INTRODUCTION

The NNRTI Efavirenz is an important component of the treatment of HIV infection for many years and has contributed significantly to the evolution of HAART. Currently Efavirenz is a recommended option for initial therapy and is usually regarded as the preferred NNRTI.1-4 Indeed, the combination of Efavirenz plus two NRTIs is recommended as the regimen of choice for initial therapy in the current UK guidelines.1 Peak Efavirenz plasma concentrations are reached by 5 h following single oral doses in uninfected volunteers.5 The time to peak plasma concentrations is ∼3−5 h and steady-state plasma concentrations of efavirenz are reached in 6−7 days.6 The bioavailability of a single 600 mg dose of Efavirenz hard capsules in uninfected volunteers is increased by 17%−22% by food.5 Efavirenz is highly bound (∼99.5%−99.75%) to human plasma proteins, predominantly albumin.5,6 Efavirenz has a long half-life of 52 h after single doses, and 55 h after multiple doses.7 The long half-life of Efavirenz makes it suitable for once-daily dosing. The recommended dosage in adults is 600 mg once daily. Many

Histopathological effect of co-administration of efavirenz and vitamin E on the liver, and biochemical parameters in wistar rats

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ABSTRACT

Background: Efavirenz is a drug used singly and in combination as highly active antiretroviral therapy (HAART) used for the treatment of human immunodeficiency virus (HIV). Aims and Objectives: To investigate the effects of Efavirenz and vitamin E on the histopathology and biochemical parameters in the liver of Wistar rats. Materials and Methods: Twenty five Wistar rats were divided into five groups. Group A were administered with 1 ml of distil water; group B animals were administered with 8.57 mg/kg of Efavirenz, group C were administered with 17.14 mg/kg Efavirenz; group D were administered with 8.57 mg/kg of Efavirenz and 14.82 mg/kg Vitamin E, and group E were administered with 14.82 mg/kg of Vitamin E for 32 days. On day 33, the rats were sacrificed using chloroform inhalation method. The liver were excised, routinely processed, stained using haematoxylin and eosin method, and viewed in DPX medium under light microscope. Blood samples from the rats were collected and then centrifuged after 30 minutes to obtain the serum for analysis of aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB). Results: The liver of Wistar rats administered with Efavirenz, showed distortions with various degree of vacoulations, dilatation of sinusoidal spaces and nuclei pyknotic changes. In the group where Efavirenz was combined with vitamin E this changes were ameliorated. This also agreed with the biochemical changes which showed significant levels of increase in AST and ALT in Efavirenz groups, this changes were also ameliorated in the group that Efavirenz was combined with vitamin E. Conclusion: Efavirenz administration can damage the liver, vitamin E can ameliorate this effect, therefore Vitamin E should be prescribed for patients on Efavirenz administration.

Key words: Histopathological, Efavirenz, Liver, Vitamin E, Biochemical parameters
studies comparing different NRTI combinations have used Efavirenz as the common third agent.7-11

Adherence is a major predictor of the success of HAART12,13 with higher adherence rates leading to a lower risk of viral rebound and resistance development.14 The complexity of the treatment regimen is an important barrier to good adherence13,15 and patients generally prefer the simplicity of once-daily regimens.16,17 The use of once-daily agents and the co-formulation of multiple anti-retrovirals in fixed-dose combinations have simplified HAART regimens in recent years. As well as providing simplified initial HAART regimens, these approaches have been used in switch strategies to improve convenience for patients stabilized on more complicated regimens.

Efavirenz has been generally well tolerated in clinical trials. According to the systematic review by Bartlett et al. 4%-16% of patients treated with Efavirenz plus two NRTIs discontinued treatment due to adverse events;18 the NRTI combinations of Lamivudine plus Zidovudine or Abacavir were associated with the higher discontinuation rate.

