Effects of Two Months Durational Vitamin E Therapy on Paraquat-inflicted Liver Damage in Wistar Rats (*Rattus norvegicus*)

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Author's contribution

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

Original Research Article

ABSTRACT

Paraquat is a highly poisonous chemical that is commonly used as a herbicide (plant killer) to control weeds and grasses. It has been found to disrupt the levels and activities of some liver enzyme parameters. This experimental study was aimed at assessing the effect of Vitamin E in restoring liver physiology in paraquat-induced liver poisoning in rats. In this study, 200 male rats with a mean weight of 0.20.02kg, with 50 rats in each group of four, labeled A, B, C, and D. The "A" group received no paraquat, while the "B", "C", and "D" groups received 0.02g, 0.04g, and 0.06g of paraquat per kilogram of rat every two weeks for three months respectively. Each group also had a subgroup treated only with vitamin E and the Vit. E + paraquat subgroup B_{VE}, C_{VE}, and D_{VE} but A_{VE} was treated with paraquat but only Vit E. The blood was taken and the liver function was assayed. The result showed that there was a significant dose-dependent increase (P-value<0.05) in the levels liver enzymes in the subgroups treated with paraquat alone (A_0, B_0, C_0 and D_0). After treatment with vit E, the result revealed that there a significant decrease (P-value<0.05) in the level of the liver enzymes when compared to the corresponding subgroups without Vit. E treatment. This study has shown that vit E can ameliorate the effect of paraquat liver poisoning particularly when the treatment is administered weekly for two months.

Keywords: Paraquat; liver enzymes; vitamin E; toxicity.

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1. INTRODUCTION

Paraquat is a highly poisonous chemical that is commonly used as a herbicide (plant killer) to control weeds and grasses [1,2]. Paraquat is typically sold as a liquid in various strengths in the United States. Paraquat is classified as "restricted use" by the US Environmental Protection Agency. This means that only licensed applicators are allowed to use it [1,3].

In the United States, paraquat is one of the causes of poisoning and has been linked high number of mortality [4,5]. Paraquat causes cellular damage in various organs by causing lipid peroxidation, activation of $NF_{-K}B$, mitochondrial damage, and apoptosis [6]. Jacob *et al.,* (2019) in their study reported that inhalation of paraquat could bring about the damage of the lungs and result to the disease paraquat lung [7]. Ingestion of paraquat has also been reported to be the lead cause of damages to the linings of the mouth, stomach, intestine, liver, kidney, and esophagus while long term exposure could lead to lung scarring and even death [7]. According to the research by Gawarammana et al., [6] and Suntres [8], paraquat causes damages to the organs of the body through the generation of the superoxide anion, which can lead to the formation of more toxic reactive oxygen species like hydrogen peroxide and hydroxyl radical; and the oxidation of cellular NADPH, which is the major source of reducing equivalents for the intracellular reduction of paraquat, which disrupts important NADPH-requiring biochemical processes [6,8]. Respiratory failure caused by an oxidative assault to the alveolar epithelium, followed by obliterating fibrosis, is the leading cause of death in paraquat overdose [8].

Paraquat has been found to disrupt the levels and activities of some liver enzyme parameters such as alanine transaminase, aspartate aminotransferase, alkaline phosphatase levels, and Kelch-like ECH-associated protein 1 gene expression [9,10,11]. Paraquat also caused a decline in the total antioxidant capacity, total thiol group levels, Glutathione S-transferases, heme oxygenase 1 and led to histological injuries to the tissues of the liver according to a study by Kheiripour et al. [9].

Vitamin E was discovered in the 1920s and is an important nutrient. For nearly a century, scientists have examined several of vitamin E's physiological roles, including its antioxidative actions [12]. Alterations in redox balance mediated by internally and externally generated free radicals are implicated in a numerous disorders and are also a process that is thought to be necessary for survival. Due to its high concentration among the lipid soluble vitamin groups, vitamin E is known to control redox balance in the body and is found throughout the body, including cell membranes and lipoproteins [12]. In studies conducted by Watanabe *et al.*, (1986) and Harada e*t al.*, (1991), it was noted that vit. E was able to limit the impact of paraquat and to restore the normal activities of liver enzyme parameters after a period of time [13,14]. In this study, we aim to discover the ameliorative effect of vitamin E of paraquat induced toxicity on liver enzyme parameters after a two month period of treatment.

2. MATERIALS AND METHODS

2.1 Experimental Design

This chronic experimental study required the use of 200 male albino rats with a mean weight of 0.20±02kg [15]. Four groups of 50 rats were formed from the 200 rats. There were four groups: A, B, C, and D. The "A" group received no paraquat, while the "B", "C", and "D" groups received 0.02g, 0.04g, and 0.06g of paraquat per kilogram of rat every two weeks for three months, respectively. Within each of the basic categories, there were subclasses [15]. In the "A" group, there were "A_0" and "A_{VE}" subgroups; in the "B" group, there were "B_0" and "B_{VE}" subgroups; in the "C" group, there were "C_0" and "C_{VE}" subgroups; and in the "D" group, there were "D_0" and "D_{VE}" subgroups. The "A_0," "B_0," "C_0," and "D_0" subgroups did not receive vitamin E, while the "A_{VE}," "B_{VE}," "C_{VE}," and "D_{VE}" subgroups received it every week for two months after paraquat inducement. The blood was taken and the liver function was checked.

