



Effects of Vitamin E on Liver Function of Paraquat Exposed Wistar Rats (*Rattus norvegicus*)

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Author's contribution

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

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ABSTRACT

Paraquat is a weed killer used by farmers to protect their crops against evasive weed and increase crop yields. Paraquat has the feature for quick absorption into the body and being extremely toxic, it causes damages to the liver, kidney, and the lungs by releasing free radicals, NF- κ B activation, and apoptosis in many cells. The study used 200 male albino rats with a mean weight of 0.20 ± 0.02 kg in an experimental study. The 200 rats were placed into four groups, each with 50 rats. A, B, C, and D were the four groups. 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, respectively, every two weeks for three months. There were subgroups within each of the primary categories. "A₀" and "A_{VE}" subgroups existed in the "A" group; "B₀" and "B_{VE}" subgroups existed in the "B" group; "C₀" and "C_{VE}" subgroups existed in the "C" group; and "D₀" and "D_{VE}" subgroups existed in the "D" group. Vitamin E was not given to the "A₀," "B₀," "C₀," and "D₀" subgroups, but it was given to the "A_{VE}," "B_{VE}," "C_{VE}," and "D_{VE}" subgroups every week for one month after paraquat induction. Blood was obtained and tested for liver function. Apart from A₀ group which is the control group, B₀, C₀, and D₀ being the paraquat treated groups, as well as the B_{VE}, C_{VE}, and D_{VE} vit. E treatment subgroups had a statistically significant difference, p -value ≤ 0.05 , in SGOT, SGPT, ALP, and GGT, an outcome which confirmed the toxicity of paraquat and the ameliorative effect of vit. E. The results showed that vitamin E therapy is potent against paraquat induced toxicity on liver enzymes on a one-month treatment basis.

Keywords: Paraquat; toxicity; liver; vitamin E.

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1. INTRODUCTION

Paraquat is a weed killer used by farmers to protect their crops against evasive weed and increase crop yields [1]. It is a highly toxic chemical commonly used as commercial herbicide by farmers in the United States of America where only qualified and licensed persons are permitted to use it [2]. Paraquat has the feature for quick absorption into the body and being extremely toxic, it causes damages to the liver, kidney, and the lungs by releasing free radicals, NF- κ B activation, and apoptosis in many cells [2,3]. Ingestion of large quantity of paraquat leads to organ failure and even death within very short duration ranging from hours to days, while small quantity consumption leads to toxicity in two main organs; the lungs and kidney within 2-6 days. According to the Centers for Disease Control and Prevention, exposure to paraquat occurs through the ingestion of paraquat contaminated foods, inhalation, or skin exposure [4]. Consumption of a large amount of paraquat is accompanied with symptoms like mouth and throat expansion, which results to pain [4]. Gastrointestinal symptoms, such as nausea, vomiting, stomach discomfort, and diarrhea, are the next signs of sickness after consumption (which may become bloody) [4].

According to the research by Raja et al. [5], the plasma activity of transaminase enzymes, alkaline phosphatase, and liver transketolase were significantly reduced after paraquat administration [5]. In another study conducted by Noriega et al. [6], it was discovered that the administration of paraquat, on test subjects showed a significant rise in lipid peroxidation and a drop in reduced glutathione (GSH) levels. The activity of antioxidant enzymes in the liver, such as superoxide dismutase, catalase, and glutathione peroxidase, was reduced 3 hours after paraquat inducement [6].

Vitamin E is a set of eight fat-soluble molecules with rich and potent antioxidant properties [7]. Of the eight groups, humans' dietary needs are best met by alpha-tocopherol [7]. Vitamin E is well known for its ability to defend the body against oxidative stress from free radicals also known as reactive oxygen species [7]. Rizvi et al., (2014) in their research noted that vitamin E is mostly found in cell and organelle membranes, where it can provide the most protection, even if the concentration ratio is only one molecule per 2,000 phospholipid molecules [8]. It protects cell membranes from free radical attack and acts as

the initial line of defense against lipid peroxidation [8]. According to Bridges et al. (2021), vitamin E is stored in the body within the adipose tissues and the liver [9]. Vitamin E is present in a wide range of foods and oils. High levels of alpha-tocopherol can be found in nuts, seeds, vegetable oil and considerable amounts can also be found in green leafy vegetables and fortified cereals. [8].

