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# Selenium and Omega-3 Fatty Acids Ameliorate Highly Active Anti-Retroviral Therapy (HAART)-induced Reproductive Impairment in Male Wistar Rats

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**Summary:** Highly active anti-retroviral therapy (HAART) is currently the main stay in the treatment of Human Immunodeficiency Virus (HIV) disease. This treatment regimen typically combines three or more antiretroviral drugs. Like most drug combinations or polypharmacy, HAART has side effects including those on reproductive function which could place HIV patients on HAART under double risk in terms of reproductive function. Part of tissue damage following HAART administration is blamed on oxidative stress. We therefore sought to explore effects of Omega 3 and Selenium, two common antioxidants on HAART-induced male reproductive impairment in a non-HIV animal model. Sixteen male adult Wistar rats weighing 120g to 250g used for the study were divided into 4 groups of four rats each (control, HAART-only, HAART + Omega 3 and HAART + Selenium groups). Duration of daily administration was six weeks. Results showed no significant changes in pH of epididymal semen among the groups. Sperm count and viability were significantly reduced in HAART-only compared with control ($p<0.05$) but increased in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group ($p< 0.05$). Sperm motility was significantly reduced in HAART-only compared with control group ($p< 0.05$). A significantly higher percentage of total sperm defects was observed in HAART-only group compared with control ($p <0.05$) but significantly lower in the HAART + Selenium compared with HAART-only groups ($p<0.05$). Serum testosterone was significantly reduced in HAART-only compared with control groups ($p<0.05$) but significantly increased in HAART + Omega 3 and HAART + Selenium groups compared with HAART- only group ($p<0.05$). Serum concentration of luteinizing and follicle stimulating hormones were not significantly different among the groups. Testicular concentration of malondialdehyde was significantly increased in HAART-only compared with control ($p<0.05$) but significantly reduced in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group ($p<0.05$ in each). Testicular glutathione peroxidase activity was significantly reduced in HAART-only and HAART + Selenium groups compared with control ($p< 0.05$), but significantly higher in HAART + Omega 3 compared with HAART-only groups ($p<0.05$ each). Testicular superoxide dismutase activity was significantly lower in the HAART-only and HAART + Selenium compared with control ($p<0.05$) but significantly higher in HAART + Omega 3 and HAART + Selenium compared with HAART-only groups ($p<0.05$ each). Level of tumour necrosis factor – alpha in testes was significantly higher in HAART-only ($p<0.05$) compared with control but lower in the HAART + Selenium and HAART + Omega 3 ($p<0.05$) groups compared with HAART-only groups. It was also significantly lower in HAART + Selenium compared with control ($p<0.05$ each) groups. Interleukin-6 levels were significantly increased in all HAART-administered groups compared with control ($p<0.05$ each) though significantly lower in HAART + Omega 3 and HAART + Selenium compared with HAART-only groups ($p<0.05$ each). In conclusion, co-administration of Omega 3 or Selenium with HAART ameliorates HAART-induced male reproductive impairment as well as alterations in redox and inflammatory status in male rats.

**Keywords:** *HAART, male reproductive impairment, Omega 3, Selenium*

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## INTRODUCTION

Human immunodeficiency virus (HIV) infection and AIDS are associated with systemic affectations in virtually every body system and majorly indirectly (Kibaru *et al*, 2015, WHO 2021). This pandemic has sub-Saharan region of Africa being worse affected having over two thirds of the world's HIV infection cases (WHO, 2021; Kalling, 2008; UNAIDS 2007). The reproductive system is not spared in the complications of HIV infection (Kushnir and Lewis, 2011).

The effect of HIV infection on the reproductive system could be due to biological changes like weight loss, systemic illnesses, and stress (Khawcharoenpom and Sha, 2016). The effect on the reproductive system could also be due to AIDS-related co-morbidities like orchitis, epididymitis, pelvic inflammatory disease as well as co-infection with

sexually transmitted pathogens and from opportunistic microbes (Bezold *et al*, 2007). Infection with HIV has also been linked with hypogonadism which could be primary or secondary (Raffi *et al*, 1991).

