



## Histomorphological Effects of Co-Administration of Efavirenz and Vitamin E on the Hippocampus of Wistar Rats

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### Keywords

Histomorphology  
; Efavirenz;  
Hippocampus;  
Vitamin E.

**ABSTRACT:** Efavirenz is an oral non-nucleotide reverse transcriptase inhibitor (NNRTI) used as a part of highly active antiretroviral therapy (HAART) for the treatment of type 1 human immunodeficiency virus (HIV-1). Thirty Wistar Rats of weight 140 g–192 g were used for the study. The rats were divided into six treatment groups. Control (group) A were administered with 1 ml of distil water; group B were administered with 8.57mg/kg body weight of Efavirenz ; group C were administered with Double dose of 17.14 mg/kg body weight of Efavirenz; group D were administered with Triple dose of 25.71 mg/kg body weight of Efavirenz; group E were administered with 8.57mg/kg body weight of Efavirenz and 14.82 mg/kg body weight of Vitamin E; and group F were administered with 14.82 mg/kg body weight of Vitamin E. drug administration lasted for 32 days. The animals were handled according to the guidelines for the treatment of laboratory animals. On the 33rd day, the rats were sacrificed using chloroform inhalation method. The hippocampi were excised, routinely processed, stained using haematoxylin and eosin method, and viewed in DPX medium under light microscope. Findings revealed that the percentage change in body weight was higher in rats treated with EFV and approximately normal in rats treated with a combination of EFV and Vitamin E when compared with the control animals. The hippocampus of Wistar rats administered with efavirenz, showed distortions and neurodegeneration in the cell layers; the pyramidal cells were shrunken with various degree of vacuulations. In the group where efavirenz was combined with viamen E this changes were slightly ameliorated. The cells of the polymorphic (PM) pyramidal and molecular (M) layers appeared normal in the control groups A and group F. Efavirenz administration can damage the hippocampus. Vitamin E has the potential of ameliorating this effect. Therefore, Vitamin E should be prescribed for patients on efavirenz administration. © 2015 iGlobal Research and Publishing Foundation. All rights reserved.

## INTRODUCTION

Efavirenz (EFV), is an oral non-nucleotide reverse transcriptase inhibitor (NNRTI) used as a part of highly active antiretroviral therapy (HAART) for the treatment of type 1 human immunodeficiency virus (HIV-1) [1]. Efavirenz has been found to be effective in many combination regimes for the treatment of HIV infection, both in previously untreated and in treated individuals. It is combined in regimens with other HAART agents [2-4] Efavirenz has high specificity against HIV-1 reverse transcriptase with no inhibitory effect on human cellular

DNA polymerases and HIV-2 reverse transcriptase [5]. Its antiviral activity is dependent on intracellular conversion to the active triphosphorylated form. In the active form, EFV binds to and deactivates HIV-1 reverse transcriptase preventing the formation of viral double-stranded DNA from the single-stranded viral RNA genome [6]. Efavirenz is principally metabolized by the cytochrome P450 system (in the liver) to hydroxylated metabolites which are subsequently glucouronidated for excretion in the urine [7]. As such, patients with liver disease have

greater risk for adverse effects from efavirenz due to decreased drug clearance [7].

Most antiviral agents do not efficiently penetrate the blood brain barrier or are actively transported out of the central nervous system. Hence, even after antiviral treatment that successfully controls virus in the treatment compartments, the central nervous system may suffer continuing damage induced by HIV infection. Efavirenz is one of the HAART agents that can penetrate the central nervous system and spinal fluids with a capacity to strongly inhibit multi-drug resistant proteins [8-14]. Thus, efavirenz has emerged as cornerstone of most HIV regimens [15].

The following nervous system disorders have been reported with the use of efavirenz; abnormal dreams, disturbance in attention, dizziness, headache, insomnia, somnolence, agitation, amnesia, ataxia, epilepsy, abnormal coordination, confusional state, convulsions, and abnormal thinking [4, 16-18].

It is known that efavirenz has the potential to cause toxicity in the central nervous system, but this is yet to be elucidated [19]. In cellular necrosis, the rate of progression depends on the severity of the environmental insults. The prime candidates for inducing the massive cell destruction observed in neurodegeneration are neurotoxins. These may be substances present in small amounts in the environment, or even naturally occurring chemicals such as glutamate used by the brain as transmitter's substances. The latter when present at a critical level can be toxic to the brain cells in which they normally excite [20]. Prolonged administration of efavirenz has been reported to cause toxic effects on the lateral geniculate body [4].

