International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Cardiotoxic evaluation of some antiretroviral drug regimens in Wistar rats

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International Journal of Science and Research Archive, 2025, 14(03), 1522-1531

Publication history: Received on 21 August 2024; revised on 21 March 2025; accepted on 24 March 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.14.3.1795

Abstract

Highly active antiretroviral therapy (HAART) and antiretroviral therapy (ART) are implicated in drug-associated toxicities. The study investigated the cardiotoxic effect of some HAARTs and ARTs drug combinations using biochemical and histological parameters. Forty-eight male Wistar rats were randomly grouped into eight groups (n=6). Group 1 received 2 ml distilled water while Groups 2 and 3 received HAART: Efavirenz + Lamivudine + Tenofovir disoproxil fumarate (ELT 17.14 mg) and Lamivudine + Nevirapine + Zidovudine (LNZ 9.29 mg) respectively, while Groups 4 to 8 received ART: Sulfate d'Abacavir + Lamivudine (SL 12.85 mg); Lamivudine + Zidovudine (LZ 6.42 mg); Lamivudine + Tenofovir disoproxil Fumarate (LT 8.57 mg); Atazanavir + Ritonavir (AR 5.71 mg) and Liponavir + Ritonavir (LR 3.57 mg) per kg body weights respectively for 30 days. There was significantly ($p < 0.05$) increased cardiosomatic index in LNZ, LZ, LT and AR-administered groups compared to control. All drug-administered groups had elevated lipidemia, with marked increase in ELT and LR-administered groups compared to control. Superoxide dismutase and glutathione peroxidase showed significant elevation in ART-administered groups compared to control. Cardiac troponin I showed marked elevation in SL and LZ-administered groups compared to control. Cardiac histology demonstrated varying anomalies ranging from abnormal branching in groups 2, 3 and 5, to altered striations in groups 3, 4, 5 and 6, hypertrophy in groups 6 and 7, and irregularly shaped nuclei in groups 4, 7 and 8. In conclusion, while all test groups revealed varying degrees of cardiac injury, ELT, LT and AR regimens are more cardiotoxic while LNZ, SL and LZ possess better cardiac safety profiles.

Keywords: Antiretroviral therapy; Highly active antiretroviral therapy; Cardiotoxicity; Cardiac safety; Oxidative stress

1. Introduction

Latest statistics have it that an estimated 39.9 million (36.1 million – 44.6 million) people were living with human immunodeficiency virus (HIV) at the end of 2023, with about 65 % residing in the African region. About 30.7 million of these individuals had access to antiretroviral therapy [1]. The most advanced stage of the viral infection is acquired immunodeficiency syndrome (AIDS). Currently, there is no cure or preventive vaccine against HIV infection. However, combinations of two antiretroviral (ARV) drugs, designated as antiretroviral therapy (ART), and ~~of~~ three ARV drugs, designated as highly active antiretroviral therapy (HAART), have been utilized for the management of the disease [2]. For HAART, at least two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) combined with a protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitors (NNRTI) had been recommended [3]. With these

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treatment regimens, morbidity and mortality associated with HIV/AIDS and the single ARV treatment measures have been dramatically reduced, resulting in improved life expectancy and quality of life for people living with HIV [4].

Despite the outstanding feat achieved by ARTs and HAARTs in the combat of HIV and longevity of HIV patients, its cardiotoxicity raises concern [5]. The HAART itself may be associated with an increase in coronary artery diseases. Moreover, there are claims of potential risks for cardiovascular diseases threaten the successful maintenance of HIV therapies [2,6]. Some cardiovascular side effects reported include atherosclerosis, myocarditis, endocarditis, pericardial effusion, congestive heart failure, and dilated cardiomyopathy [7]. Certain classes of ARVs such as the PIs are strongly implicated in these adverse effects [8,9].

Lipid metabolism complications of varying degrees have been reported following long-term exposure to components of some ARVs [10]. Reactive oxygen species (ROS) are generated as by-products of several cellular metabolic activities and instances of redox disturbance are the main culprits in the cardiovascular complications associated with the administration of ARVs [5].