The advent of highly active antiretroviral therapy (HAART) or combination antiretroviral therapy (cART) was therefore great news with potential to improve management of HIV and its complications including those on reproductive function. This drug treatment regimen or cocktail contains at least three drugs from two or more classes of antiretrovirals given in combination (Mulata *et al*, 2015; Oyeyipo *et al*, 2018). The introduction of HAART therefore heralded an important gain in the fight against HIV/AIDS as the combination therapy targets different stages in the life cycle of the Human Immunodeficiency Virus and so reducing the tendency for drug resistance.

However, like most drugs, drug combinations or polypharmacy, the administration of HAART has been associated with several unwanted effects including those on reproductive function. These effects could be a primary effect of a particular drug component or a synergistic effect of two or more drugs in the cocktail. Administration of HAART has been associated with gonadotoxicity in male rats (Osonuga *et al*, 2010; Bakere *et al*, 2020) as well as impaired sperm motility (Oyeyipo *et al*, 2018; Akhigbe *et al*, 2021; Akang, *et al*, 2022; Savasi *et al*, 2019). Other effects on the reproductive system induced by HAART include reduced serum testosterone, reduced semen volume and impaired sperm count, viability and morphology (Akhigbe *et al*, 2021, Kushnir and Lewis, 2011). Other complications of HAART are sperm mitochondrial deletion (White *et al*, 2001) as well as increased sperm DNA fragmentation (Akang *et al*., 2020, Savasi *et al*, 2018). Oxidative stress has also been noted in testes of patients treated with HAART (Akang *et al*, 2022; Oyeyipo *et al*., 2018) and this can be measured either directly by evaluatng the products of processes like malondialdehyde (MDA), hydrogen peroxide, or indirectly by assessing the concentration or activities of antioxidant systems like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) etc. (Marrocco *et al*, 2017; Yoshikawa and Naito, 2002).

From the above literature, one can observe that though the introduction of HAART has great potential to reduce morbidities and mortalities associated with HIV infection, it is not without its own side effects including those on the reproductive function, the pathophysiology of which is partly attributed to oxidative stress (Akang *et al*., 2022; Oyeyipo *et al* 2018). It was based on this that the possible effects of Omega 3 and Selenium, two commonly available and cheap antioxidants on HAART-induced reproductive toxicity were evaluated.

## MATERIALS AND METHODS

**Ethical Approval:** Ethical consent with approval number 108PHY3821, was granted by the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar – Nigeria.

**Drugs:** A combined antiretroviral therapy (cART) drug (Mylan Laboratories Ltd, India) composed of 50mg Dolutegravir-DTG (as dolutegravir sodium), 300mLamivudine-3TC and 300mg Tenovir Disoproxil Fumarate-TDF per tablet was obtained from the Antiretroviral Unit of Infections Disease Hospital (IDH), Calabar, Cross River State, Nigeria. Omega 3 fatty acid (Emzor Omega-3® fish oil, 1000mg per capsule) manufactured by Gujarat Liqui Pharmacaps Pvt Ltd – GLPL, India) containing Eicosapentaenoic Acid NLT (18%), Docosahexaenoic Acid NLT (12%) and Selenium as Sodium Selenite (200mcg per capsule), made by Bactolac Pharmaceutical Inc. USA were obtained from Bez Pharmacy, Eta Agbor Road, Calabar.

Extrapolation of HAART dosage: The dosage for rats was extrapolated from human doses using the formular developed by Nair and Jacob (2016). Animal equivalent dose – AED (mg/kg, Human dose (mg/kg) x Kin ratio

Where (Km) = corrected factor estimated by dividing the average body weight (kg) of species to its body surface area (m2).

**Preparation of drugs/stock solutions/dosages:** Tween-80\water solution: Ten drops of the solvent (Tween-80) were placed on a graduated bottle and made up to 40mls with portable water.

**Stock solution of HAART.** This was prepared by reducing two tablets of HAART to powder, then dissolving it in 8 drops of Tween-80 and the resulting solution made up to 40mls with water to give concentrations of 2.5mg, 15mg and 15mg of Dolutegravir, Lamivudine and Tenovir Disoproxil Fumarate respectively per ml of solution.

**Stock solution of Omega 3:** This was done by dissolving the content of six capsules of Omega 3 (6000 mg) in 4 drops of Tween-80 and the solution marked up to 40mls giving a concentration of 150mg/ml of solution.