Less common side-effects of efavirenz include headache, alcohol intolerance, fever, aches, pains and fatigue, fluid retention in the hands and feet, dry mouth, elevated lipid levels, pancreatitis, skin problems, asthma and changes to vision and taste. Interestingly, reports of lipid elevations have included rises in high-density lipoprotein (HDL) or 'good' cholesterol, particularly in people with a genetic polymorphism in the multidrug resistance gene [21, 22]. However, levels of total cholesterol also increase, resulting in a small net increase in the ratio of total to HDL cholesterol [23]. This study also found that efavirenz causes elevations in triglyceride levels.

Vitamin E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities [24]. Naturally occurring Vitamin E exists in eight different forms: alpha-, beta-, gamma-, and delta-tocopherol; and alpha-, beta-, gamma-, and delta-tocotrienol. Although tocopherols and tocotrienols are available from the diet, alpha-tocopherol is the most active form in humans [24]. The liver metabolizes and excretes the other vitamin E forms but preferentially resecretes alpha-tocopherol via the hepatic alpha-tocopherol transfer protein. As a result, blood and cellular concentrations of other forms of vitamin E are lower than those of alpha-tocopherol. Vitamin E has many biological functions, the antioxidant function being the most important and best known. Other functions include enzymatic activities, gene expression, and neurological function(s) [24]. Another important function of vitamin E has been suggested in cell signaling. Some studies have suggested that tocotrienols have specialized roles in protecting neurons from damage [25] and cholesterol reduction. Oral consumption of tocotrienols is also thought to protect against stroke-associated brain damage. There is some evidence that all-rac alpha-tocopherol (man-made vitamin E) is effective for slowing cognitive function decline in people with moderately severe Alzheimer's disease.

This study therefore was designed to investigate the effect of combined administration of Vitamin E and efavirenz on the histology of the hippocampus of Wistar rats.

## **METHODOLOGY**

Thirty Wistar Rats of weight 140 g–192 g were used for the study. The rats were divided into six treatment groups. Control (group A) were administered with 1 ml of distilled water; group B were administered with 8.57mg/kg body weight of Efavirenz; group C were administered with Double dose of 17.14 mg/kg body weight of Efavirenz; group D were administered with Triple dose of 25.71 mg/kg body weight of Efavirenz; group E were administered with 8.57 mg/kg body weight of Efavirenz and 14.82 mg/kg body weight of Vitamin E; and group F were administered with 14.82 mg/kg body weight of Vitamin E. drug administration lasted for 32 days. The animals were handled according to the guidelines for the treatment of laboratory animals. On the 33rd day, the rats were sacrificed using chloroform inhalation method. The hippocampi were excised, routinely processed, stained

using haematoxylin and eosin method, and viewed in DPX medium under light microscope.

## RESULTS & DISCUSSION

All the rats had increased body weight at the end of the treatment period as shown in table 1 and figure 1 below.

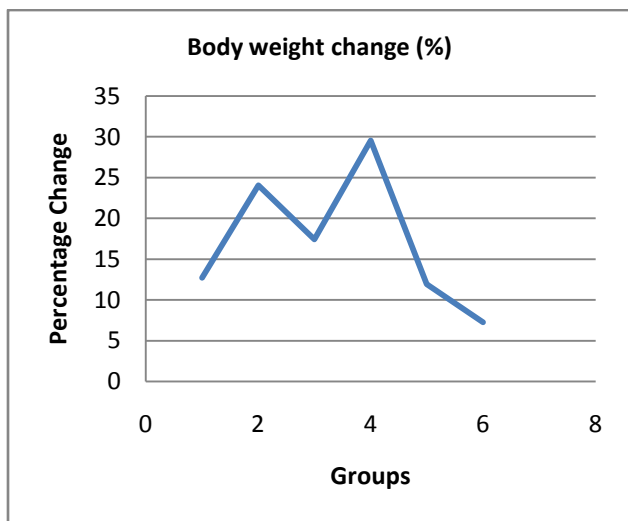
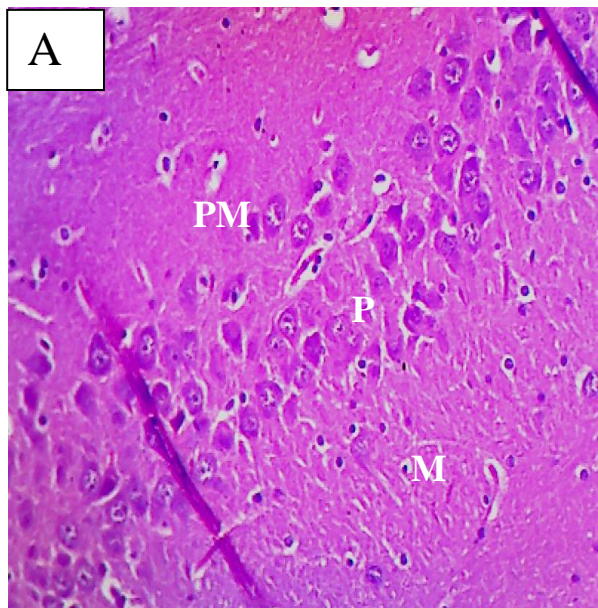


