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Full length Research Article

Hippocampus and Prefrontal Cortex Following the Use of Anti-Retroviral Therapy in Adult Wistar Rats: Therapeutic Role of Epigallocatechin Gallate

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Summary: The contribution of prefrontal-hippocampal interactions to brain function of people infected with HIV may be aggravated by toxicities due to long-term use of antiretroviral agents. This study was designed to investigate the curative potential of Epigallotatechin gallate (EGCG) in the treatment of neurodegenerative disorders as a possible consequence of antiretroviral toxicity. Twenty-four adult male Wistar rats, weighing 80~100g, were divided into four groups and treated as follows: control A (distilled water), B (HAART), C (EGCG 2.5mg/kg), D (EGCG 2.5mg/kg) + HAART) Brain histology, immunohistochemistry, and oxidative stress markers such as superoxide dismutase (SOD), glutathione (GSH),catalase (CAT) and malondialdehyde (MDA) were examined. The use of highly active antiretroviral therapy (HAART) showed extensive architectural deformation with pyknotic neuronal cells and obliterated neurons in the hippocampus and prefrontal cortex. Expression of inflammasome cells was also evident in this group. MDA levels increased significantly with a significant reduction in the levels of GSH, as well as antioxidant enzyme (SOD and CAT) activities compared to other treatment groups. Treatment with EGCG resulted in partial neuronal restoration of histopathological alterations, and modulation of NLRP3 inflammasome in the hippocampus and prefrontal cortex. EGCG also showed significant improvements in terms of increased antioxidant levels of SOD, GSH, CAT and a reduced MDA level and well-preserved brain architecture. Epigallocatechin gallate improves brain morphology and function with a reversal of HAART-induced alterations.

Keywords*: Antioxidants, Antiretroviral, Inflammasome, Epigallocatechin gallate*

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INTRODUCTION

The HIV/AIDS epidemic remains one of the most devastating health challenges that has had a significant impact on health outcomes and life expectancy in recent decades (Assefa and Gilks, 2020). There has been tremendous progress in the fight against this epidemic in recent years, with antiretrovirals serving as the standard treatment, thanks to the global commitment of the Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO) (Cobb *et al*., 2020, Zhang *et al*., 2020, Mai and Nguyen, 2022). In the global treatment of HIV/AIDS, highly active antiretroviral therapy (HAART) has been effective in revolutionizing the treatment of HIV infections and the quality of life of infected patients (Pongbulaan and Triyono, 2021). HAART, a tailor-made treatment regimen, consists of an alloy of various classes of antiretroviral drugs including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), fusion inhibitors (FIs) and chemokine receptor antagonists (CCR5 antagonists) (Ru and Tang, 2017). Current antiretroviral drugs are not curable and people with HIV will need to take them for the rest of their lives (Pasternak *et al*., 2021, Stern *et al*., 2021). However, the optimization of HAART, which requires long-term use, is not excluded from drug-related side effects (Matuszewska *et al*., 2021). HIV infection is associated with comorbidities that can be caused not only by HIV itself, but also by the

toxicity of long-term use of antiretroviral therapy (ART) (Ogedengbe *et al*., 2018b).

Previous studies have shown deleterious effects of HAART on normal brain function, resulting in subclinical brain lesions related to HIV (Makhathini *et al*., 2018, Sanford *et al*., 2018). HIV replication in the brain may be responsible for neurocognitive changes in infected individuals, and the toxicities of antiretroviral drugs is also expected to contribute to neurodegenerative disorders in HIV infected patients (Manda *et al*., 2011). Vital parts of the brain, such as the hippocampus and prefrontal cortex have long been recognized for playing a central role in various behavioral and cognitive functions (Sigurdsson and Duvarci, 2016). The contributions of pre-frontal hippocampal interactions to the brain function of people infected with HIV can be compounded by toxicities resulting from the long-term use of antiretroviral drugs (Akang *et al*., 2019, Prats *et al*., 2021). Antiretroviral drugs cross the blood brain and blood cerebrospinal fluid barriers to the central nervous system (CNS), causing significant adverse effects attributed to their presence in these tissues (Streck *et al*., 2008). However, this galvanizes in patients susceptibility to the development of mild to severe degrees of neuropathy, neuropsychiatric, cerebrovascular toxicity, neurodegenerative disorders, with negative effects on brain development and cognitive behavior after the long-term use of antiretroviral drugs in the hippocampus and prefrontal cortex (Garcia *et al*., 2020, Bertrand *et al*., 2021).