**Stock solution of Selenium:** This was prepared by dissolving the content of 10 capsules (2000µg) in 4 drops of Tween-80 and the solution marked up to 40mls to give a concentration of 50µg/ml of solution.

**Experimental Design:** Sixteen male Wistar rats weighing 120 – 250g were randomly divided into four groups of 4 rats per group and raised in wooden cages which were cleaned regularly. Animals were given food and water ad libitum. Group 1 (control) was given 0.003ml/g of the Tween-80/water solution daily. Group 2 (HAART-only) was given 25mg/kg, 25mg/kg and 3.8mg/kg (ie 0.00167ml/g) of Lamivudine, Tenovir and Dolutegravir respectively of the HAART solution. Group 3 (HAART + Omega 3) received 0.00167ml/g (i.e. 3TC-25mg/kg, TDF-25mg/kg and DTG-3.8mg/kg ) of HAART solution + 600mg/kg of Omega 3. Rats in group 4 (HAART + Selenium) received 0.00167ml/g (3TC-25mg/kg, TDF-25mg/kg and DTG-3.8mg/kg) + 0.3mg/kg (0.0075ml/g) of Selenium. Drugs were administered daily by gavaging for duration of six (6) weeks. Reweighing of animals was done on a regular basis to take care of needed adjustments in the amount of drugs administered.

**Collection of samples:** At the end of administration period (six weeks) animals were anaesthesized under chloroform and blood samples collected via intracardiac puncture for

assay of relevant parameters. Animals were then sacrificed and testes dissected out for determination of oxidative stress, seminal and inflammatory parameters.

**Seminal fluid analysis:** Epididymal seminal fluid analysis was carried out following standard procedures outlined by the World Health Organization WHO (2006) summarized below:

**Seminal Ph:** Semen was aspirated from epididymis with a sterile needle and the pH measured directly using a hand-held pH meter (Zellik, Belgium)

**Sperm count:** This was done using improved Neubeur counting chamber. A sample of 0.5ml of sperm suspension was diluted with 9.5ml of sperm-diluting solution (5g NaHCO3, 1ml formalin (35%) and 25mg Eosin per 100ml distilled water). Ten microlitre (10µl) of the diluted sperm suspension was transferred to each counting chamber and allowed to stand for 5 minutes. The concentration of sperms was evaluated as million sperm cells per ml of sperm solution under x 400 magnification using light microscope (Olympus, Tokyo, Japan).

**Sperm viability:** One (1) drop of semen was mixed with one (1) drop of 0.5% Eosin solution on a slide. The preparation was left for two (2) minutes after which x10 objective was used to focus the specimen while x40 objective was used to evaluate the per centage of viable (unstained) and nonviable (stained) sperm cells.

**Sperm morphology:** A thin smear of well mixed semen was made on a slide and while still wet fixed with 95% v/v ethanol for five (5) minutes and allowed to air-dry. The smear was then covered with carbol Fushcin (1 in 20), allowed to stand for 30mins and then washed off with water. It was then counter-stained with diluted Leoffler methylene blue for two (2) minutes. Using x40 objective lens, morphology of sperm cells was assessed and expressed in percentage.

**Sperm motility:** One (1) drop of well mixed semen was placed on a slide and covered with a cover glass. A light microscope (Olympus, Tokyo, Japan) was then used to focus the specimen using x10 objective lens. A x40 objective lens was thereafter used to examine several fields for motility with results expressed in percentage (%).

**Determination of serum concentration of follicle stimulating hormone (FSH):** This was determined in triplicated samples by Chemiluminescent Microparticle Immunoassay (CMIA) technique using ARCHITECT FSH Kit (7K75) obtained from Abbott Laboratories Diagnostics Division, Abbott Park, IL 60064, USA- and as used by Tan *et al* (2018).

**Evaluation of serum concentration of luteinizing hormone (LH):** This was done using Chemilumiscent Microparticle Immunoassay using ARCHITECH LH Kit (6C25) obtained from Abbott Laboratories Diagnostics Division, Abbott Park, IL USA. This evaluation was in tandem with similar method as demonsrated by Steyn *et al* (2013).

