

Original Research Article

THERAPUETIC EFFECTS OF VITAMIN E ON PARAQUAT INDUCED LIVER TOXICITY IN MALE ALBINO RATS (*Rattus norvegicus*)

Abstract

Paraquat is a high toxic organic chemical capable of causing organ and tissue damage by generation of free radicals. Vitamin E is a powerful antioxidant which is domiciled within the cells and organelles of the body and functions by acting as an initial responder against oxygen reactive species called free radicals which attack and destroy the tissues. The study was aim at assessing a short term therapeutic effect of vitamin E on paraquat induced male albino rat. A total of 200 male albino rats were used for the study. In the experimental design, the 200 rats were divided into four main groups (A, B, C, D) and each group had 50 rats and was further subgrouped into two, 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 represented the sub-group not treated with Vit E and the subgroup treated with Vit E (500mg) respectively. This design also applied to group "B", "C" and "D". paraquat induction frequency was fortnightly for three month followed by weekly treatment for one month. Blood sample and liver were collected and harvested respectively for liver function test (TB, CB, albumin, total protein and globulin) and histological assessment of the liver. There was a significant difference in liver function markers among the "Ao", "Bo", "Co" and "Do", p-value<0.05 except for globulin marker. There was a significant difference in liver function markers among the "Ave", "Bve", "Cve" and "Dve", p-value<0.05 except for globulin marker. The result also showed that there were significant differences in intra-group comparison in total bilirubin and conjugated bilirubin markers, p-value<0.05 while other markers were not significantly different. This study has shown that vitamin E is has a therapeutic effect in male albino rats on one month of weekly treatment. Therefore, a weekly treatment with Vit E, can treat liver toxicity in rats.

Comment [AME1]: Aims to assess the short term

Comment [AME2]: Which were divided

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Comment [AME4]: "A" group is without paraquat induction

Comment [AME5]: and paraquat induction was done fortnightly

Comment [AME6]: This study observed that vitamin E has therapeutic effect on paraquat induced rats. Therefore, it can be used to reverse liver toxicity.

Keyword: Vitamin E, paraquat, rat, antioxidant, liver

1.0 Introduction

Vitamin E is a fat soluble vitamin which belongs to a category of eight lipid-soluble compounds with antioxidant properties with the capability of shielding the cells and tissues from oxidative stress [1,2]. The eight groups are further divided into two groups comprising of tocopherols (a class of organic compounds having many vitamin E activity) and tocotrienols [2].The tocopherol group is made up of the alpha-, beta-, delta-, and gamma-tocopherols with each possessing a unique quality. Alpha (α)-tocopherol is the chemical form of vitamin E suitable for the nutritional need of the human body as well as the most active form of vitamin E [3]. α -tocopherol plays several roles in the human body which ranges from the neutralization of free radicals which sponsors oxidative damages, to the protection of the cells against damages and the maintenance of the integrity of the cell membranes via preventing protein oxidation and fat peroxidation [3].The alpha- form of vitamin further plays key roles such as gene control, participation in neurological performance, platelet aggregation inhibition, and vasodilation enhancement [3]. Vitamin can be sourced naturally from foods like seeds, nuts, some vegetables, and fortified products. It can also be sourced from dietary supplement [4].

Comment [AME7]: Alpha (α) tocopherol

Comment [AME8]: It also

Albino rats (*Rattus norvegicus*) mainly known as laboratory rat is a species of rat reared for scientific studies. They have played a key role in various fields of scientific studies such as seen in medical sciences, pharmacology, physiology, genetics and neuroscience [5].

Toxicant has been reported by many studies to be present in the environment due to increased industrial activities, human activities, poor waste management and agricultural chemical [6,7]. Paraquat is an organic compound also known as methyl viologen, a chemical herbicide or weed killer with highly toxic effect on ingestion or exposure [8,9,10]. According to Gotter (2022), paraquat is also known by the common name gramoxone, which is an extremely fatal poison on ingestion [9]. A report by National Center for Biotechnology and Information, 2022 on paraquat compound cited that a concentrated solution of paraquat causes skin irritation among other damages such as nail shedding and cracking, and delayed wounds and cut healing [8]. According to Thomas (2018), exposure to paraquat can be confirmed through urinary dithionite test [10]. According to several studies carried out on the toxicity of paraquat, it was discovered that paraquat poisoning altered the levels of certain biochemical parameters [11,12]. Zhou et al. (2016) in their research highlighted the toxic impact of paraquat in both survivors and non-survivors where it led to significant alteration in the concentrations of some biochemical parameters [12].