The use of natural antioxidants to treat human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) in developing countries remains a controversial issue due to their unknown safety profiles (Saeidnia and Abdollahi, 2014). One of the antioxidants that has a positive effect on the brain is Epigallocatechin gallate, which protects against cellular damage caused by free radicals (Perdices *et al*., 2020). Epigallocatechin gallate is found predominantly in green tea. It can also be seen in small quantities in other foods such as: white and black teas, strawberries and blackberries, and nuts (Singh *et al*., 2015). Early research indicates that Epigallocatechin gallate can play a role in improving the neurological function of cells and in preventing degenerative brain disease (Perdices *et al*., 2020). Studies have shown its ability to exert mitigation potentials against a range of disorders including HIV, neurodegeneration, muscular atrophy of the spine, cancer and cerebral vascular insult due to its high perceived therapeutic values (Priya *et al*., 2017, Nair *et al*., 2021).

Epigallocatechin gallate also has a strong antioxidant capacity and potential to reduce stress and inflammation (Shukla *et al*., 2019). Therefore, we seek to evaluate the curative potential of Epigallocatechin gallate in the treatment of neurodegenerative disorders as a possible consequence of antiretroviral toxicity.

MATERIALS AND METHODS

Animals and treatment: For this experiment, twenty-four (24) male Wistar rats weighing between 80 and 100 g were used. They were held in the animal house unit of Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. All animal procedures were conducted in accordance with the National Council for Medical Research principle on Laboratory Animal Care and the Guide for the care and use of laboratory animals (Council, 2010). The protocol of the study was approved by the Animal Ethics Committee with the protocol reference number: AB/EC/17/01/294. The rats were acclimated for 2 weeks prior to the start of the experiment. The animals had unrestricted access to rodent food and water and were exposed to a 12-hour cycle of darkness and light.

Acriptega containing Dolutegravir (DTG, 50mg), Lamivudine (3TC, 300mg) and Tenofovir (TDF, 300 mg) (Li *et al*., 2021) was obtained from the Pharmaceutical Department University of Lagos Teaching Hospital (LUTH), Lagos State, Nigeria. The administered dose of Acriptega was calculated for the animal on the basis of the therapeutic equivalent dose for the human dose of a rat model (Ogedengbe *et al*., 2018a). Epigallocatechin gallate used was purchased from Nature's Nutrition, Porter Rockwell, United States.

Experimental design: Animals were randomly selected and divided into four (n = 4) (A-D) groups with six rats in each group and treated as follows:

- Group A: distilled water received as a control
- Group B: received HAART cocktail-Acriptega using human therapeutic equivalent doses (50mg, 300mg, and 300 mg/day of dolutegravir, lamivudine and tenofovir respectively was dissolve in 100 ml of distilled water and reduce it to the equivalent animal dose of 0.12, 0.74 and 0.74 mg/kg body weight, respectively (Ogedengbe *et al*., 2016).
- Group C: received 2.5 mg/kg of Epigallocatechin gallate (EGCG)
- Group D: received 2.5 mg/kg EGCG + HAART

All treatments were administered orally daily and, after 6 weeks the animals were killed 24 hours after the last treatment by cervical displacement (Adana *et al*., 2018).

Body weight determination: The animals were weighed on the first day before treatment, then weekly, and then on the last day of the study. Body weights were recorded in the morning between 8:00 a.m. and 10:00 a.m. using an electric scale (HX-T electronic weighing balance HX-302 T, China).

Sample collection: Animals were killed son day 43 by cervical dislocation. Blood (approximately 3 mL each) was collected from the heart by means of a cardiac puncture in plain tubes and clotted at room temperature for 2 h. It was centrifuged for 15 minutes at 1000 g and the supernatant (Serum) was separated after centrifugation for biochemical analyses (Eze and Ekundina, 2018, Kehinde *et al*., 2021).
Brain samples were also collected from each animal and their weights were recorded using an electronic scale (HX-T electronic weighing balance HX-302 T, China). They were then perfused with10% neutral buffered formalin for histological and immunohistochemical analyses.

