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Original Research Article

Remedial Effects of Vitamins C and E on Atazanavir/Ritonavir-Induced Alterations in Biomarkers of Renal Function and Oxidative Stress of Male Albino Rats

Elias Adikwu[*1], Odoko Joseph Onyedenyifa[2] and Akpe Anthony Bibowei[2]

[1]*Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria*
[2]*Department of Community Health Sciences, College of Health Technology, Otuogidi, Bayelsa State, Nigeria*

***Correspondence Info:**
Elias Adikwu,
Department of Pharmacology,
Faculty of Basic Medical Sciences,
University of Port Harcourt, Choba, Rivers State, Nigeria
E-mail: adikwuelias@gmail.com

Abstract

Background: Atazanavir/ritonavir (ATV/r) combination is used for the management of human immunodeficiency virus and could be associated with renal toxicity characterized by oxidative stress.
Objective: This study investigated the effects of vitamins C and E on baseline and ATV/r- induced serum levels of creatinine, urea, uric acid, total protein, albumin and kidney levels of malondialdehyde, catalase, superoxide dismutase, glutathione and glutathione peroxidase of male albino rats.
Materials and Methods: Fifty four male albino rats used for this study were divided into 9 groups, (A – I) of 5 animals each. Group A (placebo control) and group B (solvent control) received water and arachis oil orally for 30 days respectively. Groups C- F received vitamin C (20mg/kg), vitamin E (20mg/kg), combined dose of vitamin E+C and ATV/r (90/30mg/kg) orally for 30 days respectively. Groups G – I were pretreated with vitamin C, vitamin E and vitamin C+E orally before the oral administration of ATV/r for 30 days respectively.
Results: Baseline serum creatinine, urea, uric acid and kidney MDA levels were significantly (p<0.05) decreased while kidney SOD, CAT, GSH, and GPX levels were increased in vitamins C and E treated animals when compared to the control. In contrast, ATV/r treatment significantly (p<0.05) increased serum creatinine, urea, uric acid and kidney MDA levels while serum total protein, albumin, kidney SOD, CAT, GSH, and GPX levels were decreased when compared to the control. However, ATV/r –induced changes in the above parameters were attenuated in vitamins C and E pretreated animals with maximal attenuation observed in animals pretreated with combined doses of vitamin C and E.
Conclusion: Observations in this study could be attributed to the oxidative effect of ATV/r and the antioxidant effects of vitamins C and E.
Keywords: Atazanavir, Ritonavir, Kidney, Oxidative Stress, Vitamins

1. Introduction

The kidney performs essential functions which include clearance of endogenous waste products, maintenance of electrolyte, endocrine function, metabolism and excretion of exogenously administered therapeutic and diagnostic agents [1]. In the elimination of exogenous drugs and toxins, the kidney is vulnerable to various forms of injury. Kidney injury due to drugs and chemicals is highly variable and is influenced by several factors. Among these is the direct toxic effect of drugs and chemicals on the nephron, the effects on renal function, the high metabolic activity of particular segments of the nephron, the multiple transport systems which can result in intracellular accumulation of drugs and chemicals, and the high concentrations in the kidney with possible precipitation and crystallization of particular drugs [1,2]. Several therapeutic agents have known nephrotoxic potential; examples include anti-microbial agents, analgesics, and immunosuppressive, anti-hypertensive and antiretroviral agents [3].

Incidence of kidney injury due to antiretroviral drugs is on the increase due to reports from several studies. Prolonged used of antiretroviral drugs could contribute to renal dysfunctions like acute tubular necrosis, acute interstitial nephritis, and crystal nephropathy [4,5]. Atazanavir boosted ritonavir is an antiretroviral drug combination used in the management of human immunodeficiency virus. In 2004, it was approved in Japan and later in Europe for the management of HIV

infection [6]. It is used as one of the first-line antiretroviral drug due to its efficacy and tolerability [7, 8]. However, the use of ATV/r, could be associated with renal toxicity which is a limiting factor [9]. Atazanavir has the potential to yield its crystalline precipitation in urine and renal interstitial tissues, leading to crystalluria, urolithiasis, acute kidney injury and chronic kidney disease [10, 11]. Renal pathological findings due to treatment with ATV/r showed interstitial nephritis, tubular atrophy, interstitial fibrosis, infiltration of lymphocyte and plasma cells into interstitial tissues [12]. In addition, oxidative damage characterized by increase in malondialdehyde level with decreases in antioxidant levels were reported in the kidneys of ATV/r treated animals [13].