Figure 1: Graphical representation of percentage change in body weight.

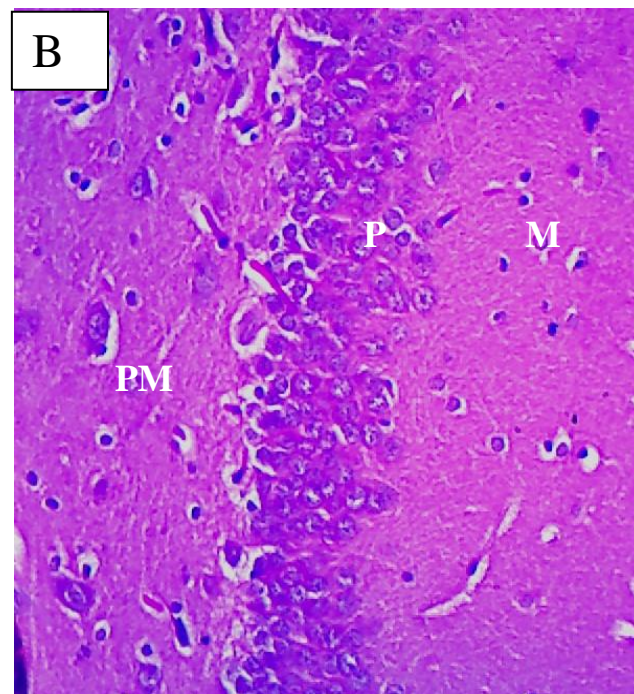
Photomicrographs are as shown in plates I to vi below:

### Plate I



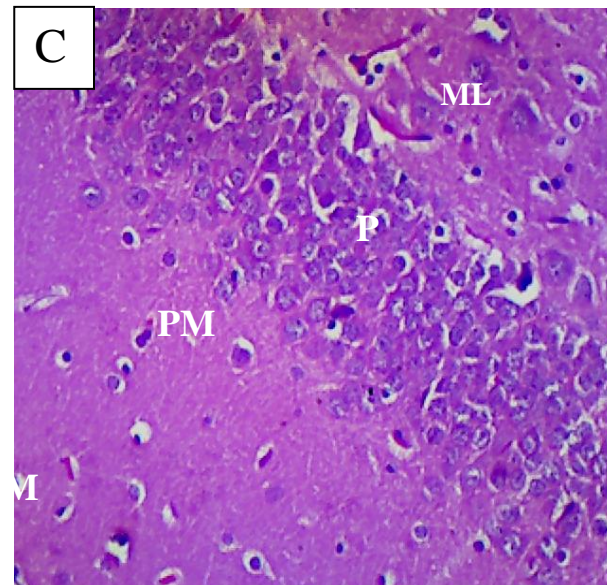
Photomicrograph of the histology of the hippocampus of rats in control group-1, showing the polymorphic (PM), pyramidal (P), and molecular (M) layers

### Plate II



Photomicrograph of the histology of the hippocampus of rats treated with 8.57mg/kg efavirenz (group 2) showing the polymorphic (PM), pyramidal (P), and molecular (M) layers.