Histological studies: The tissue samples of the prefrontal cortex and the hippocampus were fixed in 10% of neutral buffered formalin and processed for histology. Fine sections were obtained at 5 µm using Rotary Microtome MicromGmbH, Serial No 42861, CAT. No 02100). The sections were stained using hematoxylin and eosin (H&E)

staining methods for general assessment of tissue structures. The interpretation of slides was performed by a histopathologist blinded to the study protocol.

Immunohistochemical analysis: Paraffin sections were deparaffinized and hydrated. Thermal repair or digestive treatment was performed on antigens in the tissue section. Incubation was performed with E-IR-R217C (3% H2O2) for 10 minutes to eliminate endogenous peroxidase activity. It was then washed with PBS for 2 min x 3 times. E-IR-R217A normal goat serum (ready for use) was added and incubated at 37^0C for 30 minutes. The excess fluid was removed. Primary antibody (NLRP3 inflammasome : 1:100-1:200) was added with the corresponding dilution ratio and then incubated at 20-37^0C at 4^0C overnight (then heated at 37^0C for 30 minutes)(Xue *et al*., 2019). It was then washed with PBS for 2 minutes x 3 times. The section is dried with absorbent paper. The 1E-IR-R217B (Rabbit anti-mouse polyperoxidase/IgG) was added and incubated at room temperature or 37^0C for 20 minutes. It was washed 2 minutes x 3 times with PBS. One drop (approximately 50μL) of E-IR-R217D (concentrated OBD) was added in every 1 ml of E-IR-R217E (DAB substrate), completely mixed and the mixed reagent is the DAB working solution. A fresh solution was prepared prior to use and the prepared solution was stored in the dark. The newly prepared DAB working solution is valid within 4 hours and the unused solution was abandoned. The DAB coloring time was kept under control, and a color of tan or brownish yellow was seen the positive signal. Excessive reaction was avoided. The section was washed with deionized water to stop the chromogenic reaction, the procedures of counter-staining, dehydration, transparency and sealing were applied. The sections were viewed and photographed with a Leica microscope (DM 750, Germany) and an ICC HD 50 camera. The images were captured at a magnification of x400.

Measurement of serum malondialdehyde concentration in **the brain:** The serum concentration of malondialdehyde (MDA) was analyzed by the method of Albro *et al*. (1986) and Das *et al*. (1990). 0.1 ml of serum with 2 ml of (1:1:1 ratio) tert butyl alcohol (TBA)– trichloroacetic acid (TCA)–hydrochloric acid (HCl) reagent (TBA 0.37%, 0.25N HCl and 15% TCA) and 15 min in water bath, cooled and centrifuged and then clear supernatant was measured at 535nm against reference blank.

Measurement of serum reduced glutathione (GSH) concentration: The measurement was carried out according to the method described by Sedlak and Lindsay (1968) with slight modifications, using the serum samples. The principle was dependent on protein precipitation using tungstate/sulfuric acid solution and yellow coloration formation after reaction with 5, 5′dith-iobis-2-nitrobenzoic acid (DTNB), and the absorbance was read within 30–60s at 412 nm against the blank. Glutathione (GSH) concentrations were extrapolated from a standard GSH curve.

Measurement of serum superoxide dismutase activity: The superoxide dismutase (SOD)was determined by the method described by Fridovich (1975). Serum sample of 0.1 ml was diluted in 0.9ml of distilled water to make 1:10 dilution of microsome. An aliquot mixture of 0.2 ml of diluted microsome was added to 2.5 ml of 0.05M carbonate buffer. The reaction began with the addition of 0.3 ml of 0.3 mM adrenaline. The reference mixture contained 2.5 ml 0.05 M carbonate buffer, 0.3 ml 0.3 mM adrenaline and 0.2 ml distilled water. Absorbance was measured over 30 seconds to 150 seconds at 480nm.

Measurement of serum catalase activity: The serum catalase (CAT) activity as carried out according to the method described by Aebi (1974).10μl of serum was added to a test tube with 2.80ml 50mM potassium phosphate buffer (pH 7.0). The reaction was started by adding 0.1 ml of 30mM H2O2 freshly prepared and the decomposition rate of H2O2 was measured at 240nm for 5 min on a spectrophotometer.