Antioxidants are chemical substances that protect cells against oxidative stress via scavenging of free radical and activation of other antioxidants [14]. Vitamins C and E are water and lipid soluble antioxidants respectively which can inhibit oxidative stress and lipid peroxidation in vivo and in vitro. Vitamin C is a monosaccharide oxidation-reduction (redox) catalyst which can scavenge and neutralize reactive oxygen species [15]. In addition to its direct antioxidant effect, vitamin C is a substrate for redox enzyme ascorbate peroxidase, a function that is particularly important in oxidative stress resistance [16]. Vitamin E as a fat-soluble antioxidant that could prevent the production of reactive oxygen species formed when fat undergoes oxidation and it protects cell membrane from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [17, 18]. Synergy in antioxidant activities have been reported with concurrent use of vitamins C and E [19]. Pretreatments with vitamins C and E have been reported to attenuate alterations in biochemical parameters and prevented oxidative damage induced by xenobiotics [20]. This study was designed to investigate the effects of vitamins C and E on baseline and ATV/r- induced serum levels of creatinine, urea, uric acid, total protein, albumin and kidney levels of malondialdehyde, catalase, glutathione, superoxide dismutase and glutathione peroxidase in male albino rats.

2. Materials and Methods

2.1 Animals

Adult male albino rats of average weight 250 ± 5.0g were used for this study. The rats were obtained from the animal house of the Department of Pharmacology and Toxicology, Madonna University Elele. Animals were allowed to acclimatize for 14 days and had free access to food and water *ad libitum*.

2.2 Drugs

ATV/r was manufactured by Mylan laboratories limited, India while vitamins C and E were obtained from Shijiazhuang *Aopharm* Import & Export Trading *Co*., *Ltd*. Shijiazhuang, *China*. All other chemicals used for this study were of analytical grade. Doses of vitamin C (20mg/kg), and vitamin E (20mg/kg) were used for this study [21].

2.3 Experimental Design

Fifty four (54) male albino rats were divided into 9 groups, A – I of 5 animals each. Group A (placebo control) and group B (solvent control) orally received water and arachis oil for 30 days respectively. Groups C- F orally received vitamin C (20mg/kg), vitamin E (20mg/kg), a combined doses of vitamin E+C and ATV/r (90/30mg/kg) for 30 days respectively. Groups G – I were orally pretreated with vitamin C, vitamin E and vitamin C+E before the oral administration of ATV/r for 30 days respectively.

2.4 Collection of Samples

Animals were sacrificed at the end of 30 days of drug treatment with the aid of diethyl ether. Blood sample was collected and serum extracted for evaluation of renal function parameters. Animals were dissected; kidneys were collected and washed in an ice cold 1.15% KCL solution. Kidneys were then homogenized with 0.1M phosphate buffer (pH 7.2). The resulting homogenates were centrifuge at 2500 rpm speed for 15minutes and the supernatants were decanted and assayed for oxidative stress biomarkers

2.5 Evaluation of Renal Function Parameters

Total protein and albumin were evaluated as reported by Ibiam et al., 2013 [22] while Creatinine, urea and uric acid were evaluated as reported by Prabu et al., 2014 [23]

2.6 Oxidative Stress Indices Assay

Glutathione peroxidase [23], Superoxide Dismutase [24], Catalase [25], Reduced Glutathione [26] and Malondialdehyde [27]

