

Beneficial Effects of Vitamins C and E on Tenofovir and Nevirapine-Induced Hepatorenal Toxicity in Male Albino Rats

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Abstract

Background: Tenofovir and nevirapine are associated with kidney and liver toxicity respectively; hence concurrent use might be associated with hepatorenal toxicity. Therefore, the present study evaluated the effects of pretreatments with vitamins C and E on tenofovir and nevirapine (TDF-NVP) -induced serum levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin (TB), creatinine (Cr), urea (U) and uric acid (UA) in male albino rats. Effects of treatments with vitamins C and E were also evaluated on baseline serum levels of the above parameters.

Methods: Forty five adult male albino rats which were divided into nine groups (A-I) of five animals each were used for this study. Animals in group A (placebo control) and group B (solvent control) were treated orally with water and arachis oil respectively. Animals in groups C-F were treated with vitamin C (20 mg kg⁻¹), vitamin E (20 mg kg⁻¹), vitamin C+E, and TDF-NVP (90 mg/60 mg kg⁻¹) for 30 days respectively. Animals in groups G-I were pretreated with vitamin C, vitamin E and combined doses of vitamin E and C prior to treatment with TDF-NVP for 30 days respectively. Animals were sacrificed and the serum levels of the above parameters were evaluated.

Results: Treatment with combined doses of vitamin E and C significantly ($p < 0.05$) decreased baseline serum levels of all evaluated parameters while TDF-NVP treatment significantly ($p < 0.05$) increased serum levels of all evaluated parameters when compared to the control. However, TDF and NVP -induced increases in serum levels of all evaluated parameters were decreased in animals pretreated with individual doses of vitamins C and E with maximal decreases observed with pretreatment using combined doses of vitamin C and E.

Conclusion: These vitamins could be beneficial in the treatment of liver and kidney damage associated with tenofovir-nevirapine.

Keywords: Antiretroviral, Toxicity, Pretreatments, Vitamins, Rats

1. Introduction

Tenofovir (TDF) is a nucleotide analog of deoxyadenosine monophosphate, with activity against HIV-1 and 2 [1, 2]. It was approved by the FDA in 2001, as an ester prodrug of tenofovir which is hydrolyzed to tenofovir intracellularly and phosphorylated to the active metabolite, tenofovir diphosphate [3,4]. Several clinical trials indicated that TDF is highly potent in combination with other antiretroviral drugs in the treatment of both naïve and experience patients at reducing HIV viral load significantly [5, 6]. However, the use of tenofovir could be associated with renal toxicity marked with proximal tubular dilatation and necrosis [7]. This may involve oxidative stress characterized by mitochondria damage and the generation of oxidative radicals [8].

Nevirapine (NVP) due to its efficacy was approved in 1996 as the first non-nucleoside reverse transcriptase

inhibitor for the management of HIV [9]. The use of NVP in current practice confers some important clinical advantages in treating patients with particular comorbidities. Nevirapine could be highly suitable for the treatment of naïve patients with an elevated cardiovascular risk profile, patients with a history of mental illness, especially depression or substance abuse, and women who are pregnant or planning to become pregnant [10, 11]. However, the use of nevirapine has been trailed by reports of hepatotoxicity characterized by liver necrosis and inflammation [12,13]

Tenofovir – Nevirapine (TDF-NVP) combination is concurrently used as a component of highly active antiretroviral therapy and has become a standard regimen for HIV management. It is commonly use in the management of HIV especially in poor resource settings with several recent studies with large patient populations showing beneficial

outcomes. It has contributed to marked decrease in viral load, morbidity, mortality and improvement in the quality of life of HIV patients [14, 15]. However, the use of NVP-TDF could be associated with hepatorenal toxicity characterized by mitochondria damage and oxidative stress [16, 17].

Vitamins C and E are water and lipid soluble antioxidants respectively [18]. Their antioxidants properties are associated with the prevention of peroxidation, quenching, mopping up of oxidative radicals and breakage of peroxidation chain reactions. In addition these vitamins could regenerate other antioxidants, inhibit protein kinase C and calcium metabolism thereby blocking the initiation and spread of oxidative mechanisms [19, 20]. Furthermore, synergy in antioxidant activities were reported with concurrent use of vitamins C and E which could be attributed to the sparing effects these vitamins have on each other [21, 22]. In animal studies, both vitamins have been shown to attenuate toxicological effects induced by xenobiotics [23, 24]. Therefore, the present study was designed to evaluate the effects of pretreatments with vitamins C and E on TDF and NVP-induced serum levels of alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, creatinine, urea and uric acid in male albino rats. Effects of treatments with vitamins C and E on the baseline serum levels of the above parameters were also evaluated.