Statistical analysis: Morphometric data were analyzed using standard parametric tests. The results were expressed as mean ± standard error of the mean, then subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test with Graphpad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Organ-body weight changes: An insignificant overall increase in final body weight was observed in groups A (Control), B (HAART) and C (EGCG) when compared to their corresponding initial body weights. This increase was least recorded in control group A, with group C having the highest value.

Table 1:
Body weight (BW), Brain weight (BrW), BrW/BW ratio in groups

Groups	Initial BW (g)	Final BW (g)	Weight diff (g)	Difference (%)	BrW (g)	(BrW/BW) x 100
A	216±3.70	310±1.79	94	43.52	1.65±0.07	0.53
B	221±1.20	324±1.79	103	46.61	1.61±0.03	0.49
C	223±1.30	317±7.02	91	40.81	1.67±0.04	0.53
D	217±1.60	335±3.64[a,b]	118	54.37	1.58±0.04	0.47

Values were expressed as mean ± SEM (all values compared were p<0.05); A; (control); B; (HAART), C; (EGCG), D; (EGCG+HAART). Groups compared are as follows B, C, D vs A; D vs B and D vs C. For Initial weight, D vs A[a](P<0.01), B vs A[b](P<0.05), D vs C [c](P<0.01) & D vs B [d](P<0.05). For final weight, D vs A[a](P<0.01), D vs C [b](P<0.05).

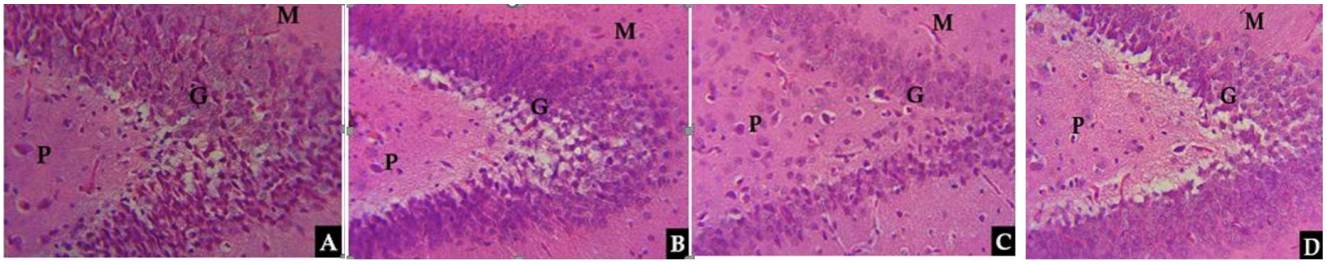

Plate 1:
Photomicrograph of a section of the hippocampus. A (control) and C (ECGC) showing dentate gyrus with Molecular cell layer, polymorphic cell layer, normal and uniform distribution of granular cell layer. B (HAART) revealed pyknotic neuronal cells. Group D (HAART+ECGC) shows partial restoration of the granular cell layer. Stained with H&E, Mag x800

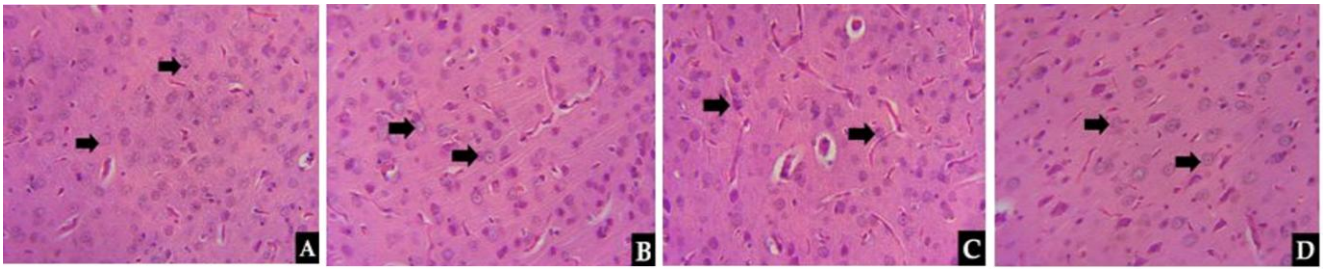

Plate 2:
Photomicrograph of a section of the prefrontal cortex showing the morphology and distribution of neuronal cells. A (control) and C (ECGC) revealed normal distribution of neuronal cells, while B (HAART) showed pyknotic neuronal cells with obliterated neurons when compared to A. Group D (HAART+ECGC) shows only a small and sparse distribution of the neuronal cells. Stained with H&E, Mag x800.