2.7 Statistical Analysis

Results are expressed as Mean + SEM. One way analysis of variance (ANOVA) was used for the analysis of the results and statistical significance was set at $p<0.05$

3. Results

3.1 Renal Function Parameters

This study observed significant ($p<0.05$) decreases in baseline serum creatinine, urea and uric acid levels in animals treated with individual doses of vitamins C and E when compared to the control. Maximal and significant ($p<0.05$) decreases in these parameters were obtained in animals treated with combined doses of vitamin C and E when compared to the control (Table1). Treatment with vitamins C and E did not produce significant ($p>0.05$) effects on serum total protein and albumin levels when compared to the control (Table 1). On the contrary, serum creatinine, urea and uric acid levels were increased significantly ($p<0.05$) in animals treated with ATV/r when compared to the control. However, these serum parameters were significantly ($p<0.05$) decreased by pretreatments with individual doses of vitamins C and E when compared with ATV/r treatment (Table 2). Further and significant ($p< 0.05$) decreases in these serum parameters were obtained with pretreatment using combined

doses of vitamin C and E when compared to pretreatments with individual doses of vitamins E and C. Furthermore, this study observed significant ($p< 0.05$) decreases in serum total protein and albumin levels in ATV/r treated animals when compared to the control. However, these serum parameters were significantly ($p< 0.05$) increased with pretreatments using individual doses of vitamins C and E when compared to treatment with ATV/r. Interestingly, pretreatment with combined doses of vitamin C and E produced maximal and significant ($p< 0.05$) increases serum levels of total protein and albumin when compared to serum levels obtained by pretreatments with individual doses of vitamins C and E (Table 2).

3.2 Oxidative Stress Indices

Furthermore, treatment with individual doses of vitamins C and E produced insignificant ($p>0.05$) increases in baseline kidney SOD, CAT, GSH and GPX levels with decrease in MDA level when compared to the control. Maximal and significant ($p<0.05$) increases in baseline kidney SOD, CAT, GSH and GPX levels with decrease in MDA level were obtained in animals treated with combined doses of vitamin C and E when compared to the control (Table 1). On the contrary, treatment with ATV/r significantly ($p<0.05$) decreased kidney SOD, CAT, GSH and GPX levels to 1.90 ± 0.01, 5.45 ± 0.01, 4.10 ± 0.02 and 2.00 ± 0.01 U/mg protein with increase in MDA level to 2.61 ± 0.03 when compared to the control. However, SOD, CAT, GSH, CAT and GPX levels were increased to 3.70 ± 0.01, 13.7 ± 0.03, 7.93 ± 0.02 and 3.24 ± 0.02 U/mg protein while MDA level was decreased to 1.50 ± 0.03 nmole/mg protein in vitamin C pretreated animals. Vitamin E pretreatment increased SOD, CAT, GSH and GPX levels to3.81 ± 0.03, 13.83 ± 0.02, 8.82 ± 0.01 and 3.57 ± 0.04 U/mg protein with decrease in MDA level to 1.42 ± 0.03 nmole/mg protein respectively. These pretreatments values were significantly ($p< 0.05$) different when compared to values obtained in ATV/r treated animals. This study noticed that pretreatment with a combination of both vitamins further increased SOD, CAT, GSH, CAT and GPX levels to 5.90 ± 0.03, 25.9 ± 0.01, 11.4 ± 0.07 and 6.97± 0.01 U/mg protein with decrease in MDA level to 0.71 ± 0.02 nmole/mg protein respectively. These values were significantly ($p< 0.05$) different when compared to values obtained with pretreatments using individual doses of vitamins C and E (Fig 1-5).