2. Materials and methods

2.1 Animals

Rats of average weight 330 ± 5 g used for this study were obtained from the animal house of the Department of Pharmacology and Toxicology Madonna University Elele, Rivers State. Animals were allowed to acclimatize for 14 days and had free access to food and water *adlibitum*.

2.2 Drugs

Pure samples of tenofovir, nevirapine, vitamins E and C were purchased from Shijiazhuang Aopharm Import &

Export Trading Co., Ltd. Shijiazhuang, China. Tenofovir and nevirapine powder were suspended in arachis oil while vitamin C was dissolved in water. Vitamin C (20mgkg^{-1}) and vitamin E (20mgkg^{-1}) were used for this study [25].

2.3 Experimental Design

Animals were divided into 9 groups (A-I) of five animals each. Animals in group A (placebo control) and group B (solvent control) were treated with water and arachis oil for 30 days respectively. Animals in groups C-F were treated with vitamin C (20mgkg^{-1}), vitamin E (20mgkg^{-1}), combined doses of vitamin C +E and $90/60\text{ mgkg}^{-1}$ of TDF-NVP for 30 days respectively. Animals in groups G-I were pretreated with vitamin C, vitamin E and combined doses of vitamin C and E prior to treatment with TDF-NVP for 30 days respectively.

2.4 Collection of Sample

Animals were sacrificed at the end of 30 days of treatment with the aid of diethyl ether. Blood sample was collected in a sterile sample container via cardiac puncture. Blood sample was centrifuged at 1200 rpm for 15 min and serum was collected and evaluated for liver and renal function parameters.

2.5 Biochemical Analysis

Serum alanine aminotransferase, aspartate aminotransferase, and total bilirubin were evaluated according to methods reported by Ogbuehi et al [26]. Estimation of alkaline phosphatase, Serum creatinine, urea and uric acid were evaluated as reported by Kind and King [27], Annino and Giese [28], Toro and Ackermann [29] respectively

2.6 Statistical Analysis

Results are expressed as mean +SEM. Results were analyzed using one way analysis of variance (ANOVA) and statistical significance was set at $p < 0.05$

Table 1: Effects of pretreatments with vitamins C and E on baseline serum liver and renal function parameters of male albino rats

Drugs	U (mg/dL)	UA (mg/dL)	CR (mg/dL)	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	TB ($\mu\text{mol/L}$)
Control	37.5 ± 0.02	1.39 ± 0.05	1.21 ± 0.06	36.9 ± 0.02	55.3 ± 0.03	46.5 ± 0.06	9.75 ± 0.06
Vit + C	32.4 ± 0.02	1.30 ± 0.01	1.18 ± 0.02	32.7 ± 0.79	51.5 ± 0.82	40.7 ± 0.88	8.53 ± 0.08
Vit + E	30.0 ± 0.09	1.32 ± 0.03	1.15 ± 0.04	32.4 ± 0.90	48.4 ± 1.01	39.1 ± 1.33	8.50 ± 0.03
Vit + C+E	$20.1 \pm 0.03^*$	$1.02 \pm 0.04^*$	$0.81 \pm 0.01^*$	$20.4 \pm 0.94^*$	$35.3 \pm 0.90^*$	$25.6 \pm 0.75^*$	$6.10 \pm 0.05^*$

Vitamin C (20mgkg^{-1}), Vitamin E (20mgkg^{-1}). Data are expressed as means \pm standard error of mean. *Significant ($p < 0.05$) difference when compared to the control