Histopathological examination of the hippocampus and prefrontal cortex: The cross section of the hippocampus of group A (Control) and group C (EGCG) revealed a well-preserved cyto-architecture of the dentate gyrus showing the molecular cell layer, the polymorphic cell layer with a normal and uniform distribution of the granular cell layer (Plate 1). A significant increase was observed in final body weight of group D (HAART+EGCG) when compared with the Control group A [a](P<0.01) and C [b](P<0.05) respectively. The brain weight (BrW) and relative organ weight (BrW/BW X 100) of all groups were similar, without reaching statistical significance between the group differences (p< 0.05) (Table 1). Sections of the prefrontal cortex of groups A (control) and C (ECGC) also showed normal morphology and uniform distribution of neuronal cells (Plate 2). Group B (HAART) histological examinations revealed the presence of pyknotic neuronal cells with obliterated neurons in the hippocampus and prefrontal cortex. The group D (HAART+ECGC) showed less architectural deformation with partial neuronal restoration, which became evident in the prefrontal cortex and granular cell layer of the hippocampus.

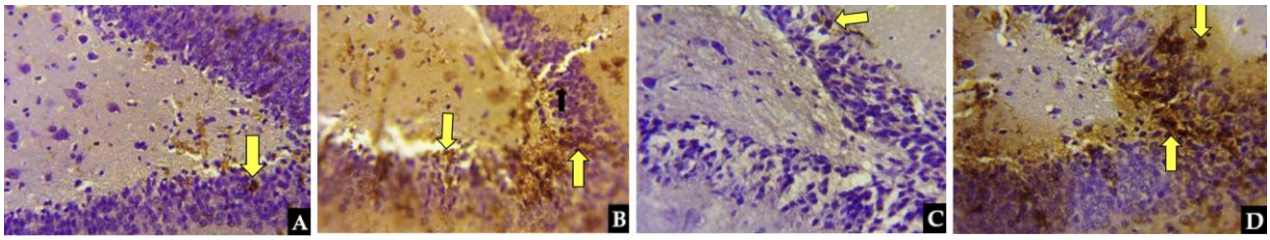

Plate 3:
Inflammasome immunopositive cells in the dentate gyrus of the hippocampus. A (Control) and C (ECGC) revealed less or no expression of inflammasome. Expression of inflammasome cells was seen in the B (HAART) group. Group D (HAART+ECGC) cells showed less expression of inflammasome as compared to the group B (Dark brownish round cells indicate the immunopositive cells), Mag x800.

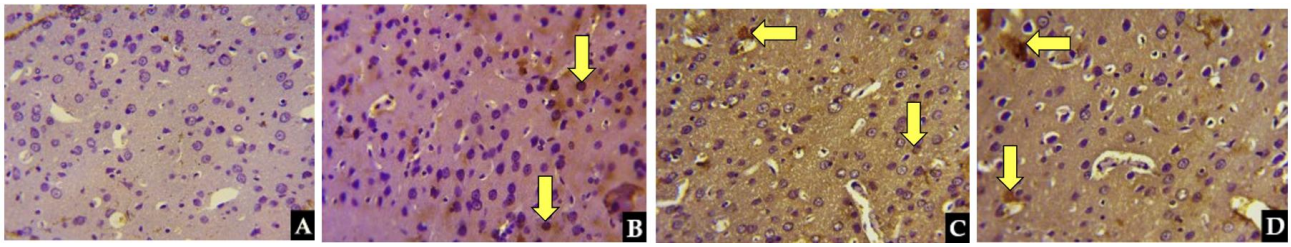

Plate 4:
Inflammasome immunopositive cells in the prefrontal cortex. A (Control) and C (ECGC) revealed less or no expression of inflammasome. Expression of inflammasome cells was seen in the B (HAART) group. Group D (HAART+ECGC) cells showed less expression of inflammasome as compared to the group B (Dark brownish round cells indicate the immunopositive cells), Mag x800.

