Antioxidants as recipes for efavirenz-induced liver damage: A study in albino rats

Elias Adikwu,[a] Bonsome Bokolo[b]

[a]Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria
[b]Department of Pharmacology, Faculty of basic medical Sciences, Niger Delta University Wilberforce Island, Bayelsa State, Nigeria
Correspondence to Elias Adikwu (email: adikwuelias@gmail.com).
(*Submitted: 02 September 2017 – Revised version received: 24 September 2017 – Accepted: 11 October 2017 – Published online: 26 March 2018*)

Objective Hepatotoxicity is a clinical challenge associated with the use of efavirenz (EFV). This study investigated the effects of *n*-acetylcysteine (NAC), vitamins C and E on EFV-induced hepatotoxicity in albino rats.
Methods Rats were divided into groups and administered with NAC (20 mg/kg), vitamin C (50 mg/kg), vitamin E (50 mg/kg), vitamins C + E and 60 mg/kg of EFV, respectively. Rats were also divided into groups and pretreated with NAC, vitamins C, E, and combined doses of vitamins C + E prior to treatment with EFV for 15 days, respectively. After drug administration rats were sacrificed and serum was collected and evaluated for liver function parameters. Rats were dissected, liver was collected weighed and, homogenized and evaluated for alkaline phosphatase (ALP), alanine aminotransferase (AST), aspartate aminotransferase (ALT), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), malondialdehyde (MDA), super oxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPX) levels and pathological damage.
Results Effects were not significant ($p > 0.05$) on body and liver weights, however, the levels of AST, ALT, AST, GGT, LDH, CB, TB and MDA were increased significantly ($P < 0.05$) whereas SOD, CAT, SOD, GSH and GPX were decreased significantly ($P < 0.05$) in EFV-treated rats in comparison to control. The liver of EFV-treated rats showed necrosis of hepatocytes hepatocytes necroses. Nevertheless, EFV-induced alterations in the above parameters were significantly ($P < 0.05$) ameliorated in antioxidants pretreated rats. The combined doses of vitamins C and E producedbest and significant ($P < 0.05$) in comparison to their individual doses.
Conclusion This study shows the showed prospects of antioxidants as candidates for the treatments of efavirenz-induced hepatotoxicity.
Keywords antioxidants, efavirenz, liver, toxicity, mitigation, rat

Introduction

The liver plays vital role in the biotransformation of drugs and toxins.[1] In view of the function of the liver, in the biotransformation of drugs and toxins, liver cells which include Kupffer, hepatic stellate, endothelial and parenchymal cells are major target of oxidative radicals produced by drugs and toxins.[2,3] Oxidative stress has been shown to be an essential originating factor in the pathogenesis of drug-induced hepatic damage.[4,5] The over-production of oxidative radicals is toxic to hepatocytes and initiates reactive oxygen species (ROS)-mediated cascade causing hepatocyte death, and leading to acute or chronic hepatic damage.[6,7]

The use of Efavirenz (EFV) a member of the non-nucleoside reverse transcriptase inhibitors (NNRTIs) has dramatically reduced human immunodeficiency virus (HIV)-related morbidity and mortality in the world.[8] However, studies have associated the use of EFV with hepatic damage which is a frequent class problem of the non-NNRTIs.[9,10] The molecular pathogenesis of this effect is poorly understood, but recent reports have highlighted features of mitochondrial dysfunction in hepatic cells exposed to clinically relevant concentrations of EFV.[11] Also, recent studies have demonstrated endoplasmic reticulum stress responses involving mitochondrial dysfunction and oxidative stress in human hepatic cell lines.[12]

N-acetylcysteine (NAC) is the acetylated derivative of the amino acid L-cysteine. It is frequently employed as a source of sulfhydryl groups to cells as an acetylated precursor of reduced GSH. It is an antioxidant that interacts directly with reactive oxygen and nitrogen species and up-regulate endogenous GSH status, thereby preventing oxidative stress-induced alterations in biomolecules.[13] Furthermore, in experimental animal model and clinical studies, NAC showed ameliorative effect on drug-induced acute liver injury and has been clinically adopted for the treatment of acetaminophen associated hepatotoxicity.[14,15] Moreover, animal and human studies of NAC suggest that it is a very safe as an effective tool for the treatment of many diseases considered to be mediated by oxidative radical and it has been used therapeutically in several disorders related to oxidative stress.[16]

Vitamin E is the major lipid-soluble and potent chain-breaking antioxidant that inhibits the production of oxidative radicals when fat undergoes oxidation and during the propagation of free radical reactions.[17] It is primarily located in cell and organelle membranes where it can exert its protective effect. It acts as the first line of defence against lipid peroxidation, by protecting polyunsaturated fatty acids present in membrane phospholipids and in plasma lipoproteins from free radical attack.[18,19] It is the only major lipid-soluble, chain breaking antioxidant found in plasma, red cells and tissues, allowing it to protect the integrity of lipid structures, mainly membranes.[20] In addition, previous studies have demonstrated the potential of vitamin E in the amelioration of drug-induced hepatotoxicity in animal model .[21,22]

Vitamin C is a six-carbon compound structurally related to glucose. It consists of two inter-convertible compounds: L-ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L-dehydroascorbic acid.[23] It is a water-soluble antioxidant with diverse biological functions. Its actions include acting as a cofactor for the enzymes involved in collagen hydroxylation, biosynthesis of carnitine and norepinephrine, tyrosine metabolism and peptide hormone amidation.[24] Its antioxidant property includes inhibition of lipid peroxidation, oxidative cell damage and stimulatory effects on other antioxidants especially

vitamin E.[25,26] Furthermore, vitamin C has shown ameliorative effect on drug-induced hepatotoxity as shown in *in-vivo* animal studies.[27,28] The present study was therefore designed to evaluate the effects of NAC, vitamins C and E on efavirenz-induced hepatotoxicity in albino rats.

