Original Article

Supplementation of Vitamins C, E and its Combination on Paraquat-Intoxicated Rats: Effects of some Biochemical and Markers of Oxidative Stress Parameters

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ABSTRACT
Paraquat (PQ) is a toxic chemical that is widely used as an herbicide in developing countries. It has been described as a major suicide agent, thus leading to its ban or restriction in use by Environmental Protection Agency in some countries. There is no known chelating agent or antidote for PQ. This study investigated protective effects of antioxidant vitamins C, E and its combination in both pre-treatments and post-treatments. Pre-treatment of rats with vitamins C, E and C+E gave survival rates of 40%, 20%and 20% respectively while post-treatment gave 80%, 20% and 20% respectively when lethal dose (150mg/Kg) of PQ was administered. However, when sub-lethal dose (75mg/Kg) was administered, biochemical investigations revealed a significant (p<0.05) increase in cholesterol, SOD, CAT, POD and GPx activities, decreased total protein and triglyceride in PQ treated rats. The extent of lipid peroxidation as measured by malondialdehyde (MDA) concentration was more pronounced in the lung than in the liver. Histopathological investigations revealed proliferation of the bile duct and severe centrilobular necrosis in the liver and severe haemorrhage in the lungs of rats treated with PQ alone compared to the control. No visible lesion except hepatic regeneration and mild congestion of the liver and kidney of vitamin C post-treated rats were observed. The results also provided some evidence in respect of the potency of vitamin C post-treatment in conferring some level of protection against PQ-induced oxidative stress by modulating the extent of lipid peroxidation and antioxidant enzyme activities.
Keywords: vitamins C and E, paraquat, oxidative stress, rats.

INTRODUCTION

PQ has been described as a major suicide agent in many countries not only because it is highly acutely toxic, but also because it is readily available, relatively cheap and had no known antidote [1]. Fatality rate for intentional PQ ingestion ranges from 58% in Fiji [2] to nearly 80% in Southern Mexico [3]. Each year around the world, there are estimated to be more than thousands of suicides due to PQ ingestion, though problems with poor reporting and data collection in developing countries made the real data and magnitude of the problem unknown. Forensic analysis of fatal intentional poisoning in South Trinidad showed that in 105 deaths from poisoning in 1996-97, PQ was the causative agent in 80 cases (76%) [4]. In Samoa, PQ was banned for being used as a suicide agent in 70% of all suicide causes between 1996 and 2000 [5]. In Sri Lanka, it was observed that two of the three peaks of seasonal incidence of PQ poisoning (intentional and unintentional use) are related to the most common time of use of PQ [6]. A link was also revealed between the monthly incidence of PQ poisoning and the volume of monthly PQ imports in Surinam [7]. In Costa Rica, hundreds of PQ poisoning occur each year, most of them in the banana producing atlantic region [8]. In a review of poisoning incidents, Garnier *et. al.,* [9] concluded that poisoning as a result of accidental ingestion of paraquat was now rear in Europe not only because of improved training, but also due to addition of emetic agent to commercial products. A twenty year survey from the National Poison Information Centre (London), noted in 2001 that most of the cases of poisoning from mistaken ingestion occurred at the start of the study in the early 1980s with the last one recorded in 1992, confirming the virtual disappearance of accidental fatalities since their peak in the early 1970s [10]. Surprisingly, to the best of our knowledge, there is no known chelating agent or antidote against paraquat toxicity. However, since PQ mechanism of action involved generation of free radicals that causes oxidative stress, this study was therefore carried out to investigate ameliorating effect of antioxidant vitamins (vitamins C and E) and its combination in both pre- and post-treatments against PQ toxicity.

MATERIALS AND METHODS

White albino rats of both sexes weighing between 150-250g purchased from the College of Veterinary Medicine, University of Agriculture, Abeokuta, were used in this study. They were housed in wood chips cages, allowed to acclimatize for a week with free access to tap water and feeds (Vital feeds Nig. Ltd.) throughout the period of the study. Care and treatment of the rat followed the Guiding Principles in the use of Animals in Toxicology by the Society of Toxicology. In the first experiment, 48 rats divided into groups of 8 were used. The control was given only feed and water while group 2 were challenged with lethal dose of paraquat (150mg/Kg), groups 3, 4 and 5 were also challenged with lethal dose of PQ after which they were post-treated with mega doses of vitamins C (1000mg/Kg), E (300mg/Kg), C+E respectively. Groups 6, 7 and 8 were pre-treated with mega doses of vitamins C, E, C+E respectively before being challenged with lethal dose of PQ. The survival rate for each group was calculated and plotted against days.