Table 1: Effects of treatments with vitamins C and E on baseline serum renal function parameters and kidney oxidative stress indices of male albino rats

Parameters	Urea mg/dl	Uric acid (mg/dL)	Creatinine (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)
Control	36.8 ± 0.08	1.50 ± 0.03	1.72 ± 0.04	7.60 ± 0.05	3.32 ± 0.04
VIT C (20mg/kg)	31.8 ± 1.12	1.13 ± 0.01*	1.15 ± 0.02*	7.68 ± 0.01	3.40 ± 0.06
VIT E (20mg/kg)	29.2 ± 1.05	1.10 ± 0.03*	1.10 ± 0.05*	7.71 ± 0.07	3.43 ± 0.01
VIT C+ E	20.9 ± 1.39**	0.70 ± 0.05**	0.67 ± 0.06**	7.90 ± 0.03	3.41 ± 0.02
Parameters	**MDA nmole/mg protein**	**SOD U/mg protein**	**CAT U/mg protein**	**GSH U/mg protein**	**GPX U/mg protein**
Control	0.67 ± 0.01	6.30 ± 0.07	9.70 ± 0.04	6.30 ± 0.06	8.82 ± 0.09
VIT C (20mg/kg)	04.8 ± 0.06*	7.13 ± 0.05	10.0 ± 0.01	7.10 ± 0.05*	10.15 ± 0.02
VIT E (20mg/kg)	0.40 ± 0.04*	7.17 ± 0.03	10.9 ± 0.02	7.15 ± 0.09*	10.10 ± 0.05
VIT C+ E	0.21 ± 0.01**	13.4 ± 0.55**	14.7 ± 0.07**	8.24 ± 0.09*	12.67 ± 0.06*

MDA; Malondialdehyde, SOD; Superoxide Dismutase; CAT; Catalase, GSH; Glutathione, GPX; Glutathione peroxidase, VIT; Vitamin, Data are expressed as Mean± SEM, n=5, * Significant ($p<0.05$) difference when compared to the control. ** Significant ($p<0.05$) difference when compared to treatments with individual doses of vitamins C and E

Table 2: Effects of pretreatments with vitamins C and E on serum renal function parameters of ATV/r treated male albino rats

Parameters	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Total Protein (mg/dL)	Albumin (mg/dL)
Control	1.32 ± 0.04	36.8 ± 0.08	1.53 ± 0.03	7.60 ± 0.05	4.32 ± 0.04
ATV/r	4.20±0.01	78.8±0.07	4.61±0.06	2.21±0.07	1.06±0.07
ATV/r + VIT C	2.71± 0.04*	50.3 ± 1.16*	2.35 ± 0.07*	4.29 ± 0.05*	2.20 ± 0.01*
ATV/r + VIT E +	2.65 ± 0.03*	52.2 ± 1.01*	2.25 ± 0.01*	4.41 ± 0.07*	2.41 ± 0.01*
ATV/r + VIT C+ E	1.27 ± 0.08**	35.3 ± 0.19**	1.30 ± 0.03**	6.90 ± 0.03**	4.11 ± 0.02**

Data are expressed as mean± SEM. n=5 * Significant ($p<0.05$) difference when compared to ATV/r treated rats. **Significant ($p<0.05$) difference when compared to pretreatments with individual doses of vitamins C and E