Materials and Methods

Drugs and Chemicals

The vitamin E used for this study was manufactured by strides pharmaceuticals India while vitamin C was manufactured by Emzor pharmaceuticals industries Lagos, Nigeria. Efavirenz was manufactured by Miland laboratory, India. The present study used 20 mg/kg/day of NAC, 50 mg/kg/day of vitamin C and 50 mg/kg/day of vitamin E[29,30] and 60 mg/kg/day of EFV. NAC was dissolved normal saline, vitamin C was dissolved in water while arachis oil was used as the vehicle for vitamin E and EFV.

Grouping of Rats and Drug Administration

Fifty five albino rats were randomized into six groups A-E. Groups A, B, D contained five rats each while group C and E contained twenty rats each which were further divided into four sub-groups of five rats each. The rats in group A served as placebo control whereas group B served as the solvent control and were administered with normal saline and arachis oil respectively. The rats in group C were orally administered with NAC (20 mg/kg/day), vitamin C (50 mg/kg/day), vitamin E (50 mg/kg/day) and a combination of vitamins C+ E respectively. The rats in group D were orally administered with 60 mg/kg/day of EFV. The rats in groups E were orally pretreated with NAC, vitamin C, vitamin E, and combined doses of vitamins C+E prior to the administration of EFV for 15 days respectively.

Collection of Samples and Biochemical Analysis

After the termination of drug administration on day 15, the rats were fasted overnight and sacrificed under diethyl ether. The blood was collected via cardiac puncture; serum was extracted and evaluated for alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, gamma glutamyl transferase conjugated and total bilirubin using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.). Liver was excised, weighed and washed in cold 1.15% KCL solution, then homogenized and centrifuged. The supernatant was decanted and used for the evaluation of oxidative stress indices. Glutathione peroxidase (GPX) activity was evaluated as reported by Rotruck et al., 1973[31] SOD activity was determined by the method of Sun and Zigma (1978)[32] while CAT activity was measured according to Sinha et al., 1972.[33] Reduced Glutathione was analyzed according as described by Sedlak and Lindsay (1968)[34] while malondialdehyde (MDA) was measured using the method of Buege and Aust (1978).[35]

Histological Examination of the Liver

The histological examination of the liver was performed in Anatomical Pathology Department of University of Port Harcourt Teaching Hospital, Choba, Rivers State, Nigeria. The liver tissue was excised and rinsed in normal saline. Liver sections were taken and processed and embedded in paraffin. Liver tissues were then processed into sections 4-5 µm thick, stained with Hematoxylin and Eosin and observed under a light microscope for any morphological changes.

Statistical Analysis

Results are expressed as mean ± SD. Differences among the experimental groups were identified by one-way analysis of variance and Tukey's multiple comparison test and a $p < 0.05$ was considered significant.

Results

Effects on Body, Liver Weights and Liver Function Parameters

The administration of NAC, vitamins C and E for 15 days did not produce significant ($p > 0.05$) effects on serum levels of AST, ALT, ALP, GGT, LDH, TB and CB when compared to control (Table 2 and 3).On the other hand, the above parameters were significantly ($p < 0.05$) increased in rats administered with 60mg/kg/day of EFV for 15 days with no significant ($p > 0.05$) effects on the body and liver weights in comparison to control (Table 1-3). However, rats pretreated with individual doses of NAC, vitamins C and E showed significant ($p < 0.05$) reductions in the serum levels of AST, ALT, ALP, GGT, LDH, CB and TB when compared to EFV-treated rats (Table 2 and 3). Furthermore, a combination of vitamins C and E produced most and significant ($p < 0.05$) reductions in the serum levels of AST, ALT, ALP, GGT, LDH, CB and TB when compared to their individual doses, but effects did not differ significantly ($p < 0.05$) from pretreatment with NAC (Table 1and 2). Furthermore, treatment with NAC, Vit C and E did not produce significant ($p < 0.05$) effects on liver levels of AST, ALT, ALP, GGT, and LDH in comparison to control. On the contrary, these parameters were significantly ($p < 0.05$) increased in EFV-treated rats in comparison to control (Table 1 and 2). However, the liver levels of AST, ALT, ALP, GGT, and LDH were decreased significantly ($p < 0.05$) in rats pretreated with NAC, vitamins C and E prior to treatment with EFV. The observed decreases in these parameters were most and significant ($p < 0.05$) in rats pretreated with combined doses of vitamins C and E when compared to their individual doses. However, decreases observed in these parameters in rats pretreated with combined doses of vitamins C and E did not differ ($p > 0.05$) when compared to NAC pretreated rats (Table 2 and 3).

