The Effect of Long-Term Vitamin E Therapy on Hepatotoxic Injury in Male Wistar Rats

Abstract

Paraquat (1, 1-dimethyl-4, 4- bipyridilium dichloride: PQ) is a commercial herbicide which increases agricultural yield by killing weeds worldwide. Vitamin E (α-tocopherol) is a lipid soluble antioxidant that is found in all cellular membranes. It helps in protecting the cell against lipid peroxidation. This study was aimed at determining the effect of Vitamin E on paraquat-induced liver damage on rat. A total of 200 male albino rats were used for the study. The rats were divided into four groups of 50 rats in each group (A, B, C, D) and was further subgrouped, having 25rats per subgroup. "A" group was not induced with paraquat while "B", 'C" and "D" groups were induced in increasing dose of 0.02g, 0.04g and 0.06g respectively. "A" group had two subgroups; "Ao" and "Ave" which were the sub-group not treated with Vit E and the subgroup treated with Vit E (500mg) respectively. This also applied to group "B", "C" and "D". Paraquat was administered every forth night for three months followed by weekly treatment with vitamin E for three month. Blood samples were collected and analysed for liver function test (total and direct bilirubin, total protein, albumin and globulin) and the liver harvested for histological examination. There was a significant difference in the level of all the parameters among the "Ao", "Bo", "Co" and "Do", p-value<0.05 and also among the "A_{VEC}", "B_{VEC}", "C_{VEC}" and "D_{VEC}", p-value<0.05. The result also showed that there were significant differences in intra-group comparison in all the liver biochemical parameters, p-value<0.05 while there was no significant difference among the A_O and A_{VE} subgroups for all parameters. There was also no significant difference in the concentration of globulin among the subgroups. This study has confirmed that vitamin E has a therapeutic effect in male rats on three month of weekly treatment. Therefore, a weekly treatment with Vit E can ameliorate some liver damage caused by paraquat in rats.

Keyword: *Vitamin E, paraquat, rat, antioxidant, liver markers.*

1.0 Introduction

paraquet (1, 1-dimethyl-4, 4- bipyridilium dichloride: PQ) is a commercial herbicide which increases agricultural yield by killing weeds worldwide [1]. It has a fast action and it is non selective, on contact with green plants, it destroys the plant tissues and by translocation in the plant [1]. Intentional or accidental ingestion of PQ in humans is usually toxic as it brings about multi-organ failure [1]. PQ has been shown by previous studies to be very toxic in humans and animals . it has also been reported that PQ toxicity caused increased mortality in past decades [1]. Because of the roles played by reactive oxygen species (ROS), antioxidants can be used as a therapeutic tool against PQ-induced toxicity because there is scarcity of effective treatment and specific antidote for it [2]. Studies have shown the importance of paraquat and the antioxidant system as well as the roles played by antioxidants [3, 4]. The roles of environmental changes on the mechanisms of PQ toxicity and effective treatment is not yet clear. Even though there is a strong evidence base and recommendations, any form of treatment should put into consideration, the mechanism of paraquat toxicity and accepted treatment of patients with PQ.

The liver has a very great role in the metabolism of xenobiotics with some changes in biochemical parameters seen in some chronic conditions [5]. Cytochrome P450 (CYP) is forms especially CYP1A1, CYP1A2 and CYP2E1 have been implicated in the facilitation and formation of ROS during xenobiotic metabolism consequently contributing to oxidative stress induced damage [6]. Studies have shown that CYP-mediated free radical generation is primarily involved in pesticides [7]. However, the metabolism of PQ is very poor and it is therefore excreted almost unchanged in the urine. Studies hve reported that PQ metabolism is via methylation (monomethyl dipyridone ion) or oxidation (PQ pyridine ion and PQ dipyridone ion) [8]. Additionally, CYP2E1 mediated production of superoxide radicals and hydrogen peroxide in vitro and in transected cultured cells has been reported in previous studies [8].

Vitamin E (α-tocopherol) it is a lipid soluble antioxidant that is found in all cellular membranes. it helps in protecting the cell against lipid peroxidation [9]. One of its functions is to act as a chain-breaking antioxidant this is done by preventing chain initiation and propagation of free radical reaction and lipid peroxidation in cellular membrane. Another function of vitamin E is to influence the cellular response to oxidative stress through signal-transduction pathway modulation. Vitamin E also functions to stabilize membranes [10-14]. Vitamin E sources include vegetable oil and wheat-germ oil. Dietary vitamin E is transported via special mechanism in aqueous environment of the plasma, body fluids and cells because it is hydrophobic.

