Antioxidant effects of vitamin e on liver enzymes activities in rats under paraquat insult

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This study determined the effect vitamin E (VE) has on liver enzymes of rats under paraquat (PQ) toxicity. 96 male rats, 12 per sub-group (A₀, A₉, AVE, B₀, BVE, C₀, CVE, D₀ and DVE) were injected monthly, intra-peritoneal, with different sub-lethal doses (0, 0, 0.02, 0.02, 0.04, 0.04, 0.06 and 0.06g of PQ/kg BW) respectively. The sub-grouped animals (AVE, BVE,CVE and DVE) were orally given 500mg/L VE, while the other sub-grouped animals (A₀, B₀, C₀ and D₀) received no vitamin E for three months. Each month’s end, 5 mls of blood from 4 animals per sub-group were taken by Cardiac puncture procedure, processed and analyzed for the Liver enzymes. PQ dose and exposure-time dependent toxicity effects resulted in the highly elevated liver enzymes activity values of the test sub-grouped animals (B₀, BVE, C₀, CVE, D₀ and DVE) when compared to the Control sub-grouped animals (A₀ and AVE) at P ≤ 0.001; the high enzymes values of the test sub-grouped animals were more on the animals with no VE (B₀, C₀ and D₀) than the animals with VE medications (BVE, CVE and DVE) at P ≤ 0.001. Vitamin E could be a lifesaving adjunct to toxic insult.

Key words: Vitamin E, liver, enzymes, antioxidant, paraquat, rat, toxicity.

INTRODUCTION

Paraquat is a synthetic product. It was first described in 1882 by Weidel and Russo. In 1933, Michealis and Hill discovered its redox properties of paraquat and called the compound methyl viologen. The herbicidal properties of paraquat (PQ) were first described in 1958 (summers, 1980) and it became commercially available in 1962 (Summers, 1980). This bipyridylium herbicidal compound (PQ) is effective as a non-selective herbicide when applied to leaves (Mees, 1960). Because PQ has a redox potential of − 446 mV, any reducing agent with sufficient energy can donate an electron to the bipyridylium divalent cation (PQ²⁺) to form a free radical (PQ⁺). The oxidation of the bipyridylium radical to form the original paraquat (PQ²⁻) results in the transfer of an electron to oxygen and the formation of the superoxides (Punchard and Kelly, 1996). Subsequent Haber Weiss and Fenton reactions yield toxic hydroxyl radicals (Punchard and Kelly, 1996), thus PQ, functions as a catalyst to transfer reducing equivalent to oxygen. These reactive oxygen species (ROS), so formed, may escape the electron transport chain causing damage to cellular components (Davies, 1995). Paraquat modes of action are by Redox cycling and it is believed to cause cell death by lipid peroxidation or NADPH depletion (Smith, 1987).

In general, antioxidant systems either prevent these ROS from being formed, or remove them before they can damage vital components of the cells (Rhee, 2006). Vitamin E, a fat soluble chemical, is found in the diet in varying amounts. Its most active form is the alpha-tocopherol (Springboard, 2004). One of the most well established mechanisms is its capacity to destroy free radicals generated as part of the oxidation reaction in the human body or by exogenous agents. Vitamin E has also been shown to stabilize membranes by physicochemical interaction between its phytol side chain and the fatty acid chain of polyunsaturated phospholipids at the cells membrane bilayers. It also inhibits the synthesis of prostaglandins and prevents platelet aggregation in-vitro and in-vivo (Springboard, 2004).

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Several studies have shown that vitamin E protects cells from the toxicity of many compounds; therefore, this study is centered on how to enhance liver function activities using vitamin E, as a potent antioxidant, in paraquat toxicity. We aim to achieve results by studying the effects vitamin E has on Liver enzymes activities highly affected by PQ insult.

**MATERIALS AND METHODS**

**Rats**

A total of 120 male albino rats (Rattus norvegicus), weighing between 180 to 220g (average body weight [BW] of 0.2 ± 0.02 kg), obtained from the animal house of the Department of pharmacology and toxicology, College of Health Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria, were fed *ad libitum* with animal pelletized finisher feed with negligible vitamin E content, and allowed to acclimatize for two weeks in metabolic cages before the commencement of the study.