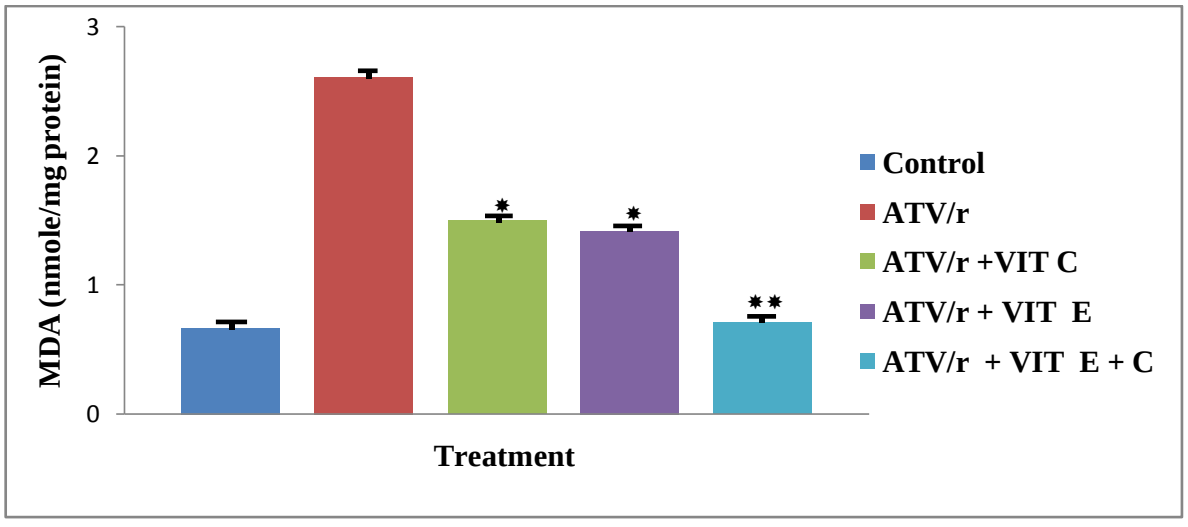

Fig 1: Effects of pretreatments with vitamins C and E on ATV/r-induced kidney level of malondialdehyde in male albino rats. * Significant (p<0.05) difference when compared to treatment with ATV/r. ** Significant (p<0.05) difference when compared to pretreatments with individual doses of vitamins C and E

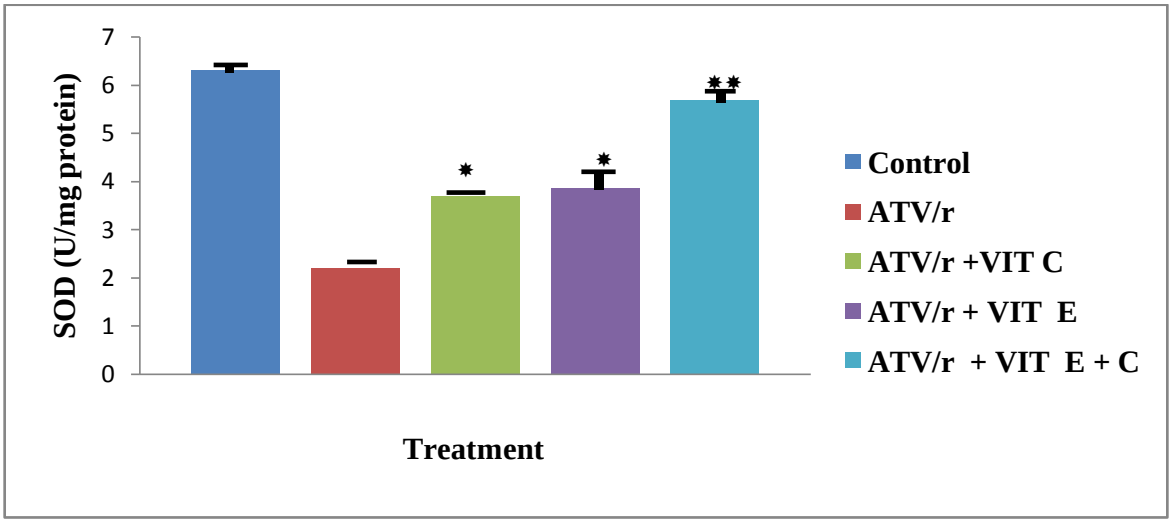

Fig 2: Effects of pretreatments with vitamins C and E on ATV/r- induced kidney level of superoxide dismutase in male albino rats. * Significant (p<0.05) difference when compared to treatment with ATV/r. ** Significant (p<0.05) difference when compared to pretreatments with individual doses of vitamins C and E