Other sources of Vitamin E are in fruits, vegetables, legumes, oilseeds, grains, and other foods. Vitamin E is considered important to health because of its antioxidant property. It neutralizes free radicals, which destroys cellular molecules, and also preserves the integrity of renal tubules [11]. Vitamin E has been studied severally because of its reported hepatoprotective effects in animals, because of its ability to attenuate the induced oxidative stress in various tissues by reducing Malondialdehyde (MDA) levels, thereby restoring the levels of Glutathione (GSH), and Superoxide dismutase (SOD), and the recovery of impaired hepatic cells [9].

The role of vitamin E in PQ toxicity was demonstrated in several studies where deficiency of vitamin E induced the development of acute PQ toxicity in animals. It was shown shortened and reduced survival was brought about by PQ toxicity [4]. Moreover, the potentiating of acute PQ toxicity by vitamin E deficiency was reversed by administration of antioxidant Vitamins [5]. Although the mechanism the mechanism of Vitamin E protection against PQ toxicity is not yet clear, it may be attributed to its antioxidant properties in preventing Lipid

Peroxidation or the inhibition of superoxide anion generation and its toxicity [6]. Results from another study showed that PQ has the ability to induce the formation of micronuclei, commonly used to assess chromosomal damage, in polychromatic erythrocytes (PCE), both in the bone marrow and in the peripheral blood of mice, this is a treatment effect brought about by the generation of ROS [7].

Administration of melatonin to these mice conferred protection against the PQ induced micronuclei and this effect was attributed to the antioxidant properties of the pineal secretary product [8].

It has been documented that some vitamins have a protective effect against PQ-induced biochemical toxicity in albino rats [12]. It must be noted that literature on effect Vitamin E therapy on the chronic toxicity of paraquat on the Liver in albino rats is very rare; therefore it is imperative to carry out study on the subject.

2.0 Material and Methods

2.1 Study Area/Population

The study was carried out in the medical Laboratory Science Departmental Laboratory of Rivers State University. The study was a biological trial with Albino rats which were considered the choicest animals for this experiment because of their availability, cost, genetic makeup, handling technique and nature of the study. Two hundred (200) healthy mature male albino rats with a mean weight of 0.2±0.02kg were used in this study. The rats were obtained from Animal House, Department of Biology, Rivers State University. The rats were transported to the study site and allowed to acclimatize for two week before proceeding with the study. The rats were housed in conventional wire mesh cages under standard laboratory conditions and were allowed free access to water and feed throughout the experiment.

2.2 Grouping and Treatment of Animals

Two hundred (200) male Albino Rats were used for this research and were divided according to their body weight into 4 groups with each group containing fifty (50) Rats each.

Group A: This was the control group. They were not induced with paraquat

Group B: This group were induced every two weeks with 0.02g of paraquat per kg of rat for three months.

Group C: This group were induced every two weeks with 0.04g of paraquat per kg of rat for three months.

Group D: This group were induced every two weeks with 0.06g per kg of paraquat for three months.

Each of the main groups had two subgroups. “A” group had “Ao” and “Bve” subgroups; “B” group had “Bo” and “Bve” subgroups; “C” group had “Co” and “Cve” subgroups; “D” group had “Do” and “Dve”

“Ao”, “Bo”, “Co” and “Do” subgroups: were not treated with vitamin E

“Ave”, “Bve”, “Cve” and “Dve” subgroups: were treated orally with 500mg of vitamin E every week for three months.

However, treatment with Vit E commenced after the three months paraquat induction. After three month of weekly treatment with Vit E, the rats were sacrificed and their blood samples were analyzed for liver parameters and histological examination carried out.

2.3 Procedures for Administration of Toxicant and Vitamin E

Toxicant was administered via oral gavage route. The rats were held at the skin over the head and turned so that the mouth was faced upward and the body lowered towards the holder. The syringe needle bevel was then placed into the mouth of the rat a bit laterally in a way to avoid the teeth which are located centrally. The contents of the syringe were then emptied into the mouth of the rat gradually [15].

2.4 Sample Collection

The blood samples were collected via cardiac puncture from the animals and sacrificed under 70% chloroform anesthesia into the lithium heparin specimen bottle and used for analysis of liver parameters and liver harvested for histological examination.

2.5 Laboratory analysis

2.5.1: Bilirubin method (Mallor, *et al.*, 1937; Martinek, 1966 and Young, 1997).

