IBRO Neuroscience Reports 12 (2022) 210–216

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

Brain antioxidants and hippocampal microanatomical alterations following the administration of Efavirenz/Lamivudine/Tenofovir disoproxil fumarate and Lamivudine/Nevirapine/Zidovudine in adult male Wistar rats

Innocent A. Edagha [a,*], Akpan U. Ekanem [a], Itoro F. Usoh [b], Victor A. Umoh [c], Ataben M. Ataben [a], Anietie A. Akpan [a]

[a] Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Nigeria
[b] Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Nigeria
[c] Department of Internal Medicine, Faculty of Clinical Sciences, University of Uyo Teaching Hospital, Nigeria

ARTICLE INFO

Keywords:
Highly active antiretroviral therapy
Hippocampus
Neurodegeneration
Oxidative stress

ABSTRACT

Highly active antiretroviral therapies (HAARTs) are used for the management of human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS). The present study was designed to characterize the neurotoxicity profile of two popular HAARTs on the brains' antioxidants and hippocampal microanatomical alterations in an in vivo model. Fifteen adults male Wistar rats, were assigned to three groups (n = 5); group I the normal control (NC) received distilled water (5 mL/kg b.wt), groups II administered with oral therapeutic doses of Efavirenz/ Lamivudine/ Tenofovir disproxil fumerate (TLE 17.14 mg/kg b.wt), and group III with Lamivudine/ Nevirapine/ Zidovudine (LNZ 9.28 mg/kg b.wt), respectively which were available for use in University of Uyo Teaching Hospital Nigeria at the time of this experiment. After a 30-day administration, biochemical parameters (catalase, superoxide dismutase, reduced glutathione, glutathione S-transferase, malondialdehyde, glutathione peroxidase, vitamins A, C and E) were determined via serum from blood of ketamine (100 mg/kg, i.p) anesthetized rats. Brains were carefully removed and post-fixed for tissue processing employing hematoxylin and eosin (H&E), cresyl fast violet (CFV) stains, and glial fibrillary acidic protein (GFAP) antibody expression. Results revealed significantly ($p < 0.05$) decreased antioxidant concentrations and increase in oxidative markers in HAART-administered groups. Normal histoarchitecture was shown in NC, but TLE-administered group demonstrated some neuronal atrophy, and degeneration of pyramidal neurons, with milder distortions in LNZ. TLE-administered group demonstrated intense Nissl substances with chromatolysis compared to LNZ and NC, while GFAP was strongly expressed in TLE-administered group compared to LNZ. In conclusion, TLE is more neurotoxic compared with LNZ.

Introduction

Human immunodeficiency virus, and acquired immunodeficiency syndrome (HIV/AIDS) is one of major health challenges with an estimated 37.7 million people living with HIV/AIDS [PLWHA] (World Health Organization, 2020; Joint United National Programme on HIV/AIDS (UNAIDS), 2021) and about 680,000 death globally resulting from AIDS related illness in 2020 (Joint United National Programme on HIV/AIDS (UNAIDS), 2021). Africa is home to about 15.2% of the world's population, and about 25.7 million people are living with HIV and has recorded about 470, 000 AIDS-related illness resulting to death as of 2018, with northern Africa having a lower rate of HIV prevalence while southern Africa has over 10% of the population infected with HIV (World Health Organization, 2020; Joint United National Programme on HIV/AIDS (UNAIDS), 2021). In Nigeria the estimate is about 1.7 million, with about 160,000 AIDS-related death in 2020, accounting for 9% of the world PLWHA (Joint United National Programme on HIV/AIDS (UNAIDS), 2021). Notably HIV can be transmitted through exchange of a variety of body fluids from infected individuals but individuals cannot become infected through ordinary day-to-day contact such as kissing, hugging, shaking hands, or sharing personal objects, food or water (World Health Organization, 2021).

* Corresponding author.
E-mail address: innocentedagha@uniuyo.edu.ng (I.A. Edagha).

https://doi.org/10.1016/j.ibneur.2022.03.004
Received 26 November 2021; Accepted 10 March 2022
Available online 13 March 2022

Highly active antiretroviral therapy (HAART) is a multiple antiretroviral drug therapy used in the management of HIV/AIDS which help to decrease patient burden of HIV, maintain immune system function, and prevent opportunistic infections and as of 2021, 28.2 million people were accessing antiretroviral therapies (World Health Organization, 2021; Joint United National Programme on HIV/AIDS (UNAIDS), 2021).

Although HAART helped to improve the quality of life for PLWHA, it exposes patients to various diseases including neurological diseases, hyperglycemia, gastrointestinal, and lipodystrophy symptoms after longterm treatment (Chowta et al., 2018; Tshikuka et al., 2018; Lawal et al., 2021). Long term exposure of the entire body to multiple drugs including HAARTs will lead to drug toxicity (Naidu et al., 2021).