In another experiment, seventy seven (77) rats were divided into eleven (11) groups consisting of seven rats per group. The first group was given only water and feeds throughout the experimental period. The second group, paraquat group (PQ), received sub-lethal dose of PQ (70mg/Kg) orally. The third, vitamin C group, received mega dose of vitamin (1000mg/.kg) while the fourth group received mega dose of vitamin E (300mg/kg). Group 5, vitamin C and E group, received mega doses of both vitamins. Group 6, vitamin C and PQ group, were pre-treated with mega dose of vitamin C for seven days before being challenged with paraquat while group 7, vitamin E and PQ group, were pre-treated with vitamin E for seven days after which they were challenged with PQ, group 8 were given mega doses of vitamin C and E prior to administration of PQ. Group 9, PQ-vitamin C group received paraquat and were post-treated with vitamin C while group 10, PQ - vitamin E group, were administered with PQ and thereafter post-treated with vitamin E, group 11 received PQ and then post-treated with combination of vitamins C and E.

Twenty four hours after the last treatment, two sets of blood were collected, one from the tail vein, into heparinised glass capillary tube and used for determination of packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count and white blood cell (WBC) count using standard methods [11]. Another set was collected by cardiac puncture into heparinised tubes. These were centrifuged at 5000 rpm for 30 minutes to separate the plasma and red blood cells meant for biochemical assay. The animals were then sacrificed, dissected and section of the liver was excised and used for the preparation of cellular fraction. The lung, kidney and other parts of the liver were fixed in Bouin solution prior to histopathological examinations.

Biochemical Assays

Liver marker and antioxidant enzymes assays.

The activities of alanine transaminase (ALT), aspartate transaminase (AST) and acid phosphatase (AP) were determined using Randox reagent diagnostic kits. The activities of CAT, SOD and GPx were determined following the methods described by Aebi [12], Winterbourm *et. al*., [13] and Rotruck *et al*., [14] respectively.

Reduced Glutathione (GSH) Determination.

Glutathione level was measured according to the method of Ellman [15].

Estimation of Lipid Peroxidation.

Lipid peroxidation in the lung and liver was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) method of Draper *et al.,* [16].

Determination of some biochemical parameters.

The determination of total protein (TP), triglyceride (TG), total cholesterol (TC), creatinine, albumin and bilirubin were determined according to the methods of Gornall *et. al.,* [17], Allain *et.al.,* [18], Jaffe [19], Doumas *et. al.,* [20] and Jendraski and Groff [21] respectively. Very low density lipoprotein (v LDL) and low density lipoprotein (LDL) were estimated using modified Friedwald formula [22].

Histopathological procedures

Lung, kidney and liver harvested from the sacrificed rats and the fragments from tissues were fixed in Bouin fluid (picric acid + formalin + acetic acid) embedded in paraffin and then stained with hemotoxylin and eosin (HE). Preparations were evaluated with a light field microscope and were photographed (Olympus, CS21).

Statistical Analysis

Values were expressed as Mean±Standard Error of Mean. The level of homogeneity among the groups was tested using Analysis of Variance (ANOVA). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). All analysis was done using Statistical Package for Social Science (SPSS) version.

RESULTS

The results of the effects of treatments of the survival rate of rats given lethal dose (150mg/Kg) of paraquat are shown in figure 1.