Principle: Bilirubin is converted to coloured azobilirubin through diazotized sulphanilic acid and measured photometrically. Of the two fractions existing in serum (bilirubin-glucuronide and free bilirubin) solely the sure bilirubin reacts without delay in aqueous solution (Direct bilirubin), whilst free bilirubin requires solubilization with dimethylsulphoxide (DMSO) to react (Indirect bilirubin). In the determination of indirect bilirubin the direct is also

determined, the results corresponds to Total bilirubin. The intensity of the colour formed is proportional to the bilirubin concentration in the sample.

Procedure:

Total Bilirubin: 1.5mls of reagent-1 (Sulphanilic acid, HCl and Dimethylsulphoxide) was added to two glass-tubes labeled 'Blank' and 'Test' respectively. 50*µL* of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently 100*µL* of sample was added to the 'Blank' and 'Test' tubes, mixed and incubated for exactly 5 minutes at room temperature. After which the absorbance were read spectrophotometrically at 530 – 580nm and 15 – 25^0C, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 19.1 = Result in (mg/dL). Conversion factor: mg/dL X 17.1 = Result (*µL*/L).

Direct Bilirubin: 1.5mls of reagent-2 (Sulphanilic acid and HCl) was added to two glass-tubes labeled 'Blank' and 'Test' respectively. 50*µL* of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently 100*µL* of sample was added to the 'Blank' and 'Test' tubes, mixed and incubated for exactly 5 minutes at room temperature. After which the absorbance were read spectrophotometrically at 530 – 580nm and 15 – 25^0C, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 14 = Result in (mg/dL). Conversion factor: mg/dL X 17.1 = Result (*µL*/L).

2.5.2: Total protein (Biuret colorimetric method by Burtis, *et al.*, 1999)

Principle: Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is protected as an antioxidant. The intensity of the colour formed is proportional to the complete protein concentration in the sample.

Procedure: 1mL of Biuret reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 25*µL* each of Standard (7g/dL) and Sample added to the 'Standard' and 'Test' tubes respectively. The contents were mixed and incubated for 10 minutes at room temperature, after which, the absorbance (A) of the 'Test' and 'Standard' were read against the 'Blank'. The colour produced is stable for at least 30 minutes at room temperature.

Calculation: [A(Test) ÷ A(Standard)] X 7(Standard concentration)

= Result in g/dL

2.5.3: Albumin (Bromocresol green method by Grant, *et al.*, 1987)

Principle: The measurement of serum albumin is primarily based on its quantitative binding to the indicator 3,3!,5,5!-tetrabromo-m-cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Procedure: 3mls of Bromocresol green reagent was each introduced to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 10µL each of Water, Standard (7g/dL) and Sample introduced to the 'Blank', 'Standard' and 'Test' tubes respectively. The contents were mixed and incubated for 10 minutes at 20 – 250C, after which, the absorbance (A) of the 'Test' and 'Standard' were read against the 'Blank'. The coloration produced is stable for at least 30 minutes at room temperature.

Calculation: [A (Test) ÷ A (Standard)] X 7 (Standard concentration)

= Result in g/dL

2.5.4: Globulin calculation method by Grant, *et al.*, 1987.

In this approach globulin value are calculated as a difference when albumin value are subtracted from the value of the total protein gotten from the same sample.

Globulin (g/dl) = Total protein (g/dl) – Albumin (unit in g/dl).

2.6 Histopathological analysis.

After the three month study duration, the liver harvested from each subgroup was dissected and their tissues were histologically examined.

2.7 Statistical analysis

The data generated from this study was analyzed using SPSS version 23.0 for descriptive and inferential statistics (ANOVA) for inter-group comparison and T-test for intra-group (sub-group) comparison at test significance, P-value<0.05.

3.0 Result

Table 1 below shows the inter group comparison of liver markers after three months paraquat induction. It is observed from the result that there was a significant increase in concentrations of total and direct bilirubin among subgroups A_O, B_O, C_O and D_O. But there was significant decrease in concentrations of total protein and albumin among the subgroups A_O, B_O, C_O and D_O. There was also no significant difference in the concentration of globulin among the subgroups

Table 1: ***Inter group comparison of liver markers after three months paraquat induction***.