The most notable adverse events associated with Efavirenz are rash and central nervous system (CNS) symptoms. Rash is common, but led to discontinuation in <2% of patients and was severe in <1%.19 When Efavirenz and Nevirapine were directly compared (each plus Lamivudine and Stavudine) discontinuations due to adverse events or HIV events occurred in 15.8% of patients treated with efavirenz and 24.1% of patients treated with nevirapine once daily.20 The difference between the groups in adverse event-related discontinuations was mainly due to a greater incidence of rash and hepatobiliary toxicity with Nevirapine.20

CNS or neuropsychiatric disturbances have been reported in ~25%–70% of patients receiving Efavirenz.21-25 Symptoms include dizziness, headache, confusion, impaired concentration, agitation, amnesia, psychotic symptoms, sleep abnormalities, abnormal dreams and insomnia. These symptoms usually arise within the first few days of treatment and lead to early discontinuation of Efavirenz in ~4%-10% of patients, although some investigators have reported higher discontinuation rates.26 The prevalence of most neuropsychiatric symptoms declines within a few weeks if therapy is continued.21,22,24,25,27

Studies in animals have suggested that the effects of Efavirenz on cytokines may play a role in depression associated with Efavirenz.27 Sleep disturbances may play a role in the development of neuropsychiatric symptoms.26

While exposure to HAART increases the risk of myocardial infarction,28 this appears to be due to PIs and not to NNRTIs.29 Renal toxicity has been reported, albeit rarely, with Tenofovir administration.30 Although small differences in glomerular filtration rate have occurred over time when Tenofovir was combined with Efavirenz over 144 weeks in HAART-naïve patients, no clinically relevant renal disease or adverse events were observed.31 Approximately 75% of the idiosyncratic drug reactions result in liver injury or death.32 Drug-induced hepatic injury is the most common reason cited for withdrawal of an approved drug. Physicians must be vigilant in identifying drug-related liver injury because early detection can decrease the severity of hepatotoxicity if the drug is discontinued. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure.32 The objective of this study was to investigate the effects of combined administration of Efavirenz and vitamin E on the histopathology and biochemical parameters in the liver of Wistar rats.

MATERIALS AND METHODS

Twenty five Wistar rats weighing between 140g−192g were used for the study. The rats were divided into five groups. Control (group) A were administered with 1 ml of distil water; group B were administered with 8.57 mg/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 8.57 mg/kg body weight of Efavirenz and 14.82 mg/kg body weight of Vitamin E; and group E 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 day 33 the rats were sacrificed using chloroform inhalation method. The liver were excised, routinely processed, stained using haematoxylin and eosin method, and viewed in DPX medium under light microscope. Blood samples from each rat were collected using syringes and needles and separated into sample bottles and allowed to stand for 30 minutes for clotting to take place and then centrifuged. The serum extracted into fresh test tubes and stored in a refrigerator for analysis of aspartate amino transaminase test (AST), alanine amino transaminase test (ALT), alkaline phosphotase (ALP) and total bilirubin (TB).

Measurement of alkaline phosphatase

This was by the optimized standard method recommended by the deutsche Geseitschage fur Klinische Chemie GSCC (1972). P-nitrophenyl phosphate is hydrolysed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01 ml in a test tube was mixed with reagent (0.5 ml) containing the substrate p-nitrophenyl
phosphate and brought to room temperature. The solution was mixed, initial absorbance read after 1 minute. The reaction was allowed to stand for 3 minutes and the absorbance read again at 405 nm. Alkaline phosphates activity was calculated from.

\[ UL = 2760 \times \Delta A \text{ nm/minute micro} \]

UL = Unit of Alkaline Phosphatase affinity

\[ \Delta A = \text{Change in absorbance} \]

**Measurement of alanine and aspartate transferase**

The measurement of ALT and AST activities in the serum were done using endpoint colorimetric-diagnostic kit (Randox; Labouratories UK) based on Reitman and Frankel (1952) method. The pyruvate produced by transamination reaction between L-alanine and ketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a colored hydrazone, which represents alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine is used to measure aspartate amino transferase (AST). Both AST and ALT were read at 540 nm wavelength.

All results were analyzed using one way Analysis of Variance (ANOVA) and post hoc test values.

**RESULT**

Group A: Photomicrograph of the histology of the liver administered with distilled water. Showing normal liver architecture; the central vein (V), hepatocytes plates (H), sinusoidal spaces (S) and nuclei (N) are all normal H & E, × 100 and X 400 as shown in Figure 1.