Effects on Oxidative Stress Indices and Liver Histology

The administration of NAC, vitamins C and E did not produce significant ($p > 0.05$) effects on the liver levels of SOD, CAT, GSH, GPX and MDA when compared to control. In contrast, liver levels of SOD, CAT, GSH and GPX were decreased significantly ($p < 0.05$) whereas MDA levels were increased significantly ($p < 0.05$) in rats treated with EFV when compared to control (Table 4). However, rats pretreated with NAC, vitamin C, and E prior to treatment with EFV showed significant ($p < 0.05$) increases in SOD, CAT, GSH and GPX levels whereas MDA levels were decreased significantly ($p < 0.05$) when compared to EFV-treated rats. Interestingly, pretreatment with a combination of vitamins C and E produced the most and significant ($p < 0.05$) effects on SOD, CAT,

Table 1. **Effects of *n*-acetylcysteine, vitamin C and E on body and liver weights of efavirenz- treated albino rats**

Treatments	Initial BW (g)	Final BW (g)	BW gain (g)	Liver W (g)	Relative liver W(%)
Control	220 ± 12.0	250 ± 13.3	18.0 ± 2.00	6.95 ± 0.31	2.78 ± 0.18
EFV	230 ± 11.2	248 ± 10.6	18.0 ± 2.11	7.00 ± 0.19	2.82 ± 0.47
EFV +NAC	234 ± 12.7	253 ± 12.9	19.0 ± 1.00	6.70 ± 0.10	2.64 ± 0.84
EFV+Vitamin C	225 ± 12.0	243 ± 14.2	18.0 ± 3.00	7.05 ± 0.37	2.90 ± 0.51
EFV+Vitamin E	240 ± 11.7	260 ± 10.5	20.0 ± 3.13	6.90 ± 0.23	2.65 ± 0.24
EFV+Vitamins C + E	248 ± 9.66	268 ± 9.06	21.0 ± 2.20	7.01 ± 0.42	2.61 ± 0.89

W,weight; BW,Body weight; Data expresses ad Mean ± SD, $n = 5$.

Table 2. **Effects of *n*-acetylcysteine, vitamins C and E on serum liver function indices of efavirenz-treated albino rats**

Treatments	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)	GGT (U/L)	TB (g/dL)	CB (g/dL)
Control	38.8 ± 2.09^a	32.7 ± 4.12^a	34.2 ± 3.38^a	50.2 ± 5.22^a	0.85 ± 0.08^a	9.20 ± 1.44^a	4.78 ± 0.08^a
NAC	35.3 ± 3.09^a	28.3 ± 3.09^a	29.3 ± 3.00^a	46.9 ± 3.11^a	0.77 ± 0.06^a	8.79 ± 0.09^a	4.00 ± 0.12^a
Vitamin C	36.7 ± 5.06^a	30.9 ± 3.00^a	32.3 ± 0.23^a	49.3 ± 4.20^a	0.83 ± 0.08^a	8.92 ± 0.31^a	4.53 ± 0.06^a
Vitamin E	35.6 ± 3.04^a	31.4 ± 3.06^a	31.4 ± 2.11^a	48.0 ± 5.20^a	0.82 ± 0.05^a	8.85 ± 0.22^a	4.27 ± 0.01^a
Vitamins C + E	33.8 ± 4.20^a	28.3 ± 2.00^a	30.6 ± 3.01^a	46.3 ± 4.35^a	0.78 ± 0.01^a	8.75 ± 0.05^a	4.09 ± 0.05^a
EFV	114.8 ± 7.13^b	130.0 ± 9.34^b	120.7 ± 8.55^b	160.1 ± 9.23^b	3.97 ± 0.02^b	21.1 ± 0.11^b	14.9 ± 1.15^b
EFV + NAC	40.3 ± 3.09^a	38.3 ± 3.09^c	40.3 ± 3.09^c	55.6 ± 5.40^a	0.90 ± 0.01^c	10.3 ± 1.52^a	5.31 ± 0.09^c
EFV + Vitamin C	69.8 ± 5.14^c	70.2 ± 6.21^d	75.6 ± 9.13^d	110.6 ± 9.07^c	1.73 ± 0.06^d	15.6 ± 1.21^c	10.7 ± 0.13^d
EFV + Vitamin E	67.4 ± 4.05^c	62.5 ± 5.15^d	70.3 ± 7.24^d	100.1 ± 8.22^c	1.56 ± 0.05^d	15.1 ± 1.31^c	10.3 ± 0.24^d
EFV + Vitamins C + E	41.2 ± 2.08^a	38.6 ± 4.06^c	37.5 ± 4.01^c	57.7 ± 6.14^a	0.87 ± 0.06^a	10.7 ± 1.05^c	575 ± 0.01^c

NAC, *n*-acetylcysteine; EFV, efavirenz. Data expressed as mean ± SD, $n = 5$. Values with different superscripts on the same column differ significantly at $P < 0.05$ ANOVA.