Vitamin E is a powerful antioxidant which is domiciled within the cells and organelles of the body and functions by acting as an initial responder against oxygen reactive species (free radicals) which attack and destroy the tissues [13]. Howard et al. (2011) revealed that vitamin E also promotes the repair of the plasma membrane [14]. This study was aimed at assessing the ameliorative effect of vitamin E therapy on certain biochemical parameters of paraquat induced male albino rats. (The justification is not well captured to lead to this study. Kindly reframe.)

2.0 Material and Methods

2.1 Study Design

The study was a chronic experimental design of biological trial on 200 male albino rats with a mean weight of 0.2 ± 0.02 kg. The 200 rats were divided into four main groups of 50 rats each. The groups were A, B C and D. The "A" group was not induced with paraquat; "B" group was induced every two weeks with 0.02g of paraquat per kg of rat for three months; "C" group was induced every two weeks with 0.04g of paraquat per kg of rat for three months; "D" group was induced every two weeks with 0.06g per kg of paraquat for three months. Each of the main groups had subgroups. "A" group had "Ao" and "Ave" subgroups; "B" group had "Bo" and "Be" subgroups; "C" group had "Co" and "Cve" subgroups; "D" group had "Do" and "Dve" subgroups. "Ao", "Bo", "Co" and "Do" subgroups were not treated with vitamin E while "Ave", "Bve", "Cve" and "Dve" were treated orally with 500mg of vitamin E every week. However, treatment with Vit E commenced after the three months paraquat induction. After one month of weekly treatment with Vit E, the rats were sacrificed and their samples (blood and liver organ) were analyzed for liver function.

Comment [AME9]: According to a report from National centre for biotechnology and information (2022)

Comment [AME10]: delete

Comment [AME11]: Is it Be or Ave

Comment [AME12]: Is it Be or Bve

2.2 Animal source

Two hundred rats of average weight of 0.2 ± 0.02 kg were obtained from Animal House, Department of Biology, Rivers State University of Science and Technology. The rats were transported to the study site and allowed to acclimatize for two week before proceeding with the study. The study was conducted in Department of Medical Laboratory Science, Rivers State University of Science and Technology.

Comment [AME13]: Rivers State University

2.3 Sample Collection method

Blood sample was collected for liver function test and liver was harvested for histological analysis. Using syringe and needle, 2mls of blood was collected through cardiac puncture and dispensed in plain bottles. The blood was allowed to clot and then spun at 4000rpm to separate the serum and kept frozen at -20°C until ready for analysis. The serum separated was analyzed for total bilirubin, conjugated bilirubin, total protein, albumin and globulin. By means of 70% chloroform anesthesia, animal sacrificed, liver organ harvested and preserved in 10% formal saline. The carcasses remaining were incinerated to avoid environmental pollution.

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2.4 Laboratory analysis

Bilirubin method [15]

Procedures:

Total Bilirubin: 1.5mls of reagent-1 (Sulphanilic acid, HCl and Dimethylsulphoxide) was added to two glass-tubes labeled 'Blank' and 'Test' respectively. $50\mu\text{L}$ of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently $100\mu\text{L}$ 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 $15 - 25^{\circ}\text{C}$, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 19.1 = Result in (mg/dL). Conversion factor: $\text{mg/dL} \times 17.1 = \text{Result } (\mu\text{L/L})$.

Direct Bilirubin: 1.5mls of reagent-2 (Sulphanilic acid and HCl) was added to two glass-tubes labeled 'Blank' and 'Test' respectively. $50\mu\text{L}$ of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently $100\mu\text{L}$ 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 $15 - 25^{\circ}\text{C}$, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 14 = Result in (mg/dL). Conversion factor: $\text{mg/dL} \times 17.1 = \text{Result } (\mu\text{L/L})$.

Total protein (Biuret colorimetric method) [16]

Procedure: 1mL of Biuret reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 25 μ L 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: $[A(\text{Test}) \div A(\text{Standard})] \times 7(\text{Standard concentration})$

= Result in g/dL

Albumin (Bromocresol green method) [17]

Procedure: 3mLs of Bromocresol green reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 10 μ L each of Water, Standard (7g/dL) and Sample added 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 colour produced is stable for at least 30 minutes at room temperature.

Calculation: $[A(\text{Test}) \div A(\text{Standard})] \times 7(\text{Standard concentration})$

= Result in g/dL

Globulin calculation method [17]

In this method 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).

