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

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

PQ (1, 1-dimethyl-4, 4-bipyridilium dichloride) is a commercial herbicide that increases agricultural yield by killing weeds all over the world. Vitamin E (-tocopherol) is a fat-soluble antioxidant found in all cellular membranes.It helps to protect the cell from lipid peroxidation. The purpose of this study was to see how Vitamin E affected paraquat-induced liver damage in rats. For the study, 200 male albino rats were used. The rats were divided into four groups of 50 rats each (A, B, C, D) and then subdivided into 25 rats per subgroup. The "A" group received no paraquat, whereas the "B," "C," and "D" groups received increasing doses of 0.02 g, 0.04 g, and 0.06 g, respectively. The "A" group was divided into two subgroups: "Ao" and "Ave," which were the subgroups that were not treated with Vit E and the subgroup that was treated with Vit E (500 mg), respectively. This was also true for groups "B", "C", and "D". For three months, paraquat was given every fourth night, followed by weekly vitamin E treatments. Blood was drawn and tested for liver function (total and direct bilirubin, total protein, albumin, and globulin), and the liver was harvested for histological examination. There was a significant difference in the level of all parameters between the "Ao," "Bo," "Co," and "Do," p-value0.05, as well as between the "AVEC," "BVEC," "CVEC," and "DVEC," p-value <0.05.The results also demonstrated that while there was no significant difference between the AO and AVE subgroups for any of the liver biochemical parameters, there were significant differences in intra-group comparisons for all of the liver biochemical parameters, with p-values<0.05. Additionally, there was no discernible variation in globulin concentration between the groupings. This study has proven that weekly treatment for three months with vitamin E has a therapeutic impact on male rats. Therefore, a weekly vitamin E treatment can lessen some of the liver damage paraquat induced in rats.

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1. INTRODUCTION

Commercial herbicide paraquet (1, 1-dimethyl-4, 4-bipyridilium dichloride: PQ) "increases agricultural productivity by eliminating weeds globally" [1].

PQ poisoning, whether intentional or unintentional, frequently results in multi-organ failure in humans [1]. Previous investigations have demonstrated that PQ is extremely harmful to both people and animals. PQ poisoning has reportedly been linked to an increase in mortality in previous decades [1]. Because of the roles of reactive oxygen species (ROS), antioxidants can be used as a therapeutic tool against PQ-induced toxicity because there are few effective treatments and specific antidotes [2].

Several studies have demonstrated the significance of paraquat and the antioxidant system, as well as the roles that antioxidants play [3,4]. The roles of environmental changes in PQ toxicity mechanisms and effective treatment are not well understood. Despite a strong evidence base and recommendations, any form of treatment should take the mechanism of paraquat toxicity and accepted treatment of patients with PQ into account.

The liver plays an important role in the metabolism of xenobiotics, with some changes in biochemical parameters seen in some chronic conditions [5]. Cytochrome P450 (CYP) is forms, particularly CYP1A1, CYP1A2, and CYP2E1, have been implicated in the facilitation and formation of ROS during xenobiotic metabolism, thereby 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. According to research, PQ is metabolized through methylation (monomethyl dipyridone ion) or oxidation (PQ pyridine ion and PQ dipyridone ion)." [8]. In addition, it has been revealed in earlier research that CYP2E1 mediates the formation of superoxide radicals and hydrogen peroxide in vitro and in transected cultured cells [8]. All cellular membranes contain vitamin E (also known as -tocopherol), an antioxidant that is soluble in lipids.

It assists in preventing lipid peroxidation and safeguards the cell [9]. As a chain-breaking antioxidant, one of its tasks is to stop the chain formation and spread of free radical reactions and lipid peroxidation in cellular membranes.

Through the modulation of signal-transduction pathways, vitamin E also affects the cellular response to oxidative stress. Additionally, vitamin E helps to keep membranes stable.

Clarke, Truber, Ambali, Azzi [10-13]. Wheat germ oil and vegetable oils are sources of vitamin E.

Due to its hydrophobic nature, dietary vitamin E is transported through a unique process in the watery environment of the plasma, bodily fluids, and cells.

Fruits, vegetables, legumes, oilseeds, grains, and other foods provide additional sources of vitamin E. Due to its antioxidant properties, vitamin E is thought to be crucial for health.