The control and management of modifiable risk factors, such as body weight, organ weight (heart), and organosomatic index for cardiovascular diseases (CVD) and metabolic disorders are crucial, especially in cohort studies using ARVs [11]. Cardiosomatic index, lipid profile, levels of serum antioxidants, and/or cardiac troponin levels are some of the key parameters in drug cardiotoxicity determination [12]. With the many available ARV drugs [13], a challenge is posed as to which drug presents the most cardiotoxicity. The importance of evaluating the safety and/or toxicity index of these drug regimens cannot be overemphasized in achieving maximal adherence and benefit of the therapy. This study aimed to investigate cardiotoxic effect of some ARV drugs using HIV-naïve Wistar rat model.

2. Material and methods

Forty-eight (48) adult male Wistar rats weighing between 150 – 267 g were obtained and housed at the Faculty of Pharmacy animal house, University of Uyo, Nigeria. These animals were weighed and housed in wooden cages bedded with dry and clean wood shavings. The rats were kept under suitable environmental conditions (room temperature and a 12-hour light and dark cycle) and had free access to standard rat pellet feed (Growers Vital feed®) and water *ad libitum*. The animals were acclimatized in a well-ventillated room for one week prior to administration of ARV drugs. The initial body weights of the animals were measured using an electronic balance (Zeiss Pty Ltd. Gottingem, West Germany), and the average weight per group was calculated.

2.1. Drug Composition

The drugs were sourced from the Pharmacy unit of the University of Uyo Teaching Hospital, Nigeria. Non-nucleoside/nucleotide reverse transcriptase inhibitors (Efavirenz and Nevirapine), Protease inhibitors (Ritonavir, Atazanavir and Lopinavir), and Nucleoside reverse-transcriptase inhibitors (Tenofovir, Zidovudine, Lamivudine, and Sulfate d'Abacavir) were the drugs used for this study.

2.2. Experimental Design

Forty-eight adult male Wistar rats weighing between 150 – 267 g were randomly divided into eight groups of 6 rats each. Daily drug administration was carried out via orogastric gavage between 7.00 am and 10.00 am for a duration of 30 days. Therapeutic doses of the drugs were administered to the animals as shown below:

- Group 1: Normal control (2 ml/kg Distilled water)
- Group 2: HAART (17.14 ml/kg b.w ELT)
- Group 3: HAART (9.28 ml/kg b.w LNZ)
- Group 4: ART (12.85 ml/kg b.w SL)
- Group 5: ART (6.42 ml/kg b.w LZ)
- Group 6: ART (8.57 ml/kg b.w LT)
- Group 7: ART (5.71 ml/kg b.w AR)
- Group 8: ART (3.57 ml/kg b.w LR)

Where:

- ELT = Efavirenz (600mg) + Lamivudine (300mg) + Tenofovir Disoproxil Fumarate (300mg)
- LNZ = Lamivudine (150mg) + Nevirapine (200mg) + Zidovudine (300mg)

- SL = Sulfate d'Abacavir (600mg) + Lamivudine (300mg)
- LZ = Lamivudine (150mg) + Zidovudine (300mg)
- LT = Lamivudine (300mg) + Tenofovir Disoproxil Fumarate (300mg)
- AR = Atazanavir (300mg) + Ritonavir (100mg)
- LR = Lopinavir (200mg) + Ritonavir (50mg)

2.3. Drug Administration

The dosage was calculated from the normal dose for a 70 kg man. The dosage of drugs administered was calculated using the formula below:

$$\frac{\text{Weight of animal}}{1000} X \frac{\text{dosage}}{stock}$$

2.4. Collection and Preparation of Samples

The animals were euthanized with ketamine (40 mg/kg) anesthesia (Sigma Aldrich, Germany). Blood was collected via cardiac puncture and transferred into plain bottles. The collected blood samples were centrifuged at 2000 rpm for 15 minutes (g = 9.78 m/s^2) using a bench top centrifuge. The straw-colored sera were thereafter used for analysis of some biochemical parameters (lipid profile, cardiac antioxidant and cardiac troponin I). The heart of each rat was excised, weighed and stored in 10% formaldehyde for histology.