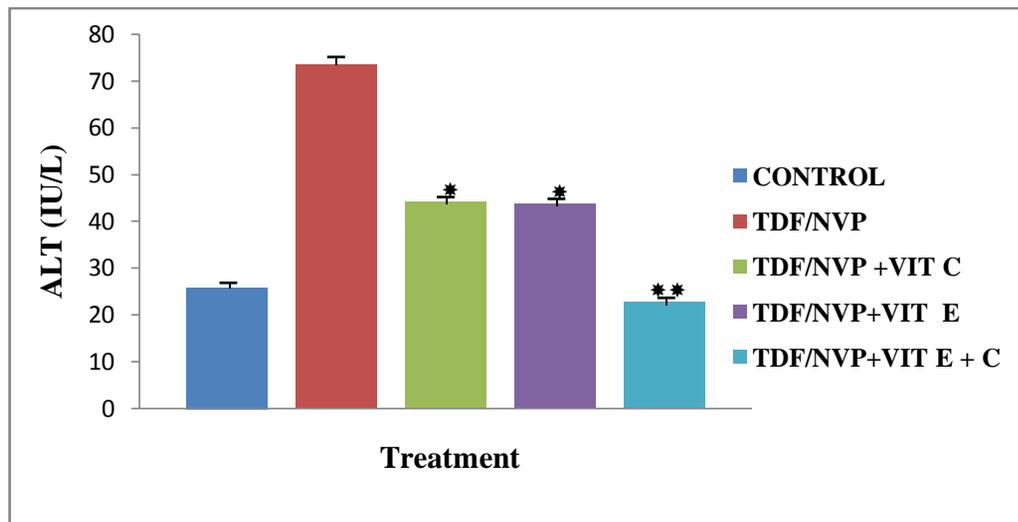


Fig 1: Effects of pretreatments with vitamins C and E on TDF and NVP-induced serum alanine aminotransferase level in male albino rats, *Significant ($p < 0.05$) difference when compared to treatment with TDF-NVP. **Significant ($p < 0.05$) difference when compared to pretreatments with individual doses of vitamins C and E

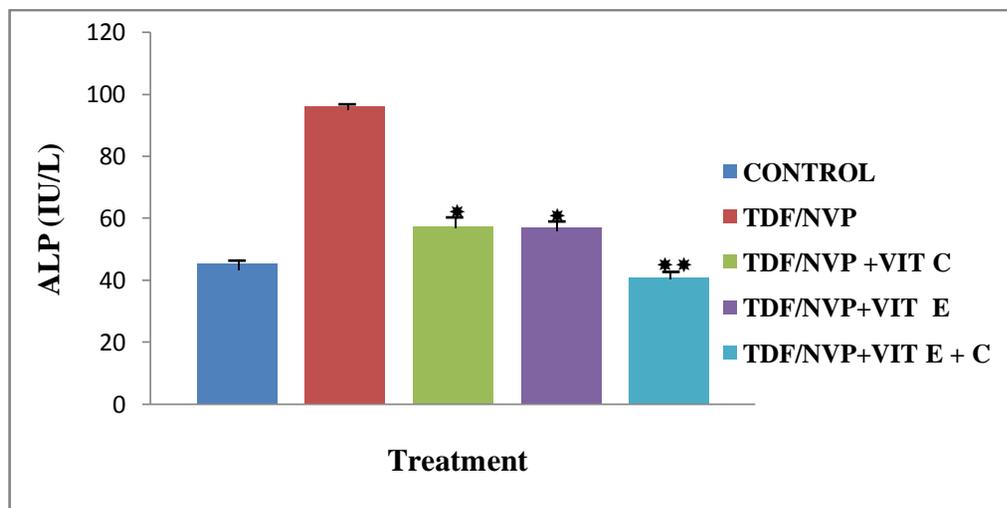


Fig 2: Effects of pretreatments with vitamins C and E on TDF and NVP-induced serum alkaline phosphatase level in male albino rats * Significant ($p < 0.05$) difference when compared to treatment with TDF-NVP. **Significant ($p < 0.05$) difference when compared to pretreatments with individual doses of vitamins C and E

