

**International Journal of Multidisciplinary Research in Biotechnology, Pharmacy,
Dental and Medical Sciences (IJMRBPDMS)****Evaluation of serum levels of oxidative stress markers and
Cardiovascular Risk Among Adult HIV Patients on Dolutegravir-
based Therapy in Benue State, Nigeria.****S.A Agada^{1,2}, D.E Uti¹, C.O Ogbu¹, V. Abah³, A.B Onoja⁴, M.C Adilije², .C.O Ezeh², J.E Ikekpeazu²**¹Department of Clinical Biochemistry, Federal University of Health Sciences Otukpo, Benue State, Nigeria¹²Department of Medical Biochemistry, University of Nigeria, Enugu Campus, Enugu, Nigeria³Department of internal medicine, Federal University of Health Sciences Teaching Hospital, Otukpo, Benue State.⁴Department of Anatomy, Federal University of Health Sciences, Otukpo, Benue State, Nigeria.**ABSTRACT****Background:**

Human Immunodeficiency Virus (HIV) remains a major global health burden, with approximately 40.8 million people affected worldwide and about 1.9 million in Nigeria as of 2024. Although dolutegravir-based antiretroviral therapy (TLD) has improved patient outcomes, concerns persist regarding its association with oxidative stress and cardiovascular risk.

Methods:

A cross-sectional study was conducted among 400 adults (≥ 18 years) recruited from three HIV treatment centers in Benue State, Nigeria. Participants were grouped into five categories ($n = 80$ each): HIV-negative controls, newly diagnosed HIV-positive individuals not yet on TLD, HIV-positive individuals on TLD for < 1 year, those on TLD for > 1 year, and individuals switched from TLE to TLD. Serum levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were measured using colorimetric assays.

Results:

Antioxidant enzyme activities (GPx, SOD, CAT) decreased significantly across the groups ($p < 0.0001$), while MDA levels increased significantly ($p < 0.0001$), indicating elevated oxidative stress.

Conclusion:

TLD-based therapy is associated with increased oxidative stress among HIV patients in Benue State. Routine monitoring of oxidative stress markers as markers of cardiovascular diseases is recommended to improve long-term clinical outcomes.

Keywords: HIV, oxidative stress, dolutegravir, TLD, GPx, SOD, catalase, MDA, antiretroviral therapy, Nigeria

1. Introduction

People living with human immunodeficiency virus (HIV) are at an increased risk of developing cardiovascular diseases (CVD), including stroke, myocardial infarction, and heart failure, compared to uninfected individuals (Feinstein *et al.*, 2021). This heightened susceptibility is associated with a combination of factors, including the direct effects of HIV infection as well as certain antiretroviral therapies (Feinstein *et al.*, 2021). Although antiretroviral therapy (ART) has significantly improved survival and quality of life among people living with HIV, it may also contribute to the development of cardiovascular risk factors (Feinstein *et al.*, 2021).

SOD is an essential antioxidant enzyme that functions as a first line of defense against harmful reactive oxygen species (ROS) particularly the superoxide radical (Silva *et al.*, 2019). It aids in the dismutation of the superoxide radical into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), which can then be broken down by other enzymes like catalase and glutathione peroxidase (Silva *et al.*, 2019). This process protects cells from oxidative damage, preserving proteins, DNA and other

vital components. NADPH from the pentose phosphate pathway reduced oxidized glutathione to reduced glutathione; the reduced glutathione is then used by glutathione peroxidase to reduce H_2O_2 to $2\text{H}_2\text{O}$ molecules that are excreted by the kidneys (Silva *et al.*, 2019). CAT breaks down H_2O_2 into H_2O and O_2 through a two-stage, two-step mechanism involving the enzyme's heme-iron center, first, a H_2O_2 molecule reacts with ferric iron (Fe^{3+}) in the enzyme to form a high-valent intermediate called compound I, and the enzyme releases one molecule of water in the second stage, a second H_2O_2 molecule reacts with compound I and the enzyme is regenerated, releasing O_2 and another molecule of water (Silva *et al.*, 2019).

Malondialdehyde (MDA) is a reactive organic compound that serves as an important biomarker of oxidative stress and lipid peroxidation in biological systems (Morales & Munné-Bosch, 2019). It is a byproduct generated during the breakdown of polyunsaturated fatty acids in cell membranes following attack by reactive oxygen species (ROS) (Morales & Munné-Bosch, 2019). Because of its stability and ease of detection, MDA is widely used in clinical and experimental studies to assess oxidative damage (Morales & Munné-Bosch, 2019).

The body's antioxidant defenses and ROS generation are in equilibrium under normal physiological settings (Valenzuela, 1991). However, excess reactive oxygen species (ROS) can harm important cellular components like lipids, proteins, and DNA when this balance is upset by things like environmental stress, toxins or illness (Valenzuela, 1991; Cordiano *et al.*, 2023). According to Valenzuela (1991) and Cordiano *et al.*, (2023), lipid peroxidation produces MDA, which can then combine with proteins and nucleic acids to form adducts that hinder normal cellular activity.

