01)$ F vs D

FSH: Group B receiving HAART had a mean serum FSH level of 1.8 ± 0.52 ng/mL, which was significantly lower ($p < 0.001$) than the control group A (5.80 ± 0.17 ng/mL). Furthermore, adjuvant *C. esculentus* treatment in groups C (7.2 ± 0.46 ng/mL) and D (7.0 ± 0.46 ng/mL) significantly increased ($p < 0.001$) this parameter as compared to HAART group B respectively. Groups E and F have mean serum FSH levels of (6.4 ± 0.16 ng/mL) and (6.5 ± 0.22 ng/mL), respectively (Fig. 2B).

Malondialdehyde: As shown in fig 3A, adjuvant co-treatment of *C. esculentus* with HAART in groups C ($p < 0.05$) and D ($p < 0.01$) significantly decreased MDA concentration compared to HAART group B. The mean MDA concentrations in groups A, B, C, D, E, and F were 0.65 ± 0.04 mol/L, 0.83 ± 0.05 mol/L, 0.61 ± 0.44 mol/L, 0.53 ± 0.03 mol/L, 0.52 ± 0.07 mol/L, and 0.56 ± 0.03 mol/L, respectively (Fig 3A).

Superoxide dismutase: The mean superoxide dismutase enzyme activity is shown in Figure 3B. SOD levels in HAART group B (42 ± 3.1 U/ml) were significantly lower ($p < 0.001$) than those in control group A (90 ± 2.9 U/ml). Meanwhile, compared to group A, this parameter increased significantly ($p < 0.05$) in group C (66 ± 5.3 U/ml). Compared to the HAART alone group B, the SOD levels in groups C ($p < 0.05$) and D ($p < 0.01$) increased to varying degrees. This parameter was significantly higher ($p< 0.01$) in Group E than in Group C. Groups D, E, and F had mean SOD levels of (72 ± 1.6 U/ml), (95 ± 5.1 U/ml), and (72 ± 4.8 U/ml), respectively (Fig 3B).

Reduced glutathione: *C. esculentus* treatment increased GSH concentrations in group E (33 ± 1.8 mmol/ml) significantly ($p < 0.01$) when compared to Control groups A (24 ± 0.6 mmol/ml) and C (25 ± 1.9 mmol/ml), respectively. This parameter increased significantly ($p < 0.01$) in Group F (29 ± 1.6 mmol/ml) compared to Group D (21 ± 0.6 mmol/ml). B's mean GSH concentration is (20 ± 0.6 mmol/ml).

DISCUSSION

When compared to their antiretroviral therapy-naive peers, HAART treated patients typically experience testicular dysfunction, which is characterized by a decline in sperm motility, count, viability, and volume of seminal fluid (Ogedengbe *et al*., 2018c, Savasi *et al*., 2019). Findings of the present study suggest that antiretroviral medications have a detrimental impact on testicular functional indicators, since HAART-treated rats showed a significant decrease in sperm count, progressive sperm motility, and an increase in the percentage of immobile spermatozoa. The risk of reduced fertility in the testes is thought to be associated with high levels of gonad permeability to antiretroviral medications (Adana *et al*., 2018). Our results also corroborate previous studies that found a favorable correlation between the reduction in sperm functional characteristics and the use of HAART (Akhigbe *et al*., 2021, Ogunlade *et al*., 2022).

Meanwhile, adjuvant *C. esculentus* co-treatment with HAART (groups C and D) resulted in considerably better semen quality (progressive and non-progressive motilities) than HAART alone (group B). These observations and the stereological data (volume density and absolute volume) showed a positive association. Sperm cells require mitochondrial energy metabolism, which can be impaired by toxicants owing to their limited glycolysis capacity. This process is crucial for motility, metabolism, and other functions (Costa *et al*., 2023). It is likely that disruption of mitochondrial energy metabolism may significantly affect sperm cell function and fertility, leading to testicular dysfunction and infertility, as seen in this protocol, thus emphasizing the importance of this crucial process in sperm cells (Ogedengbe *et al*., 2018c).

Reactive oxygen species (ROS) are byproducts of energy metabolism and regulate normal physiological activities. Excessive levels of ROS can compromise the body's ability to detoxify and fight free radicals, leading to oxidative stress and cell damage (Deluao *et al*., 2022). Free radicals induce lipid peroxidation, causing cell damage and death. Antiretroviral drugs can increase ROS generation, causing testicular imbalance, oxidative stress damage, and lower spermatogenic indices, thereby negatively affecting male fertility (Akhigbe *et al*., 2021; Ogunlade *et al*., 2022).