Sub-group	Tot. Bilirubin (µmol/L)	D. Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_0	4.35 ± 3.55	0.30 ± 0.02	7.83 ± 0.04	5.06 ± 0.04	2.78 ± 0.08
B_0	7.75 ± 0.55[a]	1.45 ± 0.04[a]	4.85 ± 0.02[a]	2.34 ± 0.01[a]	2.51 ± 0.01
C_0	9.75 ± 1.05[a]	1.70 ± 0.08[a]	4.36 ± 0.08[a]	2.12 ± 0.01[a]	2.24 ± 0.02
D_0	19.70 ± 0.80[a]	2.30 ± 0.03[a]	4.03 ± 0.02[a]	1.68 ± 0.02[a]	2.35 ± 0.02

Statistical significance: $P \leq 0.05$.

- Index (a) = represents a statistically significant difference between the test subgroups and the control subgroups at each treatment month.

Table 2 below shows the intra group comparison of liver markers after three months of Vitamin E treatment. The result shows a significant increase in concentrations of total and direct bilirubin among subgroups A_{VE}, B_{VE}, C_{VE} and D_{VE}. But there was significant decrease in concentrations of total protein and albumin among subgroups A_{VE}, B_{VE}, C_{VE} and D_{VE}. There was also no significant difference in the concentration of globulin among the subgroups.

Table 2: ***Intra group comparison of liver markers after three months of Vit E treatment.***

Sub-group	Tot. Bilirubin (µmol/L)	D. Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_{VE}	3.15 ± 1.65	0.25 ± 0.01	7.19 ± 0.01	4.84 ± 0.04	2.35 ± 0.05

B_{VE}	4.00 ± 1.10[a]	0.50 ± 0.01[a]	5.10 ± 0.01[a]	2.82 ± 0.01[a]	2.28 ± 0.01
C_{VE}	10.40 ± 0.70[a]	0.95 ± 0.02[a]	4.75 ± 0.01[a]	2.39 ± 0.01[a]	2.36 ± 0.02
D_{VE}	12.60 ± 1.80[a]	1.35 ± 0.05[a]	4.30 ± 0.04[a]	2.02 ± 0.00[a]	2.28 ± 0.01

Statistical significance: $P \leq 0.05$

- Index (a) = represents a statistically significant difference between the test subgroups and the control subgroups at each treatment month.

Table 3 below shows the inter and intra group comparison of liver markers after three months of treatment with Vitamin E. It shows a significant difference in concentration of total Bilirubin between subgroups B_O, and B_{VE}, C_O and $C_{VE,}$ and D_O and D_{VE} at $p<0.05$. It also showed a significant decrease in direct bilirubin among subgroups B_{VE}, C_{VE} and D_{VE} at $p<0.05$. There was however no significant difference in the concentrations of all the parameters in subgroups A_O and A_{VE} at $p<0.05$. It also showed a significant increase in concentrations of total protein and albumin in the B_{VE}, C_{VE} and D_{VE} subgroups at $p<0.05$. There was also no significant difference in the concentration of globulin across the groups at $p<0.05$.

Table 3: ***Inter and Intra group comparison of liver markers after three months treatment***

Sub-group	Tot. Bilirubin (µmol/L)	D. Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_0	4.35 ± 3.55	0.30 ± 0.02	7.83 ± 0.04	5.06 ± 0.04	2.78 ± 0.08
A_{VE}	3.15 ± 1.65	0.25 ± 0.01	7.19 ± 0.01	4.84 ± 0.04	2.35 ± 0.05
B_0	7.75 ± 0.55[a]	1.45 ± 0.04[a]	4.85 ± 0.02[a]	2.34 ± 0.01[a]	2.51 ± 0.01
B_{VE}	4.00 ± 1.10[a,b]	0.50 ± 0.01[a,b]	5.10 ± 0.01[a,b]	2.82 ± 0.01[a,b]	2.28 ± 0.01
C_0	9.75 ± 1.05[a]	1.70 ± 0.08[a]	4.36 ± 0.08[a]	2.12 ± 0.01[a]	2.24 ± 0.02
C_{VE}	10.40 ± 0.70[a,b]	0.95 ± 0.02[a,b]	4.75 ± 0.01[a,b]	2.39 ± 0.01[a,b]	2.36 ± 0.02
D_0	19.70 ± 0.80[a]	2.30 ± 0.03[a]	4.03 ± 0.02[a]	1.68 ± 0.02[a]	2.35 ± 0.02
D_{VE}	12.60 ± 1.80[a,b]	1.35 ± 0.05[a,b]	4.30 ± 0.04[a,b]	2.02 ± 0.00[a,b]	2.28 ± 0.01

Statistical significance: $P \leq 0.05$

- Index (a) = represents a statistically significant difference between the test subgroups and the control subgroups at each treatment month.

- Index (b) = represents a statistically significant difference observed within each group (i.e. Group B: B_0Vs B_{VE}) at each month.