Malondialdehyde (MDA) is commonly measured using assays such as the thiobarbituric acid reactive substances (TBARS) test (Valenzuela, 1991). Increased levels of malondialdehyde (MDA) have been linked to various disease states, such as cardiovascular disease, diabetes, neurodegenerative disorders, and cancer (Valenzuela, 1991). Its presence reflects the extent of oxidative damage and can be used to monitor disease progression or the effectiveness of antioxidant therapies (Valenzuela, 1991). Despite its usefulness as a biomarker, MDA is not entirely specific, as other compounds can interfere with its measurement (Valenzuela, 1991; Cordiano *et al.*, 2023). Nevertheless, it remains a valuable indicator of oxidative stress in both research and clinical practice.

Materials and Methods

Materials

1. Plane sample collection tubes, EDTA test tubes
2. Conical flasks (Pyrex, England)
3. Vacutainer needles
4. ILab 300 Plus Chemistry Analyzer (Instrumentation Laboratory Company, Milan, Italy)
5. IMMAGE Immunochemistry System (Beckman Coulter, USA)
6. Thermocycler (Thermoscientific, U.K)
7. Realtime PCR (Applied Biosystems, U.S.A)
8. Electrophoresis cell (Embi Tec, U.S.A)
9. Micro centrifuge (Eppendorf, UK)
10. Pipettes (Fisher Scientific, England)
11. Hand gloves
12. Tunicate
13. Cold chain box

Study Area

This study was conducted in Benue State, Nigeria. Benue state has a prevalence rate of HIV of 4.8% which is next to Akwa Ibom state in Nigeria with the highest HIV prevalence rate of 5.5% (Premium Times, 2022). Benue State, located in north central Nigeria, has historically faced a significant HIV burden (Premium Times, 2022). In 2018, an estimated 35,623 people living with HIV (PLHIV) in the state had not begun antiretroviral treatment (ART), contributing to an estimated 11% of Nigeria's national ART coverage gap. In response, the Benue ART Surge (BAS) program was launched

between May 2019 and September 2021 across 252 health facilities. The initiative aimed to enhance HIV case identification, ART initiation, and viral load suppression (Premium Times, 2022). Over 893,000 individuals were tested, resulting in 60,297 new HIV diagnoses. Remarkably, 99.8% of these individuals were promptly linked to ART (Premium Times, 2022). This intervention led to a 467% increase in monthly HIV case identification, from 650 in 2019 to a peak of 3,685 in August 2020. By September 2021, viral load suppression rates had risen to 96%, surpassing the UNAIDS 95-95-95 targets (Premium Times, 2022). Despite these advancements, challenges persist. As of 2023, an estimated 188,000 PLHIV in Benue remained untreated, based on projections from the Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS, 2018). Additionally, the prevalence rate has decreased from 16.8% in 2018 to 4.9% in 2023, yet the state continues to experience new infections, particularly among young adults aged 20–24, often due to unprotected sexual activities (NAIIS, 2018).

Ongoing efforts by the Benue State AIDS Control Agency (BENSACA) and international partners aim to address these gaps (NAIIS, 2018). Though challenges such as insecurity, displacement, and limited healthcare infrastructure hinder consistent access to HIV services. Nevertheless, the BAS program's success underscores the potential for targeted interventions to significantly reduce HIV prevalence and improve treatment outcomes in Benue State.

The adoption of Tenofovir/Lamivudine/Dolutegravir (TLD) as the recommended first-line ART regimen has been pivotal in the state's efforts to combat HIV/AIDS (NAIIS, 2018). This single-pill regimen offers enhanced efficacy, improved adherence, and a reduced risk of resistance compared to previous treatments (NAIIS, 2018). The state's HIV response has been significantly bolstered by the collaboration between local health practitioners and international partners (NAIIS, 2018). Through the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), implemented via the Centers for Disease Control and Prevention (CDC) and APIN Public Health Initiatives, significant support has been provided to this collaboration (NAIIS, 2018). This assistance has enabled the distribution of Tenofovir/Lamivudine/Dolutegravir (TLD) in more than 264 health facilities across all 23 local government areas of Benue State, ensuring that over 180,000 people living with HIV can access life-saving antiretroviral therapy (ART) (NAIIS, 2018).

Additionally, the establishment of a state-of-the-art molecular laboratory at the Federal Medical Centre in Makurdi, accredited by ISO 15189:2012, has enhanced diagnostic capabilities, enabling timely and accurate monitoring of viral load suppression among patients (NAIIS, 2018). These collaborative efforts have not only improved treatment outcomes but also contributed to the prevention of HIV transmission from mother-to-child, with approximately 8,000 HIV-negative babies born annually to HIV-positive mothers in the state (NAIIS, 2018). Through sustained partnerships and the effective use of TLD, Benue State is making significant strides toward achieving HIV epidemic control (NAIIS, 2018). As a result, Benue State is one of the best States in the Nation for this study.

This was a cross-sectional study that was carried out within 6 months (between January 2024 and July 2024) at 3 selected HIV care units: The Federal Medical Centre, Makurdi, Benue State University Teaching Hospital, Makurdi and Bishop Murray Medical Centre, Makurdi, Benue State. These hospitals were selected because they have special units for the management of HIV/AIDS in the State.

Sample Size

The sample size for this study was determined using the Yamane formula (1967) for calculating the sample size. The formula is:

$$n = N / \{1 + N(e)^2\}$$

Where:

n = Sample size

N = the population of interest for the study = 184,745

e = margin error in the calculation = 0.05

Therefore, n = 399.