As well as protecting the health of renal tubules, it eliminates free radicals, which break down biological components [11]. The reported hepatoprotective effects of vitamin E in animals, as well as its capacity to lower levels of malondialdehyde (MDA) and restore levels of glutathione (GSH) and superoxide dismutase (SOD), as well as to improve the recovery of damaged hepatic cells, have all led to numerous studies on vitamin E. [9]. "Several investigations where vitamin E deficiency caused the development of acute PQ toxicity in animals showed the role of vitamin E in PQ toxicity. PQ poisoning was demonstrated to shorten and impair survival. [4]. Additionally, the introduction of antioxidant vitamins reversed the potentiating of acute PQ toxicity caused by vitamin E deficiency. [5]. The antioxidant effects of vitamin E in avoiding lipid peroxidation or the suppression of the formation of superoxide anion and associated toxicity may be responsible for its protection against PQ toxicity, even though the exact mechanism is still unclear [6]. The findings of a different study demonstrated that PQ can cause the creation of micronuclei, which are frequently used to evaluate chromosomal damage, in mice's bone marrow and peripheral blood. This treatment effect is caused by the generation of ROS [7]. The antioxidant capabilities of the pineal secretary product were thought to be responsible for the protective effects of melatonin on these animals when it

was administered to them [8]. It has been established that several vitamins can protect albino rats from PQ-induced metabolic damage [12]. It should be mentioned that there is very little research on the effects of Vitamin E therapy on the chronic liver toxicity of paraquat in albino rats, therefore further research is absolutely necessary.

2. MATERIALS AND METHODS

2.1 Study Area/Population

The investigation was conducted at Rivers State University's medical laboratory science departmental laboratory in Port Harcourt, Nigeria. Albino rats were used in the biological testing because they were readily available, inexpensive, and suitable for the experiment due to their genetic makeup, handling methods, and availability.

In this investigation, 200 healthy mature male albino rats weighing an average of 0.2±0.02 kg were employed [14]. The rats came from Rivers State University's Animal House in the department of biology. Before beginning the trial, the rats were brought to the study location and given two weeks to acclimate**.** The rats were kept in typical wire mesh cages in a laboratory setting with free access to food and water for the duration of the experiment.

2.2 Grouping and Treatment of Animals

For this study, two hundred (200) male Albino Rats were divided into four groups based on body weight, with each group containing fifty (50) Rats.

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 [14].

Group C: This group were induced every two weeks with 0.04g of paraquat per kg of rat for three months [14].

Group D: This group were induced every two weeks with 0.06g per kg of paraquat for three months [14].

Each of the main groups had two subgroups. “A” group had “Ao” and “Ave” 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 [14].

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

"The toxin was administered orally via gavage. The rats were held by the skin over their heads and turned so that their mouths were facing up and their bodies were lowered towards the holder. The syringe needle bevel was then inserted slightly laterally into the rat's mouth, avoiding the centrally located teeth. The contents of the syringe were then gradually emptied into the rat's mouth" [15].

2.4 Sample Collection

The animals were euthanized under 70% chloroform anesthesia, and the blood samples were drawn from their hearts into a lithium heparin specimen vial. The liver was then excised for histological study and the liver parameters were analyzed [15].

2.5 Laboratory Analysis

2.5.1 Bilirubin method as described by Okolonkwo et al. [14,15]

Procedure: Total Bilirubin: Two glass tubes marked "Blank" and "Test" were each given 1.5 ml of reagent 1 (sulphanilic acid, HCl, and dimethylsulphoxide).

Following the addition of 50 mL of reagent 3 (sodium nitrite) to the tube designated for the test only, 100 mL of sample was added to the "Blank" and "Test" tubes, mixed, and incubated for precisely 5 minutes at room temperature.

Following that, the absorbance was measured spectrophotometrically between 530 and 580 nm at temperatures between 15 and 250°C, with the instrument set 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 as described by Okolonkwo et al. [14,15]

Procedure: Three glass tubes with the labels "Blank," "Standard," and "Test" were each filled with 1 mL of the biuret reagent. Then, the "Standard" and "Test" tubes were filled with 25 L of the Standard (7g/dL) and the "Sample," respectively.

The mixture was incubated at room temperature for 10 minutes, after which the absorbances (A) of the test and standard samples were compared to the blank sample. At room temperature, the resulting color is stable for at least 30 minutes.

Calculation:

[A (Test) ÷ A (Standard)] X 7 (Standard concentration) = Result in g/dL

2.5.3 Albumin (Bromocresol green method as described by Okolonkwo et al. [14,15]

Principle: Serum albumin concentration is primarily determined by how well it binds quantitatively to the indicator 3,3!,5,5!-tetrabromo-m-cresol sulphonephthalein (bromocresol green, BCG).

The albumin-BCG complex absorbs most effectively at a wavelength of 578 nm, with the absorbance directly proportional to the albumin content in the sample.

Procedure: 3 mls 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 as described by Okolonkwo et al. [14,15]

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 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. RESULTS

After three months of paraquat induction, the liver indicators are compared between groups in Table 1 below. The findings show that the subgroups AO, BO, CO, and DO had significantly higher quantities of both total and direct bilirubin. However, the concentrations of total protein and albumin were significantly lower in the subgroups AO, BO, CO, and DO. Additionally, there was no discernible variation in the subgroups' globulin concentration.