Group B: Photomicrograph of the histology of the liver administered with 8.57 mg/kg body weight of Efavirenz showing moderate distortion of liver cellular architecture; the central vein (V) are dilated, hepatocytes plates (H) are swollen, sinusoidal spaces (S) dilation and pyknotic nuclei (N) H & E, × 100 and x 400 as shown in Figure 2.

Group C: Photomicrograph of the histology of the liver administered with double dose 17.14 mg/kg body weight of Efavirenz; showing severe distortion of liver cellular architecture; the central vein (V) are dilated, hepatocytes plates (H) are swollen, sinusoidal spaces (S) and nuclei (N) are all normal H & E, × 100 and X 400 as shown in Figure 3.

Group D: Photomicrograph of the histology of the liver administered with 8.57 mg/kg body weight of Efavirenz and 14.82 mg/kg body weight of Vitamin E showing mild distortion of liver cellular architecture; the central vein (V) are normal, sinusoidal spaces (S) are dilated with normal nucleus (N) when compared with the control group A. H & E, × 100 as shown in Figure 4.

Group E: Photomicrograph of the histology of the liver administered with 14.82 mg/kg body weight of Vitamin E. Showing normal liver architecture; the central vein (V), hepatocytes plates (H), sinusoidal spaces (S) and nuclei (N) are all normal H & E, × 100 and X 400 as shown in Figure 5.

**DISCUSSION**

Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20-40% of all instances of fulminant hepatic failure. Knowledge of the commonly implicated agents and a high index of suspicion are essential in diagnosis. The current study was designed to investigate
the effects of Efavirenz and its co-administration with vitamin E. The results obtained from this study revealed that oral administration of Efavirenz had toxic effects on the liver, with moderate to severe distortion of liver cellular architecture; dilatation of the central vein, sinusoidal spaces and pyknotic nuclei changes. This finding is supported by clinical findings that Efavirenz can compromise the liver and renal functions.3,4 Other adverse effects include; insomnia, nightmares, confusion, memory loss, and depression5 and more serious symptoms such as psychosis may occur in patients with compromised liver or kidney function.3,4 In general, severe hepatic injuries have been documented to occur in HAART patients, regardless of their treatment.5,6 ALT and AST are liberated into the blood whenever liver cells are damaged and increased plasma enzymes activity is a sensitive index of hepatic damage.3,4 Neither of these enzymes is specific to the liver but ALT occurs in much higher concentration in the liver than elsewhere.3,4 Therefore, the increased serum ALT and ALP activity in this study, as shown in table 1 more specifically reflects hepatic damage.3,4 This agreed with the histological findings which revealed liver distortions. The changes caused by the administration of Efavirenz were ameliorated by vitamin E in the group that Efavirenz was combined with vitamin E. This must have been as a result of the antioxidant activity of vitamin E. This was shown both in the histological appearance which only had mild distortions and the liver function test that was not significantly different from control group (Table 1).

Drug-related toxicity leads to poor medication adherence and ultimate virological failure.6 Hepatic and cerebellar toxicity due to antiretroviral drug was also observed in some studies.6,7 This is why studies on adverse drug reactions are very important and relevant, to identify potential risk and prevent of drug toxicity. For HIV treatment it will help to improve compliance and reduce the incidence of drug resistance.

In conclusion, chronic administration of Efavirenz leads to toxic changes in the histology and biochemical indices in the liver of Wistar rats, its combined administration with vitamin E ameliorated these effects. Therefore Efavirenz should be given with vitamin E and strict monitoring of liver function should be a norm in patients taking Efavirenz.

REFERENCES


40. Peter AI, Ekanem BT, Ekong MB, Oguedemod HE and Archibong AM. Ameliorative effects of Neuromite™ on
histopathological changes of the cerebellum of lamuvudine treated Wistar rats. Journal of Neurochemistry 2013; 125(Suppl. 1):70.


Authors Contribution:
AIP - Concept and Design of the study, Experimentation, Review of literature, Manuscript preparation and statistical analysis; IAE - Experimentation, Review of literature and Final approval of manuscript.

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