Inclusion and Exclusion Criteria

The inclusion and exclusion criteria are:

Inclusion Criteria

The study included only:

1. Newly diagnosed HIV positive patients who are 18 years and above and are to commence TLD as first line ART in Benue state, Nigeria.
2. Newly diagnosed HIV positive patients who are 18 years and above and are to commence TLD as first line ART and HIV positive patients on TLD in Benue state, Nigeria who consented to the study.
3. Newly diagnosed HIV positive patients who are to commence TLD as first line ART in Benue state, Nigeria with no known history of heart related diseases.
4. HIV-uninfected patients who are 18 years and above as negative control.

Exclusion Criteria

The study excluded:

1. HIV positive patients on ART other than TLD in Benue state, Nigeria.
2. HIV positive pregnant women or desired to become pregnant during the study period in Benue state, Nigeria.
3. HIV positive patients aged less than 18 years who are to commence TLD as first line ART in Benue state, Nigeria.
4. HIV positive patients who are to commence TLD as first line ART or on TLD in Benue state, Nigeria who do not consent to the study.
5. HIV positive patients who are to commence TLD in Benue state, Nigeria with a known history of heart related diseases.

Ethical Clearance

Ethical clearance for this study was granted by the Research and Ethics Committee of the College of Medicine, University of Nigeria, Enugu Campus. The committee carefully reviewed the study protocol, objectives, methodology, and potential risks and benefits to ensure that the research conformed to established ethical standards. Ethical clearance was also obtained from the three hospitals (Federal Medical Centre Makurdi, Benue State University Teaching Hospital Makurdi and Bishop Murray Medical Centre Makurdi). This approval process was vital in upholding the integrity of the research and safeguarding the rights, dignity, and welfare of all participants involved.

Subjects and Methods

Study Participants

The study population comprised male and female participants aged 18 years and older (Yilmaz et al., 2017). A total of 400 individuals were enrolled in the study, including 80 HIV-negative participants who served as the control group and 80 newly diagnosed HIV-positive participants who had not yet started treatment and were scheduled to initiate Tenofovir/Lamivudine/Dolutegravir (TLD) as first-line antiretroviral therapy (ART-naïve group), 80 HIV-infected participants who have been on TLD for 0-1 year, 80 HIV-infected participants who have been on TLD for more than 1 year and 80 HIV-infected participants who were on TLE and then switched to TLD serving as a positive control in Benue state, Nigeria.

Table 1: Groups of study participants

GROUP	TITLE	SIZES	DESCRIPTION
GROUP 1	Control	80	HIV-uninfected individuals serving as the control
GROUP 2	ART-naïve	80	newly diagnosed HIV positive participants who were to commence TLD as the first line ART.

GROUP 3	< 1 year on TLD	80	HIV-infected participants who have been on TLD for less than 1 year.
GROUP 4	> 1 year on TLD	80	HIV-infected participants who have been on TLD for 1 year and above.
GROUP 5	> 1 year on TLE	80	HIV-infected participants who have been on TLE for greater than 1 year then switched to TLD.

Sample Collection

Both demographic data and blood samples were collected for this study to provide comprehensive insights into the participants' backgrounds and health status. Demographic information, such as age, gender, and socioeconomic status, helped in understanding the distribution and diversity of the study population. Blood samples were analyzed to assess biomarkers, genetic traits, or disease indicators. Together, these data enhanced the study's ability to identify meaningful health-related correlations and outcomes.

Data Collection

Newly diagnosed participants were recruited through the counselling unit of the health centre for inclusion in the study. Those already receiving treatment were approached at the adherence clinic during their routine visits. After obtaining verbal consent, a structured case record form was used to collect socio-demographic data such as age, sex, marital status, phone number, and home address from all eligible participants. Information on self-reported health conditions, including hypertension, heart disease, and diabetes, was also recorded. In addition, clinical and biological measurements, such as blood pressure, were taken for each participant.

Blood Sample Collection

Blood sample was collected from the participants in the 5 different groups for biochemical and molecular analysis. 10mls of venous blood specimen of each participant was collected using 10mls syringe: 5mls each was dispensed into a carefully labeled EDTA anti-coagulated tubes and plain tubes respectively (Cadamuro et al., 2015). The blood samples in the plane tubes were then centrifuged at 3000 rpm for 5 minutes to obtain the serum. The serum was transferred into 2ml tubes and frozen at -200 C for biochemical analysis.

Biochemical Analysis

The following biochemical parameters were assayed for in the samples collected in all five participant groups:

1. Glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), was evaluated using the colorimetric assay method.
2. Malondialdehyde (MDA), a marker of oxidative stress, was measured using a reagent kit from North West Life Science Specialties in accordance with the method outlined by Mihara and Uchiyama (1978).

Glutathione Peroxidase (GPX) Assay (U/mg protein)

The antioxidant peroxidase activity (GPx) was measured using the Glutathione peroxidase (GPx) Colorimetric Assay Kit from Elabscience (Catalog No: E-BC-K030-M) (Elabscience, 2024), through a Colorimetric method. A microplate reader (405-414 nm) with a detection range of 2-100 $\mu\text{mol/L}$ was used (Elabscience, 2024). The assay principle relies on the reaction between reduced glutathione (GSH) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which produces a yellow-colored complex measurable using a colorimetric method at 405 nm (Elabscience, 2024). The concentration of reduced GSH in the sample is then determined indirectly.