Table 3. **Effects of *n*-acetylcysteine, vitamins C and E on liver levels of biomarkers of liver function in efavirenz-treated albino rats**

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
Control	70.6 ± 8.07^a	65.6 ± 6.33^a	63.0 ± 4.12^a	80.0 ± 7.15^a	1.81 ± 4.12^a
NAC	64.3 ± 5.25^a	60.2 ± 4.00^a	59.4 ± 3.01^a	72.9 ± 6.72^a	1.72 ± 3.01^a
Vitamin C	69.4 ± 6.21^a	63.5 ± 4.20^a	61.8 ± 5.15^a	79.8 ± 4.33^a	1.79 ± 5.15^a
Vitamin E	67.9 ± 7.18^a	62.3 ± 6.06^a	60.4 ± 4.01^a	77.4 ± 5.42^a	1.77 ± 4.01^a
Vitamins C + E	65.3 ± 4.20^a	60.0 ± 4.00^a	58.6 ± 3.01^a	72.6 ± 6.11^a	1.73 ± 3.01^a
EFV	190.8 ± 9.13^b	194.7 ± 9.34^b	186.0 ± 7.55^b	200.9 ± 9.95^b	4.89 ± 7.55^b
EFV + NAC	80.5 ± 6.08^c	82.7 ± 5.00^c	80.7 ± 5.00^c	85.7 ± 6.72^c	1.75 ± 5.00^a
EFV + Vitamin C	130.6 ± 7.14^d	130.2 ± 8.21^d	127.6 ± 9.13^d	130.6 ± 9.68^d	2.99 ± 9.13^c
EFV + Vitamin E	125.4 ± 8.05^d	127.5 ± 9.15^d	120.3 ± 9.24^d	125.3 ± 8.65^d	2.73 ± 9.24^c
EFV + Vitamins C + E	84.2 ± 7.28^c	85.6 ± 7.26^c	85.5 ± 6.01^c	87.9 ± 7.32^c	1.70 ± 6.01^d

Table 4. **Effects of *n*-acetylcysteine, vitamins C and E on liver oxidative stress indices of efavirenz-treated albino rats**

Treatment	MDA (µmol/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)	GSH (µg/mg protein)	GPX (µg/mg protein)
Control	0.38 ± 0.05^a	30.1 ± 3.12^a	20.2 ± 1.19^a	8.47 ± 0.17^a	15.2 ± 1.14^a
NAC	0.30 ± 0.06^a	34.8 ± 2.05^a	23.3 ± 1.05^a	8.79 ± 0.05^a	16.0 ± 1.32^a
Vitamin C	0.36 ± 0.02^a	31.5 ± 3.23^a	21.7 ± 1.20^a	8.50 ± 0.26^a	15.5 ± 1.00^a
Vitamin E	0.35 ± 0.01^a	32.2 ± 4.23^a	21.3 ± 1.27^a	8.55 ± 0.09^a	15.8 ± 1.75^a
Vitamins C + E	0.31 ± 0.02^a	34.2 ± 2.15^a	23.5 ± 0.21^a	8.71 ± 0.18^a	17.8 ± 1.63^a
EFV	0.98 ± 0.02^b	10.7 ± 1.23^b	6.77 ± 0.24^b	1.56 ± 0.08^b	5.32 ± 0.53^b
EFV + NAC	0.48 ± 0.09^c	25.3 ± 2.23^c	15.9 ± 0.23^c	8.27 ± 0.23^a	14.0 ± 1.03^a
EFV + Vitamin C	0.69 ± 0.06^d	15.7 ± 1.20^d	10.5 ± 0.13^d	3.22 ± 0.15^c	8.42 ± 1.22^c
EFV + Vitamin E	0.61 ± 0.07^d	17.0 ± 1.29^d	11.8 ± 0.11^d	3.59 ± 0.04^c	8.99 ± 0.56^c
EFV + Vitamins C + E	0.49 ± 0.01^c	23.5 ± 1.15^c	15.5 ± 0.17^c	8.19 ± 0.13^a	13.9 ± 1.17^a

NAC, *n*-acetylcysteine; EFV, efavirenz. Data expressed as mean ± SD, $n = 5$. Values with different superscripts on the same column differ significantly at $P < 0.05$ ANOVA.

