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Effect of HAART Cocktail and Neurovite Co-administration on the Cytoarchitecture of the Cerebellum and Neurobehaviour of Adult Male Wistar Rats

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Abstract: Highly active antiretroviral therapyi (HAART), a combination of drugs (lamivudine, zidovudine and nevirapine) used for pre-exposure prophylaxis and management of Human Immunodeficiency Virus infection in sub-Saharan Africa. The objective of this research work was to investigate the potential Ameliorative effect of neuroviteon on HAART induced toxicity on the cerebellum. Thirty two Wistar rats were divided into 4 groups of 8 rats each. Group A served as the control, while group B were administered with 9.28 mg/kg of HAART, group C received 9.28 mg/kg of HAART and 0.07mg/kg of folic acid and group D received 0.07mg/kg folic acid. Drugs were administered twice daily for 30 days after which neurobehavioural test of open field maze was perform. The rats were then sacrificed and their cerebellum harvested, processed and stained using haematoxylin and eosin method and nuro-fillament (NF) immunochemistry method. The slides were viewed under light microscope. Results showed a significant reduction in the brain to body weight index between the HAART group and the control and folic acid group. There was significant reduction in locomotor activity following administration of HAART to the animals compared with control, there were also significant reduction in rearing frequency , walling frequency and freezing duration, with a significant increased in freezing duration in the HAART treatment group. The freezing frequency, central line crossing and grooming frequency were not significantly different. The cerebella were affected with mild to moderate shrinkage of pyramidal cells and distortion of the granular cells. There was increased expression of NF in the HAART group compared to controls. HAART affects the weight, histology of the cerebellum and neurobehaviour. Neurovite has the potential of ameliorating the histological distortion and may be beneficial to people taking HAART.

Keywords: HAART; Cerebellum; Neurovite; Human immunodeficiency virus; Neurobehaviour.

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system is associated with characteristic virological, clinical, and neuropathological findings in adults and children. Productive infection in the brain and spinal cord occurs in blood-derived macrophages, resident microglia, and multinucleated giant cells [1]. it does cause prominent neurological symptoms including HIV associated neurocognitive disorder (HAND) [2, 3]. Highly active antiretroviral therap (HAART) has greatly extended the lives of HIV infected (HIV+) individuals and diminished the number of opportunistic infections. However, the prevalence of HAND has remained relatively unchanged due to HAART toxicity, [4] with milder forms now predominating. Neuropsychological performance (NP) testing remains crucial for detecting neural dysfunction due to HIV. However, cognitive normality does not imply the absence of neural dysfunction. Indeed, pathophysiologic changes occur prior to changes in NP [5, 6]. The Department of Health and Human Services now recommends immediate initiation of cART with HIV positive diagnosis [7]. With these guidelines in place, it is important to determine the effects of HAART on brain functional connectivity. In general, most research groups have found HAART to have beneficial effects on cognitive status and neurobiology [8]. While HAART may have detrimental effects, these do not outweigh the tremendous benefit in restoring overall health and lifestyle [8]. The risk of specific side effects varies from drug to drug, from drug class to drug class and from patient to patient.

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Cognitive impairment occurs in a substantial (15–50%) proportion of HIV-infected patient on highly active antiretroviral therapy [9, 10]. Other side effects reported are neurologic complications such as myelopathy, neuropathy, neuropathic pain, and cognitive decline [11]. HAART toxicity is likely going to be a public health issue in Africa very soon due to the increasing number of people exposed to HIV drug as a result of social conflicts and crime leading to rape and subsequent post exposure prophylaxis (PEP). PEP is also practiced by health workers when they have occupational exposure to HIV; they are expected to commence treatment within 72 hrs after exposure and to continue with the medication for as long as 30 days. HIV positive pregnant women are also given this medication to prevent mother to child transmission of HIV thereby exposing the mother and the unborn child to this medication.