Comment [AME17]: Reframe as follows. Bilirubin was evaluated by method according to Young (1997), total protein by method of

2.5 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, 2 and 3 show the comparative effects of vitamin E on the chronic toxicity of Paraquat in Albino Rats (*Rattus norvegicus*). Intergroup comparison of Ao, Bo, Co and Do was statistically significant, p-value<0.05 in total bilirubin, conjugated bilirubin, total protein, and albumin but there globulin comparison had no statistical significance among the groups. Intergroup comparison of Ave, Be, Cve and Dve was statistically significant, p-value<0.05 in total bilirubin, conjugated bilirubin, total protein, and albumin but there globulin comparison had no statistical significance among the groups.

Comment [AME18]: (p<0.05)

Comment [AME19]: But no significant (p>0.05) difference observed in the globulin among the group

Comment [AME20]: As in comment 18

Comment [AME21]: As in comment 19

Table 1: Inter-group comparison of liver biomarkers in paraquat induced rat

| Sub-group | T. Bilirubin (µmol/L) | D. Bilirubin (µmol/L) | T. Protein (g/dL) | Albumin (g/dL) | Globulin (g/dL) |
|----------------|---------------------------|--------------------------|--------------------------|--------------------------|-----------------|
| A ₀ | 0.81 ± 0.35 | 0.14 ± 0.03 | 7.52 ± 0.25 | 3.90 ± 0.01 | 3.62 ± 0.03 |
| B ₀ | 3.53 ± 0.79 ^a | 1.10 ± 0.03 ^a | 6.05 ± 0.38 ^a | 3.16 ± 0.01 ^a | 2.89 ± 0.03 |
| C ₀ | 9.29 ± 2.53 ^a | 1.08 ± 0.03 ^a | 6.26 ± 0.57 ^a | 3.40 ± 0.03 ^a | 2.86 ± 0.03 |
| D ₀ | 13.56 ± 3.14 ^a | 1.57 ± 0.04 ^a | 6.54 ± 0.51 ^a | 3.21 ± 0.04 ^a | 3.32 ± 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.

Comment [AME22]: g/dl

Table 2: Inter-group comparison of liver biomarkers in Vit E treated rats

| Sub-group | T. Bilirubin (µmol/L) | D. Bilirubin (µmol/L) | T. Protein (g/dL) | Albumin (g/dL) | Globulin (g/dL) |
|-----------------|--------------------------|--------------------------|--------------------------|--------------------------|-----------------|
| A _{VE} | 1.13 ± 0.24 | 0.28 ± 0.01 | 6.90 ± 0.15 | 4.00 ± 0.02 | 2.91 ± 0.03 |
| BE | 2.45 ± 0.59 ^a | 0.45 ± 0.01 ^a | 6.73 ± 0.26 ^a | 3.29 ± 0.01 ^a | 3.44 ± 0.02 |
| C _{VE} | 4.21 ± 0.78 ^a | 0.67 ± 0.04 ^a | 6.29 ± 0.33 ^a | 2.99 ± 0.03 ^a | 3.30 ± 0.01 |
| D _{VE} | 6.93 ± 1.67 ^a | 1.05 ± 0.02 ^a | 6.31 ± 0.35 ^a | 3.06 ± 0.02 ^a | 3.26 ± 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.

Comment [AME23]: Is it BE or Bve

Table 3: Inter and intra groups comparison of liver markers after one month treatment

| Sub-group | T. Bilirubin (µmol/L) | D. Bilirubin (µmol/L) | T. Protein (g/dL) | Albumin (g/dL) | Globulin (g/dL) |
|-----------------|----------------------------|----------------------------|--------------------------|--------------------------|-----------------|
| A ₀ | 0.81 ± 0.35 | 0.14 ± 0.03 | 7.52 ± 0.25 | 3.90 ± 0.01 | 3.62 ± 0.03 |
| A _{VE} | 1.13 ± 0.24 | 0.28 ± 0.01 | 6.90 ± 0.15 | 4.00 ± 0.02 | 2.91 ± 0.03 |
| B ₀ | 3.53 ± 0.79 ^a | 1.10 ± 0.03 ^a | 6.05 ± 0.38 ^a | 3.16 ± 0.01 ^a | 2.89 ± 0.03 |
| BE | 2.45 ± 0.59 ^{a,b} | 0.45 ± 0.01 ^{a,b} | 6.73 ± 0.26 ^a | 3.29 ± 0.01 ^a | 3.44 ± 0.02 |
| C ₀ | 9.29 ± 2.53 ^a | 1.08 ± 0.03 ^a | 6.26 ± 0.57 ^a | 3.40 ± 0.03 ^a | 2.86 ± 0.03 |
| C _{VE} | 4.21 ± 0.78 ^{a,b} | 0.67 ± 0.04 ^{a,b} | 6.29 ± 0.33 ^a | 2.99 ± 0.03 ^a | 3.30 ± 0.01 |
| D ₀ | 13.56 ± 3.14 ^a | 1.57 ± 0.04 ^a | 6.54 ± 0.51 ^a | 3.21 ± 0.04 ^a | 3.32 ± 0.02 |
| D _{VE} | 6.93 ± 1.67 ^{a,b} | 1.05 ± 0.02 ^{a,b} | 6.31 ± 0.35 ^a | 3.06 ± 0.02 ^a | 3.26 ± 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.