**Paraquat**

A percentage of 20 w/v Dizmazone® (Paraquat solution) from Dizengoff W.A Ltd, Lagos, Nigeria, sealed in an opaque plastic container, with a shelf-life of two years.

**Vitamin E**

100 mg/ml softgel capsules as (dl-Alpha-Tocopherol-acetate), from Good 'N' Natural® manufacturing corp. Holbrook, NY 11741, USA, were used for the study.

**Animal treatments and medications**

2 mls of the sub-lethal different doses of the toxicant (PQ) were administered intraperitoneal (ip.) to the animals, under anesthetics (Animal Care and Ethics Committee, 2007) in different dose subgroups – \( A_0 / A_{VE} \) (0g/kg), \( B_0 / B_{VE} \) (0.02g/kg), \( C_0 / C_{VE} \) (0.04g/kg) and \( D_0 / D_{VE} \) (0.06g/kg) – on biweekly basis over a period of three months (in simulation of contamination from polluted feed, water or air), while the control animals (subgroups \( A_0 / A_{VE} \) ) received 2 mls of normal saline (0.98%w/v) likewise in conformity with international standard in checking injection site reaction. The sub-grouped animals were designated into: i) for no vitamin E treatment and ii) for vitamin E treatments as indicated in Table 1.

This study was conducted with eight subgroups \((A_0 / A_{VE}, B_0 / B_{VE}, C_0 / C_{VE} \text{ and } D_0 / D_{VE})\). All the subgroups had 12 animals each with \( A_0 \) and \( A_{VE} \) being the control subgroups that received no PQ treatments. \( A_{VE}, B_{VE}, C_{VE} \) and \( D_{VE} \) were placed on vitamin E medication (by oral instillation of 0.5mls of 500mg vitamin E oily solution): \( A_0, B_0, C_0, \text{ and } D_0 \) were the subgroups that received no vitamin E. The feeds and water given to the animals were negligibly vitamin E free.

On monthly intervals, four animals per sub-group were selected, anesthetized with gaseous isoflurane anesthetic machine and 10 mls of blood sample collected using cardiac puncture from each animal (Animal Care and Ethics Committee, 2007). The samples were processed and centrifuged with the serum separated and labeled accordingly; stored frozen until needed for the estimation of enzymes activity using Kodac Autoanalyser machine. Using the described study pattern in Table 1, the results were as stated in Table 2 and Figures 1 to 4.

**Animal care**

We do affirm that in carrying out this research that “The Nigerian Institutional and National Guide for the Care and Use of Laboratory Animals” were followed.

**Statistical analysis and data computation**

The Excel (2007) Windows package and two way
Table 2. Mean values of the enzyme activities during study period.

<table>
<thead>
<tr>
<th>Month</th>
<th>Rat no</th>
<th>Group</th>
<th>Sub-Group</th>
<th>SGOT (iu/L)</th>
<th>SGPT (iu/L)</th>
<th>ALP (iu/L)</th>
<th>GGT (iu/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>A</td>
<td>A&lt;sub&gt;0&lt;/sub&gt;</td>
<td>2.20 ± 0.04</td>
<td>2.52 ± 0.08</td>
<td>11.25 ± 0.30</td>
<td>13.63 ± 0.38</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>A</td>
<td>A&lt;sub&gt;VE&lt;/sub&gt;</td>
<td>5.0 ± 0.19</td>
<td>4.08 ± 0.01</td>
<td>12.69 ± 0.55</td>
<td>16.02 ± 0.43</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>B</td>
<td>B&lt;sub&gt;0&lt;/sub&gt;</td>
<td>15.35 ± 0.22</td>
<td>10.95 ± 0.09</td>
<td>53.44 ± 1.12</td>
<td>32.00 ± 0.56</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>B</td>
<td>B&lt;sub&gt;0&lt;/sub&gt;</td>
<td>15.35 ± 0.22</td>
<td>10.95 ± 0.09</td>
<td>53.44 ± 1.12</td>
<td>32.00 ± 0.56</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>C</td>
<td>C&lt;sub&gt;0&lt;/sub&gt;</td>
<td>66.22 ± 1.68</td>
<td>134.88 ± 2.34</td>
<td>82.00 ± 1.75</td>
<td>42.67 ± 0.99</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>C</td>
<td>C&lt;sub&gt;VE&lt;/sub&gt;</td>
<td>46.00 ± 0.94</td>
<td>74.13 ± 1.64</td>
<td>33.83 ± 0.50</td>
<td>33.33 ± 0.07</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>D</td>
<td>D&lt;sub&gt;0&lt;/sub&gt;</td>
<td>99.50 ± 2.43</td>
<td>155.67 ± 3.69</td>
<td>318.17 ± 3.90</td>
<td>65.00 ± 1.37</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>D</td>
<td>D&lt;sub&gt;VE&lt;/sub&gt;</td>
<td>44.50 ± 0.89</td>
<td>93.58 ± 1.97</td>
<td>205.67 ± 2.43</td>
<td>38.17 ± 0.50</td>
</tr>
</tbody>
</table>