The assay consists of the following reagents:

Phosphate buffer	500 μ l
NaN ₃	100 μ l
GSH	200 μ l
H ₂ O ₂	100 μ l
Sample	500 μ l
Distilled water	600 μ l

Procedure

1. All reagents were allowed to reach room temperature prior to use.
2. The reaction mixture was incubated at 37°C for 3 minutes.
3. After incubation, 0.5 mL of trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 3000 rpm for 5 minutes.
4. To 1 mL of the resulting supernatant, 2 mL of K₂HPO₄ and 1 mL of DTNB were added.
5. The absorbance was then measured at 412 nm against a reagent blank.
6. Glutathione peroxidase activity was determined by constructing a standard curve, from which the concentration of residual GSH was extrapolated.
7. GSH consumed was calculated as: 245.34 – GSH remaining.
8. Glutathione peroxidase activity was expressed as the amount of H₂O₂ consumed per mg of protein.

Superoxide Dismutase (Sod) Activity Assay

Superoxide dismutase (SOD) activity in participants was assessed using the method described by Misra and Fridovich (1972). This assay is based on the capacity of SOD to inhibit the autoxidation of epinephrine under alkaline conditions (pH 10.2), providing a simple means of measuring enzyme activity. In this reaction, superoxide radicals (O₂^{•-}) generated through the xanthine oxidase system promote the oxidation of epinephrine to adrenochrome. The formation of adrenochrome increases with rising pH and higher epinephrine concentrations (Valerino and McCormack, 1971), supporting the concept that epinephrine autoxidation occurs via multiple pathways, one of which is a free radical chain reaction mediated by superoxide radicals and therefore inhibitable by SOD.

The assay reagents included:

1. **0.05 M carbonate buffer (pH 10.2):** Prepared by dissolving 3.58 g of Na₂CO₃·10H₂O and 1.05 g of NaHCO₃ in 200 mL of distilled water. The pH was adjusted to 10.2 and the volume made up to 250 mL with distilled water.
2. **0.3 mM adrenaline (epinephrine):** Prepared fresh by dissolving 0.01 g of adrenaline in 200 mL of distilled water.

Procedure

A 1:5 dilution of the sample was prepared by mixing 0.2 mL of the sample with 0.8 mL of distilled water. Then, 0.2 mL of the diluted sample was transferred into a cuvette containing 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate in the spectrophotometer. The reaction was initiated by adding 0.3 mL of freshly prepared 0.3 mM adrenaline, followed by rapid mixing by inversion.

For the reference (blank) cuvette, 2.5 mL of buffer, 0.3 mL of adrenaline, and 0.2 mL of distilled water were used. The change in absorbance was recorded at 480 nm at 30-second intervals for 150 seconds.

The rate of increase in absorbance per minute was calculated using:

Increase in absorbance per minute = (A₃ – A₀) / 2.5 where A₀ represents absorbance at 30 seconds and A₃ represents absorbance at 150 seconds.

Percentage inhibition was determined as:

% inhibition = (Increase in absorbance of substrate / Increase in absorbance of blank) × 100

One unit of SOD activity was defined as the amount of enzyme required to produce 50% inhibition of adrenaline oxidation.

Catalase Activity Assay

Catalase activity was measured using the method described by Claiborne (1985). This assay is based on the decrease in absorbance at 240 nm as catalase catalyzes the decomposition of hydrogen peroxide. Although hydrogen peroxide does not have a strong absorption peak at this wavelength, its absorbance is sufficiently proportional to concentration to allow quantitative analysis. An extinction coefficient of $0.0041 \text{ mM}^{-1} \text{ cm}^{-1}$ (Noble and Gibson, 1970) was applied for calculations.

The reagents used included:

- 0.05 M phosphate buffer (pH 7.0):** Prepared by dissolving 0.336 g of dipotassium hydrogen phosphate (K_2HPO_4) and 0.417 g of potassium dihydrogen phosphate (KH_2PO_4) in 100 mL of distilled water. The pH was adjusted to 7.0, and the final volume was made up to 100 mL with distilled water.
- Hydrogen peroxide solution (0.019 M):** Prepared by adding 109 μL of 59% H_2O_2 to 50 mL of 0.05 M phosphate buffer (pH 7.0), after which the volume was adjusted to 100 mL with the same buffer.

Procedure

Hydrogen peroxide (2.95 mL of a 19 mM solution) was dispensed into a 1 cm quartz cuvette, after which 50 μL of the sample was added (as indicated in the table below). This approach was used to minimize sample dilution, consistent with the protocol in which H_2O_2 and buffer were prepared separately in distilled water (100 mL each).

The reaction mixture was immediately inverted to ensure proper mixing and then placed in a spectrophotometer. The change in absorbance was monitored at 240 nm at 1-minute intervals over a 5-minute period (i.e., at 1, 2, 3, 3:30, 4, 4:30, and 5 minutes).

Calculation:

Catalase activity was expressed as:

Catalase activity = $(\Delta\text{OD}/\text{min} \times \text{volume of assay system}) / (0.0041 \times \text{volume of sample} \times \text{mg protein})$
 = $\mu\text{mol H}_2\text{O}_2$ decomposed per minute per mg protein.

The Serum Pro-Oxidant Biomarker

Malondialdehyde (MDA) as one of the pro-serum biomarkers was assayed for all the patients.