2.2 Animal Source

200 rats weighing an average of 0.20.02kg were furnished by the Animal House, Department of Biology, Rivers State University of Science and Technology. The rats were brought to the study site and given two weeks to adapt before the trial began. The study was conducted at the Department of Medical Laboratory Science at Rivers State University of Science and Technology.

2.3 Treatment Administration

2.3.1 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 [16].

2.3.2 Procedure for vitamin administration

Vitamin E was given orally every week for two month at doses of 500mg [16].

2.4 Sample Collection Method

Liver function test was performed using a blood sample. A heart puncture was used to extract 2ml of blood, which was then poured into simple bottles with the help of a syringe and needle. After the blood had coagulated, the serum was extracted by spinning it at 4000rpm. SGOT, SGPT, ALP and GGT levels were all measured in the serum.

2.4.1 Serum glutarate-oxaloacetate-aminotransferase (AST/ SGOT) methods

Reitman and Frankel [15]

2.4.1.1 Procedure

For the enzyme's activity evaluation, 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 30 minutes at 37ºC. Then 0.5mL of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added to each test tube, mixed again, and allowed to stand for exactly 20 minutes at 20 – 25ºC. 5.0mL sodium hydroxide (0.4mol/L) was added at the end of the session to promote color development at alkaline pH. The 'Test' (Atest) tube's absorbance was compared to the 'Reagent blank' tube's after 5 minutes.

2.4.1.2 Calculation

From the table of values previously plotted against activities, determine the activity of the enzyme AST in the serum. The assay is hampered by hemolysis.

2.4.2 Serum glutarate-pyruvic-aminotransferase (SGPT)

Reitman and Frankel [15].

2.4.2.1 Procedure

To assess 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 at 37ºC for exactly 30 minutes. Then 0.5mL of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added to each test tube, mixed again, and allowed to stand for exactly 20 minutes at 20 – 25ºC. 5.0mL sodium hydroxide (0.4mol/L) was added at the end of the session to promote color development at alkaline pH. The 'Test' (Atest) tube's absorbance was compared to the 'Reagent blank' tube's after 5 minutes.

2.4.2.2 Calculation

Determine the activity of the enzyme ALT in the serum by using the table of values previously plotted against activities. Haemolysis interferes with the assay.

2.4.3 Alkaline phosphatase (ALP) method

Englehardt as described by Okolonkwo et al [15]

2.4.3.1 Procedure

Fresh double distilled water (ddH_2O) was aspirated and used to perform a new Gain calibration in flow cell mode. The equipment from the last sample run has been reset to zero. A water blank test was performed before pouring 0.02 mL of sample and 1.0 mL of reagent (Diethanolamine buffered p-nitrophenyl phosphate) into a test tube and mixing for 2 minutes. The mixture was then fed into the Rx Monza. After about 2 minutes, the test sample result was written out using a printer connected to the machine.

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.

2.4.3.2 Manual calculation

The formula: IU/L = 2760 x ΔA 405 nm/min is used in the manual calculation of ALP.

2.4.4 Gamma-glutamyltransferase (GGT) method

As described by Okolonkwo et al. [15]

2.4.4.1 Procedure

In a cuvette, 0.1ml sample and 1.0ml reagent (Buffered Glycylglycerine and L-gammaglutamyl-3-carboxy-4-nitrolide) were poured, mixed, and the first absorbance read at 400–420nm while the timer was started. The absorbance was checked again after 1, 2, and 3 minutes.

2.4.4.2 Calculation

IU/L = 1158 X ΔA (405nm/minute).

2.5 Statistical Analysis

Data collected were analyzed for descriptive and inferential statistics using SPSS 22.0. Descriptive statistics included mean and standard deviation while inferential statistics two-way ANOVA. Statistical significance was set at $p < 0.05$

3. RESULTS

The intergroup comparison of liver enzymes parameters for all subgroups treated with paraquat after 2 months is shown below in Table 1. This result shows the toxicity impact of paraquat on the liver of the rats studied. A_0 being the control group was not treated with paraquat, while B_0, C_0, and D_0 were treated and the results were found to have statistical significant difference, $P \leq 0.05$ in SGOT, SGPT, ALP, and GGT, while A_0 group was constant after the three months treatment period.

Table 2 is the intergroup analysis comparing the state of the liver enzymes parameters after two months of vitamin E therapeutic treatment. The A_{VE} is the control, while B_{VE}, C_{VE}, and D_{VE} are the treatment group. The result showed that statistically, there was a significant difference, $p \leq 0.05$ for B_{VE}, C_{VE}, and D_{VE} subgroups in all liver enzyme parameters examined, but A_{VE} had no significant difference.