Pretreatment with vitamins C, E and combination of both gave a survival rate of 40%, 20% and 20% respectively while post-treatment gave 80%, 20% and 20% respectively.
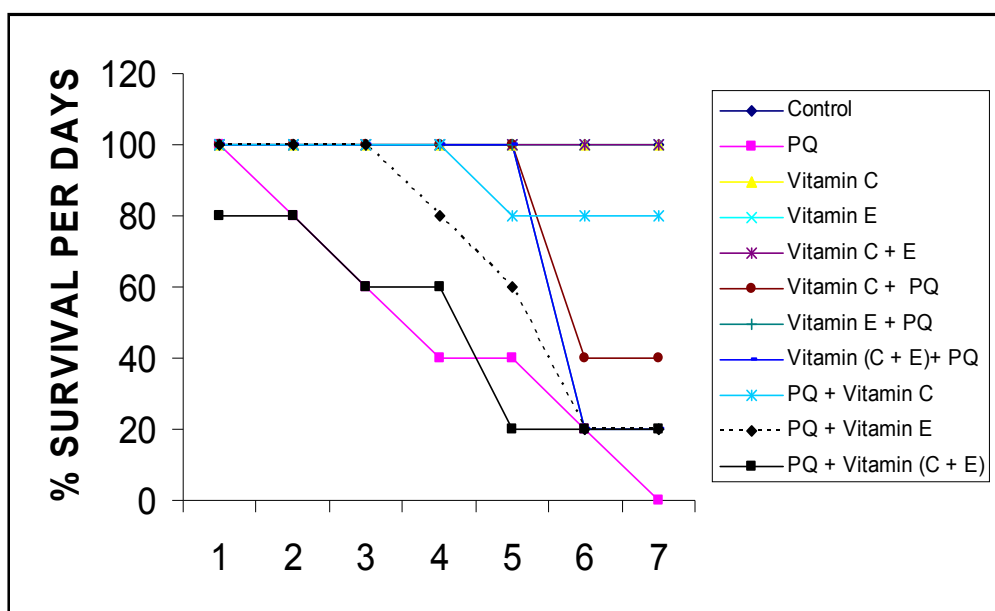

Fig1: Effects of treatments of the survival rate of rats given lethal dose (150mg/Kg) of paraquat.

Table 1: EFFECTS OF TREATMENTS ON HAEMATOLOGY OF RATS

Parameters ============ Groups	PCV	Hb (g/dl)	RBC (Mil/mm^3)	WBC (No/mm ^{3})
Control	36.25 ± 3.13[a]	12.13 ± 1.04[a]	4.50 ± 0.58[a]	4700 ± 145.62[a]
PQ	30.5 ± 3.41[b]	10.02 ± 0.3 9[b]	3.45 ± 0.13[c]	6750 ± 186.35[b]
Vit C	37.1 ± 3. 10[a]	12.40 ± 1.06[a]	3.70 ± 0.30[c]	4800 ± 140.5[a]
Vit E	36.3 ± 3.14[a]	12.10 ± 1.03[a]	3.75 ± 0.30[c]	4850 ± 142.5[aa]
Vit (C+E)	36.2 ± 3.12[a]	12.10 ± 1.02[a]	3.85 ± 0.35[c]	4950 ± 161.00[a]
Vit C+PQ	33.75 ± 2.0	11.25 ± 0.91[c]	3.58 ± 0.12[c]	5000 ± 166.72[c]
Vit E+PQ	34.25 ± 2.02	12.63 ± 1.40	3.69 ± 0.15[c]	5200 ± 168.72[c]
Vit (C+E) +PQ	34.50 ± 2.03	11.70 ± 0.96[ca]	3.62 ± 0.13[c]	5350 ± 170.32[c]
PQ+ Vit C	36.30 ± 3.06[a]	12.10 ± 1.04[a]	3.55 ± 0.23 [b]	5050 ± 156.72[a]
PQ+Vit E	35.26 ± 3.02[a]	12.00 ± 1.00[a]	3.50 ± 0.22 [b]	4750 ± 140.6[a]
PQ + Vit (C+E)	36.70 ± 3.08[a]	12.24 ± .I 0[a]	3.50 ± 0.14 [b]	4950 ± 158.25[a]

Values expressed as Mean ± SEM. Values along the same column with different superscripts are significantly different at p<0.05.

Table 1 represents the haematological parameters of each experimental group after treatments. Results of PCV which is a measure of the relative mass of red cells in the whole blood showed an increase in all rats

treated with vitamins when compared to others that were not treated. However, a significant ($p<0.05$) reduction in PCV was observed in the rats treated with PQ alone. A reduction (though not significant) in PCV value was observed in the rat pre-treated with vitamins compared to post-treated groups. Results obtained also indicate a direct relationship between Hb concentration and PCV as both parameters were observed to follow the same pattern in the entire treated group. The WBC count was highest in group 2 (PQ alone) and lowest in the control group. The RBC count was found to be lowest in the group of rats that were treated with PQ alone and this demonstrates the possibility of PQ to induce anemia. An increase in RBC count was observed in the groups of rats that were pre-treated with vitamins C, E and both when compared to post-treated groups.

