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Assessing Short-Term Vitamin E and C Treatment Effect in Paraquat Liver Poisoning

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Abstract: Paraquat has been considered as a toxicant in various countries, since it is not only intensely harmful but yet promptly accessible and generally cheap. Vitamin C and E are notable cancer prevention agents that respond rapidly to free radical and maximally forestall lipid peroxidation. The aim of this study was to evaluate the ameliorative impact of vitamin E and C in paraquat induced liver poisoning in rat. A total of 200 male wistar rats were obtained for the study. With 50 rats each, the rats were arranged into four grougs: A, B, C, and D. Each group was partitioned into two subgroups (0 and VEC), each with 25 rodents. The "A" group was not treated with paraquat, while the "B," "C," and "D" groups got 0.02g, 0.04g, and 0.06g of paraquat treatment respectively. All the "0" subgroups were those not treated with Vit E and C and all "VEC" subgroups were those treated with 500mg of vitamin E and 2000mg/dl of vitamin C. The paraquat treatment was administered once every 2 weeks for 3 months, followed by one month of week by week vitamin E and C treatment. Blood was drawn for SGPT, SGOT, ALP, and GGT testing. At p-value of 0.05, there was a significant increase in the activities of liver enzymes among the "A0", "B0", "C0", and "D0" groups. There was a significant decrease (P-value<0.05) in the activities of liver enzymes in "VEC" subgroups. This study uncovered that vitamin E and C combined treatment ameliorates treated the Paraquat induced liver injury in rats.

Keywords: paraquat, rats, vitamins, antioxidant, lipid peroxidation.

1. Introduction

Paraquat is a man-made substance. Weidel and Russo reported it in 1882. Paraquat's redox characteristics were found by Michealis and Slope in 1933, and the substance was given the name methyl viologen. Paraquat (PQ) was first perceived as having herbicidal characteristics in 1958, and it was confirmed in 1962 [1]. It has been named a significant self-destructive agent in a few countries, since it is intensely harmful, and in addition since it is generally accessible, moderately economical, and has no known benefit [2]. Most studies on paraquat poisoning has zeroed in on decreasing its usage or further enhancing its disposal [3].

PQ is converted completely to PQ mono-cation free radical by nicotinamide adenine dinucleotide phosphate (NADPH)- cytochrome P-450 reductase once it enters the cells. The electron conveyed to PQ rapidly goes to oxygen, bringing about the

development of superoxide anion, and reactive oxygen species (ROS) which causes serious oxidative harm [3]. Harm to hepatocyte brings about edema, degeneration, and fibrosis of hepatocytes because of the oxidative insults [4, 5]. ROS and other free radicals are ceaselessly made during typical physiologic cycles and altered macromolecules like proteins, lipids, and DNA, causing tissue damage [6]. The significance of oxidative stress in the etiology and movement of persistent liver illness has for some time been perceived [7, 8]. Cell repair mechanisms have recently been displayed to safeguard cells from the harming impacts of various environmental contaminations [9, 10].

Vitamins C and E are notable cell antioxidants that respond rapidly to free radicals and forestall lipid peroxidation. Therefore, the two vitamins are believed to be ingested during oxidative stress responses [11, 12]. L-ascorbic acid's ability to extinguish radicals created by paraquat redox cycling has been credited with the antioxidative capacity of vitamin C [13]. Also, studies have shown that vitamin C can prevent cancer formation especially those due to oxidative stress [14, 15].

Lack of vitamin E potentiated the advancement of intense paraquat poisoning in rodents in numerous trials, showing its capability in ameliorating paraquat poisoning. One of the most notable roles is its capacity to take out free radicals delivered by the human body's oxidation reaction or by foreign substances. [16].

Cell repair frameworks, as a general rule, either keep ROS from affecting or dispose of them before they can hurt fundamental cell parts [17].

In light of the overall significance of L-ascorbic acid and E as cell antioxidants, this study zeroed on assessing the therapeutic effect of vitamin E and C in paraquat induced liver damage in male rats.

2. Materials and Methods

2.1. Experimental Design

Two hundred (200) rats averagely weighing 0.20±0.02kg were obtained from The Animal House, Rivers State University of Science and Technology. The rats were brought to the site of study and given fourteen (14) days to acclimatize before the trial started. The rats were divided into four main groups; A, B, C and D group with each group composed of 50 rats. The A group served as the control while the B, C, and D groups served as the test groups. Only the groups were treated with 0.02g, 0.04g and 0.06g paraquat for B, C and D respectively. Each main group had two subgroups designated “V0” and “VEC” with each subgroup composed of 25 rats. “V0” subgroups had no vitamin treatment but VEC had both 500mg vit E and 2000mg/dl vit C treatment.