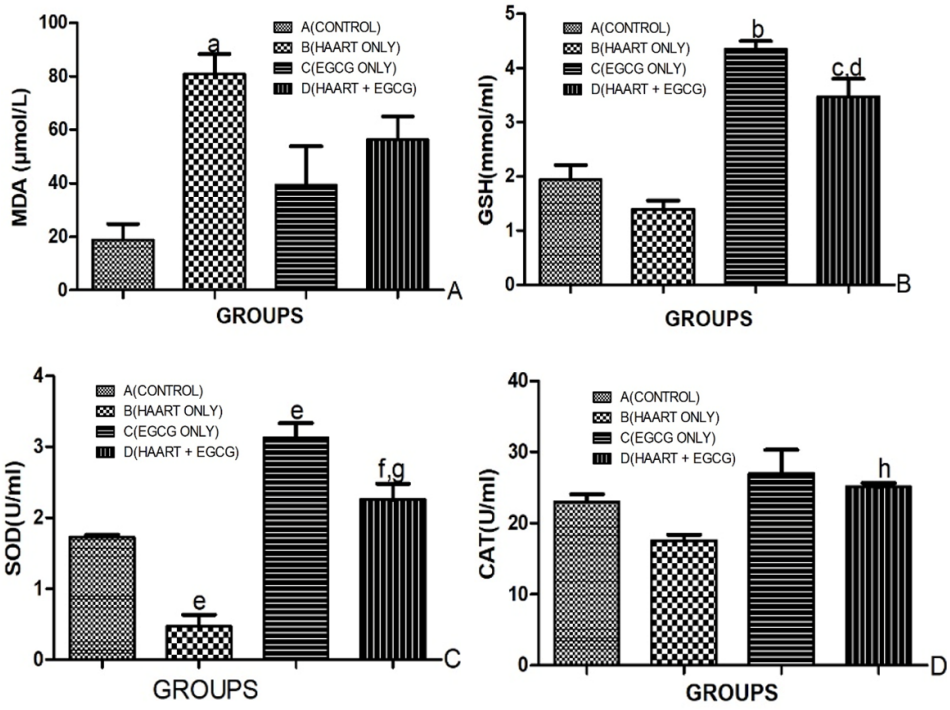

Figure 5:
Effects of EGCG and HAART treatment on the Serum (A) malondialdehyde (MDA) (B) reduced glutathione (GSH), (C) superoxide dismutase (SOD) (D) Catalase (CAT) levels in Wistar rats after 6-week treatment period. Bars indicate the mean ± SEM.
A(Control); B(HAART), C (EGCG), D (EGCG+HAART). Groups compared are as follows B, C, D vs A; D vs B and D vs C.
For MDA: [a](p<0.01) B vs A; GSH: [b](p<0.001) C vs A, [c](p<0.05) D vs A; [d](p<0.001) D vs B;
SOD: [e](p<0.001) B & C vs A; [f](p<0.001) D vs B, [g](p<0.05) D vs C;
CAT: [h](p<0.05) D vs B

Immunohistochemical examination of the hippocampus and prefrontal cortex: A (Control) and C (ECGC) showed a less or no expression of inflammasome in the prefrontal cortex and dentate gyrus of the hippocampus. However, the expression of inflammasome cells was seen in the B (HAART) group, while cells of group D (HAART+ECGC) showed less expression of inflammasome compared to the group B in prefrontal cortex and hippocampus**.**

Changes in serum malondialdehyde, reduced glutathione, superoxide dismutase and catalase concentrations.

Malondialdehyde: As shown in Figure 5A, a significant increase ($p<0.01$) was observed in mean serum MDA level of HAART-group B (80.80 ± 7.54 $x10^2$µmol/L) when compared to Control-group A (18.80± 5.92 $x10^2$µmol/L). The mean serum MDA levels ECGC-group C and D (EGCG + HAART) are (39.39 ± 14.45$x10^2$µmol/L) and (56.41 ± 8.58$x10^2$µmol/L) respectively.