World Health Organization (WHO) reported that the adverse effects of antiretroviral therapy over long term exposure is hardly differentiated from HIV complication and various research have reported side effects, toxicities and adverse effect associated with antiretroviral therapy and its combination HAART despite the numerous beneficial effects (Oyeyipo et al., 2018; World Health Organization, 2021; Naidu et al., 2021). Zidovudine, stavudine azidothymidine, tenofovir disproxil fumarate, efavirenz, and lamivudine, have been reported to cause suppression of bone marrow and stavudine has been associated with insulin resistance peripheral neuropathy, lactic acidosis and hyperlipidemia (Naidu et al., 2021).

Antiretroviral therapy used in the management of HIV/AIDS was approved by the United States' Food and Drug Agency (FDA) in 1987 and have saved lives, and which was similarly introduced in Nigeria in 2002 (National Agency for the Control of AIDS (NACA), 2021; World Health Organization, 2021). HAART is the combination of the three ARTs, having greater efficacy in treating HIV/AIDS and are classified into nucleoside analog reverse transcriptase inhibitors (NRTIs), non-nucleoside analog reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), intergrase inhibitors, fusion inhibitors and chemokine receptor and antagonist (CCR5 anatgonist), which is according to the phase of retroviral cycle that they inhibit (Saag et al., 2020; Li et al., 2021; Eggleton and Nagalli, 2021).

The hippocampus is subdivided into three cornu ammonis (CA) namely; CA1, CA2 and CA3 respectively. As part of the limbic system, the hippocampus plays an important role in the process of learning, memory consolidation, regulation of sexual and emotional behavior (Enrico and Richard, 2015; Fogwe et al., 2021; Abdullah et al., 2021; Patel et al., 2021).

Hence, with several literatures reporting the neurotoxicity of HAARTs and ARTs, this study was aimed at evaluating the toxicological profile of two commonly used HAART in University of Uyo Teaching Hospital, Akwa Ibom State, Nigeria on the histoarchitecture of the CA1 to CA3 region of the hippocampus and on alterations in biochemical parameters in Wistar rats.

Materials and methods

Experimental animals

The Department of Human Anatomy, University of Uyo, Nigeria animal experiment committee approved the protocol for all animal experiment carried out in this study. All the process carried out in this experiment follows the National Institute of Health Care Guide for the Care and Use of Laboratory Animals (NIH publication #85-23, revised in 1985). All efforts were made to minimize animal pain and to reduce the number of animals used in the experiment. Fifteen adults male Wistar rats (150–220 g) aged about 12 weeks were sourced from the Department of Zoology Animal House, Faculty of Science, University of Uyo (UNIUYO), Akwa Ibom State, Nigeria. The animals were weighed, labeled and kept in wooden cages at the Animal House, Faculty of Basic Medical Sciences (FBMS), UNIUYO, Akwa Ibom State, Nigeria and were acclimatized under optimum pathogen-free environment and maintained a 12 h light/dark cycle (light on at 6.00 a.m.) at 25–27 ^{o}C at relative humidity of 40–60% which was measured using a CEM hydrometer (DT 615, Shenzhen, China), two weeks prior to the start of the experiment to allow for free and unhindered access to diet and water. The rats were separated into cages with dimensions; length 24 cm, width 12 cm, and height 14 cm. The cages were properly maintained by daily changing the saw-dust and leftover feed. The rats were fed with pelletized growers mash (Grand Cereal Vital® Feed Ltd., Jos) and given tap water ad libitum.

Drug acquisition

Two HAARTs; LNZ and TLE were sourced from the manufacturers Strides Acrolab Limited, Bangalore-562 106, India, and Aurobindo Pharma Limited, Telangana State, India, respectively.

Experimental design

The adult male Wistar rats were weighed, marked and allotted into three groups of five (5) per group, Group one (1) served as control, groups 2–3 were given as follows:

Group	Dosage	Duration (Days)
NC- Distilled water	(5 mL/kg)	30
HAART - TLE	17.14 mg/kg	30
HAART - LNZ	9.28 mg/kg	30

Administration of experimental drugs

The experimental drugs were administered orally according to the body weight of animal. The dosage was calculated from their normal dose for a 70 kg man. The dosage of drugs administered was calculated using the formula: weight of animal/ 1000 x dosage/stock.