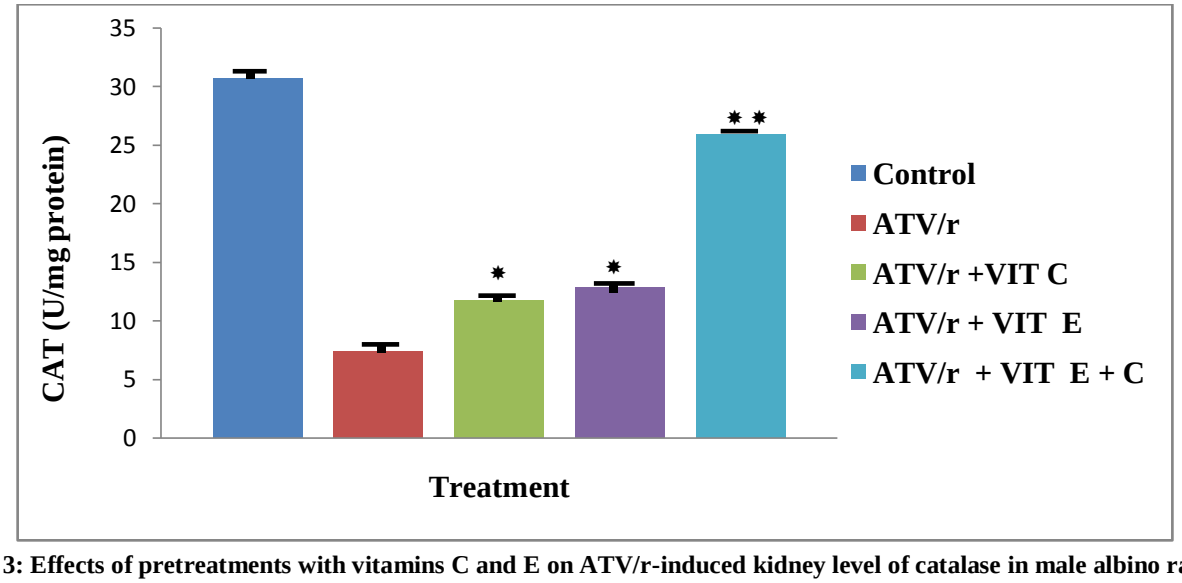

Fig 3: Effects of pretreatments with vitamins C and E on ATV/r-induced kidney level of catalase in male albino rats. * Significant (p<0.05) difference when compared to treatment with ATV/r. ** Significant (p<0.05) difference when compared to pretreatments with individual doses of vitamins C and E