n = 4; X = mean ± SEM; SGOT = Aspartate aminotransferase; SGPT = Alamine aminotransferase; ALP = Alkaline phosphatase; GGT = Gamma glutamyl transferase.

analyses of variance (ANOVA) statistical methods were used for the result analysis; with levels of significance measured at p ≤ 0.05 and 0.001 respectively.

RESULTS

The SGOT activity values of all the subgroups were as shown in Table 2 and Figure 1. The enzymes activity values were found to have significantly increased on the test animals subgroups (B<sub>0</sub>, B<sub>VE</sub>, C<sub>0</sub>, C<sub>VE</sub>, D<sub>0</sub>, D<sub>VE</sub>) when compared against the Control animals subgroups (A<sub>0</sub> and A<sub>VE</sub>), and the increases were found to be dose and exposure-time dependent (P ≤ 0.001). As the time of exposure of the toxic insult moved from month 1 down to months 2 and 3, the enzyme activities of the PQ only
treated subgroups (B₀, C₀ and D₀) increased two- to three-folds that of their corresponding subgroups that received vitamin E medication in addition to the PQ insults (B_VE, C_VE and D_VE) at the same level of significance (P ≤ 0.001), indicating that an interaction effect existed between dose of PQ given / vitamin E medication, and also with the exposure time of the toxic insult. The test subgroups with the highest sub-lethal dose (0.06g/kg BW) (D₀ and D_VE) gave an explicit picture on how vitamin E effected an improved enzyme activity when compared to the one on PQ insult only, with D₀ being higher than two times D_VE at months 1 to 3 (P ≤ 0.001).

The results of the mean SGPT enzyme activity values as shown in Table 1 and Figure 2 were similar to the ones obtained in SGOT (Figure 1) values as stated above. There existed an interaction toxicity effects between the dose of PQ given and the exposure-time (P ≤ 0.001). The test animals subgroups (B₀/B_VE, C₀/C_VE and D₀/D_VE) SGPT values were highly elevated when compared to the Control animals subgroups (A₀ and A_VE) from months 1 to 3 (P ≤ 0.001), especially the sub-grouped animals dosed 0.4 g/kg BW (D₀) at month 2. The within group comparison indicated a well-defined significant reduction in the mean values of the SGPT enzyme activity of the vitamin E medicated sub-grouped animals when compared to the sub-grouped animals on PQ insults only (B₀, C₀ and D₀) from months 1 to 3 at P ≤ 0.001. This shows that vitamin E medication reduced the elevated SGPT enzyme activity caused by PQ toxicity.

Alkaline phosphatase (ALP) enzyme activity values of the PQ treated animals were quite remarkable. At month 1 (Table 1 and Figure 3), the test animals subgroups with lower doses of PQ 0.02 and 0.04g/kg (B₀, B_VE, C₀ and C_VE) showed slight elevation of ALP activity when compared to the control subgroups (A₀ and A_VE) at P ≤ 0.05, while the sub-group animals (D₀ and D_VE) that received the highest sub-lethal dose of PQ (0.06g/kg) had highly elevated significant ALP activities at P ≤ 0.001, when compared to the Control animals subgroups.
As the time of exposure increased from month 1 to month 3, the level of enzyme activities of the test subgroups increased even more to an outrageous level at month 3 (P ≤ 0.001). The vitamin E medicated test sub-grouped animals were having an elevated ALP activity values that were lower than that of their corresponding test sub-grouped animals on only PQ insult (BVE vs B0, CVE vs C0 and DVE vs D0) at P ≤ 0.05 throughout the study period. This showed that an interaction existed between the dose of PQ given and the exposure-time of the toxic insult, and also a positive relationship also existed between vitamin E medications with PQ dosing and increase in exposure-time.