Malondialdehyde (MDA) Assay (nmol/mg protein)

The serum Pro-oxidant biomarker malondialdehyde (MDA) was assayed using the Malondialdehyde (MDA) Colorimetric assay kit from Elabscience (Catalog No: E-BC-K025-M) (Elabscience, 2024) using TBA methods described by Mihara and Uchiyama. (1978). A microplate reader with a detection range of 2.92 – 40 $\mu\text{mol/L}$ and a 530 -540 nm wavelength was used (Elabscience, 2024). This assay is based on the reaction of malondialdehyde (MDA), a breakdown product of lipid peroxidation, with thiobarbituric acid (TBA), resulting in the formation of a red-colored complex that exhibits maximum absorbance at 532 nm.

The compositions of the assay are (Elabscience, 2024):

Item	Component	Size (48 T)
Reagent 1	Clarificant	3 mL × 1 vial
Reagent 2	Acid reagent	2 mL × 1 vial

Reagent 3	Chromogenic agent	Powder x 1vial
Reagent 4	50 µmol/L Standard	5 mL × 1 vial
	Microplate	96 wells
	Plate sealer	2 pieces

Procedure

- All reagents were brought to room temperature and allowed to equilibrate before use.
- The clarifying agent was heated in a 37°C water bath until it became clear.
- The acid working solution (616 µL) was prepared by combining 21 µL of acid reagent with 595 µL of double-distilled water (Elabscience, 2024).
- The chromogenic working solution was prepared by dissolving the chromogenic reagent in 14 mL of double-distilled water at 90–100°C, followed by the addition of 14 mL of glacial acetic acid and thorough mixing (Elabscience, 2024).
- A 50% acetic acid solution (0.2 mL) was prepared by mixing 100 µL of glacial acetic acid with 100 µL of double-distilled water.
- The standard curve was generated by serially diluting a 50 µmol/L standard solution with absolute ethanol to obtain a range of concentrations as outlined below:

Item	1	2	3	4	5	6	7	8
Concentration (µmol/L)	0	5	10	15	20	25	30	40
50 µmol/L standard (µL)	0	10	20	30	40	50	60	80
Absolute ethyl alcohol (µL)	100	90	80	70	60	50	40	20

- 5ml of fresh blood sample was collected from the participants
- It was allowed to stand for 30 minutes at 25°C
- The sample was centrifuged at 2000g for 15 minutes at 4°C (Elabscience, 2024)
- The serum was preserved on ice for detection
- Standard tube:** 0.02 mL of standard with different concentrations was added into numbered 1.5 mL EP tubes.
- Sample tube:** 0.02 mL of sample was added into numbered 1.5 mL EP tubes.
- Control tube:** 0.02 mL of sample was added numbered 1.5 mL EP tubes
- 0.02 mL of clarificant was added into each tube (Elabscience, 2024)
- 0.6 mL of acid application solution was added into each tube
- 0.2 mL of chromogenic application solution was added into standard tubes and sample tubes, as well as 0.2 mL of 50% acetic acid into the control tubes.
- The mixture is mixed carefully and incubated at 100°C for 40 min
- The tube was centrifuged at 10000 g for 10 min and cooled at room temperature.
- 0.25 mL of the supernatant was taken from each tube to the microplate (Elabscience, 2024)
- The OD values was measured for each well at 532 nm with microplate reader.
- The average duplicate reading for each standard was taken.
- The mean OD value of the blank was subtracted (Standard) from all standard readings and the absolute OD value was obtained (Elabscience, 2024).
- A plot of the standard curve was obtained by using the absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively (standard curve = $y = ax + b$).

$$\text{MDA } (\mu\text{mol/L}) = (\Delta A - b) \div a \times f$$

Statistical Analysis

Statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences) version 23.0. Data were presented using tables, histograms, and bar charts. The dependent Student's t-test was applied to compare continuous variables, while the Chi-square test was used for categorical variables. A p-value of less than 0.05 was considered statistically significant.

Results

Mean Serum Levels Of Oxidative Stress Markers

Table 2: Comparative analysis of the Mean±SD of the Oxidative Stress Markers across the experimental groups

Variables	Mean ± SD GROUP 1	Mean ± SD GP 2	Mean ± SD GP 3	Mean ± SD GP 4	Mean ± SD GP 5	P-value
SOD (Umg/protein)	12.37±0.23	10.49±0.19	8.55±0.23	6.43±0.22	4.22±0.16	< 0.0001
MDA (Umg/protein)	1.69±0.12	2.43±0.17	3.30±0.18	4.59±0.21	5.79±0.19	<0.0001
CAT (Umg/protein)	15.99±0.27	12.90±0.23	10.07±0.22	7.42±0.29	5.01±0.19	<0.0001
GPX (Umg/protein)	8.83±0.21	7.35±0.21	5.93±0.18	4.22±0.23	2.71±0.19	<0.0001

Table 2 presents the mean and standard deviation (SD) values for oxidative stress markers measured across five experimental groups (Group 1 to Group 5). The analyzed variables include superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GPx). SOD activity showed a statistically significant decrease ($p < 0.0001$) from 12.37 ± 0.23 U/mg protein in Group 1 to 4.22 ± 0.16 U/mg protein in Group 5. Similarly, CAT activity decreased significantly ($p < 0.0001$) from 15.99 ± 0.27 to 5.01 ± 0.19 U/mg protein, and GPx activity also declined significantly ($p < 0.0001$) from 8.83 ± 0.21 to 2.71 ± 0.19 U/mg protein. In contrast, MDA, a marker of lipid peroxidation, showed a statistically significant dose-dependent increase ($p < 0.0001$), rising from 1.69 ± 0.12 U/mg protein in Group 1 to 5.79 ± 0.19 U/mg protein in Group 5.