The fact that NRTIs (especially didanosine, stavudine, zalcitabine, and to a lesser extent zidovudine, abacavir and lamivudine) may be responsible for CNS toxicity is based on their documented toxicity on peripheral tissues (e.g.,liver, heart, muscles). This toxicity has been shown to involve dysfunction of mitochondria resembling those in genetic mitochondrial diseases [12]. The NRTIs' pharmacologic effectiveness depends on their relative selective interference with viral DNA as opposed to host DNA, especially with long-term treatment [12]. Clinically, NRTIs can cause hematologic toxicity, myopathy,

cardiomyopathy, lactic acidosis, exocrine pancreas failure, liver failure, bone marrow failure and peripheral neuropathy. Toxicity sometimes reverses when the treatment is stopped but not always. In addition, HIV itself has been shown to cause mitochondrial toxicity [13]. While some NRTIs have poor CNS penetration, potentially leading to less mitochondrial toxicity in brain tissues, it has been shown that with advanced HIV infection, the BBB is often impaired [14], therefore increasing the likelihood of NRTIs toxicity in the brain.

Indeed, following treatment initiation some HIV-positive persons can develop a paradoxical neurological deterioration, despite improvements in HIV viral load and CD4 Tcell counts [13]. This immune reconstitution inflammatory syndrome (IRIS) has been reported in several case studies which observed the occurrence of a dementing illness [15].

The objective of this research therefore was to investigate the potential effects of cocktail of Lamivudine, zidovudine and nevirapine (Douvir-N) and the possible ameliorative effect of neurovite a free radical scavenger with reported antioxidant activity on the histology of cerebellum and locomotor and anxiety related behavior using open field maze in Wistar rats.

2. Materials and Methods

2.1. Animals

Thirty two male Wistar rats weighting 173- 240 g at the time of acquisition and acclimatization were used in this study. They were kept at the animal house of the University of Uyo. They had free access to food and water, and kept at temperature (22 ± 2°C) under a 12/12 h light-dark cycle. The guidelines of the Institutional Animal Care and Use Committee (IACUC) were strictly followed throughout in the handling the animals.

2.2. Experimental Design

The animals were divided into 4 groups of 8 rats each. Group A served as the control and received 1 ml of distilled water, while group B were administered with 9.28 mg/kg of Duovir-N, group C were administered with 9.28 mg/kg of Duovir-N and 7.05 mg/kg of Neurovite and group D received 7.05 mg/kg Neurovite . Drugs were administered twice daily for 30 days after which neurobehavioural test of open field maze was perform.

2.2.1. Open Field Test (OFT) and Animal Sacrifice

Animals were individually placed in an open field apparatus made up of Perspex plastic with dimensions (40×60×50 cm) and the floor was divided into 25 equal squares by lines. The numbers of squares crossed with all paws (frequent line crossing) were counted in a 5 min session and the following were recorded: (1) Frequency of line crossing, (2) freezing and freezing period, and (3) rearing frquency (vertical postures of the rat with its hind paws on the floor and forepaws on the wall) (4) central crossing and central crossing duration (5) grooming (6) defecation and (7) urination [16].

After the open field maze the animals were put on overnight fast before they were sacrificed under chloroform anaesthesia and their cerebellum harvested, processed and stained using haematoxylin and eosin method. The slides were viewed under light microscope.

2.2.2. Statistical Analysis

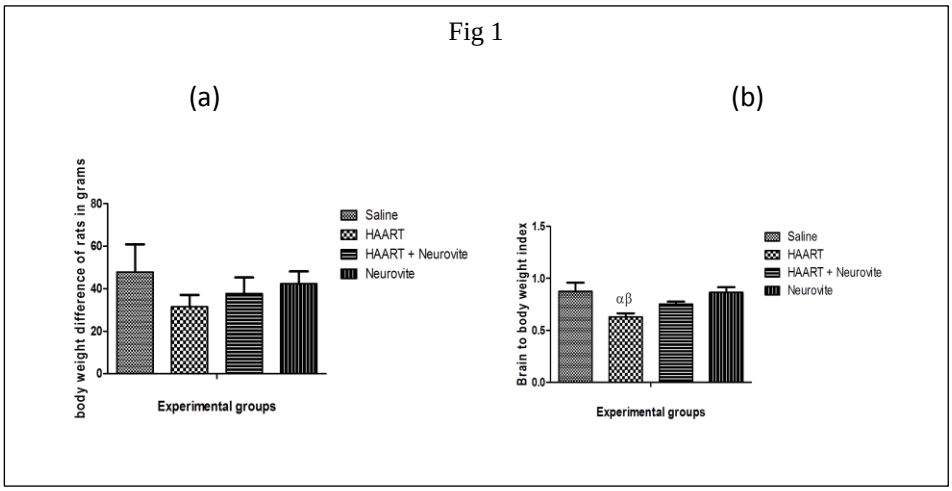
In this investigation the software package Graph Pad Prism version 6 was used for analysis and graphical representation of data. All data are presented as mean ± SEM. The data were analyzed by two-way analyses of variance (ANOVA). $P<0.05$ was considered statistically significant.

