International Journal of Health and Medicine

E-ISSN: 3047-793X
P-ISSN: 3047-7948

Research Article

Toxicity Potentials of Dolutegravir-Base Antiretroviral Therapy on the Ovary and Uterus of Adult Wistar Rats

Ogbe, O.C.[1]*, Ataman, J.E.[2],Ezeuko, V.C[3].

[1] University of Benin, Benin City, Edo State, Nigeria.
e-mail : ogbeonomeclementinahappiness@yahoo.com
[2] University of Benin, Benin City, Edo State, Nigeria.
e-mail: atamanje@yahoo.com
[3] University of Benin, Benin City, Edo State, Nigeria.
e-mail: chukwuma.ezeuko@uniben.edu

* Corresponding Author : Ogbe, O.C

Abstract: Background: Human immunodeficiency virus (HIV) remains a critical global health issue, with 88.4 million infections and 42.3 million AIDS-related deaths to date. In 2024 alone, 39.9 million individuals are living with HIV, 630,000 deaths occurred, and 1.3 million new infections were recorded. Tenofovir Disoproxil Fumarate/Lamivudine/Dolutegravir (TLD) is a widely used antiretroviral therapy, but its reproductive and oxidative effects remain underexplored in female models, particularly in the South-South region of Nigeria.Objective: This study aimed to evaluate the reproductive toxicity and oxidative stress effects of TLD on the ovaries and uterus of adult female Wistar rats.Methods: Ten adult female Wistar rats (156–187g) were divided into control and treatment groups (n=5). The control group received standard diet and distilled water, while the treated group received daily doses of TLD (Tenofovir 5 mg, Lamivudine 5 mg, and Dolutegravir 0.8 mg/kg body weight) for 90 days. At the end of the treatment, animals in estrus phase were sacrificed for biochemical, histological, and hormonal analysis.Results: The treated group showed a significant reduction in body weight but no significant changes in ovarian or uterine weight. Oxidative stress analysis revealed decreased MDA and increased SOD, GPx, and CAT in the uterus. Hormonal levels were not significantly different. Histologically, the treated group displayed impaired follicular development, atretic follicles, cysts, enlarged endometrial cavities, and thicker endometria.Conclusion: TLD administration induced notable reproductive alterations in female Wistar rats, highlighting potential implications for its use in women of reproductive age.

Keywords: HIV; Hormonal assay; Ovary and uterus; Oxidative stress; TLD

Received: March, 17th 2025
Revised: March, 31th 2025
Accepted: April, 20th 2025
Published: April, 22th 2025
Curr. Ver.: April, 24th 2025

1. Introduction

Human immunodeficiency virus (HIV) infection is a major global health concern (Iorjiim et al., 2020), primarily caused by contact with body fluids such as blood, breast milk, semen, and vaginal secretions and transmission during pregnancy and delivery (Vaishnav and Wong-Staal, 1991); Centers for Disease Control (CDC, 2018). However, the introduction of Highly Active Antiretroviral Therapy (HAART) has significantly reduced morbidity and mortality associated with HIV/AIDS (Awodele et al., 2018), making HIV a chronic illness rather than a disease with imminent death.

HIV has infected 88.4 million people globally, with 42.3 million deaths from AIDS-related illnesses. Since the start of the epidemic. In 2023, 39.9 million people were living with HIV, 630,000 died, and 1.3 million were newly infected. Women and girls have a higher proportion of new infections than men and boys, with 44% in 2023, 62% in sub-Saharan Africa, and 73% of new HIV infections occurred among men and boys in other regions (Joint United Nations Programme on HIV/AIDS (UNAIDS, 2024).

Oxidative stress (OS) is a condition characterized by an imbalance between pro-oxidant molecules and antioxidant defenses (Al-Gubory et al. (2010); Agarwal et al. (2012). It is a major factor in subfertility in both males and females and predicts mortality in HIV patients

(Masiá et al., 2016). It is commonly known that OS influences HIV infection (Salmen and Berrueta, 2012), According to one study (Awodele et al., 2012), ART was linked to a lower OS; although it is yet unknown how ART alters OS. However, other studies (Martin et al. (2001); Kwara et al. (2005); Mandas et al. (2009) revealed that it was linked to a higher OS, and can cause cardiovascular, neurological, metabolic, and hepatic problems in many people were living with HIV.

Reproductive disorders like endometriosis, polycystic ovarian syndrome (PCOS), and infertility are caused by imbalances between pro-oxidants and antioxidants, with HIV infection linked to elevated free radical generation (Jones, 2008) and suppressed antioxidant defense (Nkechi et al. (2013); Cribbs et al. (2014).

Prolonged use of antiretroviral therapy (ART) raises concerns about its adverse effects on women of reproductive age, including irregular menstrual periods and reduced ovarian function. Although ART is essential for HIV management, certain ARVs, like efavirenz, have been linked to changes in reproductive hormone profiles (Ohihoin et al., 2025).