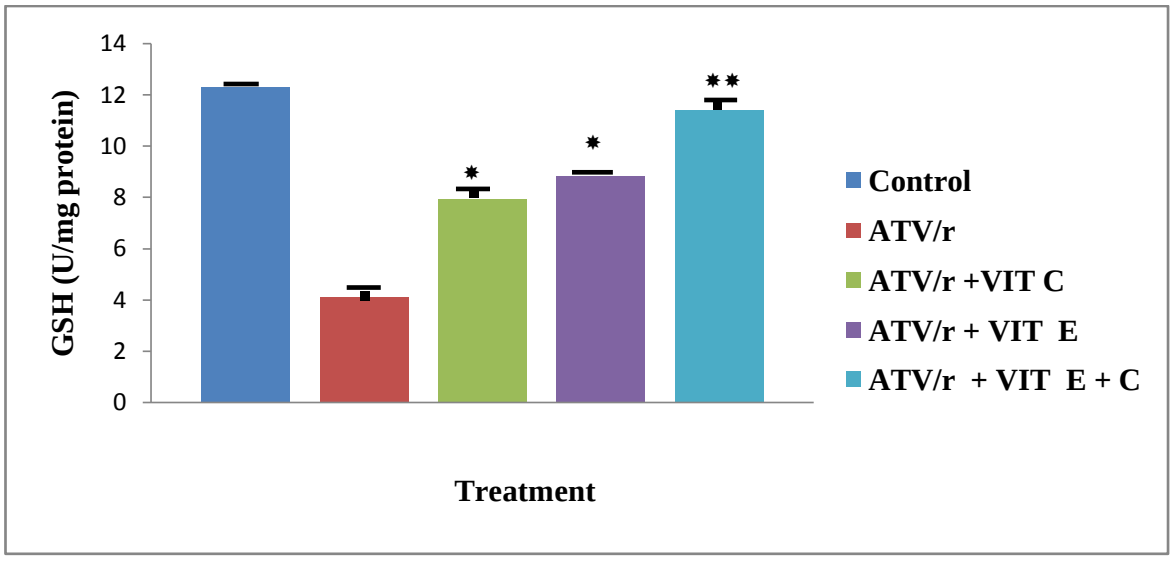

Fig 4: Effects of pretreatments with vitamins C and E on ATV/r-induced kidney level of glutathione in albino rats. *Significant (p<0.05) difference when compared to treatment with ATV/r. ** Significant (p<0.05) difference when compared to pretreatments with individual doses of vitamins C and E

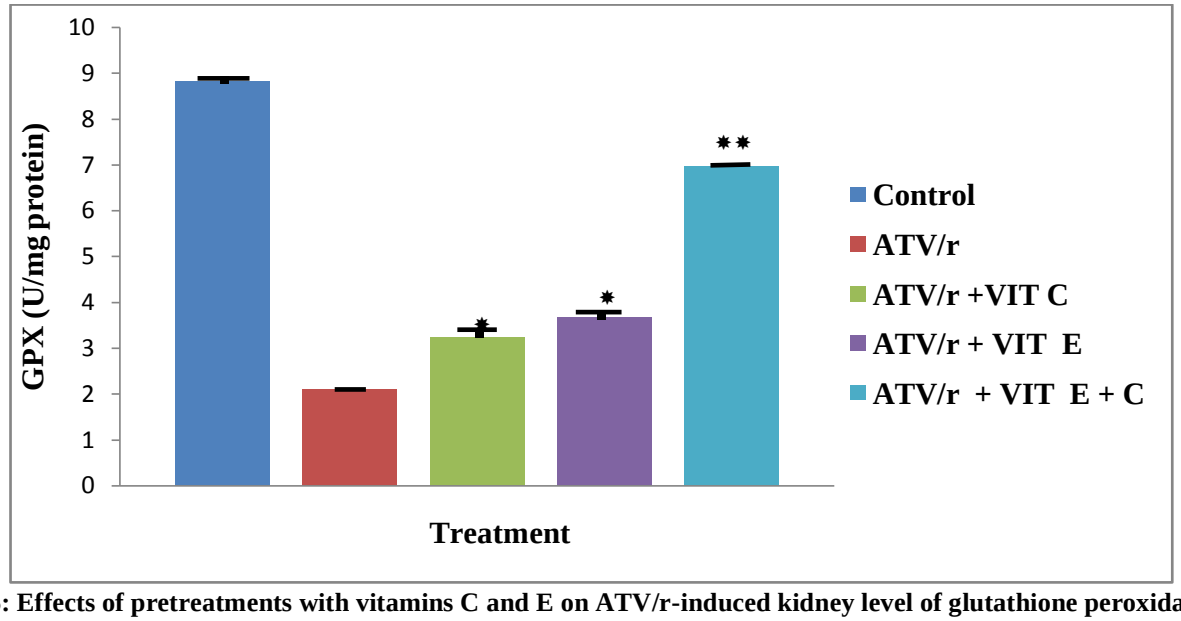

Fig 5: Effects of pretreatments with vitamins C and E on ATV/r-induced kidney level of glutathione peroxidase in albino rats. *Significant (p<0.05) difference when compared to treatment with ATV/r. ** Significant (p<0.05) difference when compared to pretreatments with individual doses of vitamins C and E