Table 2: EFFECTS OF TREATMENTS ON SOME BLOOD CHEMISTRY PARAMETER OF RATS

Parameters/ Groups	Protein (g/dl)	Bilirubin (g/dl)	Creatinine (g/dl)	Albumin (g/dl)
Control	5.38± 0.20[cd]	0.53 ± 0.01[d]	0.53± 0.04 [de]	2.84 ± 0.03[d]
PQ	3.43± 0.1[a]	2.34 ± 0.03[f]	0.64± 0.02[a]	1.46 ± 0.11[a]
Vit. C	4.80 ±0.29[bc]	0.23 ± 0.0 1[a]	0.42± 0.03[abc]	2.41± 0.02[b]
Vit. E	4.98 ±0.25[bc]	0.44 ± 0.01[c]	0.52± 0.01[de]	2.64 ±0.05[bcd]
Vit. (C+E)	4.81± 0.3[bc]	0.53 ± 0.01[d]	0.45 ±0.02[bcd]	2.86 ±0.14 [d]
Vit. C+PQ	4.95± 0.37[bc]	0.47 ± 0.02[c]	0.35 ± 0.01[a]	2.75± 0.01[bcd]
Vit. E+PQ	4.86 ±0.28[bc]	0.70 ± 0.03 [e]	0.35 ± 0.01[a]	2.80± 0.14[cd]
Vit. (C+E)+PQ	5.10 ±0.17[bc]	0.54 ± 0.01[d]	0.40± 0.03[abc]	3.68± 0.2l[e]
PQ+Vit. C	5.16± 0.07[bc]	0.72 ± 0.0l[e]	0.56±0.01[e]	2.74 0.07[bcd]
PQ+Vit E	4.57± 0.14[b]	0.32 ± 0.01[b]	0.47 ±0.06[cd]	2.39 0.02[b]
PQ+Vit (C+E)	5.95± 0.01[d]	0.45 ± 0.01[c]	0.36 ±0.02[ab]	2.44 0.02[bc]

Values expressed as Mean ± SEM. Values along the same column with different superscripts are significantly different at $p<0.05$.

Effects of treatments on some blood chemistry parameters were shown in Table 2. Except for PQ alone group, vitamin E post-treated and vitamin (C+E) post-treated groups, there was no significant ($p>0.05$) difference in the protein concentration between the control and other groups. Rats post-treated with both vitamins had the highest protein level while paraquat group had the least. A significant increase ($p<0.05$) in bilirubin and creatinine levels was observed in paraquat group compared to others. There was no significant different in the creatinine levels between vitamin C post-treated group and control as well as vitamin E post-treated group and control. A significant ($p<0.05$) reduction in albumin level was also observed in paraquat group compared to others groups. There was no significant different in the albumin level among the post-treated rats. Highest level of bilirubin and creatinine was observed in paraquat group.

Table 3: EFFECTS OF TREATMENTS ON LIPID PROFILE

Parameter/ Group	TG (mg/dl)	HDL-C (mg/dl)	Cholesterol (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dI)
Control	32.05 ± 4.75[ab]	13.70 ± 0.07[d]	66.87 ± 5.24 [d]	46.76±5.46[c]	6.41±0.95[ab]
PQ	15.55 ± 0.51[a]	12.70 ± 0.18[b]	87.72 ± 1.35 [e]	71.91±1.35[d]	3.11±0.10[a]
Vit. C	30.97 ± 2.68[ab]	13.72 ± 0.09[d]	65.00± 6.38[cd]	45.09±5.94[c]	6.19±0.54[ab]
Vit. E	44.01 ± 6.14 [b]	11.72 ± 0.07[a]	43.98 ± 2.56[a]	23.45±2.38[a]	8.80±1.23[a]
Vit. (C+E)	48.55 ± 14.32	14.22 ± 0.04 [e]	63.78 ± 4.88[cd]	39.85±4.14[bc]	9.71±2.86[a]
Vit. C+PQ	31.69 ± 2.85[ab]	12.68 ± 0.12[b]	66.03 ± 7.46 [d]	47.01±7.29[c]	6.34±0.57[ab]
Vit. E+PQ	68.76 ± 8.57[c]	13.44± 0.33[cd]	47.97± 6.13[ab]	20.78±6.66[a]	13.75±1.71[c]
Vit. (C+E)+PQ	44.87 ± 8.02[b]	13.54 ± 0.07[a]	58.88±3.56[bcd]	38.04±4.06[bc]	8.97±1.61[a]
PQ+Vit. C	40.68 ± 6.05[b]	13.02± 0.31[be]	68.01 ± 4.72[d]	46.87±5.30c	8.14±1.21[a]
PQ+Vit. E	112.66 ± 6.80[d]	13.76± 0.12[de]	50.44±4.14[ac]	14.14±5.06[a]	22.53±1.36[d]
PQ+Vit(C+E)	149.25 ± 4.19[e]	13.52 ± 0.10[a]	72.01 ± 0.81[d]	28.64±0.69[ab]	29.85±0.84[e]