The World Health Organization (WHO) recommended Tenofovir Disoproxil Fumarate (TDF) 300 mg/Lamivudine (3TC) 300 mg/Dolutegravir (DTG) 50 mg combination in 2018 as the first-line and second-line antiretroviral therapy (ART) for HIV-1 infections for adults, adolescents, and children aged 6 years and up who weigh at least 30 kg (WHO, 2018), in 2019, highlighting its potency, longevity, and tolerability (WHO, 2019).

Despite the advantages of TLD in treating HIV patients especially among women of child-bearing age, the effects of TLD on female fertility and oxidative stress have not been investigated using an animal model in the South-South region of Nigeria. Thus, this study aims to evaluate the toxicity potentials of Dolutegravir-base antiretroviral therapy on the ovary and uterus of adult Wistar rats.

2. Materials And Methods

Drugs

Tenofovir Disoproxil Fumarate 300 mg/Lamivudine 300 mg/Dolutegravir 50 mg (TDF/3TC/ DTG) (Lot:3125312, manufactured 11/2019, expired in 10/2022, manufactured by Mylan Laboratories Limited, India), were purchased from Alpha Pharmacy and Stores Ltd., located at #59 Ogbunabali Road, Port Harcourt, Rivers State, Nigeria.

ExperimentalAnimals

Adult Wistar rats used for the study were bred at the animal house of the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. The animals were kept in appropriate cages with wired open tops at room temperature and saw dusts were used as beddings for the cages. The animals were given tap water and grower mash obtained from Sa-Vee Livestock Feed Service, Isihor, Benin City. They were weighed before the commencement and also weekly throughout the duration of the experiment using electronic weighing scale balance (manufactured by Kern & Sohn GmbH, D-72336 Balingen, Germany), calibrated in gram and recorded to the nearest whole number. Protocols for these experiments were in accordance with the guidelines for the care and use of laboratory animals (National Research Council of the National Academies, 2011).

Design of Study

A total of 10 adult female Wistar rats weighing between 156g -187g, aged between 90 days and 120 days were used for this study. The animals were randomly selected and assigned into two groups, control and treated, comprising five rats each. Group 1 served as the control and was fed growers mash and distilled water only. Group 2 serves as the treated group, and was fed growers mash and distilled water and was orally administered the combination of Tenofovir Disoproxil Fumurate (5mg/kg), Lamivudine (5mg/kg) and Dolutegravir (0.8mg/kg) body weight. Human exploratory dose was calculated based on animals weight.

Sample Collection

Following ninety days (90) of oral administration of TLD, the animals in estrus phase were selected, weighed and anesthetized under chloroform and sacrificed. The ventral

abdominal walls were opened; ovaries and uterine horns were harvested, cleared of connective tissue and rinsed in 10% formal saline. The uterine horns were measured in milimeter using vernier caliper, weighed alongside with the ovaries using digital weighing balance calibrated in gram (manufactured by ECOSTAR, China), recorded to the nearest two decimal places and were preserved in 10% formal saline contained in sample bottles with the appropriate labels and was taken for histological evaluation. While the ovaries and uterus for oxidative stress were preserved in normal saline (0.9% NaCL) contained in sample bottles with the appropriate labels. Also, blood samples (5ml) were collected via the inferior venacava (IVC) in plain bottles with the appropriate labels for hormone assay by ELISA.

Histological Assessment

The procedure outlined by Drury and Wallington (1980) was followed while preparing the tissue. For histological examination, the ovarian and uterine tissue samples underwent fixation, dehydration, clearing, filtration, embedding, sectioning, and staining. The tissues were cut to a thickness of about 5 mm in order to achieve proper fusion. After the tissues were fixed with 10% formal saline, they were placed in 50% alcohol for two hours at 70%, 80%, 85%, 95%, and 100%. The treated tissues were titrated through an equal mixture of 100% (absolute) alcohol and xylene for one hour each, in order to eliminate the alcohol. Each tissue was infiltrated twice by putting it through molten paraffin wax for one and a half hours each time in an oven set to 40 oC. After being so imbedded in melted paraffin wax, the tissues were put on a wooden block and cut to size. A rotatory microtome was used to create serial sections that were 10 μm thick. After being sliced, the portions were placed on slides and heated to 40 oC in a warm water bath. From each animal's treated organ, six pieces were taken. Each slide had three samples on it. To check if the samples were correctly fixed on the slide, a microscopic examination was conducted using different magnifications of 10, 40, 100, and 400. After staining, sections were mounted using dimethyl paraffinate xylene (DPX) as a mounting agent, and microscopic analysis was performed.

Anti-Oxidants Stress

Following two cold phosphate buffered saline (PBS) washes, the tissues were homogenized in a porcelain mortar and pestle with acid-washed sand and PBS. The tissue homogenate was centrifuged for 10 minutes at 40 oC and 10,000 rpm. The supernatant was treated right away so that the endogenous antioxidant enzymes could be examined. Buege and Aust (1978) method was used to measure malondialdehyde (MDA) activity. Nyman (1959) method was used to measure glutathione peroxidase (GPx) activity. Glutathione (GSH) levels were estimated using Ellman's (1959) approach. Misra and Fridovich (1972) approach was used to measure the amount of superoxide dismutase (SOD) activity. Cohen et al. (1970) method was used to measure the activity of catalase (CAT).