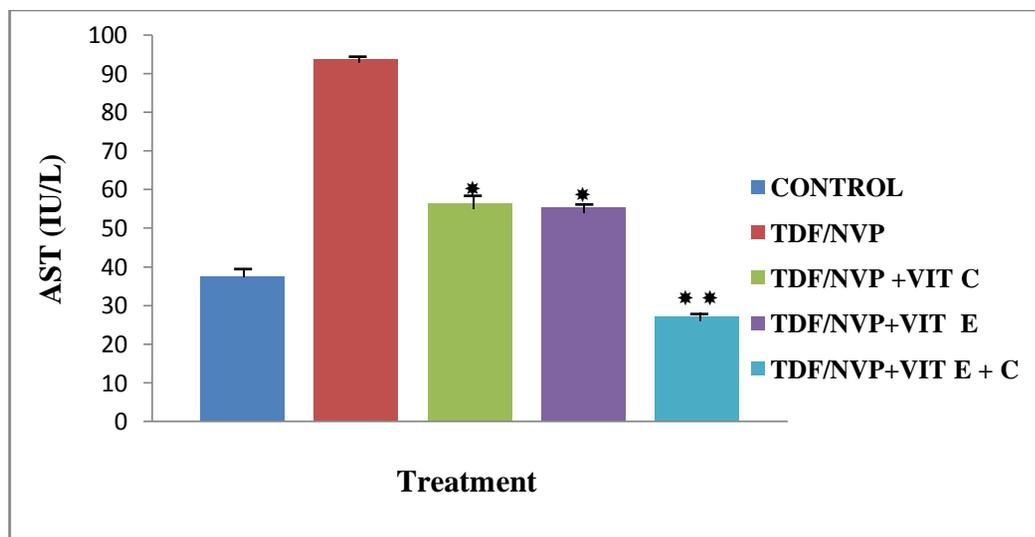


Fig 3: Effects of pretreatments with vitamins C and E on TDF and NVP -induced serum aspartate aminotransferase level in male albino rats * Significant ($p < 0.05$) difference when compared to treatment with TDF-NVP ** Significant ($p < 0.05$) difference when compared to pretreatments with individual doses of vitamins C and E

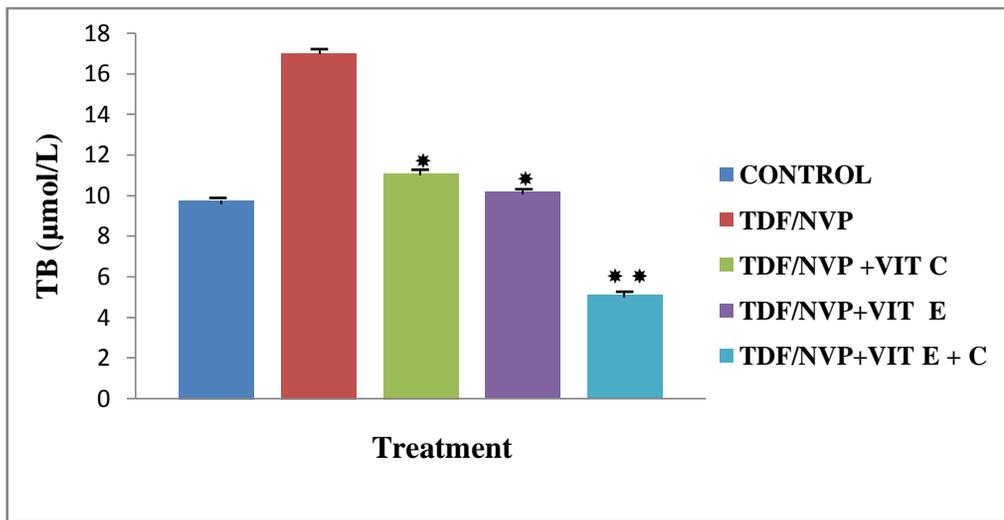


Fig 4: Effects of pretreatments with vitamins C and E on TDF and NVP -induced serum total bilirubin level in male albino rats *Significant (p<0.05) difference when compared to treatment with TDF-NVP. **Significant (p<0.05) difference when compared to pretreatments with individual doses of vitamins C and E.