2.5. Weight Determination

Animals were weighed on the first day of the experiment and then on the last day of the experiment. Percentage weight gain was calculated using the formula:

$$\frac{\text{Final body weight} - \text{ initial body weight}}{\text{initial body weight}} X\ 100$$

2.6. Cardiosomatic Index

Cardiosomatic index (CSI) of the rats was calculated using the formula:

$$\frac{\text{weight of heart}}{\text{final body weight}} X\ 100$$

2.7. Estimation of Lipid Profile

The serum cholesterol and triacyglycerol (TAG) were determined using the method described by Trinder [14]. High density lipoproteins (HDL) cholesterol low density lipoproteins (LDL) cholesterol, very low density lipoproteins (VLDL) cholesterol and chylomicron fractions were precipitated quantitatively by the action of phosphotungstic acid in the presence of magnesium ions according to the method described by Friedewald [15].

2.8. Antioxidant Determination

Superoxide dismutase (SOD) activity was determined by the method described by Beyer and Fridovich [16]. Catalase (CAT) was assayed by measuring the degradation rate of H_2O_2 using Beutler [17] method. The rate of disappearance of H_2O_2 was monitored by spectrophotometry at 230 nm. Serum malondialdehyde (MDA) for lipid peroxidation was determined by the method described by Ohkawa *et al.* [18]. Glutathione reductase (GR) and glutathione peroxidase (GPx) activity were determined by the method of Beutler [19].

2.9. Estimation of Cardiac Troponin I

Cardiac Troponin I (cTnI) was assayed by an immunoassay that uses two cTnI-specific monoclonal antibodies with independent epitopes for cTnI as described by Bodor *et al.* [20] with slight modifications.

2.10. Histological Assessment

The heart from each experimental rat was carefully excised and trimmed of all fat, connective tissues and blotted dry to remove any blood. Cardiac tissues were weighed and fixed in 10% phosphate-buffered formaldehyde. Following standard procedures [21], tissues were dehydrated, cleared, embedded in wax, sectioned on a rotary microtome,

mounted on clean glass slides, stained and counter stained with Haematoxylin and Eosin respectively [22]. Photomicrographs were generated through a digital camera AmScope® mounted on a light microscope (Olympus CX31).

2.11. Statistical Analysis

The data was subjected to analysis using the Statistical Package for Social Sciences (SPSS), Version 20.0. Data obtained were expressed as mean ± standard error of the mean (SEM). Multiple group comparison was based on one-way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparisons (post hoc test). Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of Some HAARTs and ARTs on Cardiosomatic Index of Wistar rats

Percentage changes in body weights were not significant, whereas, cardiosomatic indices of the heart were significant ($p < 0.05$) for LNZ, LZ, LT and the other ART-administered groups compared to control for the duration of the study (Table 1).

Table 1 Effect of some HAARTs and ARTs on Body and Organ Weights

Groups (n = 5)	Organ Weight (g)	Average Initial body Weight (g)	Average Final body Weight (g)	% Change in body weight	Cardiosomatic Index
1 (NC)	0.48	151.20	155.80	3.04 ± 0.26	0.30 ± 0.01
2 (ELT)	0.54	173.20	171.80	-0.82 ± 3.07[a,d]	0.31 ± 0.01[c,e,g]
3 (LNZ)	0.63	167.00	174.80	4.69 ± 5.06[d]	0.36 ± 0.02[a,b]
4 (AL)	0.56	151.80	170.80	12.29 ± 2.33[b]	0.32 ± 0.01[e,g]
5 (LZ)	0.62	156.80	159.00	1.17 ± 6.24[d]	0.39 ± 0.02[a,b,d]
6 (LT)	0.57	153.00	157.20	2.53 ± 5.16[d]	0.36 ± 0.02[a,b]
7 (AR)	0.88	224.40	234.00	4.35 ± 1.39[d]	0.37 ± 0.01[a,b,d]
8 (LR)	0.63	198.00	202.40	2.17 ± 1.73[d]	0.31 ± 0.01[c,e,f,g]