Hormonal Analysis

After collecting blood in a serum-collection tube devoid of anticoagulant, it was centrifuged for 15 minutes at 3000 rpm after coagulating for roughly 20 minutes at room temperature. Prior to the hormonal analysis (specifically, serum Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Prolactin (PRL), Progesterone (P4), Estradiol (E2), Testosterone (T) in accordance with Dorfman and Shipley, (1965); Aufrere and Benson, (1976); Danzer and Braunstein, (1980); Odell and Parlow, (1981); Kosas, (1981); Abraham, (1981) the supernatant serum samples were moved to dry, clean-capped tubes and kept at -20 °C (Accubind ELISA Microwells Monobind Inc. Lake Forest, CA 92630, USA).

Statistical Analysis

Graphpad prism Version 9 (manufactured by Graphpad Software Inc., California) was used to analyse the data. Paired-Samples T-Tests were used to compare the parameters for each group, and Mean±SEM was used to present the data. $p<0.05$ were considered significant.

Motic Images Plus 2.0 software (manufactured by Speed Fair Co., Ltd, released in 2017) was used for morphometric measurement.

3. Results

Table 1: Effect of TLD on the body weight of the Experimental Rats

Body Weight (g)	Control Group	Treated Group	P-Value
Initial body weight	165.2±3.076	168.8±3.574	0.454
Final body Weight	258.8±4.983	236.0±4.325*	0.001

Values are represented as Mean±SEM; *indicates significant decrease ($p<0.05$) in the final mean body weight of the treated group of the experimental animals compared to the control.As show in table 1, daily oral administration of the combined drugs cause a significant decrease ($p<0.05$) in the final mean body weight of the TLD-treated group compared to the control.

Table 2: Effect of TLD on ovarian weight, uterine weight and uterine horn length of the Experimental Rats

Parameters	Control Group	Treated Group	P-Value
Ovarian weight (g)	0.08±0.003	0.07±0.003	0.056
Uterine weight (g)	0.50±0.04	0.44±0.02	0.273
Uterine horn length (mm)	61.48±0.64	58.34±1.85	0.148

Values are represented as Mean±SEM; there was no significant difference ($p>0.05$) between the treated group and control in the measured parameters.

From the table 2, there was no significant difference ($p>0.05$) in the ovarian weight, uterine weight and uterine horn length TLD-treated group compared to the control (Table 2).

Table 3: Showing the measured uterine parameters of the Experimental Rats

Parameters	Control Group	Treated Group	P-Value
Height of luminal diameter of the uterus (HLD) µm	825.2±30.20	968.9±74.93	0.113
Width of luminal diameter (WLD) µm	270.9±58.99	418.2±70.66	0.148
Endometrium height (E) µm	633.1±58.59	455.8±35.21*	0.032
Myometrium height (M) µm	187.8±15.96	227.5±28.56	0.260
Perimetrium height (P) µm	179.3±28.91	161.7±20.54	0.633
Height of uterine diameter (HUD) µm	2517±57.30	2430±171.9	0.644
Width of uterine diameter (WUD) µm	1774±429.4	2258±243.9	0.356

Values are represented as Mean±SEM; *indicate significant difference ($p<0.05$) between the treated group and control group in the measured parameters [Height of luminal diameter (HLD), Width of luminal diameter (WLD), Endometrium (E), Myometrium (M), Perimetrium (P), Height of uterine diameter (HUD), Width of uterine diameter (WUD)].

From the table 3 above, morphometric analysis of the uterus shows that daily oral administration of TLD caused insignificant increase ($p>0.05$) in the height of luminal diameter, the width of luminal diameter, myometrium and width of uterine diameter. However, the endometrium was significantly reduced, while, perimetrium and height of uterine diameter were insignificantly decreased in the treated groups when compared to the control (Table 3).

Table 4: Evaluation of ovarian anti-oxidant stress of the Experimental Rats

Parameters	Control Group	Treated Group	P-Value
MDA (mole/mg protein) $*10^2$	6.639±0.813	3.452±0.125	0.054
GPx (U/mg protein)	2.789±0.532	2.311±0.198	0.299
GSH (µM)$*10^{-1}$	8.185±0.214	7.749±0.384	0.185
SOD (U/mg protein)	3.041±0.279	3.631±0.076	0.204
CAT (U/mg protein) *10	3.851±0.514	4.282±0.411	0.683

Data are represented as Mean±SEM; Absence of (*) indicate no significant difference between the measured parameters ($p>0.05$) in Malondialdehyde (MDA), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) in the treated group compared to the control.

From table 4 above, it was observed that daily oral administration of TLD in the ovary caused no significant difference ($p>0.05$) in the oxidative stress markers between the TLD-treated groups and the control.

Table 5: Uterine oxidative stress of the Experimental Rats

Parameters	Control Group	Treated Group	P-Value
MDA (mole/mg protein) $*10^2$	6.863±1.144	2.711±0.263*	0.011
GPx (U/mg protein)	1.204±0.028	1.982±0.12*	0.030
GSH (µM)$*10^{-1}$	7.675±0.352	8.258±0.199	0.358
SOD (U/mg protein)	2.295±0.063	3.356±0.096*	0.012
CAT (U/mg protein) *10	2.67±0.063	4.396±0.265*	0.031

Values are represented as Mean±SEM; *indicate significant difference ($p<0.05$) in the measured parameters [Malondialdehyde (MDA), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT)] between the treatment group and the control.