**Determination of serum testosterone concentration:** This was done using the Chemiluminescent Microparticle Immunoassay (CMIA) ELISA technique with ARCHITECT Testosterone Reagent kit obtained from Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA as demonstrated by Steyn *et al* (2013).

**Preparation of testicular homogenate:** Dissected testes were cleaned of fat and homogenized in 25ml saline-merthiolate-triton (SMT) buffer at 27,000 rpm using a Brinkmann Kinematical homogenizer for 2 minutes and used immediately for assay of various testicular parameters. This was as also demonstrated by Rodriguez-Casuriaga *et al* (2013).

**Determination of malondialdehyde (MDA) concentration in testicular homogenate:** This was determined using the method of Buege and Aust (1978). A sample of 0.1ml homogenate (in Tris-HCL buffer (at pH 7.5) was treated with 2ml (in a ratio 1:1:1) of thiobarbituric acid (TBA) – Trichloroacetic acid (TCA) and HCL reagent (TBA 0.37%, 25 NHCL and 15% TCA) after which the mixture was placed in a water bath for 15 minutes and allowed to cool. The absorbance of the clear supernatant was measured against reference blank at 535nm with a Perkin Elmer Spectrophotometer, (Lamber 25).

**Evaluation of testicular glutathione peroxidase activity:** This was assessed on testicular homogenate according to an established procedure of Weydert and Cullen (2010) using hydrogen peroxide (H2O2) as substrate. The absorbance of the product was read at 430nm.

**Determination of testicular superoxide dismutase (SOD) activity:** This was doneon testicular homogenate using biochemical method with xanthine oxidase used to generate superoxide anion (O2-) while nitroblue tetraolium (NBT) reduction was used as indicator of the O2 production. The method of Weydert and Cullen (2010) was used for this procedure. The optical density (OD) of the product was read at 450nm using a ELISA reader within 15 minutes after adding stop solution.

**Evaluation of IL- 6 and TNF-alpha:** The concentrations of IL-6 and TNF-alpha in testicular homogenate were determined using commercially available indirect sandwich ELISA kits (Bender MedSystems, Austria) for each as also done by Afshari *et al* (2005). At the end of reaction time, the absorbance for each was measured at 450nm. Analyses for each parameter were performed in duplicates following manufacturer's guidelines.

**Statistical analysis:** Data were presented as mean ± SEM. Normally distributed data were analyzed using analysis of variance (ANOVA) followed with a post hoc test of least significant differences. A value of $p<0.05$ was considered statistically significant. All analyses were conducted using Statistical Package for the Social Sciences (SPSS) software version 25.

## RESULTS

**Semen pH:** There were no significant differences in the pH of semen in various experimental groups as in Table 1.
**Sperm count:** (million/ml): Sperm count was significantly reduced in the HAART-only group (34.18 ± 7.43) compared with control (55.95 ± 5.17) ($p<0.05$) but significantly higher in HAART + Omega 3 (48.15 ± 8.54) and HAART + Selenium (48.38 ± 5.14) compared to HAART-only (34.18 ± 7,43) groups ($p<0.05$ each) as shown in Table 1.

**Sperm viability (%):** Viability of sperms was significantly reduced in the HAART-only (32.5±2.89) compared with control (87.5 ± 14.) with $p<0.05$ but increased in the HAART + Omega 3 (83.45±6.29) and HAART + Selenium (90.00 ± 4.08) groups with $p<0.05$ as shown in Table 1.

**Sperm motility (%):** Sperm motility was significantly reduced in the HAART-only group (69 ± 8.21) compared with control group (86±4.9) with $p<0.05$ as shown in Table 1.
**Morphological defects (%:)** Percentage of total morphologically defective sperm cells was significantly higher in the HAART-only (7.5±1.29) compared with control (4 ±0.82) with $p<0.05$ but lower in the HAART + Selenium (4.75±.171) compared with HAART-only (7.5±1.29) groups with $p<0.05$ as in Table 1.

**Serum FSH (mlu/ml):** Comparison of the concentrations of serum FSH of control (9.27± 0.66) HAART-only (9.12±0.29), HAART + Omega 3 (8.73±0.58) and HAART + Selenium (8.81±0.35) did not show any significant differences among the groups as shown in Table 2.