### Plate III

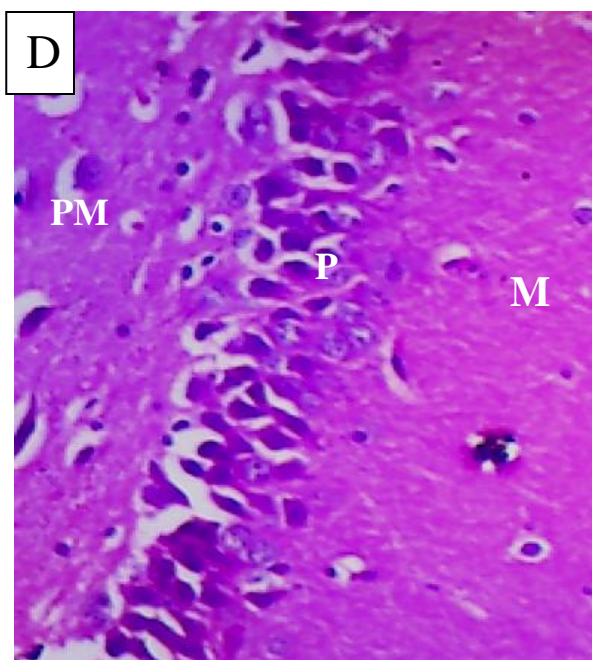


Photomicrograph of the histology of the hippocampus of rats treated with 17.14mg/kg efavirenz (group 3) showing the polymorphic (PM), pyramidal (P), and molecular (M) layers.

Table I: Body weight change of the experimental animals

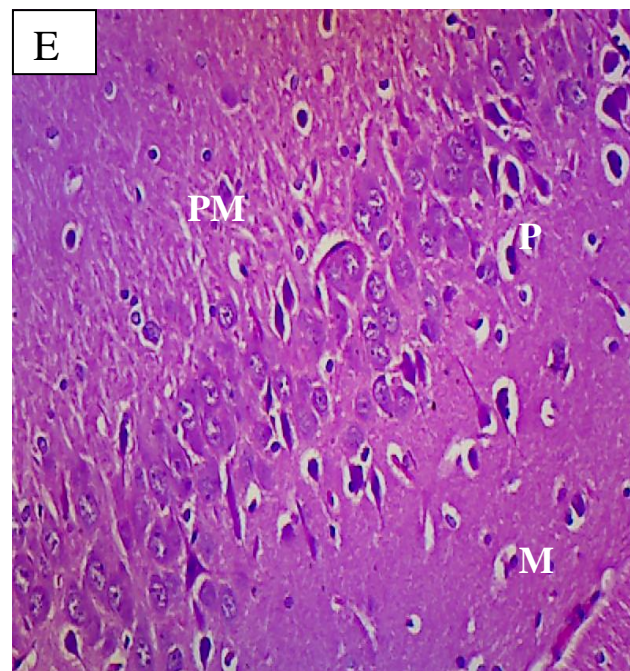
Groups	Initial body weight (g)	Final body weight (g)	Body weight change (g)	Body weight change (%)
1	140.40±5.45	158.25±11.69	17.85	12.71
2	141.40±2.96 <sup>NS</sup>	175.40±7.03 <sup>NS</sup>	34.00	24.05
3	146.00±3.11 <sup>NS</sup>	171.40±5.04 <sup>NS</sup>	25.40	17.40
4	149.60±0.40 <sup>NS</sup>	193.80±5.38 <sup>NS</sup>	44.20	29.55
5	159.40±6.41 <sup>NS</sup>	178.40±5.77 <sup>NS</sup>	19.00	11.92
6	179.40±22.68 <sup>NS</sup>	192.40±17.80 <sup>NS</sup>	13.00	7.25

Plate IV



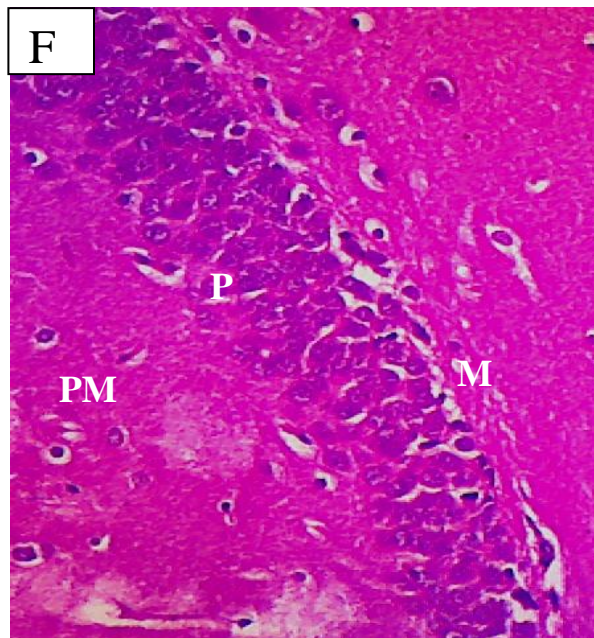
Photomicrograph of the histology of the hippocampus of rats treated with 25.71mg/kg efavirenz (group 4) showing the polymorphic (PM), pyramidal (P), and molecular (M) layers.