4. Discussion

The kidney plays a special role in concentrating toxic substances within its tubules and excreting them. These functions render it susceptible to damage by certain chemical substances. The use of ATV/r combination could be associated with renal toxicity characterized by oxidative stress [28]. Therefore, the present study investigated the effects of vitamins E and C on baseline and ATV/r- induced serum levels of creatinine, urea, uric acid, total protein, albumin and kidney levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and glutathione peroxidase (GPX) in male albino rats. Treatment with vitamins C and E decreased baseline serum creatinine, urea and uric acid levels which is consistent with some reported observations [29, 30]. Baseline Kidney MDA level was decreased while SOD, CAT, GSH and GPX levels were increased in vitamins E and C treated animals. This observation is consistent with previous reports [31]. Maximal effects on these evaluated parameters were obtained in animals treated with a combination of vitamin C and E. On the other hand, treatment with ATV/r increased serum levels of creatinine, urea, uric acid and Kidney MDA level while kidney SOD, CAT, GSH and GPX levels were decreased. However, pretreatments with individual doses of vitamins C and E decreased serum levels of creatinine, urea and uric acid and Kidney MDA level while kidney SOD, CAT, GSH and GPX levels were increased. Maximal effects on these parameters were obtained in animals pretreated with a combination of vitamin C and E.

In the present study, increases in serum levels of creatinine, urea, and uric acid observed in ATV/r treated animals are indicators of renal toxicity. This observation is consistent with previous studies [32], and could be due ATV/r-induced oxidative stress in the kidneys of treated animals which might have stimulated the activities of the mediators of renal vasoconstriction leading to decreased glomerular filtration rate [33,34]. Observed increase in MDA level with decreases in SOD, CAT, GSH and GPX levels observed in animals treated with ATV/r are indicators of oxidative stress and lipid peroxidation [35]. Studies have shown that antioxidants like SOD, CAT, GSH and GPX have been developed by mammalian cells to abrogate or prevent free radical-induced oxidative damage [36]. However, when a condition of oxidative stress establishes, the defense capacities against free radicals become insufficient [37]. This condition can stimulate depletion of intracellular concentrations of antioxidants thereby decreasing their activities [38]. Furthermore, lipid peroxidation can stimulate reactive electrophiles-induced macromolecular damage, increase membrane permeability and promote efflux of cytosolic solutes [39,40].

Atenuation of ATV/r -induced alterations in renal function parameters and kidney oxidative stress indices by pretreatments with vitamins C and E could be attributed to their abilities to inhibit ATV/r-induced oxidative stress and lipid peroxidation through scavenging of free radicals and up-regulation of the activities of other antioxidants. These vitamins scavenge free radicals by readily donating electrons to unstable and highly reactive molecules (ROS) during biological reactions and become oxidized themselves [41]. Vitamin E is an antioxidant that scavenges free radicals and is the first line of defense against the peroxidation of fatty acids in phospholipids of cell membranes [42]. It maintains the permeability and fluidity of biological membranes and prevents them from oxidative damage [43]. The antioxidant effects of vitamin E can be up-regulated by its metabolites such as carboxyethyl hydroxychromans (CEHC) that have anti-inflammatory and antioxidant properties [43]. Studies have shown that vitamin E can regenerate and sustained the activities of some endogenous antioxidants [44]. Vitamin C is a water-soluble antioxidant that mediates its effect by scavenging and neutralizing free radicals [45,46]. It has diverse biological functions which include acting as a cofactor for the enzymes involved in collagen hydroxylation, biosynthesis of carnitine and norepinephrine [47]. It could up-regulate the activities of other antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase and can regenerate oxidized vitamin E [48] .Maximal effects observed on all evaluated parameters with pretreatment using a combination of vitamin E and C could be attributed to synergy in antioxidant activities [49].

5. Conclusion

The present study showed that pretreatments with vitamins C and E mitigated ATV/r-induced alterations in kidney function and oxidative stress indices. Maximal mitigation was observed in animals pretreated with combined doses of vitamin C and E which could be attributed to synergy in their antioxidant activities. Observations in this study could be attributed to the oxidative effect of ATV/r and the antioxidant effects of vitamins C and E.

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