2. MATERIALS AND METHODS

2.1 Experimental Design

The study used 200 male albino rats with a mean weight of 0.20 ± 0.02 kg in a chronic experimental design of biological testing. The 200 rats were placed into four groups, each with 50 rats. A, B, C, and D were the four groups. 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, respectively, every two weeks for three months [10]. There were two subgroups within each of the primary categories and each subgroup was composed of 25 rats. "A₀" and "A_{VE}" subgroups existed in the "A" group; "B₀" and "B_{VE}" subgroups existed in the "B" group; "C" group had "C₀" and "C_{VE}" subgroups existed in the "C" group; and "D" group had "D₀" and "D_{VE}" subgroups existed in the "D" group. 500mg of vitamin E was not given to the "A₀," "B₀," "C₀," and "D₀" subgroups [10], but it was given to the "A_{VE}," "B_{VE}," "C_{VE}," and "D_{VE}" subgroups every week for a month after paraquat induction. The blood was obtained and tested for liver function.

2.2 Animal Source

Animal House, Department of Biology, Rivers State University of Science and Technology provided 200 rats with an average weight of 0.20 ± 0.02 kg. Before beginning the trial, the rats were brought to the study site and given two weeks to acclimate. The research was carried out at Rivers State University of Science and Technology's Department of Medical Laboratory Science.

2.3 Sample Collection Method

Blood sample was collected for a liver function test. 2ml of blood was taken through a cardiac puncture and dispensed in simple bottles using a syringe and needle. The serum was isolated by spinning the blood at 4000rpm after it had

coagulated. The serum was tested for total bilirubin, conjugated bilirubin, total protein, albumin, and globulin.

2.4 Treatment Administration

2.4.1 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 [10].

2.4.2 Procedure for vitamin administration

Vitamin E was given orally every week for one month at doses of 500mg [10].

2.4.2.1 Serum glutarate-oxaloacetate-aminotransferase (AST/SGOT) method

Serum glutarate-oxaloacetate-aminotransferase (AST/SGOT) method as described by Okolonkwo et al. [11].

2.4.2.1.1 Procedure

To determine the enzyme's activity, 0.5mL buffered-L-aspartate and -oxoglutarate solutions were added to two glass tubes labeled 'Reagent Blank' and 'Test,' respectively, followed by 0.1mL each of distilled water and sample, mixed, and incubated for exactly 30 minutes at 37°C. Then, in each of the test tubes, 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added, mixed again, and allowed to stand for exactly 20 minutes at 20 – 25°C. To increase color development at alkaline pH, 5.0mL sodium hydroxide (0.4mol/L) was added at the conclusion of the period. After 5 minutes, the absorbance of the 'Test' (Atest) tube was compared to that of the 'Reagent blank' tube. Calculation: Obtain the activity of the enzyme AST in the serum from the table of values previously plotted against activities. Haemolysis interferes with the assay.

2.4.2.2 Serum glutarate-pyruvic-aminotransferase (SGPT)

Serum glutarate-pyruvic-aminotransferase (SGPT) as described by Okolonkwo et al. [11].

2.4.2.2.1 Procedure

To determine the enzyme's activity, 0.5mL buffered-L-alanine and -oxoglutarate solution were added to two glass tubes labeled 'Reagent Blank' and 'Test,' respectively, followed by 0.1mL each of distilled water and sample, mixed, and incubated for exactly 30 minutes at 37°C. Then, in each of the test tubes, 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added, mixed again, and allowed to stand for exactly 20 minutes at 20 – 25°C. To increase color development at alkaline pH, 5.0mL sodium hydroxide (0.4mol/L) was added at the conclusion of the period. After 5 minutes, the absorbance of the 'Test' (Atest) tube was compared to that of the 'Reagent blank' tube.

2.4.2.2.2 Calculation

Using the table of values previously plotted against activities, determine the activity of the enzyme ALT in the serum. The assay is hampered by haemolysis.

2.4.2.3 Alkaline phosphatase (ALP) method

Alkaline phosphatase (ALP) method as described by Okolonkwo et al. [11].

2.4.2.3.1 Procedure

In flow cell mode, fresh double distilled water (ddH₂O) was aspirated and utilized to complete a new Gain calibration. The equipment from the last sample run is now zeroed. ALP was chosen in the Run Test Screen, and a water blank test was run before dispensing 0.02 mL of sample and 1.0 mL of reagent (Diethanolamine buffered p-nitrophenylphosphate) into a test tube and mixing for 2 minutes. After that, the mixture was pumped into the Rx Monza. The test sample result was printed out via a printer linked to the machine after about 2 minutes.

