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Research Article

Antidotal Effect of Vitamin E and C in the Management of Paraquat Intoxication in Rat

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Abstract: The liver is an essential organ involved in anabolic and catabolic processes in the body. Substance like paraquat is injurious to the liver by way of oxidative damage. Vitamin E and C are known antioxidant used to tackle oxidative stress. The aim of this study was focused at evaluating the antidotal benefit of vitamin E and C in the treatment of paraquat intoxication in rat. Exactly 200 rats were obtained and grouped into four main groups (A, B, C and D) with each group having 50 rats. Except "A" group which was the control, all groups were treated with paraquat in doses of 0.02g, 0.04g and 0.06g respectively every 2 weeks for 3 months. All groups were subdivided into two groups designated as "0" and "VEC" amounting to a total of 8 subgroups. All subgroups with "VEC" designation were treated with 500mg of vitamin E and 2000mg/dl of vitamin C weekly for 3 months. Blood samples collected after treatment duration were via cardiac puncture and blood was assayed for liver enzyme activities. Results revealed that there was a significant increase (p-value<0.05) in liver enzyme activities among the groups (A0, B0, C0 and D0). Also, there was a significant drop in liver enzyme activities after vitamin E and C supplementation.

Keywords: paraquat, liver, vitamin E and C, rats.

1. Introduction

The liver is an essential organ involved in anabolic and catabolic processes in the body. Few of the functions of liver include; synthesis of proteins. One of the main proteins it synthesizes is albumin such that a not in albumin level is a clinical indication of impaired liver function. Another key role of the liver is excretion of metabolic waste as seen in the excretion of bilirubin. The liver conjugates unconjugated bilirubin to make is soluble and excretable in urine, such that rise in bilirubin level is an indication of impaired excretory function of the liver. As much as the liver is involved in detoxification of toxic substances, certain substances when ingested still have harmful effect on the liver [1].

Paraquat is a chemical substance used in agriculture to destroy weeds in order to improve crop yield but when humans are exposed to it; it causes damage to various body organs including the liver. Paraquat induced hepatocellular damage results in elevation of liver function parameters such as bilirubin and liver enzymes while it

causes decrease in total protein and albumin levels in blood [2]. These are laboratory indications of liver damage. The mechanism by which paraquat causes hepatotoxicity is believed to lead to increases in reactive oxygen species called free radicals which give rise to oxidative stress and damage of the liver [2-4]. Since paraquat induces oxidative stress, the antidotal therapy for paraquat poisoning should be a potent antioxidant [5,6].

Vitamin E and C are enlisted amongst the potent vitamins with antioxidant activities [7-9]. Therefore, treatment with Vitamin E and C combination may provide hope for those with paraquat intoxication but few studies have looked at the antidotal therapy of vitamin E and C in paraquat intoxication. Therefore, this study seeks to assess the antidotal potential of vitamin E and C in the repair of liver damage induced by paraquat intoxication in rat.

2. Materials and Methods

2.1. Study Design

This study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria. In this study, a total of 200 rats were obtained from the Animal House in the Department of Biology and transported to the study area where it was allowed to acclimatize for 2 weeks.

After 2 weeks acclimatization, the rats were grouped into 4 main groups; A, B, C and D with each group composed of 50 rats. The “A” group served as the control while the “B”, “C” and “D” were test groups because they were treated with paraquat in doses of 0.02g, 0.04g and 0.06g respectively every 2 weeks interval for 3 months. Each group mentioned was subdivided into two subgroups designed “0” and “VEC” so that the total number of subgroups were 8; A0 and AVEC, B0 and BVEC, C0 and CVEC, and D0 and DVEC. All “VEC” subgroups (AVEC, BVEC, CVEC and DVEC) were treated with 500mg of vitamin E and 2000mg/dl of vitamin C every week for 3 months [10].

Both treatments of paraquat, and vitamin E and C were administered orally. Blood samples for liver enzyme activity were collected via cardiac puncture after the rats were sedated using chlorofoam. Blood samples were collected after paraquat treatment period (3 months). These represented the rats in A0, B0, C0 and D0. After vitamin E and C treatment (3 months), blood samples were collected and these represented AVEC, BVEC, CVEC and DVEC [10].

2.2. Laboratory Analysis

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

Procedure

Two glass tubes, one labelled "Reagent Blank" and the other "Test," were prepared with 0.5 ml of a buffered-L- aspartate and -oxoglutarate solution each, and 0.1 ml of distilled water and the sample were added to each of the four tubes. At 37˚c, the tubes were shaken for 30 minutes. After 20 minutes at 20-25°C. 0.5 ml of a 2, 4-

dinitrophenylhydrazine (2 mmol/L) solution was added to each test tube. At the end of the time period, 5.0ml of sodium hydroxide (0.4mol/L) was added to boost colouring at an alkaline pH. Once the tubes were combined, the absorbance of the 'Test' (Atest) was compared to that of the 'Reagent blank' after 5 minutes.

Calculation

Estimate the amount of AST enzyme in the blood by comparing the results with those from the activity chart. The test is invalidated by hemolysis.

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

Procedure

The enzyme's activity was measured by placing 0.5 ml of a buffered-L- alanine and -oxoglutarate solution in one glass tube, labelling the other "Reagent Blank," and adding 0.1 ml of distilled water and the sample to the other tube, before incubating at 37°C for 30 minutes. After 20 minutes of resting at 20-25°C, 0.5 ml of a 2, 4-dinitrophenylhydrazine (2 mmol/L) solution was added to each test tube. At the end of the time period, 5.0ml of sodium hydroxide (0.4mol/L) was added to boost colouring at an alkaline pH. Once the tubes were mixed together, the absorbance of the 'Test' (Atest) was measured and compared to the 'Reagent blank' after 5 minutes.