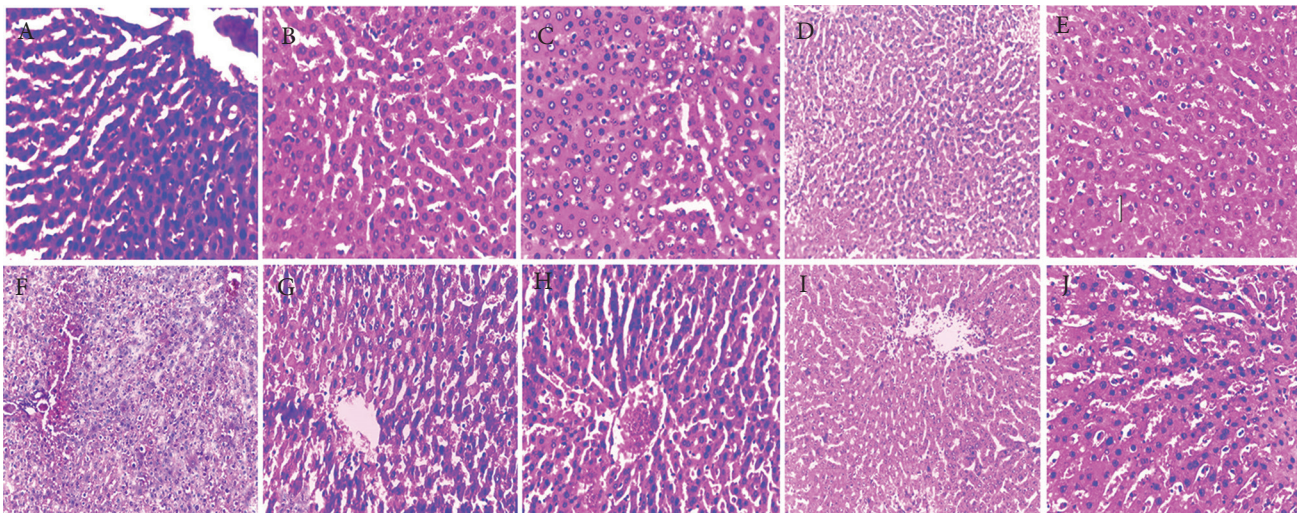

Fig 1. **(A) Liver of the control rat showing normal architecture. (B–E) Liver of rat administered with 20 mg/kg of *n*-acetylcysteine, 20 mg/kg of vitamin C, 20 mg/kg vitamin E and combined doses of vitamins C and E, respectively for 15 days showing normal architecture. (E) Liver of rat administered with 60 mg/kg/day of efavirenz (EFV) showing hepatocyte and centrilobular necrosis. (F–I) Liver of rats pre-treated with 20 mg/kg of *n*-acetylcysteine, 20 mg/kg of vitamin C, 20 mg/kg vitamin E and combined doses of vitamins C and E respectively prior to treatment with 60 mg/kg of EFV for 15 days showing normal architecture (H and EX200).**

GSH, GPX and MDA levels when compared to individual doses. However effects observed in rats pretreated with combined doses of vitamins C and E were not significantly ($p > 0.05$) different when compared to pretreatment with NAC (Table 4). Furthermore, microscopic examination of the H and E stained sections of the liver of control rat and rats administered with NAC, vitamins C and E showed normal histology (Fig 1A-E). Liver of rats treated with EFV shows necrosis of hepatocyte (Fig 1.F). However, the liver of rats pretreated with NAC, vitamins C and E and combined doses of vitamin C and E showed normal histology (Fig 1. G-J)

Discussion

Oxidative stress, a consequence of free radicals' generation is a common mechanism underlying hepatotoxicity caused by drugs and toxicants.[36] Antioxidants are chemical agents that scavenge, neutralize and mop-up free radicals, thereby preventing oxidative stress.[37] Therefore, the present study evaluated the benefits of NAC, vitamins C and E on EFV-induced liver toxicity in albino rats. In the current study, treatment with NAC, vitamins C and E did not produce significant effects on AST, ALT, ALP, GGT, LDH, CB, TB, SOD, GSH, and CAT levels. These observations are in conjunction with previous reports.[38,39] This study did not observe significant effects on the body and liver weights in EFV-treated rats, however; levels of AST, ALT, ALP, GGT, LDH, CT, TB and MDA were elevated whereas SOD, GSH, CAT and GPX levels were reduced significantly. In addition, alterations in hepatic morphology were observed in EFV-treated rats. These finding are consistent with previous reports.[40-42]

AST and ALT are enzymes involved in the transfer of amino groups of aspartate and alanine to ketoglutaric acid.[43] AST, ALT and ALP are present in the liver but, ALT is primarily located in the liver, and thus is a more specific marker of hepatocellular cell injury.[44-46] Bilirubin is produced by the normal breakdown of pigment-containing proteins, especially haemoglobin from senescent red blood cells and myoglobin from muscle breakdown.[47] AST, ALT, ALP, GGT, LDH and bilirubin are clinically used as fundamental indices for the assemement of the functionality of the liver. Elevations in the levels of these parameters typify hepatocellular injury.[48] Therefore, the observed elevations in the levels of these parameters in EFV- treated rats indicate hepatocellular injury. In various forms of liver disease, serum levels of numerous cytosolic, mitochondrial and membrane associated enzymes are increased. The detailed mechanism by which enzymes released from the cytosol and mitochondria of hepatocytes into the blood stream is not completely known. Clinical observations and experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the intracellular space.[49] A very large concentration gradient between the hepatocytes and the sinusoidal space usually exists for enzymes. Cell damage increases membrane permeability, causing cytosolic iso-enzymes to spill into the sinusoids and from there into the peripheral blood.[50]

Oxidative stress has been considered as a pathological mechanism that characterises the initiation and progression of drug-induced liver injury. A network of antioxidant defence which include SOD, GAT and GSH is structured in the liver of mammals for cellular response to down-regulate oxidative stress under physiological condition and to maintain redox homeostasis in the liver.[51] However, the stimulatory production of excessive free radicals from oxygen and nitrogen could alter liver redox homeostasis leading to antioxidant depletions.[51-53] Hence, the observed decreases in the liver levels of SOD, CAT, GSH and GPX in EFV-treated rats are evidence of hepatic oxidative stress. Oxidative stress can cause hepatic damage by inducing antioxidants depletion, irretrievable alteration of lipids, proteins and DNA contents and more importantly, modulates pathways that control normal hepatic biological functions.[54,55] MDA has been widely used for many years as a convenient biomarker for lipid peroxidation which is a free-radical-mediated chain of reactions. Its level is always elevated in a state of established oxidative stress and lipid peroxidation.[6,57] Therefore, the observed elevated liver levels of MDA in EFV-treated rats suggest lipid peroxidation which

might have resulted in oxidative deterioration of hepatic polyunsaturated lipids and increase membrane permeability.