From table 5 above, it was observed that daily oral administration of TLD significantly reduced ($p<0.05$) the concentration of malondialdehyde levels when compared with the control. However, the levels of glutathione peroxidase, superoxide dismutase, and catalase were significantly elevated ($p<0.05$) compared to the control by the oral exposure to the drug. Conversely, the concentration of reduced glutathione was elevated with insignificant difference ($p>0.05$) in the treated groups, compared to the control.

Table 6: Effect of TLD on hormonal profile of the Experimental Rats

Parameters	Control Group	Treated Group	P-Value
LH (mUl/ml)	1.03±0.092	1.27±0.047	0.226
FSH (mUl/ml)	0.793±0.038	1.053±0.123	0.092
PRL (ng/ml)	2.16±0.135	1.913±0.037	0.289
P4 (ng/ml) $*10^{-2}$	0.526±1.139	0.425±4.041	0.183
E2 (pg/ml) $*10^{-2}$	0.544±0.85	0.622±2.242	0.110
T (ng/ml)	1.15±0.096	0.3±0.09*	0.005

Values are represented as Mean±SEM; *indicate significant difference ($p<0.05$) from the control group in the measured parameters [Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Prolactin (PRL), Progesterone (P4), Estradiol (E2), Testosterone (T)].

From table 6 above, daily oral administration of TLD led to an increase with insignificant difference ($p>0.05$) in the concentration of luteinizing hormone, follicle stimulating hormone, and estradiol in the treated group when compared with the control. Conversely, the concentration of prolactin and progesterone was reduced with insignificant difference ($p>0.05$) in the treated group when compared with the control. However, the concentration of testosterone was significantly decreased ($p<0.05$) by the drug compared to its value in the control.

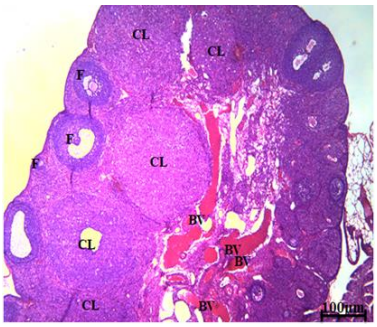

Plate 1: Photomicrograph of ovary of an adult Wistar rats of the control group showing histological features: follicle (F), corpus luteum (CL) and blood vessels (BV) within the cortex (H&E X100).

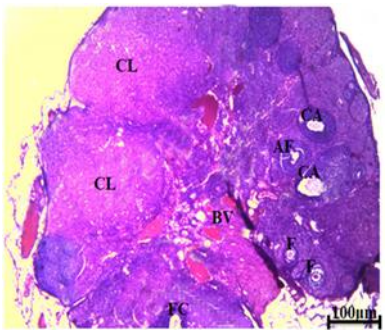

Plate 2: Photomicrographs of ovary (TLD-treated group) of an adult Wistar rats showing histological features: follicle (F), corpus luteum (CL), blood vessels (BV), atretic follicle (AF), corpus albicans (CA) and follicular cysts (FC) within the cortex (H&E X100).

Photomicrograph of the histological features of the ovary of control group of the experimental rats shows numerous growing follicles, more corpus luteum, and blood vessels in the cortex as compared to the TLD-treated group which shows a reduction in the number of growing follicles, lesser number of corpora lutea but with more corpus albicans, increased number of atretic follicles and follicular cysts in the cortex (Plate 1 and 2).

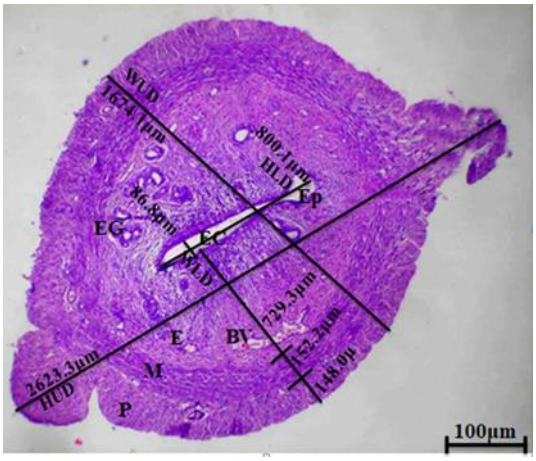

Plate 3: Photomicrographs of uterus (control) of an adult Wistar rats showing histological features: height of the uterine luminal diameter (HLD), width of the uterine luminal diameter (WLD), endometrial canal (EC), endometrium (E) lined with simple columnar epithelium (Ep), with endometrial glands (EG) and blood vessels (BV), myometrium (M), perimetrium (P), height of the uterine diameter (HUD) and width of the uterine diameter (WUD) (H&E X100).