Plate V



Photomicrograph of the histology of the hippocampus of rats treated with 8.57mg/kg of Efavirenz and 14.82mg/kg Vitamin E (group 5) showing the polymorphic (PM), pyramidal (P), and molecular (M) layers.

Plate VI



**Photomicrograph of the histology of the hippocampus of rats treated with 14.82mg/kg Vitamin E (group 6) showing the polymorphic (PM), pyramidal (P), and molecular (M) layers.**

#### Photomicrographs

Plate I shows the morphology of normal hippocampal histology from control group 1. The polymorphic (PM), pyramidal (P), molecular (M) layers and glial cells are clearly shown with no cellular distortion.

Plate II shows the hippocampus of Wistar rats administered with 8.57mg/kg of efavirenz, the pyramidal cell-mass is reduced with some areas of neurodegeneration. The cells of the polymorphic (PM) molecular (M) layers appear normal.

Plate III, Group C administered with 17.14 mg/kg of EFV, shows severe distortion of the pyramidal cell layer and shrinkage of the pyramidal cells. The cells of the polymorphic (PM) molecular (M) layers were distorted.

Plate IV Group D administered with 25.71 mg/kg of EFV shows severely atrophic hippocampus. the cell bodies in all layers are shrunken and elongated, in the polymorphic and molecular layer the cells were also shrunken.

Plate V Group E administered with 8.57mg/kg of efavirenz and 14.82mg/kg of Vitamin E shows a relatively healthy hippocampus. Some pyramidal cells are

mildly shrunken when compared with the control, but better than the hippocampus of the groups treated with efavirenz alone.

Plate VI Group F administered with 14.82mg/kg of Vitamin E shows a healthier hippocampus with distinct layers; pyramidal, polymorphic and molecular layers. The glial cells appear normal, when compared with control.

The hippocampus is involved in the consolidation of information from short-term memory to long-term memory and spatial navigation. It is important in forming new memories and connecting emotions and senses, such as smell and sound, to memories [26]. Yet there is dearth of literature on the effect of efavirenz on the hippocampus.

In this study of the effect of efavirenz on the hippocampus of Wistar rats, there was neurodegeneration of the pyramidal cells which are the cell body of neurons in the groups treated with efavirenz, the deterioration of the cell bodies and dendritic projections in these animals may lead to memory loss in patients under EFV therapy. Moreover, in Alzheimer's disease characterized with memory loss and disorientation, there is a reduced number of hippocampal dendritic spines [27] and the hippocampus is known to be one of the first regions of the brain to be damaged.

The progressive deterioration of the hippocampus in higher doses of EFV confirms the toxicity of EFV to the hippocampus. That is, cellular distortions observed in this study may have been as a result of cell death caused by the toxic effect of efavirenz. This may also lead to patients developing epileptic attack under long-term use of EFV as the atrophy of basket cells leaves the pyramidal cells to synchronous and continuous firing [28].

Vitamin E has been reported to be useful in the management of diseases of the brain and nervous system including Alzheimer's disease and other dementias, Parkinson's disease, night cramps, restless leg syndrome, and for epilepsy, along with other medications [29]. This practice is corroborated with the findings of our study on the neuro-protective potentials of Vitamin E in ameliorating the effect of efavirenz in the groups treated with vitamin E and efavirenz.

According to Tracy et al (2001), the hippocampus also functions in controlling the behaviour related to food and appetite [30]. It is crucial for the signal of satiety in humans and rats. Hippocampal-lesioned rats have been found to exhibit increased food intake and body weight [31]; and densely amnesic human patients with hippocampal damage have been reported to eat a full second meal few minutes after the first [32]. This is may be the reason the percentage change in body weight in the EFV. Groups were higher. The highest dose of EFV with the greatest hippocampal damage, also had the highest percentage change in body weight; in contrast, group V that took both EFV and vitamin E had approximately the same percentage change in bodyweight with control group A

## CONCLUSION

Chronic administration of EFV had detrimental effect on the histology of the hippocampus. Vitamin E was found to be salubrious and capable of ameliorating the detrimental effects of EFV when administered together. This was further confirmed through the percentage change in body weight that was highest in rats treated with highest dose of EFV and approximately normal in rats treated with a combination of EFV and Vitamin E. In view of this, it is recommended that Vitamin E should be prescribed for patients under EFV treatment.

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