Furthermore, in the present study pretreatment with NAC, vitamins C and E ameliorate EFV-induced hepatic damage. More so, ameliorative effects were most observed in rats pretreated with combined doses of vitamins C and E than their individual doses. The hepatoprotective effects of antioxidants observed in the present study correlate with findings by Ebuehi et al (2012)[58] who reported the palliative effects of vitamins C and E on lead-induced hepatotoxicity in rats. Similarly Awodele and co-researchers reported the ameliorative effects of vitamins C and E on nevirapine-induced hepatotoxicity in rats.[59] Also, Naglaa et al., 2015[60] reported the beneficial effect of NAC on acetaminophen-induced hepatotoxicity in rats.

In the current study, these antioxidants might have protected the liver by inhibiting the chain reactions of EFV-generated free radicals or scavenged the free radicals before they reached their hepatic targets. Also, these antioxidants might have inhibited the depletion of endogenous antioxidants or facilitate their regeneration. NAC is a direct and indirect antioxidant that scavenges, neutralizes and chelates free radicals, thereby inhibiting oxidative destruction of tissues and organs.[61] It can maintain –SH groups of enzymes and membrane proteins in their reduced state.[62] NAC can reduce extracellular cystine to cysteine, or produce SH required for the synthesis of hepatic GSH. It can prevent drug-induced hepatic GSH depletion as well; up-regulate the activity of hepatic GSH. Also, NAC can enhance hepatic glutathione-S-transferase, SOD, and CAT activities, and promote detoxification mechanisms.[63]

Vitamin C is a water soluble antioxidant that scavenges free radicals in extracellular fluid, trapping radicals and protecting biological membrane from oxidative damage.[64] It is an important source of electrons and it easily donates electrons to free radicals such as hydroxyl radicals and super oxide radicals, thereby down-regulating their activities.[65] Also, vitamin C can up-regulate the levels and activities of some endogenous antioxidants.[66] Vitamin E is an essential lipophilic antioxidant that can inhibit oxidative radical-induced damage to polyunsaturated fatty acids and act as a membrane-stabilizing agent that can prevent damage to phospholipids.[67] It is mainly found in the hydrocarbon part of membrane lipid bilayer towards the membrane interface and in close proximity to oxidise enzymes which can initiate the production of free radicals.[68,69] Studies have shown that it can inhibit peroxidation of membrane lipids by scavenging lipid peroxyl radicals, as a consequence of which it is converted into a tocopheroxyl radical a potent antioxidant.[70] Also, it can prevent xenobiotic-induced hepatic mitochondria oxidative stress by down-regulating the activities of oxidative radicals.[71] In conclusion, the present study gives an insight on the possible use of antioxidants as recipes for efavirenz-induced liver toxicity in rats.

Acknowledgments

The authors appreciate the support of Mr Eze Ihukwumere of the Faculty of pharmacy Madonna University, Elele, Rivers State

Conflict of Interest

The authors declare no conflict of interest.

Source of Funding

The authors declare no sources of funding.

References

1. Duncan SA. Transcriptional regulation of liver development. Dev Dyn. 219:131–142.
2. Sanchez-Valle V, Chavez-Tapia NC, Uribe M, Mendez-Sanchez N. Role of oxidative stress and molecular changes in liver fibrosis: A review. Curr Med Chem. 2012;194850–194860.
3. Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. World J. Gastroenterol. 2014;20:8082–8091.
4. Albano E. Free radical mechanisms in immune reactions associated with alcoholic liver disease. Free Radic Biol Med. 2002;32:110–114.
5. Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury. J Gast Hep. 15:718–724.
6. Kaplowitz N. Biochemical and cellular mechanisms of toxic liver injury. Semin Liver Dis. 2002;22:137–144.
7. Lee SJ, Kim SY, Min H. Effects of vitamin C and E supplementation on oxidative stress and liver toxicity in rats fed a low-fat ethanol diet. Nutr Res Pract. 2013;7:109–114.
8. Palella FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med. 1998;338:853–860.
9. Nunez M, Lana R, Mendoza JL, Martin-Carbonero L, & Soriano V, Risk factors for severe hepatic injury after introduction of highly active antiretroviral therapy. J Acquir Immune Defic Syndr, 2001;27:426–31.
10. Martinez E, Blanco JL, Arnaiz JA, Perez-Cuevas JB, Mocroft A, Cruceta A, et al. Hepatotoxicity in HIV-1-infected patients receiving nevirapine-containing antiretroviral therapy. AIDS. 2001;15:1261–1268.
11. Apostolova N, Gomez-Sucerquia LJ, Gortat A, Blas-Garcia A, Esplugues JV. Autophagy as a rescue mechanism in efavirenz-induced mitochondrial dysfunction: a lesson from hepatic cells. Autophagy. 2011;7:1402–1404.
12. Polo M, Alegre F, Funes HA, Blas-Garcia A, Victor VM, Esplugues JV, et al. mitochondrial (dys)function - a factor underlying the variability of efavirenz-induced hepatotoxicity? Br J Pharmacol. 2015;172:1713–1727.
13. Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci. 2003;60:6–20.
14. Baniasadi S, Eftekhari P, Tabarsi P, Fahimi F, Raoufy MR, Masjedi MR, et al. Protective effect of N-acetylcysteine on antituberculosis drug-induced hepatotoxicity. Eur J Gast Hepatol. 2010;22:1235–1238.
15. Singh S, Hynan LS, Lee WM. Acute Liver Failure Study G, Improvements in hepatic serological biomarkers are associated with clinical benefit of intravenous N-acetylcysteine in early stage non-acetaminophen acute liver failure. Dig Dis Sci. 2013;58:1397–1402.
16. Cotgreave IA. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. Adv Pharmacol. 1997;38: 205–227.
17. Burton GW, Joyce A, Ingold KU. Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Arch Biochem Biophys. 1983;221:281–290.
18. Howard AC, Anna K, McNeil AK, McNeil PL. Promotion of plasma membrane repair by vitamin E. Nat Commun. 2011;20:597.
19. Tran K, Wong JT, Lee E, Chan AC, Choy PC. Vitamin E potentiates arachidonate release and phospholipase A2 activity in rat heart myoblastic cells. Biochem. 1996;31:385–391.
20. Burton GW, Traber MG. Vitamin E: Antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr. 1990;10:357–382.
21. Fariss MW. Cadmium toxicity: unique cytoprotective properties of alpha tocopheryl succinate in hepatocytes. Toxicology. 1991;69:63–77.
22. Ben Amara N, Soudani A, Troudi H, Bouaziz T, Zegha N. Antioxidant effect of vitamin E and selenium on hepatotoxicity induced by dimethoate in female adult rats. Ecotoxicol Enviro Safe. 2011;74:811–819.