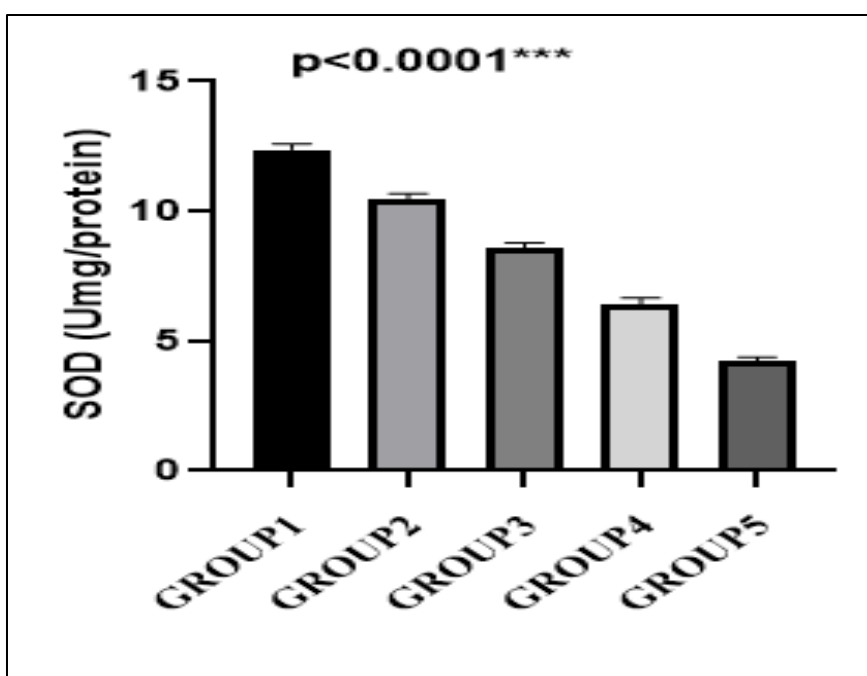


Figure 1: Bar chart showing values for the mean and standard deviation (SD) of serum oxidative stress marker (SOD) across the five experimental groups.

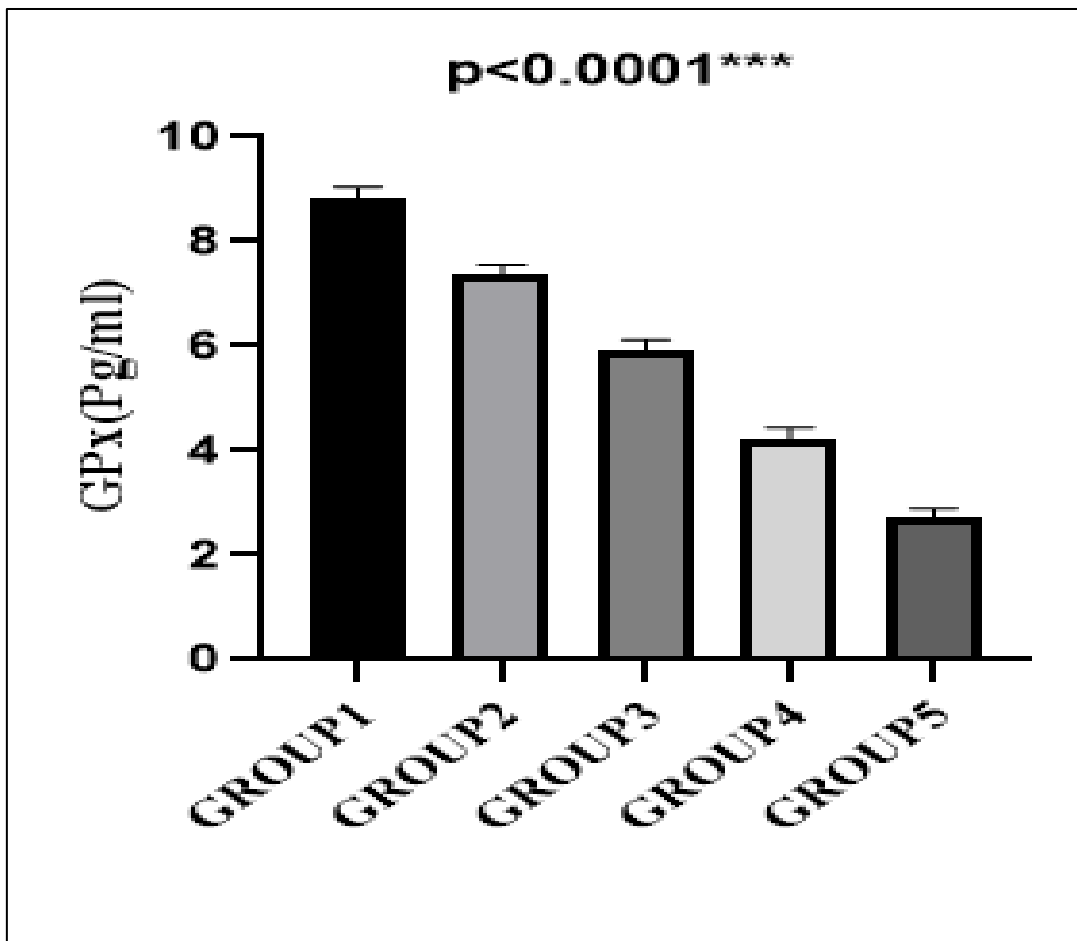


Figure 12: Bar chart showing values for the mean and standard deviation (SD) of serum oxidative stress marker (GPx) across the five experimental groups.