The amount of oxidative stress in the body is gauged by measuring malondialdehyde (MDA), a result of lipid peroxidation. Oxidative stress, which increases the susceptibility of cellular organelles to malfunctions, may be caused by high MDA levels in the body (Olojede *et al*., 2021). This can be accomplished by inhibiting the lipid metabolic pathways. Our findings, which were similar to those of previous studies, revealed higher levels of testicular MDA following HAART treatment, indicating testicular lipid peroxidation (Ogedengbe *et al*., 2018b, Kehinde *et al*., 2021). In support of the vulnerability of testes to oxidative damage, we hypothesized that the HAART Group B could be dependent on a high concentration of polyunsaturated fatty acids, which are easily damaged by free radicals, coupled with lower cytoplasmic levels of scavenging enzymes (Lenzi *et al*., 2000). Additionally, the high sensitivity of sperm cells to mitochondrial activity renders them more vulnerable to the damage caused by oxidative stress (Hussain *et al*., 2023). Thus, disturbance in the cell energy supply may result in immobilization of sperm and axonemal damage, which are required for motility (Mohlala *et al*., 2023).

Our findings also showed that the MDA levels of the *C. esculentus* and HAART co-treatment groups were significantly lower than those of the HAART alone group. *C. esculentus* maintain its capacity as a potent antioxidant with pharmacokinetic effects that may mitigate HAART oxidative stress damage, while enhancing sperm motility, viability, and morphology. Its low levels of polyunsaturated fatty acids, tocopherol, and phytosterol contribute to its high oxidative stability and inhibition of membrane lipid peroxidation (Nwangwa *et al*., 2020), thus mitigating the effects of HAART, as reflected in our study.

Biomarkers that have been found to be essential indicators of oxidative stress severity include glutathione and superoxide dismutase (Sahiner *et al*., 2018). In this experiment, an alteration in the antioxidant defense system may be shown by the lower concentrations of testicular antioxidant enzymes (GSH and SOD) in the HAART alone group. The increased GSH and SOD levels in the C. esculentus-treated groups provided additional evidence in favor of the plant's alleged antioxidative properties. These traits are essential for a successful conception.

Mitochondrial DNA denaturation experiments were not performed to uncover deficits in energy-generating pathways and structural abnormalities. Instead, we used ROS level as a universal indicator of oxidative stress. However, this study showed that *C. esculentus* may have a protective function by efficiently preventing pro-oxidants from inactivating antioxidant enzymes, which would lessen the structural abnormalities that have been reported in the mitigation of HAART effects on testicular parameters in Groups C and D. Therefore, *C. esculentus* appears to be a viable therapeutic agent for HAART-induced infertility.

Both the HAART alone and co-treatment groups C and D in the current investigation demonstrated notable spermatogenic cell degeneration according to histological findings and stereological assessments. Along with having a broader lumen and larger hypocellular interstitium, these groups also exhibited a lower volume density and absolute volume of the germinal epithelium. The decrease in seminiferous epithelial layers may be caused by inhibitory mechanisms against Spermatogonium B, which extends the G-1 phase of the spermatogenic cycle (Jedlinska-Krakowska *et al*., 2006). Mutations in mitochondria may also promote apoptosis and necrotic cell death, decreasing the number of germ cells that can progress to the next stages of spermatogenic development (Vera *et al*., 2004, Bisht *et al*., 2017). Our findings support prior investigations, indicating that HAART induction causes extensive necrosis and disruption of spermatogenesis in germinal cells, as evidenced by reduced seminiferous tubular diameter, hypocellular interstitium, and enlarged lumen (Ogedengbe

et al., 2018b, Owembabazi *et al*., 2023). The capacity of *C. esculentus* to attenuate histological alterations and loss of germ cells as a result of its concurrent use with HAART is reflected in testicular sections of groups C and D, which show some normalcy, as portrayed by the volume densities and absolute volumes of the germinal epithelium. This, in turn, improves the efficacy and viability of sperm, consequently protecting the seminiferous epithelium.

Follicle-stimulating hormone is regarded as a physiological signal to gain access to Sertoli cell activity. According to Jensen *et al*. (1997), it is also known that its effect on Sertoli cells increases the testis's capacity for sperm production. Contrary to its independent effects on spermatogenesis, testosterone works in conjunction with FSH to promote normal sperm production (Simoni and Santi, 2020). Thus, both testosterone and FSH must operate optimally during spermatogenesis (Oduwole *et al*., 2021). Comparing *C. esculentus* groups E and F to Control and HAART co-treatment group C, there were notable significant increases in testosterone levels. Additionally, increased FSH levels were observed in adjuvant *C. esculentus* co-treatment groups C and D compared to the HAART-only group B. These findings support the beneficial effects of *C. esculentus* and the detrimental effects of HAART on testosterone levels and Sertoli cell functions.

In conclusion, HAART has extended the life expectancy of AIDS patients but has detrimental consequences on usage persistence, affecting architecture, function, and semen indices. The antioxidant properties of *C. esculentus* reduced these effects and promoted spermatogenesis. Further investigations are required to quantify these effects.

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Authors Contributions

OOO conceived, designed, analyzed, supervised the experiment, and drafted the manuscript. BA and FAA were involved in methodology and project administration. AAO and ACO provided technical support for this study. KSO, ABO, and AOA provided material support and reviewed the manuscript.

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