Data expressed as mean ± SEM; a = significantly different when compared to NC at $p < 0.05$; b = significantly different when compared to Group 2; c = significantly different when compared to Group 3; d = significantly different when compared to Group 4; e = significantly different when compared to Group 5; f = significantly different when compared to Group 6; g = significantly different when compared to Group 6

3.2. Effect of Some HAART and ART on Lipid Profile

Table 2 Effects of HAART and ART on Lipid Profile

Groups (n=5)	Total Cholesterol (mg/dl)	TAG (mg/dl)	HDL– C (mg/dl)	LDL– C (mg/dl)	VLDL – C (mg/dl)
1 (NC)	164.38 ± 0.87	144.95 ± 0.13	54.21 ± 0.11	81.38 ± 0.67	28.99 ± 0.26
2 (ELT)	189.94 ± 1.63[a]	152.88 ± 0.36[a]	51.99 ± 0.53[a]	107.38 ± 1.18[a]	30.57 ± 0.03[a]
3(LNZ)	185.99 ± 1.54[a]	150.24 ± 1.92[a]	52.48 ± 0.15[a]	103.44 ± 1.52[b]	30.04 ± 0.38[a]
4 (SL)	193.50 ± 1.04[a, c]	150.27 ± 0.45[a, c, d, e, f]	52.68 ± 0.17[a, d, f]	110.77 ± 0.91[a, c]	30.05 ± 0.09[a, c, e, f]
5 (LZ)	182.68 ± 2.05[a, d, e, f]	156.56 ± 0.47[a, d]	52.92 ± 0.72[d,f]	98.44 ± 2.46[a, d, e]	31.31 ± 0.10[a, d]
6 (LT)	192.28 ± 2.06[a, f]	152.33 ± 0.96[a, e, f]	50.75 ± 0.25[a, e]	111.07 ± 2.27[a]	30.46 ± 0.19[a, e, f]
7 (AR)	193.04 ± 1.86[a, f]	157.06 ±1.25[a]	52.73 ± 0.25[a, f]	108.90 ± 1.96[a, f]	31.41 ± 0.25[a]
8 (LR)	197.98 ± 1.35[a]	157.48 ± 0.31[a]	50.64 ± 0.73[a]	115.83 ± 1.87[a]	31.51 ± 0.07[a]

Data expressed as mean ± SEM ; a = significantly different when compared to NC at $p < 0.05$; b = significantly different when compared to Group 2; c = significantly different when compared to Group 3; d = significantly different when compared to Group 4; e = significantly different when compared to Group 5; f = significantly different when compared to Group 6

Serum levels of TC, TAG, LDL-C and VLDL-C were significantly ($p < 0.05$) elevated, with significantly ($p < 0.05$) reduced HDL-C levels in all drug-administered groups, with marked elevation in ELT and LR, corresponding to HAART and ART respectively, compared to control (Table 2).

3.3. Effects of HAART and ART on Antioxidants

SOD, CAT and GPx levels showed non-significant elevation in the HAART-administered groups, while ART-administered groups showed significant ($p < 0.05$) reduction across the groups with obvious reduction in CAT level, compared to control (Table 3).