Calculation

Use the data plotted against activities table to get the serum ALT activity. The test is invalidated by hemolysis.

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

Procedure

We inhaled fresh double-distilled water to re-calibrate the Gain in flow cell mode (ddH2O). Once you press this button, the equipment will return to its original state before the last test. After dispensing a water-blank test and selecting ALP on the Run Test Screen, 0.02-ml of sample and 1.0-ml of reagent (Diethanolamine buffered p-nitrophenylphosphate) were mixed for 2 minutes. The brew was then sucked into the Rx Monza's ventilation system. After around 2 minutes, the sample test results were printed out on the machine's associated printer.

This machine approach has the benefit of producing results in a S.I. unit = IU/L within one hour for up to 200 samples.

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* [11].

Procedure

Mixing the sample (0.1 ml) and the reagent (1.0 ml) (Buffered Glycylglycerine and L-gammaglutamyl-3-carboxy-4-nitrolide) in a cuvette before reading the absorbance at 400 - 420 nm and starting the timer simultaneously yielded accurate results. After 1, 2, and 3 minutes, the absorbance was measured again.

Calculation

IU/L = 1158 X ΔA (405nm/minute).

2.3. Statistical Analysis

All collated data from the study was subjected to statistical analysis using SPSS version 25 for descriptive statistics presented as Mean ± SD and inferential statistics such as ANOVA and T-test. Result was considered significant if p-value was less than 0.05.

3. Results

The results presented in table 1.0 shows the changes in biochemical parameters after paraquat intoxication and after vitamin E and C treatment. The results shows that there was a dose-dependent, significant increase (p-value<0.05) in liver enzyme activities among the groups treated with paraquat alone. After treatment with vitamin E and C, there was a significant decrease (p-value<0.05) in liver enzyme activities in all groups except in the control group.

Table 1. Changes in Liver enzymes biochemical data after three months treatment period.

Subgroup	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
A_0	4.20 ± 0.14	1.35 ± 0.06	1.96 ± 0.02	12.75 ± 0.08
A_{VEC}	5.95 ± 0.13	5.60 ± 0.13	10.42 ± 0.04	13.15 ± 0.03
B_0	126.50 ± 2.05^{a}	99.00 ± 0.01^{a}	1028.00 ± 4.42^{a}	87.00 ± 1.30^{a}
B_{VEC}	69.00 ± 2.10^{a,b}	49.50 ± 0.15^{a,b}	691.00 ± 4.15^{a,b}	35.50 ± 2.55^{a,b}
C_0	115.50 ± 3.15^{a,b}	191.00 ± 2.40^{a}	1348.50 ± 2.75^{a}	128.50 ± 0.85^{a}
C_{VEC}	98.50 ± 0.35^{a,b}	59.50 ± 0.05^{a,b}	1297.00 ± 4.79^{a,b}	59.00 ± 1.80^{a,b}
D_0	342.50 ± 4.35^{a}	167.50 ± 1.25^{a}	1570.00 ± 6.80^{a}	170.00 ± 6.40^{a}
D_{VEC}	114.50 ± 6.05^{a,b}	70.00 ± 1.60^{a,b}	178.55 ± 0.88^{a,b}	115.00 ± 2.50^{a,b}

Statistical significance: $P < 0.05$.

“a” means significant difference among the groups (A_0, B_0, C_0 and D_0).

“b” means significant difference between the subgroups.

4. Discussion

This study focused on understanding the role of vitamin E and C in the management of paraquat intoxication in rats. First the study demonstrated that paraquat inflicted liver damage in the rats in a dose-dependent manner such that the group with the high dose of paraquat had the highest level of hepatocellular injury or hepatotoxicity while

the group with the least dose showed the least level of severity of liver damage. The order of severity of hepatotoxicity is presented as D0>C0>B0. This outcome is a clear indication that increased exposure to paraquat causes a corresponding increases in liver injury. These findings were corroborated by the works of other researchers who revealed that paraquat causes liver damage [11,12]. According to Bus and his team, the rise in liver enzyme activities in paraquat poisoning was due to the fact that paraquat damages the cell membrane of the hepatocyte due to impaired lipid bilayer induced by free radicals. The oxidative damage of the cell membrane led to leaking-out of the intracellular liver enzymes into circulation resulting in high liver enzymes [13]. This was also supported by numerous works that highlighted the damaging effect of paraquat on hepatocellular tissue [12-17].

After paraquat intoxication in the rats, the rats were treated with vitamin E and C to determine the antidotal benefits or potential of the vitamins in the treatment of paraquat poisoning and liver repair. The outcome of the study revealed that vitamin E and C combined weekly therapy for three months repaired liver damage in the rats in all groups exposed to various doses of paraquat intoxication. This was demonstrated in the laboratory assessment of liver enzyme activities. There was a significant decline in the liver enzyme activities after vitamin E and C combined treatment. This has shown that a long-term vitamin E and C treatment can ameliorate liver insults in rats poisoned with paraquat. In a similar work carried out by same author in another publication revealed that vitamin E and C combined therapy ameliorated the effect of paraquat hepatotoxicity in rats [10,11]. This supports the finding from this current study.

5. Conclusion

This study has demonstrated that paraquat ingestion causes liver toxicity in a dose-dependent fashion and vitamin E and C combined treatment can ameliorate the effect of liver damage induced by paraquat in all doses administered.

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