The mean GGT enzyme activities of the test animals had increased values that were directly proportional to the dose of the PQ given and the exposure-time of the toxic insult at P ≤ 0.05, from month 1 to 3 when compared to the Control animals sub-grouped mean values (Figure 4). Also the vitamin E medicated sub-grouped animals (BVE, CVE and DVE) all had much lower mean GGT enzyme activity values than their corresponding test sub-grouped animals under PQ insult only (B0, C0 and D0) respectively, from month 1 to month 3 at P ≤ 0.05, showing that vitamin E has an ameliorative action on the cellular enzyme activities of animals under PQ toxic insult.

Discussion

PQ studies and the toxicity effects of this compound on the organs of the body, including its mechanism of toxicity have been reported (Bus et al., 1976). Furthermore, several reports presented PQ as a hepatotoxin (Clark et al., 1966; Gibson and Cagen, 1977; Cagen et al., 1976; Murray and Gibson, 1972).

Following the findings from the acute toxicity study, where it was observed that toxicity effects existed with increasing dose and exposure time of PQ (ip.) insult on the liver cells (Dede et al., 2007). A chronic study was designed to assess the impact of PQ toxicity at long duration (3 months) and also to find the possibility of ameliorating the toxicity effects using a powerful antioxidant (vitamin E) in rats. From the liver enzymes – SGOT, SGPT, ALP and GGT – results obtained, all had changes that were highly significant (P ≤ 0.001) in both within and between subgroups (Table 2 and Figures 1 – 4), and these changes were all dose and exposure-time dependent (P ≤ 0.001). The enzymes activity values of the sub-grouped animals that received PQ only were almost two to three folds increased compared to that of the subgroups that received vitamin E medication in addition to PQ insult.

SGOT values of vitamin E medicated sub-group animals were significantly lower than those on only PQ insult (BVE < B0, CVE < C0 and DVE < D0) from months 1 to 3 (Figure 1). These changes were also observed in the values obtained for other enzymes – SGPT, ALP and GGT in Figures 2 – 4. It has been shown that PQ toxicity initiates lipid peroxidation which causes toxic destruction of lipid membrane bilayers initiating release of membrane bound enzymes – SGPT, ALP and GGT – to the cytoplasm (Wefers and Sies, 1988; Wershana, 2001; Tantcheva et al., 2003; Hassan and Fridovich, 1980). This explains the highly elevated values of these enzymes in PQ only treated animals in subgroups (B0, C0, and D0) as compared to the subgroups that received, in addition to PQ insult, vitamin E medications (BVE, CVE and DVE) in Figures 2 to 4. This indicates that vitamin E,
to a large extent, reduced the effect of PQ toxicity on the animals as seen in Figures 2 to 4.

Generally, there was improvement in enzyme activity values obtained in the sub-grouped animals on vitamin E medications during the PQ insults ($B_{VE}$, $C_{VE}$, and $D_{VE}$) in Figures 1 – 4. Though these values were still high when compared to the Control animals’ subgroups ($A_0$ and $A_{VE}$) and far above the reference values for rats liver enzymes, it’s still a pointer to health improvement. These shows that if vitamin E treatment was continued for a longer period, it could have totally repaired the liver and improved the health status of the animals.

**Conclusion**

The result of this study has demonstrated that vitamin E has the capacity to improve the health status of animals under toxic insult. Exposure of rats to PQ induced highly elevated liver enzymes-SGOT, SGPT, ALP and GGT – activities that were dose and exposure-time dependent, which on subsequent medications with vitamin E, a reduction in the activity levels were observed throughout the study, indicating that vitamin E as an adjunct medicament, could be used to treat toxic insult in patients.

**Recommendation**

Vitamin E, a potent lipid soluble antioxidant should be one of the first line treatments (Emergency procedure) for toxic insult. Vitamin E administration in PQ toxicity should not be ignored, and it should be extended even after patient’s recovery for adequate intracellular repairs to be achieved.

**REFERENCES**