Values expressed as Mean ± SEM. Values along the same column with different superscripts are significantly different at $p<0.05$.

Effects of treatments on lipid profiles of rats are presented in table 3. A significant ($p < 0.05$) reduction in triglyceride level was observed in paraquat group compared to others. There was no significant ($p > 0.05$) difference in the triglyceride levels of vitamin C pre- and post-treated groups, as well as group that were pre-treated with combination of both vitamins when compared to the control. Higher levels of triglyceride were observed in post-treated groups compared to pre-treated groups. A significant ($p < 0.05$) increase in the total cholesterol and LDL-cholesterol levels were observed in paraquat group compared to other groups. There was no significant difference in the HDL-cholesterol of vitamin E pretreated as well as post-treated groups compared to the control. Highest level of vLDL-cholesterol was observed in the group that was post-treated with combination of both vitamins.

Table 4: EFFECTS OF TREATMENTS ON GLUTATHIONE LEVELS AND LIPID PEROXIDATION IN THE LUNG AND LIVER OF RATS.

Groups	Lung			
	GSH (nmol/mg)	MDA (nmol/mg)	GSH (nmol/mg)	MDA (nmol/mg)
Control	6.42 ± 0.29[a]	0.83 ± 0.02[a]	2.33 ± 0.03[a]	0.82 ± 0.03[a]
PQ	5.25 ± 0.13[b]	4.75 ± 0.07[b]	1.64 ± 0.03[b]	3.41 ± 0.05[b]
Vit C	7.56 ± 0.14[c]	0.93 ± 0.03[c]	2.31 ± 0.03[c]	1.06 ± 0.02[c]
Vit E	7.34 ± 0.21[c]	0.83 ± 0.02[c]	2.35 ± 0.05[c]	1.09 ± 0.02[c]
Vit (C+E)	7.43 ± 0.22[c]	0.87 ± 0.01[c]	2.34 ± 0.03[c]	1.11 ± 0.03[c]
Vit C+PQ	6.71 ± 0.12[ac]	2.10 ± 0.02[d]	3.02 ± 0.01[d]	1.22 ± 0.01[c]
Vit E+PQ	6.52 ± 0.21[a]	2.01 ± 0.02[d]	2.90 ± 0.02[d]	1.40 ± 0.02[c]
Vit (C+E) +PQ	6.81 ± 0.11[ac]	2.09 ± 0.01[d]	2.80 ± 0.02[d]	1.45 ± 0.03[c]
PQ+ Vit C	7.24 ± 0.31[c]	1.22 ± 0.14[ec]	2.31 ± 0.01[ac]	0.99 ± 0.01[ac]
PQ+Vit E	7.13 ± 0.13[c]	1.33 ± 0.15[e]	2.21 ± 0.03[ac]	1.23 ± 0.04[c]
PQ + Vit (C+E)	6.91 ± 0.12[c]	1.12 ± 0.11[e]	2.28 ± 0.01[ac]	1.30 ± 0.05[c]

Values along the same column with different superscripts are significantly different at $p < 0.05$. Each value is average of three determinations

The effect of treatments on the levels of lipid peroxidation in the lung and liver is shown in table 4. There was no significant different in the glutathione levels between the control and pre-treated group. Treatment with PQ alone produced a significant ($p < 0.05$). Reduction in the glutathione levels and an enhanced level of peroxidation (as indicated by the MDA concentration) in both organs when compared to other groups. The extent of lipid peroxidation is more pronounced in the lung than in the liver as indicated by the amount of MDA formed. Post-treatment with vitamins C, E and both produced an increased in glutathione levels in the lung than what was observed in the liver. Post treatment with vitamins seems to reverse the extent of lipid peroxidation, although not to the level of control.