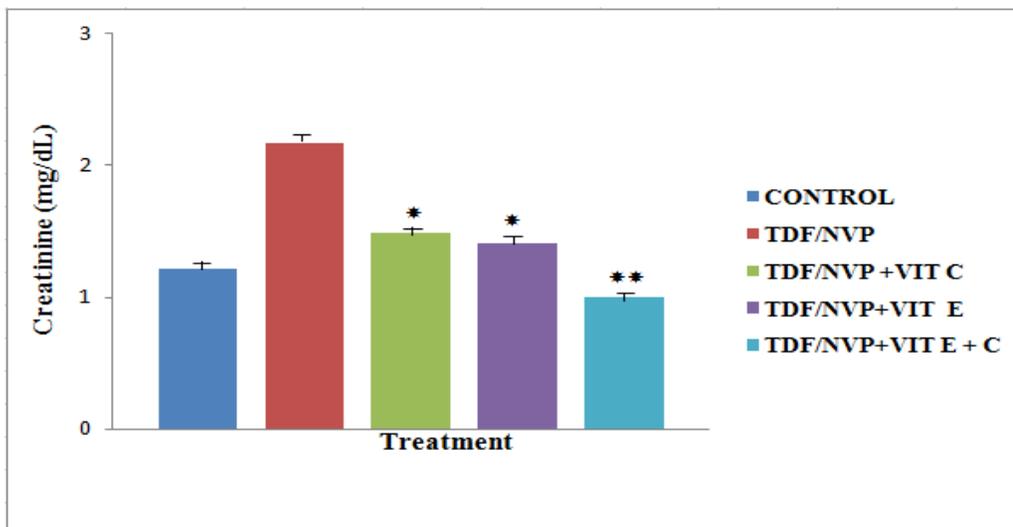


Fig 5: Effects of pretreatments with vitamins C and E on TDF and NVP-induced serum creatinine level in male albino rats *Significant (p<0.05) difference when compared to treatment with TDF-NVP. **Significant (p<0.05) difference when compared to pretreatments with individual doses of vitamins C and E

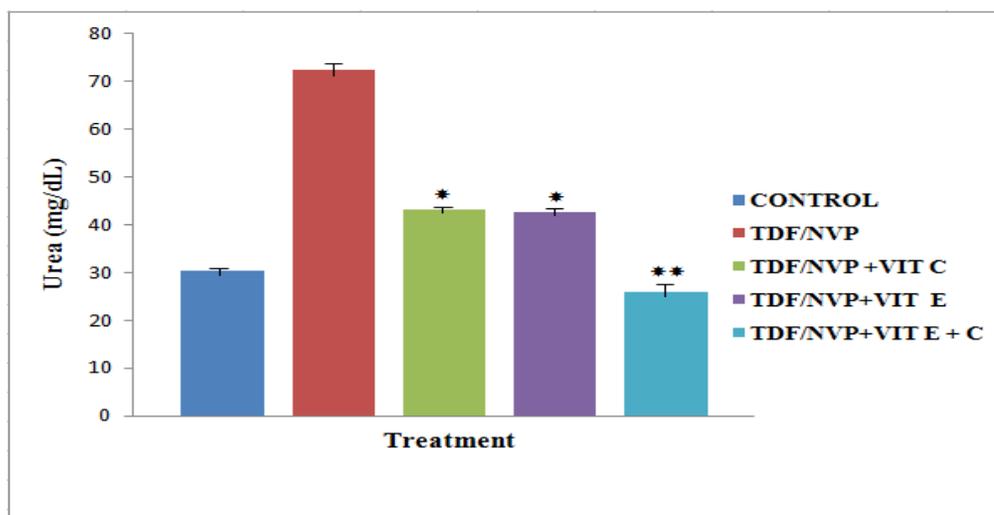


Fig 6: Effects of pretreatments with vitamins C and E on TDF and NVP-induced serum urea level in male albino rats. *Significant (p<0.05) difference when compared to treatment with TDF-NVP. **Significant (p<0.05) difference when compared to pretreatments with individual doses of vitamins C and E.