3. Result

A Effect of HAART and Neurovite group on body weight difference: Body weight of animals exposed to HAART were not significantly different when with compared with Saline control and the Neurovite as shown in fig 1a.

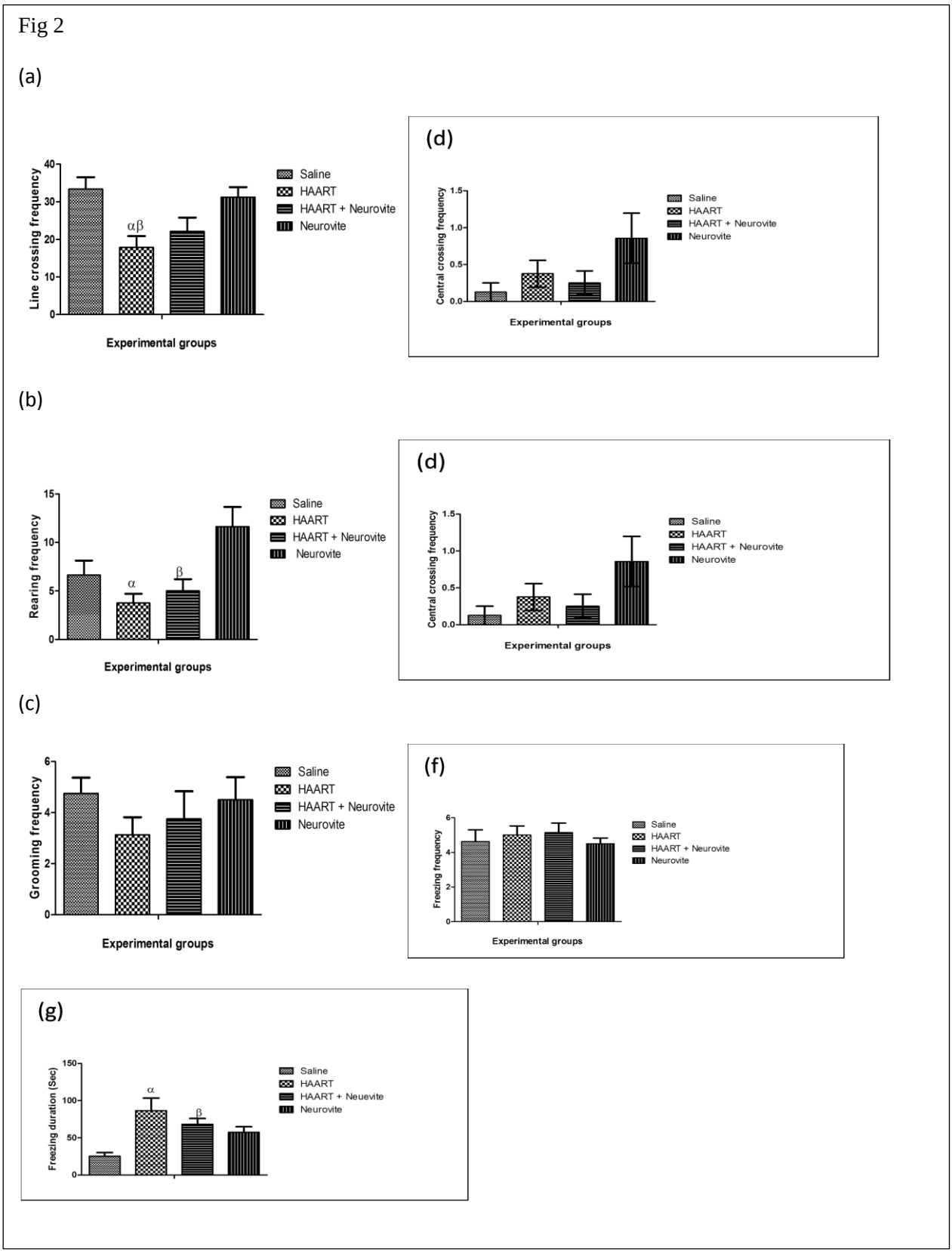
B Effect of HAART and Neurovite on brain to body weight ratio: Following exposure to HAART there was significant reduction in brain to body weight ratio compared to saline control and the Neurovite only group as shown in fig 1b

Fig 1



Note : Final weight minus initial weight equals to Weight difference

Fig-2. Effect of HAART and Neurovite group on locomotor activity in the open field



Effect of HAART and Neurovite on brain to body weight ratio in the open field box. Each bar represents Mean ± S.E.M, p≤0.05 compared to the control.