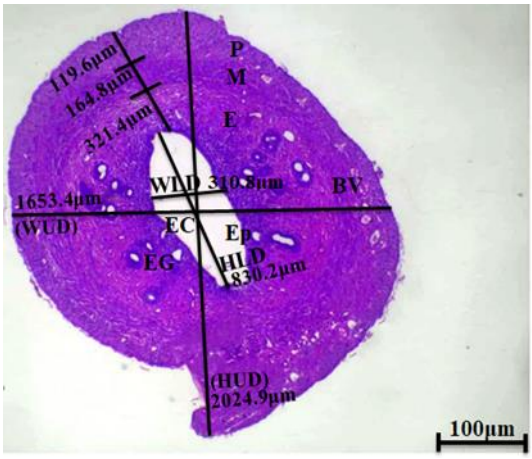

Plate 4: Photomicrographs of uterus (TLD-treated group) of an adult Wistar rats showing histological features: height of the uterine luminal diameter (HLD), width of the uterine luminal diameter (WLD), endometrial canal (EC), endometrium (E) lined with simple columnar epithelium (Ep), with endometrial glands (EG) and blood vessels (BV), myometrium (M), perimetrium (P), height of the uterine diameter (HUD) and width of the uterine diameter (WUD) (H&E X100).

Photomicrograph of the histological features of the uterus of TLD-treated group of the experimental rats shows larger endometrial canal, thicker endometrium lined with simple columnar epithelium, with endometrial glands and numerous blood vessels, myometrium and perimetrium as compared with the TLD-treated group which shows a larger endometrial canal, less thicker endometrium lined with simple columnar epithelium, with endometrial glands and blood vessels, myometrium and perimetrium (Plate 3 and 4).

4. Discussion

In this study, oral administration of Tenofovir Disoproxil Fumarate in 5mg, Lamivudine in 5mg and Dolutegravir in 0.8mg/kg body weight on the adult Wistar rats caused no mortalities in the TLD-treated as compared to the control group of the experimental animals. There was significant decrease ($p<0.05$) in the final mean body weight of the TLD-treated group of the experimental animals compared to the control. The finding of this study is in contrast with the reports that have documented no significant effect on the body weight of rats administered Tenofovir Disoproxil Fumarate, Lamivudine, Efavirenz and FDC (Ohihoin et al., 2023). The systemic metabolic effects of TLD, hormonal changes, and mitochondrial dysfunction are some of the processes responsible for the notable decrease in

the mean body weight. Conversely, the finding of this study is in contrast with some reports that have found that patients who received DTG-base regimen treatment gained more weight than those who received other ART without DTG-Base regimen (Sattler et al. (2015); Sax et al. (2020). This study was carried out in a rat model, which may explain the discrepancy in results from the work of Sattler et al. (2015) and Sax et al. (2020), which was done on human subjects.

The study on the organ weight revealed that TLD did not to significantly affect the weight of the ovary, uterus and uterine horn length. This study on ovarian weight is in contrast with Awodele et al. (2018), who reported significant reduction with oral treatment of HAART. The insignificant difference observed in this study in the TLD-treated groups as compared to the control may be due to the lack of significant endocrine disruption caused by the drugs. Reverse transcriptase inhibitors like TLD did not significantly affect ovarian function in rats not infected with HIV. The compensatory mechanisms of the hypothalamic-pituitary-ovarian axis likely neutralized minor hormonal fluctuations. However, there is scarcity of literatures to compare the effect of TLD on uterine weight and uterine horn length with other research works using antiretroviral therapy. The possible reasons for insignificant difference observed in this study in the TLD-treated groups compared to the control may be due to the dosages of TLD. Due to the remarkably tiny size, these medications may not directly affect the reproductive organs or may have more subtle effects that are undetectable by the parameters that are examined, such as horn length and uterine weight. Also, the Wistar rats may have adapted to the medication or their compensatory mechanism may have mitigated any slight alterations.

The morphometric studies revealed a significant reduction in the number of growing follicles, corpus luteum, blood vessels and increase in the number of atretic follicles and follicular cysts in the TLD-treated groups compared with the control. The finding of this study is consistent is consistent with earlier research (Awodele et al. (2018); Ohihoin et al. (2023), which attributed these effects to hormonal disruption, impaired angiogenesis, and oxidative damage. The study found that the ovarian weight of the TLD-treated group did not significantly change. The observed ovarian toxicity may result from the combined effects of the drugs. These medications cause mitochondrial toxicity, cellular stress, and interfere with hormonal signaling pathways, leading to follicular atresia and cyst development.

In this study, the uterine morphometric studies revealed insignificant increase in the height and width of luminal diameter, myometrium and width of uterine diameter in the TLD-treated groups compared with the control. However, the endometrium was significantly reduced, while, perimetrium and height of uterine diameter were insignificantly decreased in the treated groups when compared to the control. There is scarcity of literatures to compare the effect of TLD on uterine morphometric with other research works using antiretroviral therapy. The result in this study may be due to the potential pharmacological effects of TLD on the reproductive system. TDF and 3TC inhibit the reverse transcriptase enzyme, which may indirectly influence cellular proliferation or tissue remodeling. However, these drugs may have subtle effects on reproductive tissue, potentially altering hormonal regulation or immune response, leading to changes in uterine tissue structural integrity. Further investigation is needed to fully understand the underlying pharmacological mechanisms.