Table 3 Effect of HAART and ART on Enzymatic Antioxidants

Groups (n=5)	SOD (µmol/ml/min/mg pro)	CAT (µmol/ml/min/mg pro)	GSH (µmol/ml)	GPx (µmol/ml/min/mg pro)	GST (µmol/ml/mg pro)
1 (NC)	3.63 ± 0.22	35.31 ± 2.60	27.27 ± 1.22	1.11 ± 0.07	3.60 ± 0.29
2 (ELT)	3.77 ± 0.75^{e,f,g,h}	37.27 ± 4.50^{f,g}	22.87 ± 4.00^{c}	1.16 ± 0.24^{e,f,g,h}	2.39 ± 0.34^{a,c}
3 (LNZ)	4.39 ± 0.31^{d,f}	38.35 ± 5.67^{f,g}	33.08 ± 2.01^{b}	1.34 ± 0.09^{d,e,f,g,h}	4.24 ± 0.48^{a,b}
4 (SL)	3.18 ± 0.50$^{c, d, e}$	48.66 ± 7.37^{e,f,g,h}	15.82 ± 3.37^{a,c,e,f}	0.96 ± 0.15^{c}	2.57 ± 0.84^{c}
5 (LZ)	2.30 ± 0.12^{a}	26.04 ± 1.36^{d}	23.78 ± 3.75^{d}	0.72 ± 0.04^{a,b,c}	2.35 ± 0.44^{a,c}
6 (LT)	2.41 ± 0.30^{a,c}	17.12 ± 0.95^{a,b}	28.86 ± 5.33^{d}	0.71 ± 0.08^{a,b,c}	1.61 ± 0.39^{a,c}
7 (AR)	2.25 ± 0.07^{a}	18.06 ± 0.68^{a,b,c,d}	27.44 ± 3.54^{d}	0.68 ± 0.02^{a,b,c}	2.37 ± 0.23^{a,c}
8 (LR)	2.57 ± 0.05^{a}	21.38 ± 0.34^{d}	29.32 ± 1.71^{d}	0.79 ± 0.01^{a,b,c}	2.88 ± 0.60^{c}

Data expressed as mean ± SEM**;** a = significantly different when compared to NC at $p < 0.05$**;** b = significantly different when compared to Group 2; c = significantly different when compared to Group 3; d = significantly different when compared to Group 4; e = significantly different when compared to Group 5; f = significantly different when compared to Group 6**;** g = significantly different when compared to Group 7**;** h = significantly different when compared to Group 8

3.4. Effect of HAART and ART on Non-enzymatic Antioxidants

Vitamin E levels were significantly ($p < 0.05$) elevated across all treated groups compared to control (Table 4).

Table 4 Effect of HAART and ART on Non-Enzymatic Antioxidants

Groups (n=5)	MDA (µmol/ml)	Vitamin A (µg/100g)	Vitamin C (mg/g)	Vitamin E (µg/100g)
1 (NC)	9.73 ± 1.47	128.49 ± 8.74	5.02 ± 0.34	296.21 ± 33.86
2 (ELT)	8.75 ± 1.83^{e,f}	93.03 ± 17.49^{a,c,d,e}	3.22 ± 0.59^{a}	405.30 ± 24.48^{a,d,f}
3 (LNZ)	15.21 ± 4.22^{d,f}	126.93 ± 11.77^{b,d,g}	4.45 ± 0.65^{d}	467.42 ± 28.26^{a}
4 (SL)	24.52 ± 1.23^{a,c,e,f}	236.97 ± 26.63^{a,b,c,d,e,f,g}	8.33 ± 1.12^{a,c,f}	701.14 ± 26.13^{a,b}
5 (LZ)	9.58 ± 1.73^{b,d}	159.77 ± 11.45^{b,d}	5.54 ± 3.34^{e}	653.41 ± 42.61^{a}
6 (LT)	21.79 ± 4.55^{a,b,d,e,g,h}	102.65 ± 16.47^{b,d,h}	3.36 ± 0.64^{d}	670.08 ± 73.38^{a,b}
7 (AR)	12.52 ± 2.38^{d,f}	98.34 ± 18.25^{c,d,h}	2.84 ± 0.67^{d,f,h}	639.39 ± 73.38^{a}
8 (LR)	13.00 ± 1.62^{d,f}	163.64 ± 35.25^{b,d,g}	5.21 ± 0.43^{d,g}	626.83 ± 36.04^{a}

Data expressed as mean ± SEM ; a = significantly different when compared to NC at $p < 0.05$; b = significantly different when compared to Group 2; c = significantly different when compared to Group 3; d = significantly different when compared to Group 4; e = significantly different when compared to Group 5; f = significantly different when compared to Group 6; g = significantly different when compared to Group 7; h = significantly different when compared to Group 8

3.5. Effect of HAART and ART on Cardiac Troponin-I

Troponin-I (cTn-I) showed marked decrease in all drug-administered groups, except groups SL and LZ which showed non-significant elevation, compared to control (Table 5).