3.1. Effect of HAART and Neurovite on Horizontal Locomotion

Following exposure to the open field, there was significant reduction in locomotor activity following administration in the HAART compared to control, there were also significant reduction in rearing frequency , walling frequency and freezing duration , with a significant increased in freezing duration in the HAART treatment group. As shown in fig 2. The freezing frequency, central line crossing and grooming frequency were not significantly different

4. Histology

Photomicrograph of the histology of the cerebellum of group A animals treated with 1 ml of normal saline shows the three cerebellar cortical areas: molecular (M) layer, granular (G) layer, and Purkinje (P) cells. The granular cells aggregate, and the Purkinje cells are single-lined arrow. H & E, ×400.

Photomicrograph of the histology of the cerebellum of group B treated animals treated with 9.28 mg/kg of Duovir-N shows the three cerebellar cortical areas: molecular (ML) layer, granular (GL) layer, and disrupted and shrunken Purkinje (Pk) cells. The granular cells appear swollen and cells appear scanty. H & E, ×400.

Photomicrograph of the histology of the cerebellum of groups C animals treated with 9.28 mg/kg of Duovir-N and 7.05 mg/kg of neurovite shows the three cerebellar cortical areas: molecular (ML) layer, granular (GL) layer cells appear healthier than only Dourvir-N treated group H & E ×400.

Photomicrograph of the histology of the cerebellum of group D animals treated with 7.05 mg/kg of neurovite shows the three cerebellar cortical areas: molecular (M) layer, granular (G) layer, and Purkinje (P) cells the cells appear normal H & E X 400.

Photomicrograph of the histology of the cerebellum of groups A,B,C,D treated animals treated with Distil water , 9.28 mg/kg of Duovir-N, 9.28 mg/kg of Duovir-N and 7.05 mg/kg , 7.05 mg/kg of neurovite respectively shows the three cerebellar cortical areas: molecular (ML) layer, granular (GL) layer There is increased expression of NF in group B taking Dourvir –N only compared to controls NF ,× 100

Fig-3. Photomicrograph of the histology of the cerebellum of groups A,B,C,D treated animals treated with Distil water , 9.28 mg/kg of Duovir-N, 9.28 mg/kg of Duovir-N and 7.05 mg/kg , 7.05 mg/kg of neurovite respectively shows the three cerebellar cortical areas: molecular (ML) layer, granular (GL) layer H & E X 400