Oxidative status is a very important marker for the evaluation of overall health status of a living organism. In this study, daily administration of TLD had no significant alteration in the levels of ovarian oxidative stress markers. This study on ovarian oxidative stress markers is in contrast with Awodele et al. (2018), who reported significant reduction in GSH and significant increase in SOD levels in the adult Wistar rats treated with HAART as compared to the control. Awodele et al. (2018), suggests a potential disruption in the oxidative balance. The reduction in GSH, a key antioxidant, indicates increased oxidative stress, potentially due to the metabolic burden imposed by HAART drugs. These drugs generate reactive oxygen species (ROS) as byproducts of their metabolism, depleting GSH stores. The compensatory increase in SOD, an enzyme that catalyzes the dismutation of superoxide radicals, reflects the body's attempt to mitigate the elevated ROS levels. However, the concurrent decrease in GSH suggests that the oxidative challenge overwhelmed the antioxidant defenses. Specific HAART components may induce mitochondrial dysfunction, leading to increased ROS production and subsequent GSH depletion. Additionally, HAART

interfere with the synthesis or recycling of GSH, further contributing to its reduction. The observed changes in SOD and GSH are indicative of a state of oxidative stress induced by HAART, highlighting potential toxic effects on cellular redox homeostasis (Awodele et al., 2018). The result in this study which shows no significant difference in ovarian oxidative stress markers between TLD-treated groups and the control, despite potential drug-induced oxidative stress could be due to several factors, including the dosage, compensatory upregulation of antioxidant enzymes, the resilience of ovarian tissue, timing of assessments and duration of exposure, and individual variations within the adult Wistar rats population. The study suggests that these factors may have mitigated the potential pro-oxidant effects of TLD.

Daily administration of TLD in this study reveals significantly reduced the concentration of malondialdehyde levels in the uterus. However, the levels of glutathione peroxidase, superoxide dismutase, and catalase were significantly elevated. Conversely, the concentration of reduced glutathione was elevated with insignificant difference in the TLD-treated groups, compared to the control. There is scarcity of literatures to compare the effect of TLD on uterine oxidative stress markers with other research works using antiretroviral therapy. The study found that malondialdehyde (MDA) levels in TLD-treated rats decreased, suggesting a protective effect against oxidative stress. The rats showed elevated levels of antioxidant enzymes, which neutralize reactive oxygen species, preventing lipid peroxidation and MDA formation. The cells maintained their antioxidant capacity without significant changes, indicating a balance between increased oxidative stress and compensatory activation of antioxidant systems.

There was an insignificant increase in the serum concentration of luteinizing hormone, follicle stimulating hormone, and estradiol but insignificant decrease in prolactin, progesterone and decrease in testosterone level with daily administration of TLD. This finding is in contrast with previous studies. For instance Awodele et al. (2018), who reported significant reduction in prolactin, progesterone and estradiol in the adult Wistar rats treated with HAART as compared to the control. Ohihoin et al. (2023) who reported that administration TDF/3TC/EFV and FDC causes a reduction in the level of estradiol, no significant change in serum level of LH, but elevated serum level of FSH in rats model treated with EFV in the combinations. Awodele et al. (2018) attributed significant reduction in the levels of these hormones in the adult Wistar female rats in their study to a significant reduction in reproductive competency and possibly, potentially damaging adverse effect of HAART. Ohihoin et al. (2023) attributed a reduction in the level of estradiol, no significant change in serum level of LH, but elevated serum level of FSH to hormonal changes, particularly the decline in estradiol, was due to the drugs potential toxic effects on the ovaries, leading to follicular damage and a reduced capacity for estradiol production. This damage impairs the negative feedback loop that normally regulates the levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH), which explains the observed elevations in FSH in treated groups. The no significant change in serum level of LH was due to the compensatory mechanisms of the hypothalamic-pituitary-gonadal axis, which continue to regulate LH despite ovarian dysfunction. The elevated FSH levels are indicative of a reduced ovarian reserve, suggesting a disruption of ovarian function, though the effect was not as pronounced, similar to what is observed in menopause, where low estradiol levels lead to a lack of feedback inhibition on FSH production. In this study the insignificant increase in LH, FSH, and estradiol suggests that TLD affect the hypothalamic-pituitary-gonadal axis, but their impact on gonadotropin release may not be significant enough to cause significant changes. The slight changes may be due to moderate endocrine disruptions, while the significant decrease in progesterone may be due to disrupted ovarian steroidogenesis or reduced corpus luteum function. Additionally, the decreases in testosterone levels in the TLD-treated group indicate a suppression of ovarian androgen production or an alteration in the steroidogenic pathways, with the drugs affecting the endocrine feedback loops. In this case, the drugs might have a combined effect on the synthesis and release of these hormones, resulting in subtle but significant hormonal disruptions that manifest in changes to the serum concentrations observed.