**Serum LH (mlu/ml):** Comparison of concentrations of serum LH in control (7.45±1.17) and HAART-only (7.55±0.68), HAART + Omega 3 (7.36±0.75) and HAART + Selenium (6.67±0.12) groups did not show any significant differences among the groups as in Table 2.

**Serum testosterone (ng/ml):** Concentration of serum testosterone was significantly lower in the HAART-only (2.29±0.85) compared with control (4.94±0.78) with $p<0.05$ but higher in the HAART + Omega 3 (4.11±0.55) and HAART + Selenium (4.20±0.60) compared with HAART-only (2.29±0.85) groups with $p<0.05$ each as in Table 2.

**Testicular concentration of malondialdehyde (nmol/ng):** Concentration of testicular malondialdehyde (MDA) was significantly increased in the HAART-only (65.53±3.53) compared with control (47.89±3.28) with $p<0.05$ but decreased in HAART + Omega 3 (43.20±3.74) and HAART + Selenium (47.59±3.72) with $p<0.05$ compared with HAART-only groups as in Table 3.

**Testicular glutathione peroxidase – GPx activity (pg/mg protein):** Glutathione peroxidase activity was significantly reduced in the HAART-only (28.94± 2.56) compared with control (43.60±4.81) with $p<0.05$ but higher in HAART + 0mega 3 (47.17±2.88) and HAART + Selenium (35.21±4.31) when compared with HAART-only (28.94±2.56) with $p<0.05$ each. It was also significantly lower in HAART + Selenium compared with HAART + Omega 3 groups ($p<0.05$) as shown in Table 3.

**Testicular superoxide dismutase activity (pg/mg protein):** Superoxide dismutase activity in HAART-only (57.23±2.19) was significantly decreased compared with control (110.61±8.27) with $p<0.05$ but higher in HAART + Omega 3 (97.67±7.58) and HAART +Selenium (84.06±7.19) compared with HAART-only (57.23±2.19) groups with $p<0.05$ in each case. It was also significantly lower in HAART + Selenium (84.06±7.19) compared with control ($p<0.05$) as shown in Table 3.

**Table 1:**
Sperm quality parameters of the different experimental groups

| Group | pH | Sperm count ($x10^6$cells/mL) | Motile sperm (%) | Viable sperm (%) | Total sperm defects (%) |
|---|---|---|---|---|---|
| Control | 6.70±0.24 | 55.95±5.17 | 86.00±4.97 | 87.50±6.45 | 4.00±0.82 |
| Haart | 7.05±0.19 | 34.18±7.43[a] | 69.00±8.21[a] | 32.50±2.89[a] | 7.50±1.29[a] |
| Haart + Omega 3 | 6.83±0.25 | 48.15±8.54[b] | 80.00±9.13[b] | 83.75±6.29[b] | 5.00±0.82 |
| Haart + Selenium | 6.98±0.17 | 48.38±5.14[b] | 82.50±8.66[b] | 90.00±4.08[b] | 4.75±1.71[b] |

*Values are expressed as mean ±SD; a = p<0.05 vs control; b = p<0.05 vs Haart*

**Table 2:**
Reproductive hormones concentration in the different experimental groups

| Group | FSH (mIU/mL) | LH (mIU/mL) | Testosterone (ng/mL) |
|---|---|---|---|
| Control | 9.27±0.66 | 7.45±1.17 | 4.94±0.78 |
| Haart | 9.12±0.29 | 7.55±0.68 | 2.29±0.85a |
| Haart + Omega 3 | 8.73±0.58 | 7.36±0.75 | 4.11±0.55b |
| Haart + Selenium | 8.81±0.35 | 6.67±1.12 | 4.20±0.60b |

*Values are expressed as mean ±SD*
*a = p<0.05 vs control*
*b = p<0.05 vs Haart*

**Table 3:**
MDA, GPx and SOD concentration in the different experimental groups

| Group | MDA (nmol/mg Protein) | GPx (pg/mg Protein) | SOD (pg/mg Protein) |
|---|---|---|---|
| Control | 47.89±3.28 | 43.60±4.81 | 110.61±8.27 |
| Haart | 65.57±3.53[a] | 28.94±2.56[a] | 57.23±2.19[a] |
| Haart+Omega 3 | 43.20±3.74[b] | 47.17±2.88[b] | 97.67±7.58[b] |
| Haart+Selenium | 47.59±3.72[b] | 35.21±4.31[a,c] | 84.06±7.19[a,b] |