Reduced glutathione: Figure 5B shows mean serum reduced glutathione concentrations. Significant increase ($p<0.001$, $p<0.05$ respectively) were observed in group C (4.385 ± 0.15mmol/mlµ) and D (3.47 ± 0.34mmol/ml) when compared to control group A (1.94 ± 0.27mmol/ml). Treatment with EGCG also resulted in a significant increase ($p<0.01$) in GSH of group D when compared with the HAART group B (1.40 ± 0.15mmol/ml).

Superoxide dismutase: Figure 5C shows the mean superoxide dismutase enzyme activities. Treatment with HAART in group B (0.47 ± 0.17U/ml) showed a significant decrease ($p<0.001$) in SOD concentration when compared with the control group-A (1.70 ± 0.03U/ml). Consequently, a significant increase ($p<0.001$) was observed in the EGCG treated group C (3.10 ± 0.21U/ml) when compared with the control group A. Significant increase ($p<0.001$) was also observed in the EGCG + HAART co-treatment D (2.30 ± 0.21U/ml) when compared with HAART treated group B, while there was significant decrease ($p<0.05$) in group D compared to C.

Catalase: Figure 5D shows the mean concentration of serum catalase activity. Adjuvant treatment of EGCG + HAART in group D (25.1 ± 0.58 U/ml) showed a significant increase ($p<0.05$) in CAT levels when compared with HAART treated group B (17.6 ± 0.83U/ml). The mean CAT concentrations for groups A and C are (23.00 ± 1.08U/ml) and (27.0 ± 3.31 U/ml) respectively.

DISCUSSION

The present study shows the alterations in the cytoarchitectural patterns of the hippocampus and prefrontal cortex caused by HAART treatment. Examination by qualitative light microscopy (H&E stain) indicated disordered and pyknotic neuronal cells with obliterated neurons in the hippocampal and prefrontal cortex sections of the HAART-treated group. These results support previous findings that confirm that HAART causes degenerative changes in the hippocampus, some of which include cytoplasm removal, very dark pyknotic nuclei, and increased neuronal ferrugination in the animal's internal granule cells (Akang *et al*., 2019, Kim *et al*., 2019). This indicates that there has been chromatin condensation, which will eventually lead to cell death due to the toxicity of HAART in cells. Consequently, the long-term effect of

HAART on the prefrontal cortex causes progressive neurological lesions and cognitive impairment in HIV-infected individuals, with significant structural changes in cortical pyramidal cells, pronounced astrogliosis with elevated glial fibrillary acidic protein (Dosumu *et al*., 2021). Obliteration and pyknosis of neurons can also lead to reduced myelin levels in the frontal cerebral cortex, which can contribute to neurocognitive dysfunction, as the myelin membrane produced by oligodendrocytes is essential for rapid signal transduction and axonal retention (Jensen *et al*., 2015).

We report that treatment with EGCG reduces severe histopathological changes and significant neuronal cell loss by HAART. , EGCG had a protective effect on the prefrontal cortex and hippocampus with indication of normal distribution of neuronal cells in EGCG treated only group and gradual restoration of neuronal cells in the EGCG + HAART group. We suggest that EGCG retain its role as a potent antioxidant compound to mitigate the devastation of HAART and its interactions. Together with other studies, this demonstrates the significance of EGCG in bringing high ROS levels within physiologic bounds (Tanaka *et al*., 2022, Tian *et al*., 2022). Although no mitochondrial DNA denaturation test was performed to indicate specific deprivation in energy production pathways and structural changes, it is likely that EGCG may have a positive effect on the prevention of antioxidant enzyme inactivation by pro-oxidants, thereby improving observed structural changes. This is consistent with studies on the pro-oxidative effects of Epigallotatechin gallate (He *et al*., 2018, Xu *et al*., 2021).

Inflammasomes are intracellular multiprotein complexes that act as sensors of DAMPs or PAMPs, leading to the activation of pro-inflammatory caspases, cleavage and release of pro-inflammatory cytokines (Inoue *et al*., 2014). While Inflammasome immunopositive cells in the prefrontal cortex and hippocampus showed little or no inflammatory expression in the control and EGCG treated group C; increased expression of inflamasome cells was observed in HAART treated group B. Repeated or severe stress by stressors such as HAART, in the absence of pathogenic diseases, increases emotional dysregulation and inflammatory proteins in tissues and blood in humans and animals (Fleshner *et al*., 2017). This explains the crucial role played by HAART in the induction of neuroinflammation by excessive exposure to stress, resulting to morphological changes in the glial population, increased circulating pro-inflammatory cytokines and increased production of these mediators in the brain (Yang *et al*., 2018). With adjuvant EGCG + HAART, these negative effects were mitigated in this study, indicating the rich antioxidant, anti-inflammatory and free radical scavenging properties of EGCG (He *et al*., 2018).