Sacrifice of experimental animals

After thirty (30) days of administration, on day 31 of the research, the rats were anaesthetized and euthanized with ketamine (100 mg/kg, i.p). Whole brain perfusion was performed with phosphate buffered saline for 2 mins and thereafter with 4% paraformaldehyde until tail stiffness in approx. The brains were then dissected out, and then processed for light microscopy within 72 h. The paraffin wax blocked tissues were sectioned at 5 μm sampling 5 ribbons sections per tissue blocks using the rotary microtome (Microtome Thermo Scientific – Microm HM 325, England) and stained with haematoxylin and eosin, cresyl fast violet, and immunohistochemical method for glial fibrillary acidic protein antibody (GFAP-Dako, Lot 00083681).

Histopathological assessment

Haematoxylin and eosin (H&E) stain

The histological features were assessed using formalin fixed paraffin embedded tissues. The hippocampal regions of the brain were assessed after brain section was immersed in haematoxylin for about 30 s, and then rinsed in a running tap water, which was then transferred to eosin stain for about 1 min. The tissue section was rinsed again before dehydrating in alcohol concentration of 50%, 60%, 70%, 80%, 95%, and 100%, respectively. Sections of the brain tissues were cleared in xylene, and mounted using DPX (Dibutylphthalate Polystyrene Xylene) (Suvarna et al., 2019).

Cresyl violet stain

The section of the brain tissues were mounted on a slide and then immersed in chloroform/ethanol solution in a ratio of 4:1 for one hour (1 h), and placed in a solution of cresyl fast (Sigma-Aldrich, Germany)

for ten minutes (10 min). Sections were thereafter dehydrated in alcohol concentrations of 50%, 60%, 70%, 80%, 95%, and 100%, respectively, and thereafter cleared in xylene and mounted using DPX (Suvarna et al., 2019).

Glial fibrillary acidic protein (GFAP)

The sections were first treated in 0.1% H_2O_2 (Deventer, The Netherland) for 15 mins to inactivate endogenous peroxidase and then incubated for 1 h in 5% normal goat serum in PBS. The section was then incubated overnight at 4 ^{o}C in PBS containing 1% normal goat and the following antibodies: rabbit anti-glial fibrillary acidic protein (GFAP) (1: 500; Dako, Lot 00083681), to visualize astrocytes. After washing, the sections were incubated for 2 h at room temperature in the appropriate biotinylated secondary antibodies (diluted 1:200). The sections were then reacted in avidin biotin solution (ABC kit, Vectastain) and finally using 3, 3'-diaminobenzidine as chromogen and peroxide. Sections were then dehydrated in alcohol concentrations of 50%, 60%, 70%, 80%, 95%, and 100%, respectively, and then cleared in xylene, mounted on gelatinized slides, and cover-slipped using DPX (Taylor, 1978; Tascos et al., 1982).

Photomicrographs of all slides were obtained using light microscope (Olympus - CX31, Japan). All images were captured with Amscope digital camera (MU 1000, China) attached to the microscope, and they were blindly assessed by three independent histopathologists.

GFAP immunostaining score

In order to demonstrate the extent of immunostaining with anti-GFAP antibody, a semi-quantitative score was utilized using the method described by Klein et al., 1999. To achieve this purpose, each sample was scored: (a) for the percentage of labeled reactive astrocytes (0 = absence of labeling over astrocytes; 1 = less than 30% of astrocytes labeled; 2 = 30–60%; and 3 = more than 60%); and (b) and for the intensity of the immunostaining (0 = no staining; 1 = weak; 2 = mild; and 3 = strong staining). Both scores were tallied to obtain the final scoring of samples, ranging from 0 to 6. To avoid bias, the analysis was performed by at least two independent pathologists.

Biochemical analysis

The bloods of the experimental animals were obtained through cardiac puncture to the right ventricle using a 5 mL syringe. Antioxidant activities in the brain homogenates of Wistar rats were determined for superoxide dismutase (SOD) as described by Sun and Zigma (1978), catalase (CAT) as described by Sinha (1971), reduced glutathione (GSH) as described by Sedlak and Lindsay (1968), glutathione S-transferase (GST) as described by Habig et al. (1974), glutathione peroxidase (GPx) as described by Rotruck et al. (1973), malondialdehyde (MDA) an index of lipid perioxidation as described by Buege and Aust (1978), vitamins C, E and A as described by Omaye et al. (1979) and Rutkowski et al. (2006).

Statistical analysis

Data in this study were expressed as mean ± standard error of mean (SEM) and analyzed using one-way analysis of variance (ANOVA) to determine the difference in the means across the three groups, and the post-hoc test (Tukey test) was performed for between groups comparison. Values were regarded as statistically significant at $p < 0.05$.

Results

Effect of HAARTs on the body and brain weights

There was slightly increase in body and brain weights of animal following a 30-day administration of the two HAARTs (TLE and LNZ) as shown in Table 1.