Table 5 Effect of HAART and ART on Cardiac Troponin I (cTnI)

Groups (n=5)	Troponin I (mg/g)
1 (NC)	0.93 ± 0.14
2 (ELT)	0.34 ± 0.09 [a,d,e]
3 (LNZ)	0.64 ± 0.22 [d,e]
4 (SL)	1.07 ± 0.29 [b,c,f,g,h]
5 (LZ)	1.07 ± 0.36 [b,c,f,g,h]
6 (LT)	0.57 ± 0.17 [a,d,e]
7 (AR)	0.12 ± 0.01 [a,d,e]
8 (LR)	0.56 ± 0.20 [a,d,e]

Data expressed as mean ± SEM; a = significantly different when compared to NC at $p < 0.05$; b = significantly different when compared to Group 2; c = significantly different when compared to Group 3; d = significantly different when compared to Group 4; e = significantly different when compared to Group 5; f = significantly different when compared to Group 6; g = significantly different when compared to Group 7; h = significantly different when compared to Group 8

3.6. Histopathological Assessment

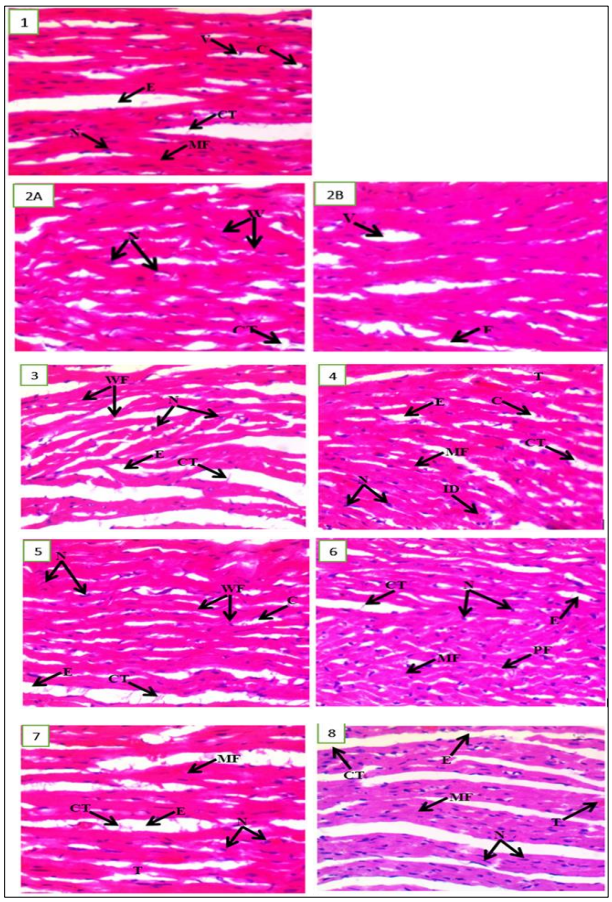

Figure 1 Representative Photomicrographs of H and E stained cardiomyocyte of adult male Wistar rats, Groups 1 - 8 (Mag. X400). N- nucleus, CT- connective tissue, MF- muscle fibre, E- endomysium, C- capillary, V- venule, WF- wavy muscle fibres, T- thrombose, ID- intercalated disc, PF- purkinje fibres. Photomicrograph observations revealed a normal myofibrillar structure with striations in {1}. Wavy myocardial fibres with increased branching, hypertrophy and reduced endomysium in {2}. Alterations of the striated morphology with abnormal branching and diffused interstitial fibrosis with wide and evenly spread endomysium in {3}. Atrophying muscle fibres with wavy striations and appearance of irregular shaped nuclei in {4}. Hypertrophy and wavy striation of muscle fibres in {5}. Distorted striation, increased branching and hypertrophic cardiomyopathy in {6}. Hypertrophic muscle fibres with large endomysium, irregular shaped nuclei and presence of binucleate bodies in {7}. Enlarged and branched myofibrils, interstitial fibrosis and increased number of nuclei with some enlarged in {8}