Oxidative stress is known to have negative effects on the brain and causes neurodegenerative disorders. Neuronal damage may reflect both an increase in oxidative processes and decreased antioxidant defenses (Edagha *et al*., 2022). Lipid peroxidation, a negative consequence of oxidative damage, free radical attacks, and excessive ROS formation, is considered to play an important role in the pathophysiology of neurodegeneration (Angelova *et al*., 2021).

Malondialdehyde (MDA) remains an appropriate indicator for determining the degree of lipid peroxidation in oxidative and redox stress signaling (Morales and Munné-Bosch, 2019). Our results indicated an elevated MDA levels in group B treated with HAART when compared with the control group. This is consistent with previous studies indicating increased levels of neuronal MDA following antiretroviral drugs administration (Edagha *et al*., 2022, Lawal *et al*., 2022). Prolonged use of antiretroviral agents is an important factor in the enhanced generation of reactive oxygen species (ROS), which leads to mitochondrial dysfunction and thus promotes cell aging and declination (Velichkovska *et al*., 2018). HAART interference with mitochondrial electron transport chain can adversely impact ATP energy production and cell respiration by complex inhibition I attributed with the pathogenesis of neurodegenerative diseases (Bertrand *et al*., 2021). Additionally, our findings support earlier research showing that HAART decreased cell proliferation by prolonging the cell cycle, increased apoptosis, and induced neuronal loss in neurons (Chang *et al*., 2012)

SOD, CAT and GSH are essential measures that reflect the potential antioxidant ability of the body (Javed *et al*., 2016). They can represent the lipid peroxidation rate and body intensity, as well as the degree of tissue oxidation (Jafari *et al*., 2012). In this study, SOD, GSH and CAT are used to measure the response to oxidative stress in the prefrontal cortex and hippocampus after HAART and EGCG co-treatments. Concentrations of SOD, GSH, and CAT were significantly reduced in the HAART group B and significantly elevated in groups co-treated with EGCG therapy. In addition, EGCG exerts its antioxidant activity on HAART-induced ravages by scavenging free radicals and preventing damage from oxidative DNA mediated by lipid peroxidation in all the treated groups. We suggest that the amplitude of EGCG phenolics favour its role as a potent antioxidant compound and can function within a favorable pharmacokinetic in this protocol to mitigate the depredation of HAART, and plausible its interactions with it. Oxidative stress, inflammation, and mitochondrial dysfunction by HAART therapy play a decisive role in the progression of cognitive impairment. EGCG on the other hand, has shown to interact with a variety of signaling molecules, resulting in a wide range of pharmacological effects, including antioxidants, anti-inflammatory, which in turn can reduce neurodegeneration and also restore neuronal development (Sebastiani *et al*., 2021). EGCG can also act as an anti-apoptotic drug in neurodegeneration restoring neuronal loss and damaged molecular targets (Reznichenko *et al*., 2010). Reports on the clinical use of EGCG have recently been published. The use of EGCG as a dietary supplement, complemented by multimodal interventions in life habits, has shown some positive planes in reducing cognitive impairment and improvement of brain connectivity in subjects with SCD and APOE ε4 carriers (Forcano *et al*., 2021). EGCG has also been potentially useful as therapy for the elimination of Alzheimer's-related complications by disaggregating tau fibrils (El Khoury *et al*., 2021). However, our study has demonstrated the neuroprotective potential of EGCG in the attenuation of HAART-induced inflammatory responses and augmentation of brain functions.

In conclusion, the use of Epigallocatechin gallate has been explored to mitigate the neurodegenerative effects of

HAART and restore/enhance memory. The results of this research also clearly show an endogenous antioxidant enzyme enhancement in the hippocampus and prefrontal cortex by Epigallocatechin gallate mitigating lipid peroxidation by neutralizing HAART-induced free radicals**.** Additional studies are needed to fully quantify these effects for clinical progress.

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