Effect of HAARTs on the enzymatic brain antioxidants

Enzymatic antioxidants; superoxide dismutase, catalase, glutathione peroxidase, were significantly ($p < 0.05$) decreased in HAART-administered groups compared to NC. Glutathione-S-transferase showed increase in the test groups compared to the NC (Table 2).

Effect of HAARTs on the non-enzymatic brain antioxidants

Vitamins A and C indicated decreasing trends in the TLE and LNZ groups compared to the NC. However, vitamin E which is a liposoluble vitamin was significantly increased in the test group compared to NC (Table 3).

Effect of HAARTs on the hippocampal histopathology

The photomicrographs of the test groups revealed pale staining hippocampal neuropil of the TLE-administered group with neuronal shrinkages, distortion of neuron morphology, atrophy, vacuolations compared to NC. The LNZ demonstrated mostly hypertrophy of pyramidal neurons across the CA1 - CA3 (Fig. 1).

Nissl substances were up-regulated with higher intensity in the HAART-administered groups particularly in the CA1 and 2 of the test groups (Fig. 2).

The GFAP antibody expression for reactive astrocyte was strongly demonstrated in the HAART-administered groups compared to NC (Fig. 3). Greater activation of astrocyte in the test groups indicates signs of reactive astrogliosis (neurotoxicity) in the hippocampal sections across the CA1 to CA3. This is shown with larger astrocytic cell bodies and thick processes (inclusive of dendritic spines) in the test groups, with the LNZ group demonstrating the most intensity (Table 4).

Discussion

Highly active antiretroviral therapy is used for management of HIV/AIDS, and the devastating effect of HIV required infected individual to use HAART for the management of the virus, especially in Africa where HIV is predominant (World Health Organization, 2021, Joint United National Programme on HIV/AIDS (UNAIDS), 2021). Many studies (Peter and Udoh, 2015; Peter et al., 2017; Lawal et al., 2021) have mentioned that antiretrovirals drugs are neurotoxic and can lead to various neurologic diseases including memory impairment and cognitive disorder (Dong et al., 2021).

In this study we investigated the neurological profile of two commonly used HAARTs in the University of Uyo Teaching Hospital Nigeria on the CA1 to CA3 region of the hippocampus. These regions

Table 1
Effect of HAARTs on the body and brain weights.

Group	Initial Body weight (g)	Final Body Weight (g)	Body Weight Change (g)	% Change in Body weight	Brain Weight (g)	Organo somatic Index
NC	151.20 ± 0.37	155.80 ± 1.71	4.60 ± 1.34	+ 2.96	1.51 ± 0.03	0.96
TLE	173.20 ± 4.19	175.80 ± 4.58**	2.80 ± 0.39	+ 1.47	1.52 ± 0.09	0.86
LNZ	167.00 ± 2.85	176.00 ± 7.54**	9.00 ± 4.69	+ 5.20	1.55 ± 0.01	0.88

Data is expressed as Mean ± Standard error of mean (SEM). NC = Normal control; TLE = Efavirenz/ Lamivudine/ Tenofovir disproxil fumarate; LNZ = Lamivudine/Nevaripine/Zidovudine. **Slight increase in body weight.

Table 2
Effect of HAARTs on the enzymatic brain antioxidants and oxidative stress markers.

Group	SOD (μmoL/mL/min/mg/pro)	CAT (μmoL/mL/min/mg/pro)	GPx (μmoL/mL/mg/pro)	GST (μmoL/mL/mg/pro)	GSH (μmoL/mL)	MDA (μmoL/mL)
NC	4.61 ± 0.25	58.57 ± 5.50	1.42 ± 0.07	4.21 ± 0.65	20.85 ± 2.28	11.29 ± 1.09
TLE	1.89 ± 0.13[a]	18.10 ± 1.28[a]	0.58 ± 0.04[a]	1.80 ± 0.17[a]	24.28 ± 1.14	14.37 ± 0.62
LNZ	3.06 ± 0.19[a,b]	36.72 ± 3.44[a,b]	0.92 ± 0.05[a,b]	3.13 ± 0.20[a,b]	25.83 ± 0.88	10.33 ± 1.62
F value	46.95	28.17	58.46	9.06	2.68	3.19
P value	0.0001	0.0001	0.0001	0.004	0.109	0.078

Data is expressed as Mean ± Standard error of mean (SEM) and considered significant at ($p < 0.05$). a = significant compared with NC; b = statistically significant compared with TLE. NC = Normal control; TLE = Efavirenz/ Lamivudine/ Tenofovir disproxil fumarate; LNZ = Lamivudine/ Nevaripine/ Zidovudine.GSH = Reduced glutathione, SOD = Superoxide dismutase, CAT = Catalase, MDA = Malonaldehyde, GPx = Glutathione peroxidase, GST = Glutathione S-transferase.