After three months of vitamin E administration, the intragroup comparison of liver markers is shown in Table 2 below. As a result, the subgroups AVE, BVE, CVE, and DVE all had significantly higher quantities of total and direct bilirubin. However, the levels of total protein and albumin were significantly lower in the subgroups AVE, BVE, CVE, and DVE.

Additionally, there was no discernible variation in the subgroups' globulin concentration.

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 ≤ 0.05

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

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 ≤ 0.05

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

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 ≤ 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*

Following a three-month course of vitamin E therapy, the liver indicators are compared across and within groups in Table 3. It demonstrates a statistically significant difference in total bilirubin concentration between the subgroups BO and BVE, CO and CVE, and DO and DVE at p 0.05. Additionally, it demonstrated a significant decline in direct bilirubin among the BVE, CVE, and DVE subgroups at p 0.05.

However, at p0.05, there was no discernible difference between the concentrations of all the measures in subgroups AO and AVE. Additionally, it demonstrated a statistically significant rise in total protein and albumin concentrations in the BVE, CVE, and DVE subgroups at p 0.05. Additionally, there was no p0.05 significant difference in the globulin concentration across the groups.

4. DISCUSSION

The objective of this study was to assess how vitamin E affected the toxicity of paraquat in albino rats' cell and membrane organelles. Paraquat was administered to several test subject subgroups, and an analysis of the toxicities that were significant and non-significant was conducted.

However, paraquat is a highly poisonous organic and chemical molecule for weed control that has

been shown to have toxic effects when exposed to cells or other tissues [16,17].

The results of the study above, which involved treating paraquat-induced subjects with paraquat alone at various concentrations and vitamin E treatment, proved paraquat's toxicity effects as well as the potency of vitamin E's ameliorative properties. Comparison on the significance of toxicity among the various subgroups and within the same subgroups was carried out also.

As indicated in Table 3 above, groups B0, C0, and D0 were contrasted against the control group A0 to see how paraquat affected the biochemical parameters total bilirubin, direct bilirubin, total protein, albumin, and globulin. This indicates that paraquat poisoning caused an increase in the concentrations of total and direct bilirubin. The results obtained also indicated a significant rise in the concentrations of total and direct bilirubin. This might be as a result of the liver harm brought on by paraquat poisoning.

However, the levels of total protein and albumin for all of the categories taken into account significantly decreased. This suggests that the quantities of total protein and albumin were decreased as a result of paraquat poisoning. This is also related to the harm paraquat does on the liver. The outcome also demonstrated that the globulin levels of all the subgroups did not differ significantly from one another. This finding partially supports research by Rizvi et al. [18] and Howard et al. [19,20,21], which found an increase in total and direct bilirubin and no discernible impact on globulin, but it contradicts their findings on total protein and albumin concentration.

Comparing the effects of vitamin E against the control group AVE, it was found that vitamin E was significantly more effective at repairing and preventing the oxidative damage that paraquat caused to the test subjects' cells and membranes. However, the levels of total bilirubin and direct bilirubin in the subgroups BVE, CVE, and DVE were significantly lower, as were the concentrations of total protein and albumin.

However, there was no discernible difference in the globulin concentration. A high intake of vitamin E is responsible for these metrics' rise. The outcome of this study also agrees partly with the studies conducted by (National Center for Biotechnology and Information [16], Rizvi et al. and Howard et al. [16-21] but disagrees with their result on total protein and albumin.

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, an intra-comparison between the control groups A0 and AVE and the test subgroups B0 and BVE, C0 and CVE, and D0 and DVE was made. The subgroups Ao and AVE did not show any discernible differences, it was found.

This demonstrates that vitamin E therapy had no impact on the total bilirubin levels in rats that were not given paraquat to induce them.

This can be the result of excessive but safe vitamin E consumption.

Additionally, it revealed that the subgroups BO and BVE, Co and CVE, and DO and DVE had significantly different total bilirubin concentrations.

This demonstrates how vitamin E therapy affects the level of total bilirubin.

The direct bilirubin concentrations among the subgroups BO, CO, and Do as well as BVE, CVE, and DVE both significantly decreased as a result of the study.

This suggests that the reduction in the levels of direct bilirubin in these groups was caused by the vitamin E therapy. This demonstrates the therapeutic value of vitamin E.

However, the direct bilirubin concentration did not differ significantly between subgroups Ao and AVE. This demonstrates that the direct bilirubin of the stated group was unaffected by Vitamin E therapy.

The outcome also demonstrated that there was no discernible variation in globulin concentration between the groupings. The findings of this investigation are somewhat consistent with those of studies carried out by Rizvi et al. and Howard et al. [18,19]. When the same groups' protein, albumin, and globulin levels were evaluated, there was no discernible difference in how well vitamin E worked to mitigate the harmful effects of paraquat on the parameters. This may imply that, as of the time of this investigation, vitamin E

is not sufficiently effective to reverse the paraquat toxicity 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.

5. 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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