*Values are expressed as mean ±SD*
*a = p<0.05 vs control; b = p<0.05 vs Haart*
*c = p< 0.05 vs Haart + Omega-3*

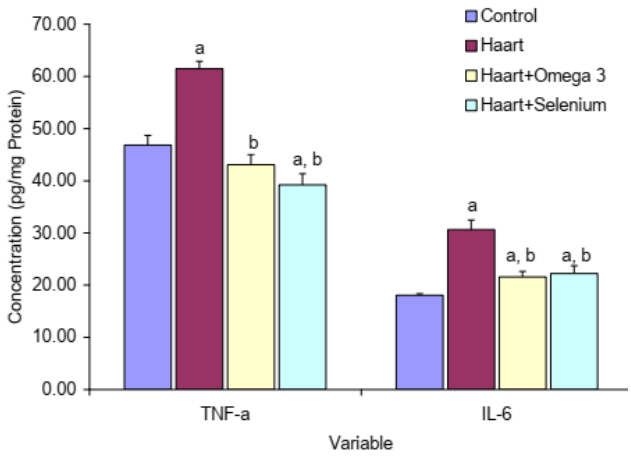


**Figure 1**
Tissue necrosis factor-alpha and interleukin-6 activity in the different experimental groups.
*Values are expressed as mean±SD, n=5*
*a = p<0.05 control; b = p<0.05 HAART*

**Testicular concentration of tumor necrosis factor – alpha (pg/mg protein):** Tumor necrosis factor-alpha (TNF-α) was significantly increased (p<0.05) in HAART-only (61.45±1.44) compared with control (46.83±1.86) but significantly decreased (p<0.05 each) in HAART + Omega 3 (43.05±1.93) and HAART + Selenium (39.20±2.16) groups compared with HAART-only group. There was also significant decrease (p<0.05) in this index in HAART + Selenium compared with control groups as shown in Fig. 1

**Concentration of testicular interleukins-6 (pg/mg protein):** There were significant increases (p<0.05 each) in the concentrations of interleukin-6 (IL-6) in HAART-only (30.64±1.83), HAART + Selenium (22.21±1.51) groups compared with control (18.05±0.32). it was however significantly decreased in HAART + Omega 3 and HAART + Selenium groups (p<0.05 for each) compared with HAART-only group as shown in Fig. 1.

## DISCUSSION

In this study, the effects of co-administration of HAART with Omega 3 or Selenium on some male reproductive and related parameters were evaluated. pH affects the physiological functions of tissues and cells including semen (Zhou *et al*, 2010). Our results however did not show any significant differences in seminal pH among all groups indicating that any abnormality in the seminal fluid parameters might not have been due to seminal pH.

The observed significant decrease in sperm count in the HAART-only group when compared with the control strongly suggests that HAART affects sperm count. Our observation is similar to the findings by Savasi *et al* (2019), Akang *et al* (2022), Alugbe *et al* (2021), Bakare *et al* (2020), Osonuga *et al* (2010) and Oyeyipo *et al* (2018)). The decrease in sperm count could have been due to direct testicular toxicity from oxidative stress (Oyeyipo *et al* 2021), sperm DNA fragmentation (Savasi *et al*, 2018; Osonuga *et al*, 2010; Bakare *et al*, 2020) or HAART-induced sperm mitochondrial DNA fragmentation and deletion (White *et al*, 2001). The significantly higher sperm count in the groups co-administered with antioxidants (HAART + Omega 3 and HAART + Selenium groups) shows the ability of the antioxidants to ameliorate the impairment induced by HAART (Oyeyipo *et al*, 2018).