4.0 Discussion

The aim here is to evaluate the effect of vitamin E on paraquat toxicity within the cell and membrane organelles of albino rats. Different subgroups of the test subjects were treated with paraquat, with an inter- and intra-relationship comparative analysis of significant and none significant toxicity carried out. However, paraquat is highly lethal chemical and organic compound for weed control, with records of toxic effect when exposed to the cells or other parts of the tissues [16,17].

The result obtained from the study above with paraquat treatment alone at various concentrations, and with vitamin E treatment on paraquat induced subjects, confirmed the toxicity effects of paraquat and also the ameliorative potency of vitamin E. Comparison on the significance of toxicity among the various subgroups and within the same subgroups was carried out also.

Groups B_0, C_0, and D_0 were compared against the control group A_0 for the effect of paraquat on biochemical parameters T. bilirubin, D. bilirubin, T. protein, albumin, and globulin as shown in Table 3 above. The result obtained showed that there was a significance increase in the concentrations of T. bilirubin, D. bilirubin, this shows that paraquat toxicity brought about an increase in the total and direct bilirubin concentrations. This may be due to the damage caused by the paraquat poisoning on the liver. There was however a significant decrease in the concentrations of T. protein, and albumin for all subgroups under consideration. This indicates that paraquat toxicity brought about a decrease in the total protein and albumin concentrations. This also can be attributed to the damage caused by paraquat on the liver. The result also showed that there was no significant difference in the globulin levels of all the subgroups. This result partly agrees with the study of Rizvi et al. (2014) and Howard et al. (2011) [18, 19] where they discovered an increase in the total and direct bilirubin and no significant effect on globulin, but disagrees with their results on the total protein and albumin.

Vitamin E's ability to repair and prevent the oxidative effect of paraquat on the cells and membranes of the test subjects was compared against the control group A_{VE} and the result revealed a significant increase in the levels of the parameters of subgroups B_{VE}, C_{VE}, and D_{VE} for T. Bilirubin and D. Bilirubin but showed a Significant decrease in the concentration of T.

Protein and albumin. There was however no significant difference in the concentration of globulin.

This increase in parameters can be attributed to excess Vitamin E intake. The outcome of this study also agrees partly with the studies conducted by (National Center for Biotechnology and Information (2022), Rizvi et al. (2014), and Howard et al. (2011) [16-21] but disagrees with their result on total protein and albumin.

Finally, an intra-comparison was made between the control groups A_0 and A_{VE} and the test subgroups B_0 and B_{VE}, C_0 and C_{VE}, D_0 and D_{VE} to determine the difference in the levels of the parameters under study, between the test groups administered with various doses of paraquat without vitamin E treatment, and the groups given paraquat with vitamin E treatment. it was observed that there was no significant difference among subgroups A_o and A_{VE}. This shows that Vitamin E therapy had no effect on the total bilirubin of rats that are not induced with paraquat. This could be because of high dosage of Vitamin E without toxicity. It also showed that there was a significant difference in the total bilirubin concentration among the subgroups B_O and $B_{VE,}$ C_o and C_{VE} and D_O and D_{VE}. This shows that Vitamin E therapy has an effect on the total bilirubin. The result also showed a significant decrease in the direct bilirubin concentrations among subgroups B_{VE}, C_{VE} and D_{VE} and B_O, C_O and D_o respectively. This indicates that Vitamin E therapy brought about the decrease in the direct bilirubin concentrations in these groups. This shows the ameliorative effect of Vitamin E. There was however no significant difference in the direct bilirubin concentration among subgroups Ao and A_{VE}. This shows that Vitamin E therapy had no effect on the direct bilirubin of the mentioned group. The result also showed that there was no significant difference in the globulin concentration among the subgroups. The results of this study corresponds partly with results of studies conducted by Rizvi et al. (2014), and Howard et al. (2011) [20,21]. When the parameters; protein, albumin and globulin of the same groups were examined, they showed no significant difference in terms of the effectiveness of vitamin E on correcting the toxicity of paraquat on the parameters. This may suggest that as at the time of this study that vitamin E alone has no significant effect in repairing the toxicity of paraquat on total protein, albumin and globulin. More studies should be conducted in this area to provide solution to the impact of paraquat toxicity on the parameters without vitamin E effectiveness.

Conclusion

This result of this study has demonstrated that Vitamin E therapy had an ameliorative effect on some liver makers against paraquat toxicity such as total and direct bilirubin but had no ameliorative effect on the total protein, albumin and globulins.

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