4. Discussion

HIV is still a major public health concern, particularly in Nigeria, where young adults in their productive years (aged 15-49) are mostly affected, with a prevalence rate put at 1.3 [23]. The effectiveness of ART and HAART in improving the life expectancy of people living with HIV is well documented [4]. However, concerns regarding the cardiotoxic effects of these therapies persist. In this study, one case each of mortality was reported in ELT and LT administered groups. This suggests that these drug regimens are potentially toxic. The toxicity of ELT may be due to the adverse drug reactions induced by the higher dose of Efavirenz (EFV-600 mg) used in the ELT cocktail in this study. Studies by Dickinson *et al.* [24] and Xiao *et al.* [25] reported reduced adverse effects, cost-effective yet non-inferior antiviral activity for treatment options using EFV-400 mg when compared to EFV-600 mg. This suggests that an EFV-based HAART cocktail may elicit dose-dependent adverse reactions, whereas, lower doses than 600 mg may be safer. Nevirapine (NVP) and Zidovudine (ZDV) regimens, notwithstanding the dose, have been linked to an increased risk of cardiotoxicity, but the effect is drastically reduced when administered in combination with low-dose LMV [26,27]. From this study, oral administration of LNZ containing low-dose lamivudine (LMV-150 mg) revealed reduced cardiotoxicity. This is consistent with the previous report that Lamivudine (3TC) possesses lower cardiotoxicity risk [28]. It can imply that the LMV-150 included regimen is potentially safe. In addition, SL containing LMV-300 mg appeared safe which may be due to the established lower risk of LMV cardiotoxicity [28].

Organosomatic indices indicate the relative sizes of organs to body weight, which depict the status of organ systems that may change in size more rapidly than organism's weight [29]. They can also be used to underscore differences in nutritional and energy status [30]. Cardiosomatic index (CSI) is one of the key parameters in drug cardiotoxicity determination. Results showed a significant ($p < 0.05$) elevation in CSI in the LNZ, LZ, LT, and AR-administered groups when compared with the ~~normal~~ control. The raised CSI are suggestive of physiological adjustments of the heart to compensate for proportionate increase in body weight of animals and/or lipohypertrophy (increased adipose tissue) [31].

Changes in serum lipid profile are evaluated to determine the possible existence of cardiovascular abnormalities and related complications. HIV-infected patients have been reported to exhibit multiple anomalies in lipid metabolism in post-HAART eras [32]. Dyslipidaemia and/or elevated levels of total cholesterol are induced by most antiretroviral drugs [10,33], especially PI included cohorts [32,34]. Hyperlipidemia, defined as an increase in TAG and TC levels, was observed in this study, consistent with previous reports [32,34]. Prominence in elevated lipidemia was seen in ELT and LR-administered groups, while LNZ and LZ least altered lipid metabolism compared to control. LNZ's least alteration effects on lipids may be due to anti-hyperlipidemic effect of Nevirapine-based regimens reported, especially against low HDL-C [32].