Table 3
Effect of HAARTs on the non-enzymatic brain antioxidants.

Group (n = 5)	Vitamin A (ng/100 g)	Vitamin C (mg/ g)	Vitamin E (ng/100 g)
NC	189.85 ± 40.46	6.11 ± 1.47	287.88 ± 18.14
TLE	101.14 ± 12.08	3.23 ± 0.64	473.11 ± 109.61
LNZ	144.20 ± 29.42	4.63 ± 1.02	399.62 ± 68.62
F value	2.23	1.72	1.53
P value	0.150	0.220	0.256

Data is expressed as Mean ± Standard error of mean (SEM) and considered significant at ($p < 0.05$). NC = Normal control; TLE = Tenofovir disproxil fumarate / Lamivudine/ Efavirenz; LNZ = Lamivudine/ Nevaripine/ Zidovudine.

(CA1 - CA3) are the principal pyramidal cell fields in the hippocampus and play important role in cognitive activities and memory (Song et al., 2020; Dong et al., 2021).

The result of the test groups' antioxidant defense state is shown in Tables 2 and 3, it demonstrated that HAART induced oxidative stress in Wistar rat, this is associated with a significant decrease in CAT, GSH and SOD and a decrease in the concentration of vitamin C (ascorbic acid) when compared with the NC. The concentration of MDA which is a marker for lipid peroxidation was significantly elevated in test group II confirming that the brain tissue was susceptible to oxidative stress, which may be due degradation of immune system due to generation of oxyradicals, inflammation to initiate neutrophils and macrophages subsequently enhancing lipid peroxidation which often led to cell apoptosis and oxidative stress (Abduljalil et al., 2015; Irato and Santovito, 2021). Accumulation of reactive oxygen species (ROS) is as a result of decrease in antioxidant enzyme activities, as shown in the result in Tables 2 and 3. When the production of ROS in the greater than anti-oxidant potential, lipid peroxidation occurs (Irato and Santovito, 2021).

In the brain tissue, SOD, CAT and GSH are mainly responsible for detoxification of ROS (Halliwell, 2006). Deficiency of non-enzymatic antibodies can lead to oxidative stress increasing the probability of cell death (Irato and Santovito, 2021). The administration of HAART has associated with decreasing SOD, CAT and GSH concentrations, this decrease is as a result of this antioxidants protecting the body cells from increase ROS resulting from oxidative stress (Suresh et al., 2009). The HAART uptake caused increase of ROS in circulation by producing more oxidized metabolites, through mitochondrial interference by nucleoside

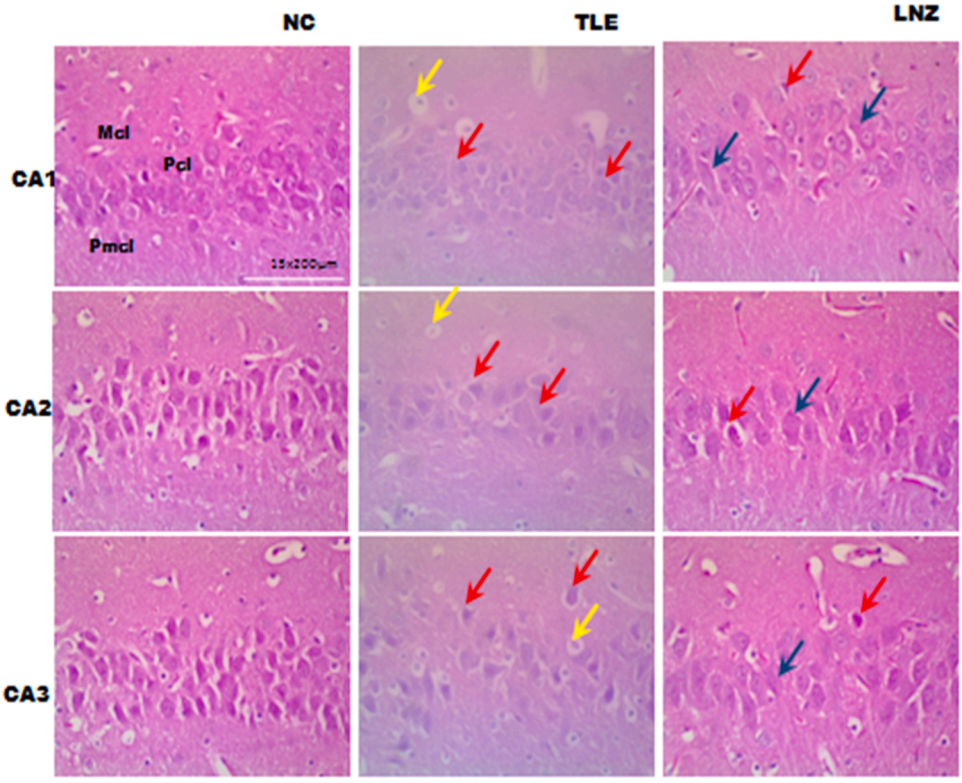

Fig. 1. Photomicrograph showing the effect of HAART (TLE versus LNZ) on the hippocampus (cornu ammonis 1–3). A = atrophy; blue arrow head = hypertrophy; red arrow head = neuronal shrinkage, H&E x400. The LNZ demonstrated neuronal shrinkage compared to the TLE-treated group having pale staining neuropil. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