The effect of treatments on the activities of some antioxidant enzymes and markers of hepatotoxicity is shown in table 5. Treatment with PQ alone results in a significant increase in the activities of all the enzymes determined namely ALT, AST, AP, SOD, CAT and GPx when compared to the control. There was a significant ($p < 0.05$) difference in the activities of these enzymes in the pre-treated groups of rats compared to the post-treated ones. Although, none of the treatments was able to bring the activities of these enzymes to the level of control, post treatment with vitamin C produced a greater reduction in the activities of ALT, AST, AP, SOD and CAT when compared to pre-treatment effects, whereas, the reverse was the case in GPx activities.

DISCUSSION
The results from this studies demonstrates that PQ treatment alters enzymatic antioxidant profiles of rat and also that pre-treatment with vitamin C confers a level protection to PQ toxicity than pre-treatment with vitamin E or their combination. Oral administration of mega dose of vitamin C (post-treatment)

Table 5: Effects of Treatments on the activities of some antioxidant enzymes and markers of hepatotoxicity

Groups	ALT (IU/L)	AST (IU/L)	AP (IU/L)	SOD (units/mg)	CAT (units/mg)	GPx (units/mg)
Control	29.4 ± 1.52[a]	52.32 ± 2.3[a]	5.50 ± 0.21[a]	56.3 ± 2.4[a]	23.1 ± 1.2[a]	1.7 ± 0.02[a]
PQ	125.16 ± 6.21[b]	143 ± 3.25[b]	12.30 ± 1.11[b]	87.2 ± 3.3[b]	31.6 ± 2.4[b]	3.60 ± 0.05[b]
Vit. C	28.1 ± 1.0[a]	50.1 ± 1.21[a]	5.51 ± 0.21[a]	57.1 ± 2.3[a]	22.6 ± 1.1[a]	2.60 ± 0.03[c]
Vit. E	28.3 ± 1.22[a]	53.1 ± 2.03[a]	5.60 ± 0.22[a]	58.2 ± 2.4[a]	23.4 ± 1.2[a]	2.71 ± 0.03[c]
Vit. (C+E)	25.1 ± 2.01[a]	53.13 ± 2.00[a]	4.96 ± 0.32[a]	56.3 ± 2.1[a]	20.1 ± 1.1[c]	2.22 ± 0.02[d]
Vit. C+PQ	42.08 ± 3.2[c]	54.16 ± 2.10[a]	10.11 ± 0.91[c]	65.5 ± 2.2[c]	28.0 ± 2.2[d]	2.81 ± 0.02[c]
Vit. E+PQ	55.14 ± 3.2[c]	56.16 ± 1.5[c]	11.01 ± 0.82[c]	66.1 ± 2.3[c]	25.6 ± 2.2[d]	2.86 ± 0.02[c]
Vit (C+E) +PQ	58.01 ± 3.2[c]	60.0 ± 1.03[c]	10.5 ± 0.81[c]	68.0 ± 2.1[c]	26.3 ± 2.3[d]	2.66 ± 0.01[c]
PQ+ Vit C	33.21 ± 3.40[a]	37.0 ± 2.10[d]	8.74 ± 0.86[d]	58.3 ± 2.3[a]	24.2 ± 1.6[a]	3.10 ± 0.03[b]
PQ+Vit. E	34.38 ± 2.62[d]	38.0 ± 2.00[d]	8.36 ± 0.81[d]	59.2 ± 2.1[d]	24.6 ± 1.8[ad]	3.61 ± 0.03[b]
PQ+ Vit. (C+E)	36.20 ± 3.04[d]	39 ± 0.30[d]	8.10 ± 1.0[d]	60.5 ± 2.4[d]	26.3 ± 2.0[d]	3.00 ± 0.03[b]

Values along the same column with different superscripts are significantly different at p<0.05.

confers the greatest form of protection from death (with survival rate of 80%) seven days after PQ treatment while those that were pre-treated with vitamin E gave 20% survival rate. This seems not surprising since vitamin E has been reported to confer protection against PQ-induced injuries mostly in vitamin E deficient animals while normal animals received little or no benefit from additional pharmacological supplementation of vitamin E [23].