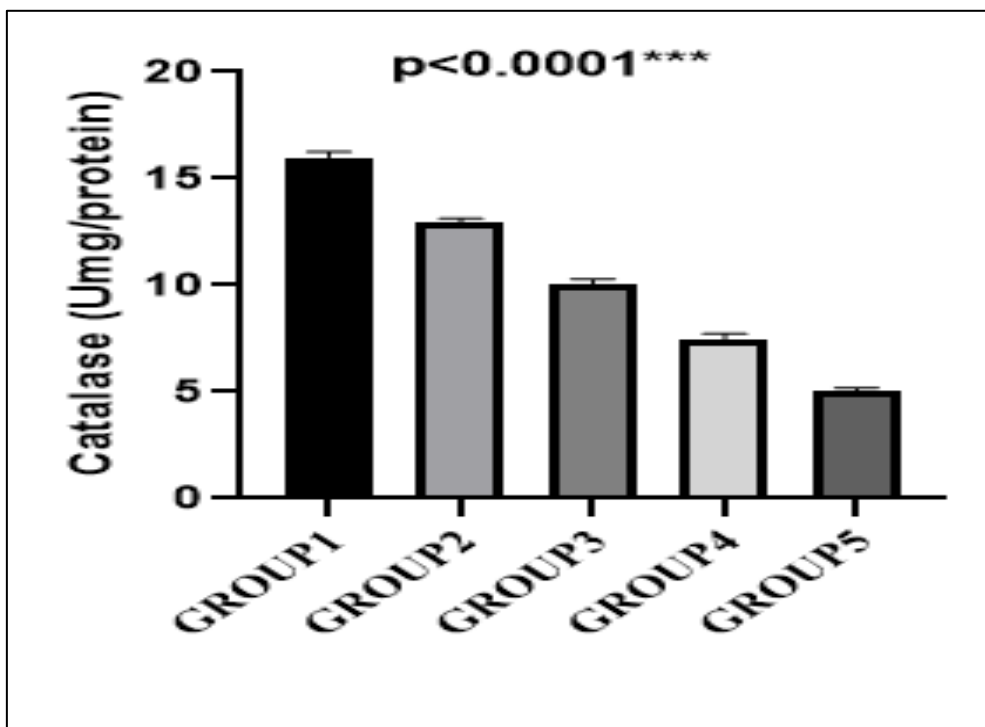


Figure 2: Bar chart showing values for the mean and standard deviation (SD) of serum oxidative stress marker (CAT) across the five experimental groups.

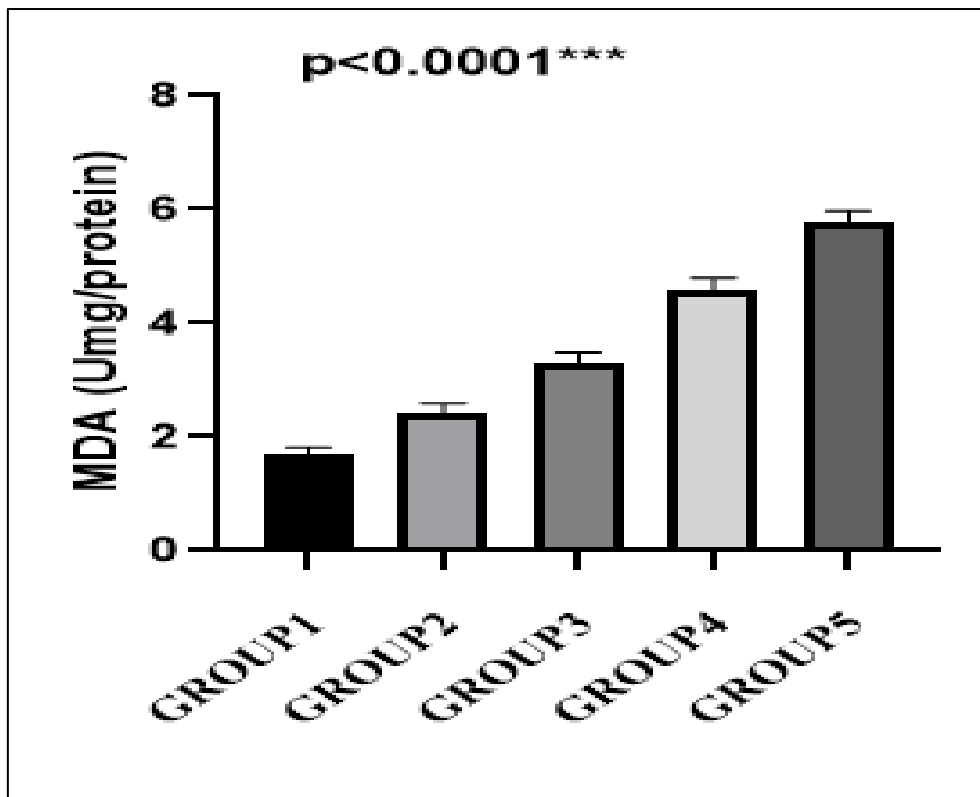


Figure 3: Bar chart showing values for the mean and standard deviation (SD) of serum oxidative stress marker (MDA) across the five experimental groups.