23. Adikwu E, Oputiri D. Hepatoprotective effect of vitamin C. Pharmacol Pharm. 2013;4:84–92.
24. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, et al. Vitamin C as anantioxidant: evaluation of its role in disease prevention. Jour of Ameri Coll of Nutri. 2001;22:18–53.
25. Frei BL, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proceedings of National Academy of Science USA. 86:6377–6391.
26. Xavier SM, Barbosa CO, Barros DO, Silva RF, Oliveria AA, Freitas RM. Vitamin C antioxidant in hippocampus of adult Wistar rats after seizures and status epilepticus induced by pilocarpine. Neurosci Lett. 2007;420:76–79.
27. Mor F, Ozmen O. Effect of vitamin C in reducing the toxicity of endosulfan in liver in rabbits. Exp Toxicol Pathol. 2010;62:75–80.
28. Al-Attar AM. Hepatoprotective Influence of Vitamin C on Thioacetamide-induced Liver Cirrhosis in Wistar Male Rats. Journal of Pharmacology and Toxicology. 2011;6:218–233.
29. Qader GI, Aziz RS, Ahmed ZA, Abdullah ZF, Hussain SA. Protective Effects of Quercetin against Isoniazid and Rifampicin Induced Hepatotoxicity in Rats." Am Jour of Pharm Scien, 2014;2:56–60.
30. Adikwu E, Onyedenyifa OJ & Samuel NK Beneficial Effects of Vitamins C and E on Tenofovir and Nevirapine-InducedHepatorenal Toxicity in Male Albino Rats International Journal of Biomedical Research. 2016;7:211–218.
31. Rotruck JT, Rope AL, Ganther HF, Swason AB. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179:588–590.
32. Sun M, Zigma S. An improved spectrophotometer assay of superoxide dismutase based on epinephrine antioxidation. Analalytic Biochemistry. 1978;90:81–89.
33. Sinha KA. Colorimetric assay of catalase. Anal Biochem. 1972;47:389–394.
34. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with ellman's reagent. Analytical Biochemistry. 1968;25:1192–1205.
35. Buege, JA, Aust SD. Microsomal Lipid Peroxidation. Methods Enzymol. 1978;52:302–310
36. Deavall GD, Elizabeth AM, Judith MH, Ruth R. Drug-induced oxidative stress and toxicity. J Toxicol. 2012 (2012), Article ID 645460, 13 page.
37. Li S, Tan H, Wang N, Zhang Z, Lao L, Wong C, et al. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci. 2015;16:26087–26124
38. Shokrzadeh M, Shobi S, Attar H, Shayegan S, Sadat S, Payam H, et al. Effect of Vitamins A, E and C on liver enzyme activity in rats exposed to organophosphate pesticide diazinon. Pak Jour of Biol Sci. 2012;15:936–941.
39. Mahmoud GS, Amer AS. Protective effects of vitamin c against nicotine-induced oxidative damage of rat liver and kidney IOSR journal of environmental science.Toxicology and Food Technology. 2014;8:50–63.
40. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. JAMA. 2000;283:74–80.
41. Adjene OJ, Avbunudiogba JA, Igbigbi PS. Oxidative stress induced by chronic administration of Efavirenz on the intracranial visual relay centers of adult Wistar rats Biology and Medicine. 2011;3;16–24.
42. Sonderup MW, Wainwright H, Maughan D, Setshedi M, Spearman CWN. Characteristics of Efavirenz drug induced liver injury: a cohort analysis. AIDS. 2016;30:1483–1485.
43. Karmen A, Wroblewski F, Ladue JS. Transaminase activity in human blood. J Clin Invest. 1995;34:126–31.
44. Dufour DR, Lott JA, Nolte FS. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin Chem. 200a;46:2027–2049.
45. Dufour DR, Lott JA, Nolte FS. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem. 200b;46:2050–2068.
46. Roberts WM. Variations in the phosphatase activity of the blood in disease. Br J Exp Pathol. 1930;11:90–95.
47. Blanckaert M. Analysis of bilirubin and bilirubin mono-and di-conjugates. Biochem J. 1985;115–28.
48. Kwo PY, Cohen SM, Lim JK. Practice Guideline: Evaluation of Abnormal Liver Chemistries Am J Gastroenterol advance online publication. 2016;517.