Sperm viability was significantly reduced in HAART-only group compared with control. This is similar to the findings of Savasi *et al* (2019) and Akhigbe *et al* (2021). This trend was however prevented with an increase in the percentage of viable sperms following co-administration with Omega 3 or Selenium

Normal sperm morphology is essential for fertilization. We noted an increase in percentage of morphologically defective sperm cells in HAART-only group compared with control which is similar to the observations of Bakare *et al* (2020), Akhigbe *et al*, (2021) and Savasi *et al* (2019). The increase in the percentage of defective sperms in the HAART-only group might have been due to increased sperm DNA fragmentation associated with HAART administration as reported by White *et al* (2001) and Savasi *et al* (2018). There was however a significant decrease in the percentage of morphologically defective sperm cells in the HAART + Selenium compared with HAART-only groups; an effect attributable to Selenium co-administration. Selenium as a trace element is the backbone of a number of Selenium–containing compounds including Selenoproteins like glutathione peroxidase enzymes involved in several physiological activities like immune modulation redox reactions, anti-inflammatory functions and inactivation of free radical to prevent oxidative stress (Brown and Authur, 2001; Yang & Liu, 2017; Constatineacu-Aruxandei *et al*, 2018; Xie *et al*, 2020).

Our results do not show any significant differences in the serum concentrations of FSH and LH among the different groups. These hormones are produced in the anterior pituitary gland (Sembulingam and Sembulingam 2012). This means that any effect of HAART might not have affected the synthesis of these hormones.

A significant reduction in the concentration of serum testosterone observed in HAART-only group compared with control is similar to the finding of Akang *et al* (2022). Akhigbe *et al* (2021) also found a similar decrease in testicular concentration of testosterone in HAART-administered rats. This decrease could have been due to direct gonadal toxicity affecting Leydig cells (Osonuga *et al*, 2010, Bakare *et al* 2020). Our observed reduction in concentration of testosterone in HAART-only group with no significant differences in FSH and LH shows that HAART induces a secondary hypogonadism. The decrease in testosterone might also have contributed to abnormalities observed in seminal parameters mentioned earlier. Co-administration of Omega 3 or Selenium with HAART prevented this trend.

Literature shows that administration of HAART is associated with oxidative stress, an imbalance between pro-oxidative and anti-oxidative mechanisms which tilts towards oxidation (Yoslukawa and Naito, 2002; Marrocco *et al*, 2017). In a similar way, we observed that testicular concentration of MDA, one of the last products of lipid peroxidation in tissues (Ivanov *et al*, 2016; HO *et al*, 2013), was significantly increased in HAART-only group compared with control which suggests an increase in lipid

peroxidation in the former group compared with the latter.This observation is also similar to that made by Oyeyipo *et al* (2018). On the other hand, our findings of significant decreases in activities of testicular superoxide dismutase and glutathione peroxidase in the HAART-only compared with control groups is similar to those of Oyeyipo *et al*, (2018); Akang *et al* (2021); Elechi-Amadi and Briggs (2018); Havlickoval (2021) and suggests elevated oxidative activities. The increase in peroxidation and oxidation observed in HAART-only group strongly agree with previous works mentioned above that HAART induces oxidative stress in testes and so can be partly responsible for the impaired testicular function seen in this study. This is especially so as these changes were prevented following co-administration of HAART with Omega 3 or Selenium which are known antioxidants.

From our results, there was a significantly increased concentration of testicular TNF-α in HAART-only compared with control groups. Tumour necrosis factor –α is a pleiotropic cytokine (Gough and Myles, 2020) and dysregulation of its production has been associated with several pathologies including inflammation, psoriasis, inflammatory blood disease, cancers etc. (Gough and Myles 2020; Brynskov *et al*, 2002; Victor and Gottlieb, 2002). The significant decrease in TNF-α observed in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group shows ability of Omega 3 and Selenium to prevent this effect of HAART if co-administered.

Interleukin-6, a multi-functional cytokine was found to be significantly increased in the HAART-only. Its dysregulated continual synthesis plays pathological role in chronic inflammation and immunity (Kimura and Kishimoto, 2010; Tanaka *et al*, 2014; Zhang *et al*, 2010). There was however a significant decrease of this index in HAART + Omega 3 and HAART + Selenium groups compared with HAART-only group'' The observed increase in testicular TNF-alpha and IL6 in HAART-only group suggests that part of mechanism of testicular injury might have been inflammation. This was however prevented following co-administration with Omega 3 or Selenium'.

In conclusion, co-administration of Omega-3 or Selenium with HAART ameliorates HAART – induced reproductive toxicity as well as testicular inflammatory status and oxidative stress in male Wistar rats.

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