Conclusions

This study shows that daily administrations of TLD caused a significant decrease in the final mean body weight of the TLD-treated group of the experimental animals compared to the control. TLD did not change the anti-oxidant status on the ovary, uterus, or hormonal level. However, histomorphological results showed a significant decrease in the rate of follicular maturation, as evidenced by the presence of atretic follicles, follicular, and luteal cysts/degeneration while the uterus showed a larger endometrial cavity and thicker endometrium in the TLD-treated group, compared to the control, a point to note in its administration, especially to females of reproductive age. This study suggests that TLD is not entirely safe due to some observed negative effects in the ovary and uterus. While its' prescription might be necessitated by medical exigency, the risks should be weighed against the benefits. Women of reproductive age who are on TLD for related ailments should be routinely monitored for ovarian functions by using ultrasound assessment and hormonal assay. Similar study can also be repeated in the male sex to evaluate the impact of TLD on the reproductive parameter as a way of ensuring overall fertility.

References

[1]. Abraham, G. E., & Saxena, B. N. (1981). Assay of estrogens. Pharmacology & Therapeutics, 12(2), 247–253.

[2]. Agarwal, A., Aponte-Mellado, A., Premkumar, B. J., Shaman, A., & Gupta, S. (2012). The effects of oxidative stress on female reproduction: A review. Reproductive Biology and Endocrinology, 10, 49. https://doi.org/10.1186/1477-7827-10-49

[3]. Al-Gubory, K. H., Fowler, P. A., & Garrel, C. (2010). The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. International Journal of Biochemistry & Cell Biology, 42(10), 1634–1650. https://doi.org/10.1016/j.biocel.2010.06.001

[4]. Aufrère, M. B., & Benson, H. (1976). Progesterone: An overview and recent advances. Journal of Pharmaceutical Sciences, 65(6), 783–800.

[5]. Awodele, O., Olayemi, S. O., Nwite, J. A., & Adeyemo, T. A. (2012). Investigation of the levels of oxidative stress parameters in HIV and HIV-TB co-infected patients. Journal of Infection in Developing Countries, 6(1), 79–85. https://doi.org/10.3855/jidc.2104

[6]. Awodele, O., Popoola, T. D., Idowu, O., Bashua, B. M., Awolola, N. A., & Okunowo, W. O. (2018). Investigations into the risk of reproductive toxicity following exposure to highly active antiretroviral drugs in rodents. Tokai Journal of Experimental and Clinical Medicine, 43(2), 54–63.

[7]. Azu, O. O., Naidu, E. C., Naidu, J. S., Masia, T., Nzemande, N. F., Chuturgoon, A., & Singh, S. (2014). Testicular histomorphologic and stereological alterations following short-term treatment with highly active antiretroviral drugs (HAART) in an experimental animal model. Andrology, 2(5), 772–779. https://doi.org/10.1111/andr.262

[8]. Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. In Methods in enzymology (Vol. 52, pp. 302–310). Academic Press.

[9]. Bujan, L., Hollander, L., Coudert, M., Gilling-Smith, C., Vucetich, A., Guibert, J., Vernazza, P., Ohl, J., Weigel, M., Englert, Y., & Semprini, A. E. (2007). Safety and efficacy of sperm washing in HIV-1-serodiscordant couples where the male is infected: Results from the European CREAThE network. Journal of Acquired Immune Deficiency Syndromes, 21(14), 1909–1914.

[10]. Centers for Disease Control and Prevention. (2018). HIV transmission - HIV basics - HIV/AIDS. https://www.cdc.gov/hiv/basics/transmission.html

[11]. Cohen, G., Dembiec, D., & Marcus, J. (1970). Measurement of catalase activity in tissue extracts. Analytical Biochemistry, 34, 30–38.

[12]. Cribbs, S. K., Guidot, D. M., Martin, G. S., Lennox, J., & Brown, L. A. (2014). Antiretroviral therapy is associated with decreased alveolar glutathione levels even in healthy HIV-infected individuals. PLoS ONE, 9(2), e88630. https://doi.org/10.1371/journal.pone.0088630

[13]. Danzer, H., & Braunstein, G. D. (1976). Serum human luteinizing hormone levels through normal pregnancy. American Journal of Obstetrics and Gynecology, 126, 678–681.

[14]. Dorfman, R. I., & Shipley, R. A. (1956). Androgens (p. 59). Chapman & Hall.

[15]. Drury, R. A. B., & Wallington, E. A. (1980). General staining procedures. In R. A. B. Drury & E. A. Wallington (Eds.), Carleton's Histological Techniques (pp. 125–150). Oxford University Press.

[16]. Ellman, G. L. (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 82(1), 70–77.

[17]. Iorjiim, W. M., Omale, S., Bagu, G. D., Gyang, S. S., & Alemika, E. T. (2020). Reproductive and oxidative stress toxicity of dolutegravir-based combination antiretroviral therapy in Drosophila melanogaster. Journal of Advances in Medical and Pharmaceutical Sciences, 22(6), 26–40. https://doi.org/10.9734/jamps/2020/v22i630190

[18]. Joint United Nations Programme on HIV/AIDS. (2024). Fact sheet 2024: Global HIV statistics. https://www.unaids.org

[19]. Jones, D. P. (2008). Radical-free biology of oxidative stress. American Journal of Physiology-Cell Physiology, 295(4), C849–C868. https://doi.org/10.1152/ajpcell.00283.2008

[20]. Kosasa, T. S. (1981). Measurement of human luteinizing hormone. Journal of Reproductive Medicine, 26, 201–206.

