The Role of Vitamin E in Hepatophysiology of Paraquat Induced Toxicity

ABSTRACT

The organic molecule paraquat, commonly known as methyl viologen, has the chemical formula [(C6H7N)2]Cl2. Paraquat is an oxidant that disrupts electron transport and other normal cell functions in all living things through the generation of free radicals. Vitamin E is a fat-soluble vitamin with powerful antioxidant properties that aid in the protection of cell membranes against reactive oxygen species (ROS). This study evaluated the ameliorative impact of vitamin E on paraquat-induced hepatotocity in rats. 200 male rats with a mean weight of 0.20.02kg were used for this study. The 200 rats were divided into four groups of 50 each. A, B, C, and D were the four sections. The "A" group was no treated with paraquat, whereas the "B," "C," and "D" groups were given 0.02g, 0.04g, and 0.06g of paraquat per kilogram of rat every two weeks for three months. Each had group had subclasses. The "A" group had "A_0" and "A_{VE}" subgroups; the "B" group had "B_0" and "B_{VE}"; "C" group had "C_0" and "C_{VE}" subgroups; and "D" group had "D_0" and "D_{VE}" subgroups. "A_0," was the control, "B_0," "C_0," and "D_0" subgroups received paraquat treatment. The "A_{VE}," was fed vitamin E only, while "B_{VE}," "C_{VE}," and "D_{VE}" were treated with paraquat and Vit E. Vit E treatment commenced weekly for two months after paraquat poisoning. The blood was taken and examined for t. protein, d. bilirubin, t. bilirubin, albumin, and globulin levels. An inter group comparative analysis of A_0, B_0, C_0, D_0; A_{VE}, B_{VE}, C_{VE}, D_{VE}, and an intra group comparison of A_0 vs A_{VE}, B_0 vs B_{VE}, C_0 vs C_{VE}, and D_0 vs D_{VE} showed a significant difference in t. protein, d. bilirubin, t. bilirubin, albumin and globulin levels. This study showed that vitamin to a great extent is effective in treating chronic toxicity of paraquat on a two month basis and is powerful is restoring balance in the levels of liver parameters disrupted by paraquat toxicity.

Keywords: Paraquat, liver, antioxidant, vitamin E

INTRODUCTION

The organic molecule paraquat, commonly known as methyl viologen, has the chemical formula [(C6H7N)2]Cl2 [1]. Paraquat is one of the most extensively used herbicides by farmers [2,3]. It's a fast-acting, non-selective pesticide that kills green plant tissue on contact. Due to its redox activity, which produces superoxide anions, it is also poisonous (lethal) to humans and animals. Paraquat has been linked to the development of Parkinson's disease [4,5]. Paraquat is an oxidant that disrupts electron transport, a process that occurs in all living things [6].

In terms of toxicity, paraquat leads to fatal damages within cells when ingested [7]. According to Suntres (2002), Paraquat and diquat are bipyridyl compounds in terms of chemistry, however, after absorption, paraquat tend to concentrate inside numerous cells, where it undergoes redox cycling, which is a process in which paraquat and paraquat radicals are cycled repeatedly by enzymes [8]. A superoxide radical, a highly reactive oxygen species that can cause direct cellular damage or react further to create additional reactive oxygen species and nitrite radicals, is a by-

product of this process [8]. NADPH, one of the cell's most important antioxidant defenses, is consumed during redox cycling. The oxidative stress caused by free radical generation and NADPH depletion induces cell damage through lipid peroxidation, mitochondrial malfunction, necrosis, and apoptosis as well as a strong secondary inflammatory response [9].

A study conducted by Ujowundu *et al*. (2018) on the hepatoxicity of paraquat, reported that paraquat was able to alter the levels of some liver parameters [10]. Another study conducted by Karima (2001) highlighted that paraquat led to significant changes in the levels of certain liver markers in the research carried out [11,12].

Four tocopherols and four tocotrienols make up vitamin E, which is a collection of eight fat-soluble molecules [13,14]. Vitamin E is a fat-soluble vitamin with powerful antioxidant properties that aid in the protection of cell membranes against reactive oxygen species (ROS) [14,15]. In a research conducted by Hobson (2016), it was reported that vitamin E was potent in influencing the healing of wound [16]. Li *et al.* (2012) revealed that vitamin E plays a significant role in internal injury the repairs [17]. Shalaby *et al*., (2020) in his work reported that vitamin E is capable of countering the oxidative stress induced by paraquat toxicity [18]. In this study, we aim to determine the ameliorative potency of two months vitamin E therapy on liver markers after paraquat toxicity in male albino rats.