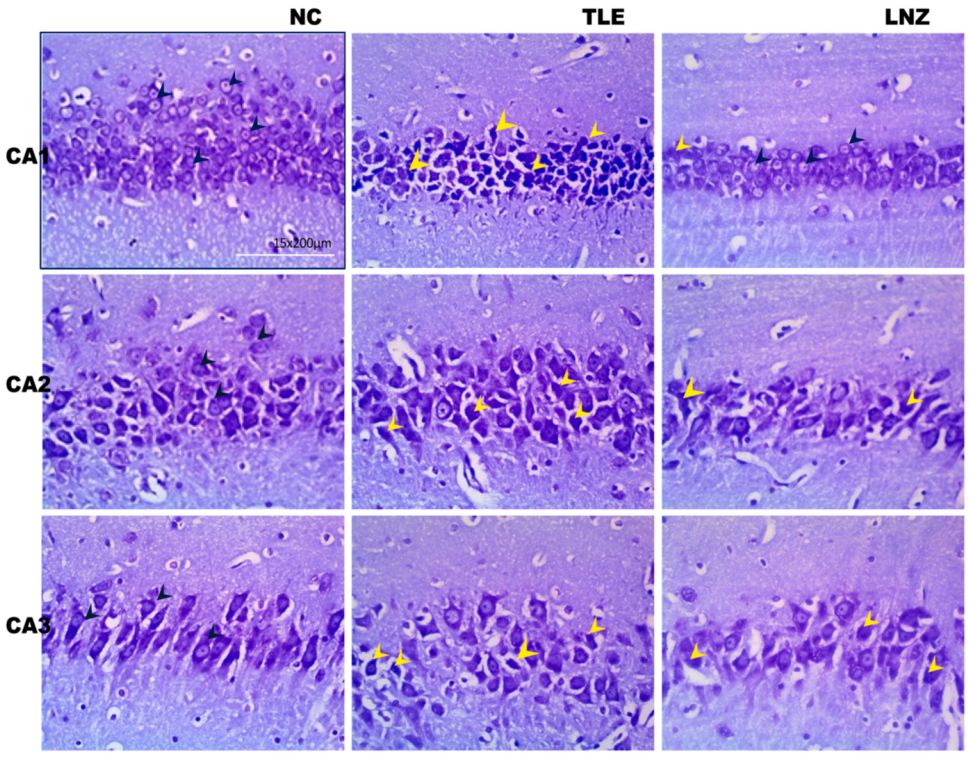

Fig. 2. Photomicrographs showing the effect of HAART (TLE versus LNZ) on hippocampal Nissl substance expression, CFV x400. The LNZ demonstrated mild to moderate Nissl substances compared to the strongly intense expression in the TLE-treated group. Black arrow head = normal Nissl substance; Yellow arrow head = strongly expressed Nissl substance. NC = Normal control. TLE = Tenofovir disproxil fumarate / Lamivudine/Efavirenz LNZ = Lamivudine/ Nevirapine/ Zidovudine. CA1 = cornu ammonis 1, CA2 = cornu ammonis 2, CA3 = cornu ammonis 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