This procedure of using machine is beneficial in that about 200 samples can be run and their results ready in 1hr using S. I. unit = IU/L.

2.4.2.3.2 Manual calculation

ALP can be calculated manually using the formula: IU/L = 2760 x ΔA 405 nm/min

2.4.2.4 Gamma-Glutamyltransferase (GGT) method

Gamma-Glutamyltransferase (GGT) method as described by Okolonkwo et al. [11].

2.4.2.4.1 Procedure

0.1ml sample and 1.0ml reagent (Buffered Glycylglycerine and L-gammaglutamyl-3-carboxy-4-nitrolide) were poured into a cuvette, stirred, and the initial absorbance read at 400–420nm with the timer started simultaneously. After 1, 2, and 3 minutes, the absorbance was measured again.

Calculation: IU/L = 1158 X ΔA (405nm/minute).

2.5 Statistical Analysis

The data generated from this study were analyzed using SPSS 24.0 for Mean \pm SD (descriptive statistics) and Two way ANOVA (inferential statistics). The level of significance was set at 0.05.

3. RESULTS

This study evaluated the ameliorative effect of vitamin E on liver enzymes in paraquat induced toxicity in male albino rats. An inter-, and intra

group comparative analysis of all the subgroups was assessed in the order of the groups treated with paraquat alone (B₀, C₀, and D₀) against the A₀ subgroup which served as the control, those treated with vitamin E of 500mg after one month of paraquat inducement (B_{VE}, C_{VE}, and D_{VE}) against the A_{VE} subgroup fed with vitamin E alone, and finally the A₀ vs A_{VE}, B₀ vs B_{VE}, C₀ vs C_{VE}, and D₀, vs D_{VE} groups respectively.

Table 1 is a presentation of the intergroup comparative analysis of the toxicity effect of paraquat on the liver enzymes parameters studied. The subgroups; A₀, B₀, C₀, and D₀ were statistically significant different, p-value \leq 0.05, in SGOT, SGPT, ALP, and GGT.

Table 2 is the intergroup comparison of the ameliorative effect of vitamin E on liver enzymes of the rats induced with paraquat. The comparison of A_{VE}, B_{VE}, C_{VE}, and D_{VE} subgroups were found to be statistically significant, p-value \leq 0.05 in all parameters (SGOT, SGPT, ALP, and GGT).

Table 3 is the intra, and inter group analyses of liver enzymes, showing the impact of paraquat inducement as well as the repairing effect of vitamin E on the toxic effect of paraquat on the test subjects (rats). Statistically, the control group A₀ and A_{VE} had no significant difference, p > 0.05 in all the parameters (SGOT, SGPT, ALP, and GGT) examined, whereas subgroups B₀ vs B_{VE}, C₀ vs C_{VE}, and D₀ vs D_{VE} expressed significant difference p \leq 0.05 statistically in all the parameters studied.

Table 1. Intergroup comparison of liver enzymes after one month treatment period

Subgroup	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
A ₀	2.20 \pm 0.04	2.52 \pm 0.08	11.25 \pm 0.30	13.63 \pm 0.38
B ₀	15.35 \pm 0.22 ^a	10.95 \pm 0.09 ^a	53.44 \pm 1.12 ^a	32.00 \pm 0.56 ^a
C ₀	66.22 \pm 1.68 ^a	134.88 \pm 2.34 ^a	82.00 \pm 1.75 ^a	42.67 \pm 0.99 ^a
D ₀	99.50 \pm 2.43 ^a	155.67 \pm 3.69 ^a	318.17 \pm 3.90 ^a	65.00 \pm 1.37 ^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 2. Inter group comparison of liver enzymes after one month of Vit E treatment

Subgroup	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
A _{VE}	5.02 \pm 0.19	4.08 \pm 0.01	22.69 \pm 0.55	19.02 \pm 0.43
B _{VE}	12.65 \pm 0.20 ^a	8.58 \pm 0.07 ^{a,b}	36.71 \pm 0.94 ^a	27.83 \pm 0.63 ^a
C _{VE}	46.00 \pm 0.94 ^a	74.13 \pm 1.64 ^a	33.83 \pm 0.50 ^a	33.33 \pm 0.70 ^a
D _{VE}	44.50 \pm 0.89 ^a	93.58 \pm 1.97 ^a	205.67 \pm 2.43 ^a	38.17 \pm 0.50 ^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 3. Inter and intra group comparison of liver enzymes after one month treatment