Discussion

Human immunodeficiency virus (HIV) infection has spread significantly over the past 30 years, exerting a profound impact on global health and economic wellbeing (Awoyemi *et al.*, 2016). In 2024, it was estimated that approximately 40.8 million people were living with HIV (PLWHIV) globally, with an incidence rate of 0.16 per 1,000 population and about 1.3 million new infections recorded within the year (WHO, 2024). Sub-Saharan Africa continues to bear the greatest burden of the epidemic, accounting for about 26.3 million cases (WHO, 2024), and roughly 66% of all people living with HIV worldwide reside in this region (UNAIDS, 2022). In Nigeria, an estimated 1.9 million individuals—about 1% of the total population—are living with HIV/AIDS, with a national prevalence of 1.4%, ranking the country fourth globally among those most affected by the epidemic (Nigerian HIV/AIDS Indicator and Impact Survey [NAIIS], 2018).

People with HIV infection may be at a higher risk of cardiovascular disease (CVD) including heart diseases such as myocardial infarction, heart failure and stroke, compared to people without HIV infection. This increased risk is attributed to combination of factors, including HIV infection itself and some HIV medications, while antiretroviral therapy (ART) has significantly improved the lives of people with HIV, it can also contribute to certain CVD risk factors (Feinstein *et al.*, 2021). Due to negative drug side effects that cause non-adherence to treatment and damage to many end organs, including the kidney and the heart, it has been challenging to fully realize the potential of ART in practice. A higher incidence of renal disease, the virus's own pathogenic effects, and possibly certain antiretroviral medication toxicities may all contribute to the elevated risk of CVD (Choi *et al.*, 2011). Research by Choi *et al.* (2011) indicates that cardiovascular disease (CVD) has become the leading cause of mortality among people living with HIV in both Africa and the United States.

Significant alterations in oxidative stress markers among people living with HIV (PLHIV) across five experimental groups was observed, suggesting a progressive redox imbalance potentially linked to disease progression, antiretroviral therapy (ART), or both (Table 3). Oxidative stress, a state where reactive oxygen species (ROS) exceed the body's antioxidant defense (Harshithkumar *et al.*, 2024), is increasingly recognized as a key contributor to HIV pathogenesis and its associated compl

ications. The observed decline in antioxidant enzyme activities—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—is consistent with previous studies that reported similar reductions in PLHIV (Dube *et al.*, 2008). SOD, which catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, showed a substantial decrease from 12.37 ± 0.23 to 4.22 ± 0.16 U/mg protein across the groups ($p < 0.0001$) (Table 3). This decline may indicate a diminished ability to neutralize superoxide radicals, leading to elevated oxidative stress. A study by Dube *et al.* (2008) also found significantly lower SOD activity in HIV-infected individuals, which was attributed to chronic immune activation and ROS overproduction during viral replication.

Similarly, the progressive reduction in CAT and GPx activities further supports the hypothesis of compromised antioxidant defense. CAT breaks down hydrogen peroxide into water and oxygen, while GPx reduces hydrogen peroxide and lipid peroxides using glutathione. In this study, CAT decreased from 15.99 ± 0.27 to 5.01 ± 0.19 U/mg protein, and GPx from 8.83 ± 0.21 to 2.71 ± 0.19 U/mg protein (both $p < 0.0001$) (Table 3). These reductions may reflect either a depletion of enzyme reserves due to ongoing oxidative challenge or enzyme inactivation by free radicals, as suggested by Elbim *et al.* (1999), who found that antioxidant defenses are impaired in HIV patients, especially during advanced disease stages.

In contrast to antioxidant enzymes, malondialdehyde (MDA), a biomarker of lipid peroxidation, exhibited a statistically significant increase from 1.69 ± 0.12 to 5.79 ± 0.19 U/mg protein ($p < 0.0001$), indicating heightened oxidative damage to cell membranes (Table 3). Elevated MDA levels are a hallmark of oxidative stress and have been widely reported among PLHIV. For instance, Oteiza *et al.* (1999) reported increased lipid peroxidation in HIV-positive individuals, which correlates with disease progression, the extent of immune dysfunction, and ART exposure. The trend observed across the groups suggests a worsening oxidative profile, possibly corresponding to disease severity or cumulative exposure to ART. While ART effectively suppresses viral load and improves immune function, certain regimens—especially those including protease inhibitors—have been implicated in mitochondrial toxicity and oxidative stress (Montoya *et al.*, 2013). This dual role of ART underscores the complexity of managing oxidative damage in PLHIV. The findings reveal a clear imbalance between oxidative stress and antioxidant defense mechanisms in PLHIV.

Conclusion

This study underscores the intricate relationship between HIV infection, antiretroviral therapy (ART), and the physiological alterations that contribute to increased cardiovascular risk among people living with HIV (PLHIV). While ART remains essential for viral suppression and improved survival, it is also associated with adverse biochemical effects, particularly heightened oxidative stress, an established contributor to cardiovascular disease (CVD). The marked alterations in oxidative stress markers observed in this study reflect a sustained state of chronic inflammation and metabolic imbalance in PLHIV. These findings emphasize the need for integrated clinical approaches that incorporate routine monitoring and management of oxidative stress and cardiovascular risk factors alongside HIV treatment.

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