The lowered level of PCV recorded in PQ alone group could be due to PQ-induced depletion of GSH on the surface of the RBC, thus making the cell liable to oxidative lysis. This probable accounted for the low Hb level and RBC count observed in this group. Also, the increased WBC count in PQ alone compared to other groups could be attributed to the effort of the rats defense systems to fight against PQ (which is foreign) and toxic to their system.

PQ was found to cause a significant increase in ALT, AST, AP, cholesterol, and creatinine levels. The liver, lung and kidney are considered to be the principal target organs for PQ. The abnormal increase in the activities of ALT and AST are sensitive indicators of hepatotoxicity or a diseased state. Generally, these results may indicate degenerative changes and hypo-function of liver and kidneys. The results also showed that PQ significantly decreased plasma levels of triglycerides, total protein and albumin. The increased level of blood creatinine with decreased level of blood protein may indicate protein catabolism and/or kidney dysfunction. These results clearly showed that PQ has a harmful and stressful influence on the hepatic and renal tissue and is consistent with those reported in the literature of paraquat toxicity [25,25].

A significant increase in total cholesterol and LDL-cholesterol level observed in PQ group compared to other groups indicated that PQ could predispose an animal to the risk of coronary heart disease. There was no significant different in the total cholesterol and LDL-cholesterol levels of groups of rats pre- and post-treated with vitamin C when compared to the control, whereas vitamin E treatment reduced these parameters below the level of control.

Overproduction of ROS or increased oxidative stress is considered a major mechanism involved in the pathogenesis of endothelial dysfunction and might serve as a common pathogenic mechanism in conditions such as hypercholesterolemia, hypertension and diabetes [26]. The high cholesterol level observed in PQ group compared to other groups may be attributed to high $0^{2-.}$generated and is also in agreement with the report of Adachi *et al.,* [26], that PQ administration in rats led to high level of cholesterol. Lee *et al*.,[27] and Kimura *et al* [28] also reported increased serum cholesterol in PQ-induced

oxidative stress condition. However, decreased HDL-cholesterol level coupled with increased total cholesterol level suggest an increase in atherogenic index in the PQ toxicity.

The level of triglyceride (TG) determined was found to be least in PQ group of rat. This is in agreement with the previous report of Igarashi *et al.,* [29] and Kimura *et al*., [28] other researchers that PQ decreases the TG level in PQ-induced oxidative stressed rat. One reason that could be adduced for this decrease in TG could be due to its use as an energy source by the stressed rat.

The increased alkaline and acid phosphatase levels in PQ group may be attributed to oxidative assault on the liver. This is in accordance with the work of Noguchi *et al.,* [30] that PQ induced damage in the rat liver in the absence of appropriate amount of antioxidant. It is imperative that peroxides are removed as they are formed in plant and animal tissues, even though they are far less toxic than other active forms of oxygen. Otherwise, potent hydroxyl radical ($OH^.$) can be formed by the non-enzymatic factor reactions. The activity of GPx was increased in PQ group of rats. This elevated level may be essential for protection of membrane from oxidative damage under stressed condition imposed or induced by PQ.

The activity CAT was found to increase in PQ group, and declined to the level of the control rats by post-treatment with vitamin C. This is similar to the observation of Tsuchiya *et al.* [31] on the protective effect of chlorogenic on PQ-induced oxidative stress in rats. The result is not in agreement with the report of Kimura *et al.* [27] who reported a decrease in catalase activities in the erythrocytes by administering PQ, the findings of which suggest possible suppression of CAT activation in prolonged PQ toxicity.

Administration of PQ alone resulted in a significant increase in SOD and CAT activities as well as MDA concentration of the liver compared to other groups. This is in agreement with the report of Ataley et *al,* [32] that under condition of oxidative stress, activities of antioxidant enzymes such as CAT and SOD increase. The increase in SOD activity following oral administration of PQ suggests that the rat liver responds to an increase in hepatic production of ROS generated by PQ. The concomitant increase in CAT activity is suggested to be induced by an increase in H_2O_2 production as a result of increase in SOD activity in detoxifying superoxide radicals. Catalase has been reported to remove H_2O_2 when present at high concentration while GPx removes H_2O_2 when present at steady state [33].