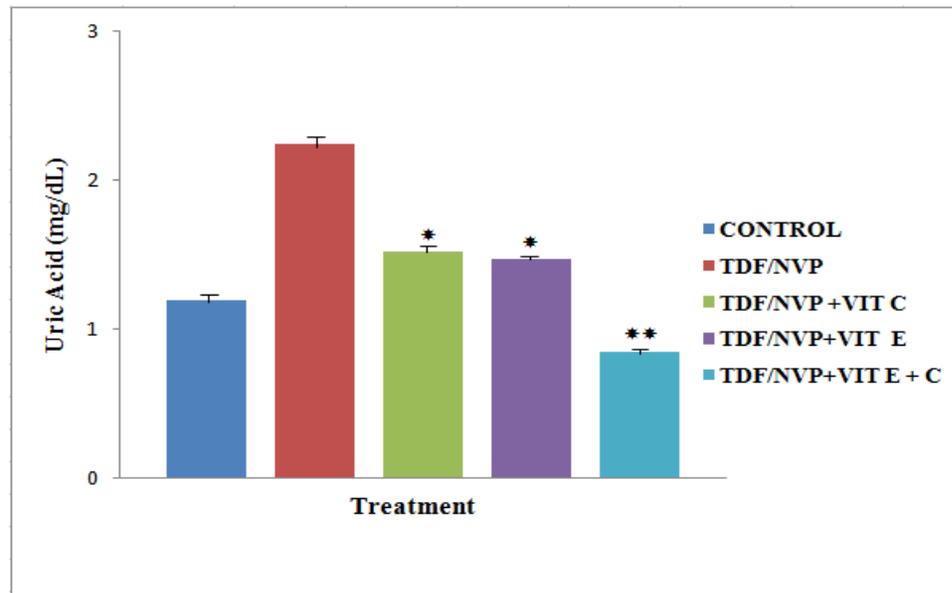


Fig 7: Effects of pretreatments with vitamins C and E on TDF and NVP-induced serum uric acid level in male albino rats *Significant ($p < 0.05$) difference when compared to treatment with TDF-NVP. **Significant ($p < 0.05$) difference when compared to pretreatments with individual doses of vitamins C and E

3. Results

The present study observed insignificant ($p > 0.05$) decreases in baseline serum levels of ALT, ALP, AST, TB, Cr, U and UA in animals treated with individual doses of vitamins C and E when compared to the control. Further and significant ($p < 0.05$) decreases in the above parameters were observed in animals treated with combined doses of these vitamins when compared to the control (Table 1). However, treatment with TDF-NVP significantly ($p < 0.05$) increased serum levels of ALT, ALP AST and TB to 73.6 ± 0.02 , 96.00 ± 0.05 , 93.6 ± 0.06 (IU/L) and 17.01 ± 0.01 $\mu\text{mol/L}$, respectively when compared to the control. These serum values were significantly ($p < 0.05$) decreased to 44.2 ± 1.89 , 57.2 ± 0.94 , 56.5 ± 0.76 (IU/L) and 11.10 ± 0.02 $\mu\text{mol/L}$ respectively with vitamin C pretreatment when compared to TDF-NVP treatment. Vitamin E pretreatment significantly ($p < 0.05$) decreased these serum values to 43.7 ± 0.29 , 57.0 ± 0.07 , 55.5 ± 0.12 IU/L and 10.2 ± 0.08 $\mu\text{mol/L}$, respectively when compared to TDF-NVP treatment. Further decreases in these serum values to 28.8 ± 0.81 , 40.8 ± 0.76 , 27.0 ± 0.84 IU/L and 5.10 ± 0.01 $\mu\text{mol/L}$ respectively were obtained with pretreatment using a combination of vitamin C and E. These serum values were significantly ($p < 0.05$) different when compared to serum values obtained with pretreatments using individual doses of vitamins C and E (Fig. 1-4).

Furthermore, significant ($p < 0.05$) increases in creatinine, urea and uric acid levels to 2.17 ± 0.03 , 2.24 ± 0.05 , 72.3 ± 0.07 mg/dL, respectively were observed in TDF-NVP treated animals when compared to the control values. These serum values were significantly ($p < 0.05$) decreased to 1.49 ± 0.06 , 1.52 ± 0.04 , 43.1 ± 0.05 mg/dL, respectively with

vitamin C pretreatment when compared to TDF-NVP treatment. Vitamin E pretreatment significantly ($p < 0.05$) decreased these serum values to 1.40 ± 0.04 , 1.47 ± 0.05 and 42.5 ± 0.07 mg/dL, respectively when compared to TDF-NVP treatment. Treatment with a combination of vitamin C and E further decreased serum creatinine, urea and uric acid values to 1.00 ± 0.07 , 0.85 ± 0.05 , 25.7 ± 0.05 mg/dL, respectively. These serum values were significantly ($p < 0.05$) different when compared to serum values obtained with pretreatment using individual doses of vitamins C and E (Fig. 5-7).