Mammalian cells are furnished with antioxidant defense systems (CAT, GST, GPx, SOD, and GSH) to combat the deleterious effects of free radicals [35]. Reactive oxygen species (ROS) affect the antioxidant defense mechanisms by perturbing the intracellular concentration of GSH, SOD, CAT, and GPx and/or increasing MDA concentrations [36]. In the present study, oxidative stress (OS) was generated during drug administration, evidenced by the general perturbation in antioxidant enzymes across all treatment groups compared with the control. There was a non-significant elevation in the serum antioxidant levels of SOD, CAT, and GPX in ELT and LNZ-administered groups compared to the control. However, ELT administered group showed a significant decrease in GST, vitamins A and C levels. The LNZ-administered group showed non-significant elevation in GSH and GST serum levels, with a non-significant decrease in serum vitamins A and C. It may be deduced that LNZ may offer some protective effect against oxidative stress, possibly due to its non-atherogenic effect as previously reported by Ibeh, *et al.* [36]. Notably, serum concentrations of SOD, CAT, GPx, and GST had an overall decrease in the AR-administered group. Overall, it can be deduced that ELT and AR are more likely to compromise antioxidant enzyme integrity due to induced oxidative stress.

Cardiac troponin I (cTnI) is a highly sensitive and specific biomarker for the diagnosis of myocardial injury [12]. cTnI are released from the necrotic myocardium both as intact protein and degradation products into circulation during cardiac injury [37]. In this study, there was a significant reduction in cTnI in the ELT group, and a non-significant increase in the LNZ-administered group relative to the control. This decrease may be due to the degradation of cTnI by intracellular proteases present in the myocardium and the blood [38]. The extent of the cTnI degradation varies during the time course of acute myocardial infarction and may influence the concentration of cTnI in the blood [39]. Strikingly, the troponin levels in the ART-administered groups showed inconsistencies when compared with the control. These anomalies in cTnI values may be due to several factors ranging from proteolytic degradation to the reaction of cTI with other proteins in the blood of the animals.

The histoarchitectural observations revealed distinct changes in cardiac muscle across the eight groups. Normal myofibrillar structure was exhibited in group 1, which indicates healthy cardiac muscle [40]. In contrast, group 2 and 5 showed evidence of cardiac remodeling evidenced by increased branching and hypertrophy [41]. This suggests an adaptive response to increased cardiac injury. Groups 6 and 7 showed hypertrophic cardiomyopathy with increased striation and branching, including large endomysium [41,42]. Furthermore, groups 3 and 8 showed cardiac fibrosis characterized by abnormal branching, altered striation and interstitial fibrosis [43], which implies a maladaptive response to cardiac injury; group 4 revealed muscle wasting and degeneration, noticed by atrophying fibres and irregular nucleus [44], which could imply a loss of muscle mass.

5. Conclusion

Evaluation of oral exposure of Wistar rats to therapeutic doses of HAART and ART revealed distinct changes which depict varying degrees of cardiac injury. However, ELT, LT, and AR are more likely to distort the cardiac architecture and, hence may compromise antioxidant enzyme integrity and induce oxidative stress than other ARV cohorts used in this study. Also, ELT and LR elicit greater hyperlipidemic effects than LNZ and other ART used in this study. For these reasons, drug regimens LNZ, SL, and LZ, including HAART containing low-dose lamivudine are recommended to people living with HIV/AIDS (PLWHA) as these drug regimens possess better cardiac safety profiles. Monitoring the extent of the cTnI degradation during the time course of acute myocardial infarction may be useful in determining the proportion of cTnI in the blood. Also, the effects of dietary and other lifestyle habits might have been underestimated in the study design and should be considered in future studies.

Compliance with ethical standards

Acknowledgments

The technical support from the following are acknowledged: Victor A. Umoh of Department of Internal Medicine, Faculty of Clinical Sciences, University of Uyo Teaching Hospital, Uyo and Edelungudi I. Edagha of Department of Family Medicine, Faculty of Clinical Sciences University of Uyo Teaching Hospital, Uyo, Nigeria.

Authors' Contributions

I.C.U and I.M.S performed the experiment. I.A.E and Late Dr. (Mrs) A.J. Ekpo conceptualized, designed and supervised the research, while E.O.N interpreted the results. I.C.U and I.A.E analyzed the data for the study. I.C.U, M.M.A and A.E co-drafted the manuscript

Disclosure of conflict of interest

Authors declare no conflicts of interest.

Statement of ethical approval

The authors declare compliance with the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) and relevant national laws. All experiments received the University of Uyo's ethics committee approval.

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