MATERIALS AND METHOD

Study Design

200 male albino rats with a mean weight of 0.20.02kg were used for this study. The 200 rats were divided into four groups of 50 each. A, B, C, and D were the four sections. The "A" group was given no paraquat, whereas the "B," "C," and "D" groups were given 0.02g, 0.04g, and 0.06g of paraquat per kilogram of rat every two weeks for three months. Each had group had subclasses. The "A" group, had "A_0" and "A_{VE}" subgroups; the "B" group, had "B_0" and "B_{VE}"; "C" group, had "C_0" and "C_{VE}" subgroups; and "D_0" and "D_{VE}" subgroups were for group “D”. "A_0," was the control and was not treated at all with paraquat, while "B_0," "C_0," and "D_0" subgroups received paraquat treatment. The "A_{VE}," was fed vitamin E only, while "B_{VE}," "C_{VE}," and "D_{VE}" subgroups stood for the vit. E $_+$ paraquat grout and were every week for two months

after paraquat inducement. The blood was taken and examined for t. protein, d. bilirubin, t. bilirubin, albumin, and globulin levels.

Animal source

The Animal House, Department of Biology, Rivers State University of Science and Technology supplied 200 rats weighing an average of 0.20.02kg. Before the commencement of the experiment, the rats were brought to the study site and allowed to adapt for 2 weeks before the trial began. The research took place at the Department of Medical Laboratory Science at Rivers State University of Science and Technology.

Treatment Administration

Procedure for Paraquat Administration

Administration of toxicant was via oral gavage route. The dose depended on the treatment group but in all, the treatment was performed every two weeks for three months.

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 content in the syringe was then emptied into the mouth of the rat gradually [19].

Procedure for Vitamin Administration

Vitamin E was given orally every week for two month at doses of 500mg [19].

Sample Collection method

Using a blood sample, liver function test was performed. 2ml of blood was extracted via heart puncture and was then epmtied into simple bottles with a syringe and needle. The serum was extracted by spinning it at 4000rpm but only after the blood had coagulated and t. bilirubin, d. bilirubin, t. protein, albumin and globulin were tested for.

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

Principle: Diazotized sulphanilic acid converts bilirubin to colored azobilirubin, which is then photometrically assessed. Only the bound bilirubin (Direct bilirubin) reacts immediately in aqueous solution, whereas free bilirubin needs to be solubilized with dimethylsulphoxide

(DMSO) to react (Indirect bilirubin). The direct bilirubin is also determined during the indirect bilirubin test, and the result is Total bilirubin. The bilirubin concentration in the sample determines the intensity of the color produced.

Procedure:

Total Bilirubin: 1.5 mL reagent-1 (Sulphanilic acid, HCl, and Dimethylsulphoxide) was added to two glass tubes labeled 'Blank' and 'Test,' respectively. 50µL of reagent-3 (Sodium nitrite) was added to the test tube and mixed; 100µL of sample was then put to the 'Blank' and 'Test' tubes, mixed, and incubated at room temperature for exactly 5 minutes. The absorbance was then measured using a spectrophotometric method at 530–580nm and 15–250°C, with the instrument set to zero using pure 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: 1.5 mL reagent-2 (sulphanilic acid and HCl) was added to two glass tubes labeled 'Blank' and 'Test,' respectively. 50µL of reagent-3 (Sodium nitrite) was added to the test tube and mixed; then 100µL of sample was put to the 'Blank' and 'Test' tubes, mixed, and incubated at room temperature for exactly 5 minutes. The absorbance was then measured spectrophotometrically at 530–580nm and 15–25ºC, with the instrument zeroed 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).