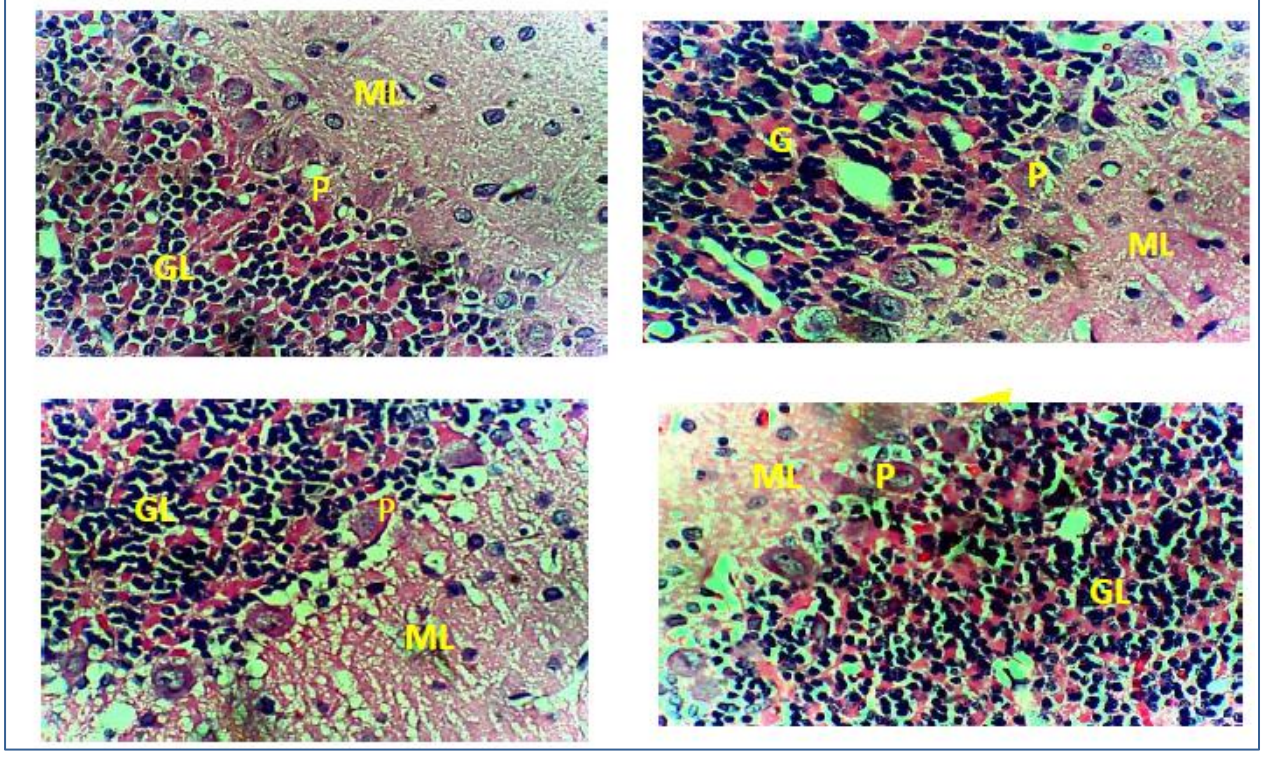
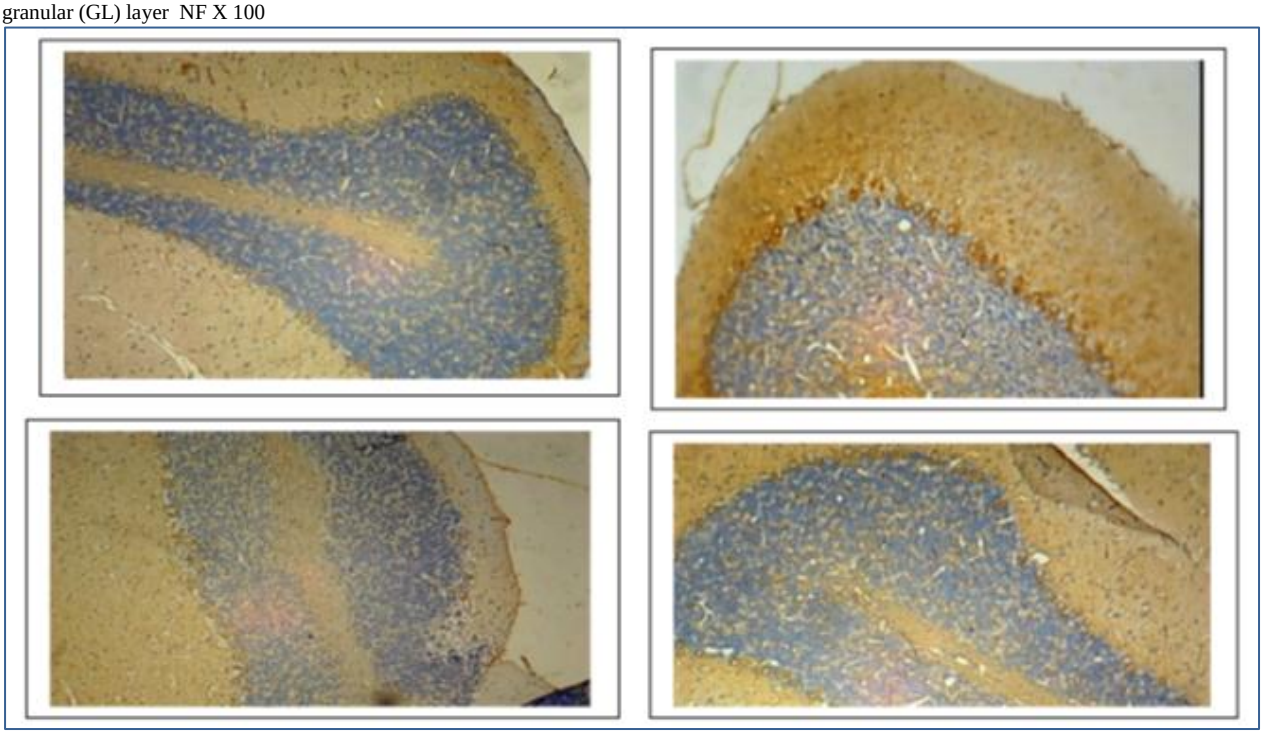