[21]. Kwara, A., Flanigan, T., & Carter, E. (2005). Highly active antiretroviral therapy (HAART) in adults with tuberculosis: Current status. International Journal of Tuberculosis and Lung Disease, 9(3), 248–257.

[22]. Mandas, A., Iorio, E. L., Congiu, M. G., Balestrieri, C., Mereu, A., Cau, D., Dessì, S., & Curreli, N. (2009). Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy. BioMed Research International, 2009, 749575. https://doi.org/10.1155/2009/749575

[23]. Martin, J. A., Sastre, J., de la Asunción, J. G., Pallardó, F. V., & Viña, J. (2001). Hepatic γ-cystathionase deficiency in patients with AIDS. JAMA, 285(11), 1444–1445.
[24]. Masiá, M., Padilla, S., Fernández, M., Rodríguez, C., Moreno, A., Oteo, J. A., ... & Gutiérrez, F. (2016). Oxidative stress predicts all-cause mortality in HIV-infected patients. PLoS ONE, 11(4), e0153456. https://doi.org/10.1371/journal.pone.0153456
[25]. Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. Journal of Biological Chemistry, 247, 3170–3175.
[26]. Morse, C. G., & Kovacs, J. A. (2006). Metabolic and skeletal complications of HIV infection: The price of success. JAMA, 296(7), 844–854.
[27]. National Research Council of the National Academies. (2011). Guide for the care and use of laboratory animals (8th ed.). National Academies Press.
[28]. Nkechi, O. F., Chinedu, O. C., Ositadinma, I. M., Ebere, A. J., Michael, E., & Priscilla, E. I. (2013). Impact of HIV and Mycobacterium tuberculosis co-infections on antioxidant status in Nigeria. Pakistan Journal of Nutrition, 12(5), 496–500.
[29]. Nyman, M. (1959). Serum hemoglobin; Methodological and clinical studies. Scandinavian Journal of Clinical and Laboratory Investigation, 11, 1–169.
[30]. Odell, W. D., Parlow, A. F., Swerdloff, R. S., Wabh, P. C., & Jacobs, H. S. (1981). Estimation of FSH test assay. Journal of Clinical Investigation, 47, 25–51.
[31]. Ohihoin, A. G., Ohihoin, E. N., Ujomu, I., Bakare, A., Olanrewaju, O., Okafor, A., ... & Taylor-Robinson, S. D. (2023). Contemporary anti-retroviral drugs (ARVDs) disrupt follicular development in female Wistar rats. Journal of Experimental Pharmacology, 15, 267–278.
[32]. Ohihoin, A. G., Ogbeifun, H. E., Ohihoin, E., Bakare, A., Mbarie, I., Herbertson, E., ... & Taylor-Robinson, S. D. (2025). Efavirenz-based highly active antiretroviral therapy disrupts folliculogenesis: Evidence from 48 women of reproductive age. Cureus, 17(2), e79597. https://doi.org/10.7759/cureus.79597
[33]. Salmen, S., & Berrueta, L. (2012). Immune modulators of HIV infection: The role of reactive oxygen species. Journal of Clinical & Cellular Immunology, 3(121), 2. https://doi.org/10.4172/2155-9899.1000121
[34]. Sattler, F. R., He, J., Letendre, S., Wilson, C., Sanders, C., Heaton, R., ... & McCutchan, J. A. (2015). Abdominal obesity contributes to neurocognitive impairment in HIV-infected patients with increased inflammation and immune activation. Journal of Acquired Immune Deficiency Syndromes, 68(3), 281–288.
[35]. Sax, P. E., Erlandson, K. M., Lake, J. E., McComsey, G. A., Orkin, C., Esser, S., ... & Waters, L. (2020). Weight gain following initiation of antiretroviral therapy: Risk factors in randomized comparative clinical trials. Clinical Infectious Diseases, 71(6), 1379–1389.
[36]. Semprini, A. E., Macaluso, M., Hollander, L., Vucetich, A., Duerr, A., Mor, G., ... & Jamieson, D. J. (2013). Safe conception for HIV-discordant couples: Insemination with processed semen from the HIV-infected partner. American Journal of Obstetrics and Gynecology, 208(5), 402.e1–402.e9.
[37]. Vaishnav, Y. N., & Wong-Staal, F. (1991). The biochemistry of acquired immunodeficiency syndrome. Annual Review of Biochemistry, 60(1), 577–630.
[38]. Van Leeuwen, E., Wit, F. W., Repping, S., Schattenkerk, J. K. M. E., Reiss, P., van der Veen, F., & Prins, J. M. (2008). Effects of antiretroviral therapy on semen quality. Journal of Acquired Immune Deficiency Syndromes, 22(5), 637–642.
[39]. World Health Organization. (2018). Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV: Interim guidelines. https://apps.who.int/iris/handle/10665/273632
[40]. World Health Organization. (2019). WHO recommends dolutegravir as preferred HIV treatment option in all populations. https://www.who.int/news/item/22-07-2019-who-recommends-dolutegravir-as-preferred-hiv-treatment-option-in-all-populations