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

Principle: In an alkaline solution, proteins form an intense violet-blue complex with copper salts. As an antioxidant, iodide is incorporated. The amount of color generated is related to the amount of total protein 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. Both contents were mixed and incubated for 10 minutes

at room temperature, followed with 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

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

Principle: The quantitative binding of serum albumin to the indicator 3,3!,5,5!-tetrabromo-m-cresol sulphonephthalein is used to determine its concentration (bromocresol green, BCG). At 578 nm, the albumin-BCG complex absorbs maximum, with the absorbance directly proportional to the albumin content in the sample.

Procedure: Three glass tubes labeled 'Blank,' 'Standard,' and 'Test' each received 3mls of Bromocresol green reagent, followed by 10µL each of Water, Standard (7g/dL), and Sample added to the 'Blank,' 'Standard,' and 'Test' tubes, respectively. After mixing the contents and incubating them for 10 minutes at 20–25ºC, the absorbance (A) of the 'Test' and 'Standard' were measured against the 'Blank'. At room temperature, the color created is stable for at least 30 minutes.

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

= Result in g/dL

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

Globulin value is calculated as a difference when albumin value is subtracted from the value of the total protein gotten from the same sample under this method.

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

RESULT

The inter group comparative analysis of each the paraquat treatment group is represented in Table 1.0 below. Groups A_0, B_0. C_0 and D_0 were compared and the result showed a statistical significant difference, $p \leq 0.05$ in T. bilirubin, D. bilirubin, T. protein, albumin and globulin for all the test groups after chronic paraquat inducement.

Table 1.0: ***Inter-group comparison of liver parameters after chronic paraquat induction***

Sub-group	Tot. Bilirubin (µmol/L)	D. Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_0	2.28 ± 0.84	0.70 ± 0.02	7.24 ± 0.36	3.93 ± 0.01	3.31 ± 0.03
B_0	9.38 ± 1.28[a]	1.60 ± 0.02[a]	5.08 ± 0.06[a]	2.82 ± 0.01[a]	2.26 ± 0.01[a]
C_0	11.45 ± 0.91[a]	1.38 ± 0.02[a]	5.00 ± 0.06[a]	2.33 ± 0.01[a]	2.68 ± 0.02[a]
D_0	16.90 ± 1.31[a]	1.48 ± 0.07[a]	4.69 ± 0.22[a]	2.25 ± 0.01[a]	2.44 ± 0.02[a]

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 1.1 represents the intergroup comparison of vit. E + paraquat treated group with B_{VE}, C_{VE}, and D_{VE} standing for vit. E + paraquat group, compared against the A_{VE} (control) treated only with vitamin E. the result showed there was a significant in the difference, $p \leq 0.05$, in all the markers studied, while there was no significant difference in the A_{VE} group.

Table 1:1. ***Inter-group comparison of liver parameters after two months 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}	1.78 ± 0.52	0.33 ± 0.01	7.03 ± 0.07	3.85 ± 0.01	3.18 ± 0.01
B_{VE}	6.65 ± 1.42[a]	0.60 ± 0.01[a]	5.67 ± 0.09[a]	3.12 ± 0.00[a]	2.55 ± 0.02[a]
C_{VE}	8.78 ± 1.10[a]	0.68 ± 0.03[a]	5.15 ± 0.14[a]	2.69 ± 0.01[a]	2.46 ± 0.01[a]
D_{VE}	10.75 ± 1.19[a]	0.63 ± 0.04[a]	5.09 ± 0.23[a]	2.89 ± 0.01[a]	2.20 ± 0.02[a]

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.

The intra group comparison of the test subjects within each group is represented in **Table 1.2**.Comparison was done on A_0 vs A_{VE}, B_0 vs B_{VE}, C_0 vs C_{VE}, and D_0 vs D_{VE}. There was significant difference, $p \leq 0.05$ in all the liver markers studied, whereas the A_0 vs A_{VE} recorded no significant difference.

Table 1.2: ***Inter and intra group comparison of liver parameters after two months treatment with Vit E***.