Fig-4. Photomicrograph of the histology of the cerebellum of groups A,B,C,D treated animals treated with Distil water , 9.28 mg/kg of Duovir-N, 9.28 mg/kg of Duovir-N and 7.05 mg/kg , 7.05 mg/kg of neurovite respectively shows the three cerebellar cortical areas: molecular (ML) layer, granular (GL) layer NF X 100



5. Discussion

Though the use of HAART in HIV management has significantly, reduced the morbidity, mortality and life expectancy of people living with HIV/AIDS, there is increasing concerns about the quality of life of these population of people due to adverse effects arising from chronic administration of these drugs.

The extensive use of highly active antiretroviral therapy (HAART) over the decade has led to a significant improvement in the survival rate and quality of life of HIV positive patients in Africa. The increased in life expectancy of people living with HIV has also raised concerns about the effect about the effects of HIV drug related toxicity on the body organs, which can directly affect the quality of life of these people.

Our study was designed to investigate the effect of combind administration of Dourvir N and neurovite on the cerebellum and neurobehavior using animal model. Morphometric findings revealed that there was no significant weight difference between the experimental groups and the control. This might suggest that the drug did not affect the feeding habit of the rats. Serotonin is involved in satiety and satiation through serotonin receptors [17]. The drug did not affect these receptors. Evidence suggests that a progressive decrease in lean body mass in the HAART era may be related to catabolic cytokines. [18, 19]. It is possible that our study did not last long enough to affect the weight.

Despite not having effect on the weight difference the result of the brain to body index ratio was significantly lower in the HAART group, implying that there was shrinkage of the brain size in this group. Organ-body weight ratio is an important indicator of organ toxicity. A researcher had earlier reported a reduction in liver size in groups of animals treated with HAART [20]. This finding is particularly important in pediatrics HIV treatment as the brain of the new born is still developing and as such more prone to toxic chemical injuries (humanillnesses.com). Studies have shown that the brain is more susceptible to drug toxicity.

The histology showed distortions in the cerebellum of the experimental groups administered with HAART only. This included shrunken Purkinje cells, granular cell swelling and loss of granular cells. This may be due to over excitation of the neurons, and gliosis, as well as depletion of myelin. These conditions may lead to cytotoxic degeneration of the neuronal cells [21]. These changes were ameliorated in the neurovite and HAART combined group which had healthy looking purkinje and granular cells when compared with the control. This could have been as a result of the antioxidant properties of neurovite. The antioxidant effect of neurovite had earlier been reported to ameliorate the toxic effects of lamivudine [22]. The toxic effect of HAART can lead to motor dysfunction as the cerebellum is the center for motor control, motor coordination, memory, cognitive processing and emotional control [23-25], This was in agreement with the immuno-staining which showed increased expression of NF in the treatment groups that was mitigated by Neurovite.

Open field maze is used to assess locomotor and exploratory behavior in a novel environment [26]. Line crossing, walling and rearing are usually used as measures of locomotor activity as well as exploration and anxiety. A higher frequency of these behavior indicates increased locomotion and low anxiety. In this study, the HAART groups had significantly lower frequencies in these parameters. These corroborated with the findings of histology

using confirming the occurrence of cerebellar toxicity. This distortions were only partially mitigated by the administration of neurovite. Central crossing and central crossing duration are also measures of exploratory behavior and anxiety with higher frequency indicating higher exploratory behavior and low anxiety, in our study there was no significant change in the frequency. Lastly the freezing frequency and grooming which are also indices of exploratory activities were not affected by HAART administration. This did not agree with the findings of Romao, *et al.* [27] that observed that Nevirapine which is a component of our HAART coctail did not affect locomotor activity in open-field test in mice.

6. Conclusion

Highly active antiretroviral therapyi (HAART), a combination of drugs (lamivudine, zidovudine and nevirapine) used for pre-exposure prophylaxis and management of Human Immunodeficiency Virus infection in Nigeria. From our findings HAART affects the weight, histology of the cerebellum and neurobehaviour, neurovite has the potential of ameliorating the histological distortion in experimental rat model.

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