4. Discussion

The kidney is an essential organ for important functions such as maintenance of homeostasis, detoxification and excretion of drugs and toxic metabolites [30]. The liver acts as a guard between the digestive tract and the rest of the body, transforming, detoxifying and accumulating metabolites [31, 32]. Kidney and liver are frequently exposed to high concentrations of potentially toxic drugs and metabolites due to their involvement in drug excretion and metabolism respectively [33]. TDF-NVP combination is a component of highly active antiretroviral therapy that could be associated with kidney and liver damage [34,35]. Therefore, this study evaluated the effects of pretreatments with vitamins C and E on TDF and NVP-induced serum levels of alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, urea, creatinine and uric acid in male albino rats. Effects of treatments with vitamins C, E and a combination of vitamin C and E were also evaluated on the baseline serum levels of the above parameters. The present study observed decreases in baseline serum renal function parameters (creatinine, urea and uric

acid) and liver function parameters (AST, ALT ALP and TB) in animals treated with individual doses of vitamins C and E. Maximal decreases in these parameters were observed in animals treated with a combination of vitamin C and E. This observation is consistent with previous study [36]. In contrast, the above parameters were increased in TDF- NVP treated animals. This observation is consistent with previous reports [37]. However, TDF and NVP -induced increases in liver and kidney function parameters were mitigated in animals pretreated with individual doses of vitamins C and E with maximal mitigation obtained in animals pretreated with a combination of vitamin C and E. In this study, increases in liver and renal function parameters observed in TDF-NVP treated animals suggest signs of hepatorenal toxicity. This could be attributed to oxidative stress induced by these agents in the liver and kidney of treated animals through the generation of free radicals [38, 39]. Studies showed that oxidative stress can cause loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability [40,41]. Another possible mechanism could be direct liver and kidney damage induced by TDF-NVP leading to dysfunction and disturbance in the biosynthesis of the evaluated biochemical parameters. Robert and others in their study reported that direct damage to tissues and organs could result in the elevation of serum concentrations of specific biochemical parameters as a result of their release or secretions from the damaged tissues/organs [42]. Kidney damage observed in this study could be attributed to the toxic effect of tenofovir on the kidney of treated animals. This is in agreement with the work of Adaramoye and others who treated rats with 50 mg/kg of tenofovir for 4 weeks and observed kidney damage marked with increases in serum levels of creatinine, urea and uric acid [43]. Abraham and others also reported tenofovir-induced kidney toxicity characterized by elevations in serum urea and creatinine levels in rats treated with 600 mg/kg of TDF for 5 weeks [44,45]. Observed liver damage in this study could be attributed to the toxic effect of nevirapine on the liver of treated animals. This is in agreement with the work of Sule and co-researchers who reported liver damage characterized by elevations in serum levels of AST, ALT and ALP in nevirapine intoxicated rats [46]. Adaramoye and others also intoxicated rats with nevirapine and reported increases in liver function parameters [47].

The protective effects of pretreatments with vitamins C and E on TDF and NVP-induced liver and renal damage observed in this study could be attributed to the abilities of these vitamins to inhibit the oxidative activities of TDF-NVP in the liver and kidney of treated animals [48]. Vitamin C is a water soluble antioxidant that scavenges free radicals by readily donating electrons to unstable and highly reactive molecules during biological reactions [49, 50]. This enables vitamin C mop up free oxygen radicals, disorganize and break peroxidation chain reactions thereby preventing oxidative

damage [51]. Vitamin E is a lipid soluble antioxidant that scavenges oxidative radicals. It has a structure that increases its efficiency as an antioxidant due to its ability to donate hydrogen from the hydroxyl group on the ring structure to free radicals thereby inactivating free radicals. Vitamin E when administered enters the phospholipids bilayer of cell membranes where it biologically protects polyunsaturated fats and other components of cell membranes from free radical-induced damage [52-54]. In the present study, maximal mitigation obtained in animals pretreated with combined doses of vitamin C and E could be attributed to synergy in the antioxidant activities of these vitamins. Studies have shown that vitamin C can regenerate oxidized vitamin E, thiol antioxidants, such as glutathione and lipoic acid can also regenerate vitamin E indirectly via vitamin C [55].

5. Conclusion

This study demonstrated that tenofovir and nevirapine- induced hepatorenal toxicity was mitigated by pretreatments with individual doses of vitamins C and E. Maximal mitigation was observed in animals pretreated with a combination of vitamin C and E which could be attributed to synergy in the activities of these vitamins.

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Conflict of Interest

The authors declare no conflict of interest.

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