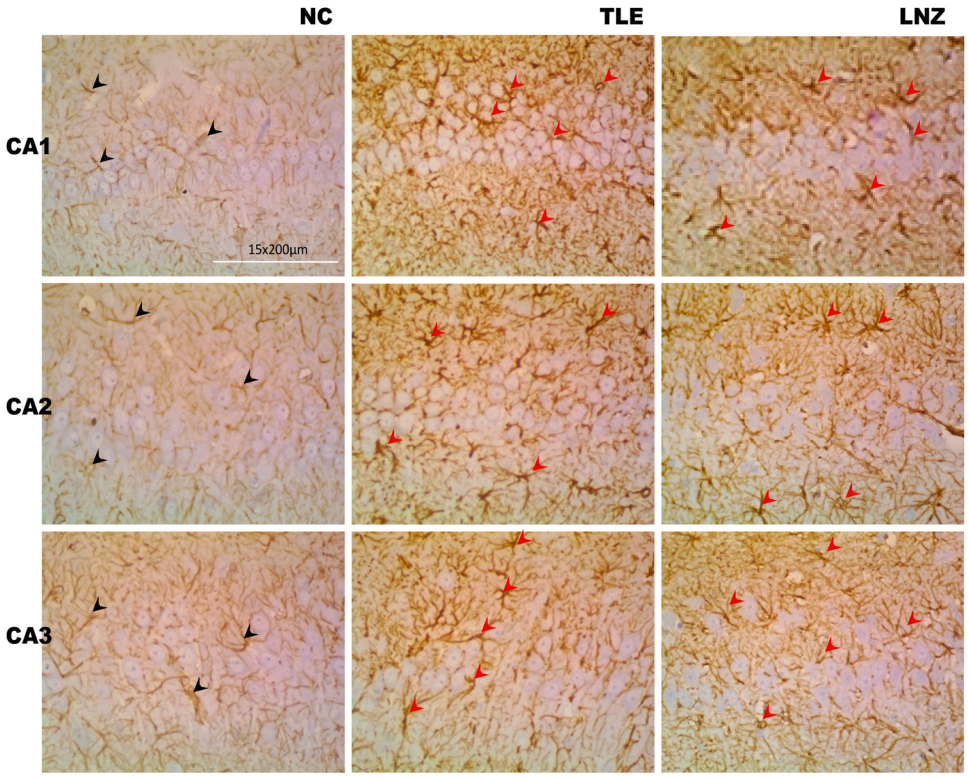

Fig. 3. Photomicrograph of the effect of HAARTs (TLE versus LNZ) on hippocampal expression of GFAP x400. The NC demonstrated mild GFAP expression (black arrow heads) compared to the strong GFAP antibody expression (red arrow heads) in the HAART-administered groups. NC = Normal control. TLE = Tenofovir disproxil fumarate/ Lamivudine/Efavirenz. LNZ = Lamivudine/ Nevirapine/ Zidovudine. CA1 = cornu ammonis 1, CA2 = cornu ammonis 2, CA3 = cornu ammonis 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reverse transcriptase inhibitor class of HAART administered in the test group (Hulgan et al., 2003).

Several studies have shown that HAART induced ROS development in the brain leading to neurocognitive decline, neurodegeneration and a progressive hippocampal dysfunction causing a deficit in memory function (Ngondi et al., 2006; You et al., 2009; Sato et al., 2010; Awodele et al., 2012; Rachel et al., 2015; Adeoti et al., 2016). In the present study, low concentration of vitamins A and C in the test groups may be as a result of detoxification of HAART-associated free radical and leading to lipid peroxidation (Pankaja, 2010; Abduljalil et al., 2015). The administered HAART is associated with fat distribution characterized by fat wasting and central abdominal fat accumulation (Daniella

Table 4
Effect of HAARTs on the hippocampal GFAP expression.

Group/ Region	% of IHC (A)	Intensity of IHC (B)	Final score (A+B)	GFAP Antibody Expression
Hippocampal CA1				
NC	< 30% (1)	Mild (2)	3	Low
TLE	> 60% (3)	Strong (3)	6	High
LNZ	> 60% (3)	Strong (3)	6	High
Hippocampal CA2				
NC	< 30% (1)	Mild (2)	3	Low
TLE	> 60% (3)	Strong (3)	6	High
LNZ	> 60% (3)	Strong (3)	6	High
Hippocampal CA3				
NC	< 30% (1)	Mild (2)	3	Low
TLE	> 60% (3)	Strong (3)	6	High
LNZ	> 60% (3)	Strong (3)	6	High

NC = Normal control; TLE = Tenofovir disproxil fumarate/ Lamivudine/ Efavirenz; LNZ = Lamivudine/ Nevaripine/ Zidovudine.
Key: % IHC: 0 = 0%; 1 = < 30%; 3 = > 60%
Intensity of IHC: 0 = No reaction; 1 = Weak; 2 = Mild; 3 = Strong
Final Score: Range = 0–6;
0/6 = Negative Reaction
1/6, to 3/6 = Low expression
4/6, to 6/6 = High expression.

et al., 2014), thus influencing the level of fat-soluble vitamin E in the test groups shown in Table 3.

The effect of HAART on the hippocampal neuronal cells as shown in Fig. 1 stained with H and E, is similar to the result observed by Emilio et al. (2008), and Peter and Udoh. (2015), who investigated the histomorphology of the hippocampus of Wistar rat administered with Efavirenz and Nevirapine. The test groups II and III histology demonstrated vacuolation and pyknotic nuclei. When neuron reacts to injury, they become swollen and rounded off, and the nucleolus is displaced toward the periphery (Kumar et al., 2014). Degeneration is simultaneously associated with inflammation which is a protective strategy or response utilized by the host cell to get rid of the cause of injury and necrotic tissue (Kumar et al., 2014). Vacuolization involves the formation of vacuoles as shown in TLE and LNZ-administered groups (see photomicrographs), which may be associated with paraptosis a cellular pathway leading to necrosis (Sperandio et al., 2004).