Lowered CAT and SOD activities in both vitamin E pre- and post-treated groups compared to PQ other groups is similar to the reported finding of Flader *et al,* (34), that mega doses of vitamin E lower the activities of antioxidant enzymes. It is also in agreement with the report of Ataley *et al.,* [32], that in rat fed with a fish oil diet increased vitamin E supply, reduced the activities of antioxidant enzymes in the liver and muscles.

PQ exposure caused a significant decrease in glutathione (GSH) level found in the liver of rat given PQ alone. This may be attributed not only to the oxidation of GSH to GSSH by activated oxygen produced by PQ but also to the high increase in the glutathione peroxidase since the reaction catalyzed by this enzyme consumes glutathione. Other reason could be due to inhibition of protein synthesis, a phenomenon consistent with the general inhibition of protein synthesis that occurs following the gradual imposition of PQ stress. The level of GSH in the group of rats pre-treated with vitamin C was quite higher than that of the control and vitamin E group. This is not surprising since vitamin C, being a strong reducing agent has been known to reduce oxidized form of glutathione (i.e. GSSH GSH). GSH has been reported to function as an antioxidant in many ways; it can react with singlet oxygen, superoxide and hydroxyl radicals and therefore function directly as a free radical scavenger. It may stabilize membrane structure by removing acylperoxides formed by lipid peroxidation reaction. GSH also recycles ascorbic acid from it's oxidised to reduced form by the enzyme dehydroascorbate reductase [35].

The highest level of MDA in group of rat given PQ alone is consistent with the findings from several studies that exposure of animals to PQ resulted in significant increase in lipid peroxidation [28, 36,37, 38]. Post treatment with vitamin C was able to reduce MDA level close to that of control. This observation supports the report of Kang *et al.,* [39] that vitamin C supplementation suppresses MDA levels during PQ induced lung damage.

Ascorbic acid, a water soluble vitamin, is effective in scavenging free radicals, including hydroxyl radicals, peroxyl radicals and superoxide anion. Ascorbic acid acts as a two electron reducing agent and confers protection by contributing an electron to reduce free radicals, thus neutralizing these compounds in

extracellular aqueous environment prior to their reaction with biological molecules [40,23]. The antioxidant potential of ascorbic acid is not only attributed to its ability to quench ROS, but also to its ability to regenerate other small molecules antioxidants such as α-tocopherol, glutathione and beta-carotene [23]. Post-treatments with vitamin C in this study resulted in marked protection against PQ-induced liver and lung injuries as evidenced by the reversal of lipid peroxidation and GSH depletion. This is in agreement with the report of Vismara *et al.* [41] that post-treatment with vitamin C protected the embryo against PQ-induced embryotoxicity. The mechanism for such a protective effect may be attributed to the ability of vitamin C to quench radicals generated by the redox cycling of PQ before they attack other biomolecules. Further protective role of vitamin C against PQ-induced toxicity is reflected in the histology of liver of vitamin C post-treated rats which revealed a relatively similar histologic pattern with the control. Vitamin E exerts its antioxidant property by preventing chain propagation as a result of its ability to transfer phenolic hydrogen to a peroxyl free radical of a polyunsaturated fatty acid, thereby minimizing the extent of lipid peroxidation. The major portion of peroxytocopherol so formed is reconverted to tocopherol by vitamin C and small amount is excreted as such in the bile [42]. The relatively lower protective role of vitamin E against PQ toxicity could be attributed to the insolubility of vitamin E, since liposoluble antioxidant are known to take relatively long to diffuse through cellular membrane. Although, it was assumed that the combined regimen of vitamin C and E would produced a synergistic protective effects which might enhanced the antioxidant capacity of the rats against paraquat toxicity. However, to our surprise, at least based on the outcome of histopathological studies of the liver, lung and kidney of rats post-treated with the combined regimen of vitamins C and E, this combined therapy does not seem to be more effective compared to individual vitamin supplementation. This might be due to less availability of vitamin C during oxidative stressed condition, in response to its shunting for the recycling of vitamin E, in reconverting peroxytocopherol to tocopherol. This observation is similar to the report of Sarita, *et. al.,* [43] on the effect of supplementation of vitamin C and E on oxidative stress in osteoporosis. The results obtained in this study suggest that vitamin C post-treatment seems to provide more protection against PQ toxicity than other forms of treatment adapted in this study

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