3. Treatment Procedure

3.1. Administration of Paraquat

Paraquat was administered by means of oral gavage. The rats received the dose of paraquat assigned to the group once every two weeks for three months.

The rodents were held at the skin over the head and turned with the goal that the mouth was looked vertical and the body brought down towards the holder. The needle slant was then positioned into the mouth of the rat a piece horizontally in a manner to stay away from the teeth. The substance in the needle was then discharged into the mouth of the rat bit by bit [18].

Administration of Vitamins

The vitamin E and C were given orally consistently for quite a long time at portions of 500mg and 2000mg/dl respectively [18].

Sample Collection technique

Blood was collected by means of cardiac puncture into plain containers (without anticoagulant) with a 2ml needle, allowed to clot to get serum, and afterward assayed for the liver catalysts GGT, SGOT, SGPT, and ALP. The rats were anaesthetized under 70% chloroform sedation. To keep away from contamination, the corpses that remained were burned.

3.2. Laboratory analysis

Serum glutarate-oxaloacetate-aminotransferase (AST/SGOT) method by Reitman and Frankel as described by Okolonkwo *et al* [18].

Procedure

In estimating the activity of this enzyme, 0.5ml of buffered-L- aspartate and α-oxoglutarate solution was each added to two glass tubes labeled 'Reagent Blank' and 'Test', followed by 0.1ml each of distilled water and sample to their respective tubes, mixed and incubated for exactly 30 minutes at 37^0C. After which, 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added to each of the test tubes, mixed again and allowed to stand for exactly 20 minutes, at 20 – 25^0C. At the end of the time, 5.0mls of Sodium hydroxide (0.4mol/L) was added to enhance colour development at alkaline pH. The tubes were mixed and the absorbance of 'Test' (A_{test}) read against that of the 'Reagent blank' after 5 minutes.

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 as described by Okolonkwo *et al* [18].

Procedure

In estimating the activity of this enzyme, 0.5ml of buffered-L- alanine and α-oxoglutarate solution was each added to two glass tubes labeled 'Reagent Blank' and 'Test', followed by 0.1ml each of distilled water and sample to their respective tubes, mixed and incubated for exactly 30 minutes at 37^0C. After which, 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) solution was added to each of the test tubes, mixed again and allowed to stand for exactly 20 minutes, at 20 – 25^0C. At the end of the

time, 5.0mls of Sodium hydroxide (0.4mol/L) was added to enhance colour development at alkaline pH. The tubes were mixed and the absorbance of 'Test' (A_{test}) read against that of the 'Reagent blank' after 5 minutes.

Calculation

Obtain the activity of the enzyme ALT in the serum from the table of values previously plotted against activities. Haemolysis interferes with the assay.

Alkaline phosphatase (ALP) method as described by Okolonkwo *et al* [18].

Procedure

Fresh double distilled water (ddH_2O) was aspirated and used to perform a new Gain calibration in flow cell mode. This zero the equipment from previous sample run. ALP was selected in the Run Test Screen and a water blank test run was carried out, after which 0.02ml of sample and 1.0 ml of reagent (Diethanolamine buffered p-nitrophenylphosphate) was dispensed into a test tube and mixed for 2 minutes. The mixture was then aspirated into the Rx Monza. After about 2 minutes the result of the test sample was then printed out from a printer connected to the machine.

The advantage of this machine procedure is that up to 200 samples can be processed and results produced within one hour in S.I. unit = IU/L.

Manual calculation

To calculate the ALP activity, using the manual method, the following formula was utilized: IU/L = 2760 x ΔA 405 nm/min.

Gamma-Glutamyltransferase (GGT) method as described by Okolonkwo *et al* [18].

Procedure

0.1ml of sample and 1.0ml of reagent (Buffered Glycylglycerine and L-gammaglutamyl-3-carboxy-4-nitrolide) were dispensed into a cuvette, mixed and the initial absorbance read at 400 – 420nm with simultaneous timer initiation. The absorbance was read again after 1, 2 and 3 minutes.

Calculation

IU/L = 1158 X ΔA (405nm/minute).

3.3. Statistical Analysis

The information created from this review was examined utilizing SPSS adaptation 23.0 for engaging an inferential insights (ANOVA) for between bunch examination and T-test for intra-bunch (sub-bunch) correlation at test importance, P-value<0.05.

Results

The result in Table 1.0 shows the mean values of liver enzymes after paraquat poisoning at various doses. The result shows that there were significant rises (P-

value<0.05) in the mean values of liver enzymes with increase dose of paraquat across the groups.