Although antiretroviral drugs have restricted capability to penetrate the blood brain barrier (BBB), but low molecular weight and hydrophobicity of drugs are factors that enhance its penetration through the BBB penetration, other multiple mechanisms include; the paracellular aqueous pathway, the transcellular lipophilic pathway, transport proteins, receptor mediated transcytosis and adsorptive transcytosis (Bertrand et al., 2021). Antiretroviral drugs often cause increased reactive oxygen species (ROS) generation during long time administration, which results in mitochondrial dysfunction, thus promoting cellular senescence (Velichkovska et al., 2018; Bertrand et al., 2021). Although not well understood, ART inhibit polymerase gamma which replicates mitochondrial DNA (mtDNA), resulting to mtDNA mutations which then induce mitochondrial dysfunction or results in a decrease in overall mtDNA levels leading to alternations of mitochondrial morphology and function, suggesting induction of multiple independent toxic mechanisms; especially under the administration of different classes of antiretroviral drugs (Bertrand et al., 2021). Also, ART can inhibit mitochondria electron transport chain which is important for mitochondria function and ATP production output through complex I inhibition which is associated with pathogenesis of neurodegenerative diseases (Velichkovska et al., 2018; Bertrand et al., 2021).

Cresyl fast violet (CFV) is useful in identifying neuronal morphology in a tissue section and also help in evaluating neuronal damage through loss of Nissl substance (Wulff et al., 2004). The result of the test groups in Fig. 2 demonstrated strongly intense Nissl substance staining and distorted neuronal morphology. Abundant Nissl substances plays a role in cellular metabolism, and which are present in the endoplasmic reticulum of neuronal cells which synthesize protein for repairs, maintenance and production of enzymes (Kumar et al., 2014). The administered HAART in the test groups have been reported to cause intense Nissl substance characterized by microcytic changes due to health diseases in the neuronal level (Adjene et al., 2010).

The GFAP is a member of filament structural protein found in astrocytes of the central nervous system (CNS). The GFAP is used as a potent bio-maker of neurotoxicity in the brain (O'Callagha and Sriram, 2005). Microglia and astrocyte activation are the main characteristic feature of lesions in the CNS, the activation of astrocyte in the test groups shown in Fig. 3 is evidenced by the presence of hypertrophied cell bodies and thickened dendritic processes. Astrogliosis characterized by hypertrophy, increased GFAP expression and increase proliferation, secreted molecule that up-regulate neuro-inflammation and memory impairment (Vandevord et al., 2008; Saija et al., 2016). Further studies with prolong oral therapeutic exposure is necessary to investigate the mobilization of microglia regulated by astrocyte during neuro-inflammation in HAARTs used for HIV/AIDS management.

Conclusion

From the result of this sub-chronic 30 days study, which investigated the comparative toxicological profile of two HAARTs; TLE and LNZ, on the brain oxidative stress concentrations and hippocampus histoarchitecture of adult male Wistar rats following administration of oral therapeutic doses, TLE regimen of HAARTs is more neurotoxic compared to LNZ.

Conflicts of Interest

Authors declare that there is no conflict of interest.

Ethical standards

The Department of Human Anatomy, University of Uyo, Nigeria animal experiment committee approved the protocol for all animal experiment carried out in this study. All the process carried out in this experiment follows the National Institute of Health Care Guide for the Care and Use of Laboratory Animals (NIH publication #85-23, revised in 1985).

Funding

This project was self-funded.

CRediT authorship contribution statement

Innocent A. Edagha: undertook the Conceptualization, Project administration/sourcing of funds/drugs and Supervision, participated in the Investigation, Methodology, Data curation, Software, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. **Akpan U. Ekanem:** undertook the Conceptualization, Project administration/sourcing of funds/drugs and Supervision. **Itoro. F. Usoh:** undertook the Conceptualization, Project administration/sourcing of funds/drugs and Supervision. **Victor A. Umoh:** Conceptualization, Project administration and Supervision. **Ataben M. Ataben:** participated in the Investigation, Methodology, Data curation, Software,

Visualization, Formal analysis, Writing – original draft, Writing – review & editing. **Anietie A. Akpan:** participated in the Investigation, Methodology, Data curation, Software, Visualization, Formal analysis, Writing – original draft, Writing – review & editing.

Author contributions

The authors contributed equally to this research.

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