Comment [AME24]: Same as comment 23

- Index (b) = represents a statistically significant difference observed within each group (i.e. Group B: B₀ Vs B_{VE}) at each month.

Author (s) are advised to use ANOVA to compare A₀, B₀, C₀ and D₀, A_{VE}, B_{VE}, C_{VE} and D_{VE} BUT student t-test to compare A₀ and A_{VE}, B₀ and B_{VE}, C₀ and C_{VE}, D₀ and D_{VE}. Author(s) should provide P or F values of the analysis as well as the Post Hoc test analysis to establish the area of difference in the means.

4.0 Discussion

In the quest to assess the therapeutic effect of Vitamin E on paraquat induced liver toxicity in male albino rats, different rat subgroups were studied based on paraquat induction dose. This was followed by inter- and intra- comparative analysis after treatment with vitamin E.

The result obtained 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 therapeutic potential of vitamin E. Comparison on the significance of toxicity among the various subgroups and within the same subgroups was carried out also. Groups B₀, C₀, and D₀ were compared against the control group A₀ for the effect of paraquat on liver biomarkers; T. bilirubin, D. bilirubin, T. protein, albumin, and globulin. The result obtained showed there was a significance difference in the concentrations of T. bilirubin, D. bilirubin, T. protein, and albumin for all subgroups under consideration. However, there was no significant difference in globulin level. This could be due to the fact that globulin proteins are not all produced in the liver. Albumin which is primarily produced in the liver was significantly affected after periods of paraquat induction. This result is in agreement with the study by Rizvi in 2014 and Howard in 2011 [13,14].

The ability of vitamin E to repair the oxidative damage caused by paraquat on the cells and membranes of the liver was studied. The Vit E treated sub-groups (A_{VE}, B_{VE}, C_{VE} and D_{VE}) were compared and the result revealed a significant difference in the liver parameters of the subgroups except for globulin. This means that vitamin E did not have any comparative effective on rats induced and rats not induced with paraquat. It may be due to the fact that paraquat also had no toxic effect on globulin concentration. This outcome also agrees with the studies conducted by (National Center for Biotechnology and Information (NCBI) in 2022, Rizvi in 2014, and Howard in 2011 [5,13,14].

Finally, an intra-comparison was done between the control groups A₀ and A_{VE} and the test subgroups B₀ and B_{VE}, C₀ and C_{VE}, D₀ and D_{VE} to determine the therapeutic potential of Vit E on increasing paraquat dosage. It was observed that there was a significant difference in the levels of T. bilirubin, and D. bilirubin of subgroups B_{VE}, C_{VE}, and D_{VE}. It means that Vit E had the potential to restore liver excretory function of bilirubin metabolism. Vit E was still therapeutically effective in restoring liver function increasing toxicant dose of 0.02g to 0.06g. The recovery of albumin level after treatment was not achieved in one month of weekly treatment with Vit E even at the least toxicity level (0.02g) used. This corresponds with results of studies conducted by Rizvi in 2014, and Howard in 2011 [13,14]. the effectiveness of vitamin E

Comment [AME25]: This is conclusion which cannot come before discussion

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.

Comment [AME26]: Author(s) should discuss findings scientifically and draw a valid conclusion.

Conclusion

This study has shown incomplete effectiveness of vitamin E in correcting the toxicity of paraquat on liver of albino rats. Although therapeutic success was achieved in the excretory function of bilirubin metabolism, a possible therapeutic success may be possible in long term treatment with Vit E.

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