Sub-group	Tot. Bilirubin (µmol/L)	D. Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_0	2.28 ± 0.84	0.70 ± 0.02	7.24 ± 0.36	3.93 ± 0.01	3.31 ± 0.03
A_{VE}	1.78 ± 0.52	0.33 ± 0.01	7.03 ± 0.07	3.85 ± 0.01	3.18 ± 0.01
B_0	9.38 ± 1.28^{a}	1.60 ± 0.02^{a}	5.08 ± 0.06^{a}	2.82 ± 0.01^{a}	2.26 ± 0.01^{a}
B_{VE}	6.65 ± 1.42^{a,b}	0.60 ± 0.01^{a,b}	5.67 ± 0.09^{a,b}	3.12 ± 0.00^{a,b}	2.55 ± 0.02^{a}
C_0	11.45 ± 0.91^{a}	1.38 ± 0.02^{a}	5.00 ± 0.06^{a}	2.33 ± 0.01^{a}	2.68 ± 0.02^{a}
C_{VE}	8.78 ± 1.10^{a,b}	0.68 ± 0.03^{a,b}	5.15 ± 0.14^{a,b}	2.69 ± 0.01^{a,b}	2.46 ± 0.01^{a}
D_0	16.90 ± 1.31^{a}	1.48 ± 0.07^{a}	4.69 ± 0.22^{a}	2.25 ± 0.01^{a}	2.44 ± 0.02^{a}
D_{VE}	10.75 ± 1.19^{a,b}	0.63 ± 0.04^{a,b}	5.09 ± 0.23^{a,b}	2.89 ± 0.01^{a,b}	2.20 ± 0.02^{a}

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.

DISCUSSION

The therapeutic efficacy of vitamin E on repairing the chronic toxicity of paraquat on liver parameters T. bilirubin T. protein, D. bilirubin, albumin and globulin after two months of treatment was examined in this study. Three comparative analyses were conducted to determine

the toxicity of paraquat on the liver parameters, the repairing effect of vitamin E on paraquat induced toxicity, and an intra group analysis within each subgroup was also conducted.

The inter group analysis of subgroups B0, C0, and D0 group treated with only paraquat was compared with the A0 (control) group and the result showed that there was a significant difference in the levels of all the liver parameters (T. bilirubin T. protein, D. bilirubin, albumin and globulin) in all the subgroups treated with paraquat alone at the end of the treatment duration, whereas the control group A_0 recorded no significant difference. The significant differences recorded in T. bilirubin T. protein, D. bilirubin, albumin and globulin among the treatment group could be as a result of the peroxidation [6] caused by the paraquat induced within in the rats, since the control group remained unaffected. It also would imply that the levels of these parameters within the test subjects which expressed the significant difference were altered on induction with paraquat [8]. The change in the liver parameter may imply a decrease in the antioxidant defense of the liver due to the depletion of the tissue's NADPH [9] This agrees with the research of Ujowundu *et al*. (2018), Karima, (2001) and Hu *et al.,* (2019) which stated in their works that paraquat alters the levels of some liver function markers via peroxidation [10,11,12].

The result from the inter comparison of the vit E + paraquat treatment groups (B_{VE}, C_{VE}, and D_{VE}) showed that there was also a significant difference in the liver parameters studied, when compared with the A_{VE} counterparts fed only with vitamin E. The significant difference reported was positive as the oxidative stress recorded in the subgroups treated only with paraquat was being corrected, wheresa the A_{VE} subgroup significantly did not record any difference after the duration of treatment. This suggests that vitamin E had antioxidative effect on paraquat poisoning [17]. This result is in consonance with the work of MIC (2015) and S.H.I.D (2022) which highlighted in their work that vitamin E is powerful against reactive oxygen species (ROS) which causes oxidative stress within cells [14,15].

The third result was the intra group comparative analysis of the groups within each subgroup. The A_0 was compared with A_{VE}, B_0 vs B_{VE}, C_0 vs C_{VE} and D_0 vs D_{VE}. Whereas the A_0 vs A_{VE} had no significant difference in the liver parameters tested, the B_0 vs B_{VE}, C_0 vs C_{VE} and D_0 vs D_{VE} showed a significant difference in the levels of T. bilirubin T. protein, D. bilirubin, albumin and globulin. The findings from this study confirm that vitamin E was able to exert ameliorate

the effect against the oxidation induced by paraquat. This in consonance with the work of Shalaby *et al*., (2020) who reported that vitamin E is effective in cushioning oxidative stress within cells [18].

CONCLUSION

In conclusion, it can be seen from this study that vitamin to a great extent is effective in treating chronic toxicity of paraquat on a two month basis and is powerful is restoring balance in the levels of liver parameters disrupted by paraquat toxicity.

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