Effects of vitamin E on Liver function of Paraquat Exposed Wistar Rats (*Rattusnorvegicus*)

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-$_K$B activation, and apoptosis in many cells.. The study used 200 male albino rats with a mean weight of 0.20.02kg 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. "A_0" and "A_{VE}" subgroups existed in the "A" group; "B_0" and "B_{VE}" subgroups existed in the "B" group; "C_0" and "C_{VE}" subgroups existed in the "C" group; and "D_0" and "D_{VE}" subgroups existed in the "D" group. Vitamin E was not given to the "A_0," "B_0," "C_0," and "D_0" 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 A_0 group which is the control group, B_0, C_0, and D_0 being the paraquat treated groups, as well as the B_{VE}, C_{VE}, and D_{VE} vit. E treament subgroups had a statistically significant difference, p-value$\leq$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

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-$_K$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 2-6days. 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.* (1992), 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. (2002), 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 [6]. 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 redicals 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 live [9]. Vitamin E can is present in a wide range of foods and oils. High levels of alpha-tocopherol can be found in nuts, seeds, and vegetable oils, and considerable amounts can also be found in green leafy vegetables and fortified cereals. [8].

MATERIALS AND METHOD

Experimental Design

The study used 200 male albino rats with a mean weight of 0.20.02kg 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. There were subgroups within each of the primary categories. "A_0" and "A_{VE}" subgroups existed in the "A" group; "B_0" and "B_{VE}" subgroups existed in the "B" group; "C" group had "C_0" and "C_{VE}" subgroups existed in the "C" group; and "D" group had "D_0" and "D_{VE}" subgroups existed in the "D" group. Vitamin E was not given to the "A_0," "B_0," "C_0," and "D_0" subgroups, 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.

Animal source

Animal House, Department of Biology, Rivers State University of Science and Technology provided 200 rats with an average weight of 0.20.02kg. 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.

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.

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 [10].

Procedure for Vitamin Administration

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

Serum glutarate-oxaloacetate-aminotransferase (AST/SGOT) method: by Reitman and Frankel, 1957.

Principle: In a pH 7.5 buffered substrate containing aspartate and -ketoglutarate, AST is incubated at 37^0C for precisely 60 minutes. The amino group from aspartate to ketoglutarate is transferred by AST, resulting in oxaloacetate and glutamate. In an alkaline media, oxaloacetate

combines with 2, 4-dinitrophenylhydrazine to yield 2, 4-dinitrophenylhydrazone, which is red-brown in color. Spectrophotometrically, the absorbance of the given color is measured at 540nm.

α-oxoglutarate + L-aspartate $\xrightarrow{\text{SGOT}}$ L-glutamate + Oxaloacetate.

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 37^0C. 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 – 250C. 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.

Serum glutarate-pyruvic-aminotransferase (SGPT) by Reitman and Frankel, 1957.

Principle: In a pH 7.5 buffered substrate containing L-alanine and -ketoglutarate, ALT is incubated for exactly 60 minutes at 37^0C. The transfer of the amino group from alanine to ketoglutarate is catalyzed by ALT, resulting in the formation of pyruvate and glutamate. Pyruvate interacts with 2, 4-dinitrophenylhydrazine to generate 2, 4-dinitrophenylhydrazone, which has a red-brown color in an alkaline medium. Spectrophotometrically, the absorbance of the color produced is measured at 540nm.

(α-oxoglutarate-L-alanine $\xrightarrow{\text{GPT}}$ L-glutamate + pyruvate

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 37^0C. 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 – 250C. 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: 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.

Alkaline phosphatase (ALP) method by Englehardt, *et al*., 1970.

Principle: This is an improved standard approach that monitors the concentration of p-nitrophenol produced with p-nitrophenlphosphate to determine ALP.

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.

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

Gamma-Glutamyltransferase (GGT) method: (Szasz and Bergmeyer, 1974 and Teitz, 1987)

Principle: n the presence of glycylglycine, the substrate L-V-glutamyl-3-carboxy-4-nitroanilide is transformed by GGT in the sample to 5-amino-2-nitrobenzoate, which can be detected spectrophotometrically at 405nm.

L-V-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\text{GGT}}$

L-V-glutamylglycylglycine + 5-amino-2-nitrobenzoate.

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).

3. RESULT

Table 1.0 is a presentation of the intergroup comparative anoupalysis of the toxicity effect of paraquat on the liver enzymes parameters studied. The subgroups; A_0, B_0, C_0, and D_0 were statistically significant different, p-value≤0.05, in SGOT, SGPT, ALP, and GGT.

Table 1.0 ***Intergroup comparison of liver enzymes after one month treatment period.***

Subgroup	Treatments (4 Rats in each subgroup) Mean ±SEM			
	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
B_0	15.35 ± 0.22[a]	10.95 ± 0.09[a]	53.44 ± 1.12[a]	32.00 ± 0.56[a]
C_0	66.22 ± 1.68[a]	134.88 ± 2.34[a]	82.00 ± 1.75[a]	42.67 ± 0.99[a]
D_0	99.50 ± 2.43[a]	155.67 ± 3.69[a]	318.17 ± 3.90[a]	65.00 ± 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 1.1 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≤0.05 in all parameters (SGOT, SGPT, ALP, and GGT).

Table 1.1: ***Inter group comparison of liver enzymes after one month of Vit E treatment.***

Subgroup	Treatments (4 Rats in each subgroup) Mean ±SEM			
	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
A_{VE}	5.02 ± 0.19	4.08 ± 0.01	22.69 ± 0.55	19.02 ± 0.43
B_{VE}	12.65 ± 0.20[a]	8.58 ± 0.07[a,b]	36.71 ± 0.94[a]	27.83 ± 0.63[a]
C_{VE}	46.00 ± 0.94[a]	74.13 ± 1.64[a]	33.83 ± 0.50[a]	33.33 ± 0.70[a]
D_{VE}	44.50 ± 0.89[a]	93.58 ± 1.97[a]	205.67 ± 2.43[a]	38.17 ± 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.

Tables 1.2 is the intra, and inter group analysis 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 A_0 and A_{VE} had no significant difference, $p > 0.05$ in all the parameters (SGOT, SGPT, ALP, and GGT) examined, whereas subgroups B_0 vs B_{VE}, C_0 vs C_{VE}, and D_0 vs D_{VE} expressed significant difference $p \leq 0.05$ statistically in all the parameters studied.

Table 1.2: ***Inter and intra group comparison of liver enzymes after one month treatment***

Subgroup	Treatments (4 Rats in each subgroup) Mean ±SEM			
	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: B_0 vs B_{VE}) at each month.

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 (B_0, C_0, and D_0) against the A_0 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 A_0 vs A_{VE}, B_0 vs B_{VE}, C_0 vs C_{VE}, and D_0, vs D_{VE} groups respectively.

The result showed that the inter group comparison of the B_0, C_0, and D_0 against the A_0 (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.*, (2014) [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 B_0 vs B_{VE}, C_0 vs C_{VE}, and D_0 vs D_{VE} all exhibited significant differences, $p \leq 0.05$ in the parameters (SGOT, SGPT, ALP, GGT) examined, whereas the A_0 vs A_{VE} remained comparatively similar. The significant differences seen in the subgroups

B_0 vs B_{VE}, C_0 vs C_{VE}, and D_0 vs D_{VE} may be as a result of the activities of vit. E in cancelling the peroxidation caused by paraquat in the B_0, C_0, and D_0 subgroups. As there was no oxidative stress recorded in the A_0 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.*, (2022) and Rizvi *et al*., (2014) [7,8].

CONCLUSION

In conclusion, 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.

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