Intragroup comparative analysis of changes in liver biochemical data in liver enzymes of the test subjects is represented in Table 3. A_0 vs A_{VE} remained the control groups, while B_0 vs B_{VE}, C_0, C_{VE}, and D_0 vs D_{VE} were the paraquat and the vitamin E + paraquat groups respectively. The

results showed that there was statistical significant difference, $p \leq 0.01$ in SGOT, SGGT, ALP, and GGT, whereas A_0 vs A_{VE} subgroups remained the same.

4. DISCUSSION

This study evaluated the chronic toxicity impact of paraquat on the levels and activity of liver enzymes parameters SGOT, SGGT, ALP, and GGT, as well as the therapeutic effect of vitamin on the toxicity after treatments for two months. Initially, an intergroup comparison was conducted on the subgroups administered only paraquat (B_0, C_0, and D_0) against their control A_0. Another intergroup comparison was conducted on the vitamin E + paraquat treated group (B_{VE}, C_{VE}, and D_{VE}) to determine the healing effect of vitamin E on paraquat toxicity. The last analysis was an intragroup comparison within the same subgroup (A_0 vs A_{VE}, B_0 vs B_{VE}, C_0, C_{VE}, and D_0 vs D_{VE}).

The results obtained showed that the intergroup comparison of the paraquat treatment subgroups (B_0, C_0, and D_0) against their control group A_0 showed there was significant increase in all the liver enzyme parameters examined. This increase was dose-dependent such that $D_0 > C_0 > B_0$. This pattern means that the group with the highest dose of paraquat treatment had the highest liver toxic insult and the group with the least dose of paraquat had the least toxic insult. This outcome could be a proof that paraquat is toxic and leads to alteration in the levels and activity of some liver enzymes parameters. This result is in agreement with the reports of studies conducted by Varma et al., (2022), Kim et al., (2017), Heller et al., (2019), Kheiripour et al., (2021), and Liu et al., (2020) which all pointed out that paraquat is a toxic chemical and has the ability to disrupt the normal functioning of some liver enzyme parameters [4,5,7,9,10].

Inter group analytic comparison of the vitamin E + paraquat treatment subgroups (B_{VE}, C_{VE}, and D_{VE}) against the neutral group fed only with vitamin E (A_{VE}) was significantly difference in all the liver enzyme parameters examined. The result obtained here is an indication of the antioxidant potency of vitamin E against the peroxidation caused by paraquat. This result is in consonance with that reported by Miyazawa et al., (2019), Watanabe et al., (1986) and Harada et al., (1991) which revealed that vitamin E possesses antioxidant ability and was able to limit paraquat peroxidation [12,13,14].

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

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
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 ≤ 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 two month of Vit E treatment

Subgroup	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	$8.58 \pm 0.07^{a,b}$	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 ≤ 0.05
Index (a) = represents a statistically significant difference between the test subgroups and the control subgroups at each treatment month

Table 3. Changes in Liver enzymes biochemical data after two months treatment period

Subgroup	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
A_0	6.17 ± 1.38	4.18 ± 0.03	1.57 ± 0.05	13.33 ± 0.17
A_{VE}	5.55 ± 1.38	3.43 ± 0.01	1.91 ± 0.06	15.75 ± 0.57
B_0	15.24 ± 1.39^a	41.15 ± 0.44^a	100.30 ± 1.00^a	63.00 ± 1.39^a
B_{VE}	$15.11 \pm 1.38^{a,b}$	$19.68 \pm 0.38^{a,b}$	$73.00 \pm 1.47^{a,b}$	$33.48 \pm 1.30^{a,b}$
C_0	99.65 ± 1.64^a	147.00 ± 1.52^a	130.68 ± 0.84^a	76.25 ± 1.30^a
C_{VE}	$94.69 \pm 1.86^{a,b}$	$131.50 \pm 2.15^{a,b}$	$72.80 \pm 4.42^{a,b}$	$43.50 \pm 0.71^{a,b}$
D_0	193.00 ± 2.70^a	258.75 ± 3.10^a	$315.53 \pm 1,63^a$	99.00 ± 0.60^a
D_{VE}	$138.50 \pm 2.97^{a,b}$	$140.25 \pm 2.11^{a,b}$	$198.78 \pm 1.87^{a,b}$	$82.00 \pm 0.84^{a,b}$

Statistical significance: P ≤ 0.01, 0.05 or 0.001.
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

Significant difference was recorded on the intra group comparison conducted within each subgroups B_0 vs B_{VE}, C_0, C_{VE}, and D_0 vs D_{VE} (treatment subgroups) except for the A_0 vs A_{VE} (control groups). This result showed that vitamin E was able to ameliorate the imbalance in the liver enzyme parameters (SGOT, SGGT, ALP, and GGT) caused by paraquat, a result which is in agreement with the report of the research done by Watanabe et al. and Harada et al., [13,14].

5. CONCLUSION

It can therefore be deduced in conclusion that paraquat induced toxicity in liver can be treated with vitamin E therapy on a two month treatment period.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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