49. Tredger JM, Sherwood RA. The liver: New functional, prognostic and diagnostic tests. Ann Clin Biochem. 1979;34:121–141.
50. Freidel R, Diedrichs F, Lindena J. Release and Extra Cellular Turnover of Cellular Enzyme. In: Schmidt E, FW Schmidt I, Transchold S. Munich (Eds.), Clinical Enzymology. Karger. 1979;70–105.
51. Li Sha, Tan H, Wang N, Zhang Z, Lao L, Wong C, et al. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci. 2015;16:26087–26124
52. Karabulut AB, Gui M, Karabulut E, Kiran TR, Ocak SG, Otlu O. Oxidant and antioxidant activity in rabbit livers treated with zoledronic acid. Transplant Proc. 2010;42:3820–3822.
53. Dey A, Lakshmanan J. The role of antioxidants and other agents in alleviating hyperglycemia mediated oxidative stress and injury in liver. Food Funct. 2013;4:1148–1184.
54. Singal AK, Jampana SC, Weinman SA. Antioxidants as therapeutic agents for liver disease. Liver Int. 2011;31:1432–1448.
55. Feng, Y, Wang N, Ye X, Li H, Feng Y, Cheung F, et al. Hepatoprotective effect and its possiblemechanism of Coptidis rhizoma aqueous extract on carbon tetrachloride-induced chronic liver hepatotoxicityin rats. J. Ethnopharmacol. 2011;138:683–690.
56. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods in Enzymology. 1990;186:407–421.
57. Esterbauer H, Schaur RJ, Zollner H. Chemistry and Biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes," Free Radical Biology and Medicine. 1991;11:81–128.
58. Ebuehi OAT, Ogedegbe RA, Ebuehi OM. Oral Administration of Vitamin C and Vitamin E ameliorates Lead-induced Hepatotoxicity and Oxidative Stress in the Rat Brain. Nig Qt J Hosp Med. 2012;22:85–90.
59. Awodele O, Popoola T, Rotimi K, Ikumawoyi V, Okunowo W. Antioxidant modulation of nevirapine induced hepatotoxicity in rats Interdiscip Toxicol. 2015;8:8–14.
60. Naglaa AB, Saad HE, Mohammed MM, & Ahmed MSH, Protective Effect of Curcumin versus N-acetylcystein on Acetaminophen Induced Hepatotoxicity in Adult Albino Rats. J Cytol Histol 2015; S3; 018. 1–8.
61. Flanagan RJ, Meredith TJ. Use of N-acetyl cysteine in clinical toxicology. Am J Med. 1991;91:131S–139S.
62. Wagner PD, Mathien-Castello O, Bebout DE, Gray AT, Natterson PD, Glennow C. Protection against pulmonary O2 toxicity by N-acetyl cysteine. Eur Respir J. 1989;2:116–126.
63. Zhao C, Shichi H. Prevention of acetaminophen-induced cataract by a combination of diallyl disulfide and N-acetyl cysteine. J Ocul Pharmacol her. 1998;14:345–55.
64. Harapanhalli RS, Yaghmai V, Giuliani D, Howell RW, Rao DV. Antioxidant effects of vitamin C in mice following X- irradiation. Res Comm Mol Pathol Pharmacol. 1996;94:271–287.
65. Bendich Antioxidant Micro Nutrients and Immune Responses. In "Micronutrients and Immune Functions" (A. Bendich and C.E. Butterworth, eds.), 87:168–180. New York Academy of Science, New York.
66. Durak D, Uzun FG, Kalender S, Ogutcu A, Uzunhisarcikli M, Kalender Y. Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro. Environmental Toxicology. 2009;24:235–242.
67. Brigelius-Flohe R. Vitamin E: the shrew waiting to be tamed. Free Radic Biol. Med. 46;2009;543–554.
68. McDowell LR. Vitamin C, A and E. In: Vitamins in Animal nutrition comparative aspects to human nutrition, pp. 93-131. Academic Press, London, UK. 1989.
69. Hosseini-Mansoub N, Chekani-Azar S, Tehrani AA, Lotfi A. Influence of dietary vitamin E and zinc on performance, oxidative stability and some blood measures of broiler chickens reared under heat stress (35°C). J Agrobiol. 2010;27:103–110.
70. Dowd P, Zheng ZB. On the mechanism of the anticlotting action of vitamin. E quinone Proc Natl Acad Sci USA. 1995;92:8171–8175.
71. Fariss MW. Oxygen toxicity: unique cytoprotective properties of vitamin E succinate in hepatocytes. Free Radic Biol Med. 1990;9:333–343.