Table 1. Changes in Liver Enzyme Levels After Three Month Paraquat Induction

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: $P \leq 0.05$

"a" represents significant difference among the groups.

Table 2.0 shows the recovery effect of paraquat poisoned liver after vitamin E and C combined treatment. The result showed that there were significant drops (P-value<0.05) in the mean values of the liver enzymes across all test groups treated with vitamin E and C.

Table 2. Changes in Liver Enzyme Levels After One Month Vitamin EC 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_{VEC}	4.65 ± 0.11	2.27 ± 0.05	13.04 ± 0.17	10.33 ± 0.23
B_0	15.35 ± 0.22	10.95 ± 0.09	53.44 ± 1.12	32.00 ± 0.56
B_{VEC}	11.38 ± 0.22[b]	11.83 ± 0.22[b]	26.78 ± 0.51[b]	18.50 ± 0.28[b]
C_0	66.22 ± 1.68	134.88 ± 2.34	82.00 ± 1.75	42.67 ± 0.99
C_{VEC}	36.33 ± 0.56[b]	68.40 ± 0.69[b]	29.33 ± 0.51[b]	23.33 ± 0.39[b]
D_0	99.50 ± 2.43	155.67 ± 3.69	318.17 ± 3.90	65.00 ± 1.37
D_{VEC}	67.67 ± 1.15[b]	86.48 ± 1.35[b]	297.67 ± 6.15[b]	31.50 ± 0.41[b]

Statistical significance: $P \leq 0.05$

"b" represents significant difference within the groups.

4. Discussion

Regardless of continual worldwide call for a worldwide prohibition on PQ use, its high weed-control viability and minimal expense are unquestionable claims for its continual use in most non-industrial countries [19, 20].

This study zeroed in on serum hepatic enzymes like ALT, AST, ALP, and GGT for the investigation of hepatotoxicity and recovery. When compared with the control subgroups (A0 and AVEC), the enzymes activities were viewed as higher in the test subgroups (B0, BVEC, C0, CVEC, D0, and DVEC), there was a link between the PQ dose given and the level of enzymes activities; in this way, the rise was viewed as straightforwardly connected with the dose of PQ administered, as seen in [21, 22]. PQ studies and the poisoning impacts of this substance in the body's organs, as well as its mechanism of action, have been accounted for [23]. Following a hepatocellular injury,

liver enzymes are discharged into the circulatory system, bringing about an increase in their activity in serum tests. These unusual elevations in the enzymes are key signs of hepatotoxicity or disorder. As a rule, these discoveries might highlight liver degeneration and hypofunction. Lipid peroxidation is known to disturb the integrity of cell layers, bringing about cytoplasmic enzyme spillage. Accordingly, rise of these enzymes in the serum could be the consequence of serious hepatocellular harm. This is likewise steady with a few examinations [22, 24-28] that viewed PQ as a hepatotoxin.

Besides, within group comparison of all the enzymes uncovered an obvious decrease in the enzyme activities of the L-ascorbic acid and E treated subgroups (BVEC, CVEC, and DVEC) when compared with their corresponding PQ treated subgroups (B0, C0 and D0). For this situation, the combined treatment of Vitamin E and C reestablished hepatocellular recovery. Vitamin E is notable for controlling all oxidation processes in the body and consequently functions as an antioxidant necessary for tissue repair. It functions as an antioxidant by forestalling chain proliferation by moving phenolic hydrogen to a peroxyl free radical of a polyunsaturated unsaturated fat, in this manner restricting the degree of lipid peroxidation. Most of the peroxytocopherol produced is reconverted to tocopherol by L-ascorbic acid, while L-ascorbic acid, generally, diminished the toxic insult and works as a substrate for the antioxidant enzyme ascorbate peroxidase, a capability that is particularly significant in oxidative stress mitigation, in this manner keeping up with and cell integrity; a role which brought about the lower values. The ability of vitamin C to extinguish free radicals made by PQ's redox cycling before they hurt other biomolecules might actually be connected to this defensive capability. This is consistent with other research works [22, 29-34].

Shockingly, the discoveries of this study are not in agreement with the works of other authors who asserted that vitamin E and C affected the biochemical response to paraquat, inferring that they had no defensive impact against paraquat inebriation [35-39].

5. Conclusion

This study has demonstrated that combined vitamin supplementation in rats poisoned with paraquat can restore liver function destroyed by the toxicant (paraquat) within a month of weekly treatment with the vitamin. It particularly revealed it can restore liver function even in cases of prolonged or chronic poisoning. At poisoning dose of 0.02g to 0.06g, vitamin E and C combined therapy can ameliorate liver function in rats.

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