Subgroup	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
A ₀	2.20 ± 0.04	2.52 ± 0.08	11.25 ± 0.30	13.63 ± 0.38
A _{VE}	5.02 ± 0.19	4.08 ± 0.01	22.69 ± 0.55	19.02 ± 0.43
B ₀	15.35 ± 0.22 ^a	10.95 ± 0.09 ^a	53.44 ± 1.12 ^a	32.00 ± 0.56 ^a
B _{VE}	12.65 ± 0.20 ^{a,b}	8.58 ± 0.07 ^{a,b}	36.71 ± 0.94 ^{a,b}	27.83 ± 0.63 ^{a,b}
C ₀	66.22 ± 1.68 ^a	134.88 ± 2.34 ^a	82.00 ± 1.75 ^a	42.67 ± 0.99 ^a
C _{VE}	46.00 ± 0.94 ^{a,b}	74.13 ± 1.64 ^{a,b}	33.83 ± 0.50 ^{a,b}	33.33 ± 0.70 ^{a,b}
D ₀	99.50 ± 2.43 ^a	155.67 ± 3.69 ^a	318.17 ± 3.90 ^a	65.00 ± 1.37 ^a
D _{VE}	44.50 ± 0.89 ^{a,b}	93.58 ± 1.97 ^{a,b}	205.67 ± 2.43 ^{a,b}	38.17 ± 0.50 ^{a,b}

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: B₀ vs B_{VE}) at each month.

4. DISCUSSION

This study evaluated the ameliorative effect of vitamin E on liver enzymes in paraquat induced toxicity in male albino rats. An inter-, and intra group comparative analysis of all the subgroups was assessed in the order of the groups treated with paraquat alone (B₀, C₀, and D₀) against the A₀ subgroup which served as the control, those treated with vitamin E of 500mg after one month of paraquat inducement (B_{VE}, C_{VE}, and D_{VE}) against the A_{VE} subgroup fed with vitamin E alone, and finally the A₀ vs A_{VE}, B₀ vs B_{VE}, C₀ vs C_{VE}, and D₀ vs D_{VE} groups respectively.

The result showed that the inter group comparisons of the B₀, C₀, and D₀ against the A₀ (control) group was significantly different in all the liver enzyme parameters (SGOT, SGPT, ALP, GGT) studied. The significant difference in the results obtained could be an outcome of the damages from oxidative stress induced by paraquat on the liver organ of the rats. This result is in agreement with works of Raja et al. (1992) and Noriega et al. (2002) which reported that paraquat created a shift in the function of some liver enzymes in test subjects used in their research works [5,6].

There was also a significant difference in the parameters (SGOT, SGPT, ALP, GGT) when the inter group comparative analysis of subgroups A_{VE}, B_{VE}, C_{VE}, and D_{VE} were evaluated. This results recorded could be attributed to the ameliorative potency of the vitamin E on the impacts created by paraquat toxicity. It also goes to prove the results of other studies such as those Richter et al., (2022) and Rizvi et al. [7,8] which revealed that vitamin E has the ability to

ameliorate injuries arising from reactive oxygen species produced by paraquat.

The intra group comparison of subgroups B₀ vs B_{VE}, C₀ vs C_{VE}, and D₀ vs D_{VE} all exhibited significant differences, $p \leq 0.05$ in the parameters (SGOT, SGPT, ALP, GGT) examined, whereas the A₀ vs A_{VE} remained comparatively similar. The significant differences seen in the subgroups B₀ vs B_{VE}, C₀ vs C_{VE}, and D₀ vs D_{VE} may be as a result of the activities of vit. E in cancelling the peroxidation caused by paraquat in the B₀, C₀, and D₀ subgroups. As there was no oxidative stress recorded in the A₀ vs A_{VE} subgroups, the administration of vitamin E yielded no result as there was no injury to heal, confirming again that vitamin E possesses ameliorative ability as shown in the research works of Richter et al. and Rizvi et al. [7,8].

5. CONCLUSION

The experiment aimed to determine the effects of vitamin E on Liver function of Paraquat Exposed Wistar Rats. The results of this experiment showed that vitamin E therapy is potent against paraquat induced toxicity on liver enzymes on a one-month treatment basis.

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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