The Effect of Combined Vitamin Treatment on Paraquat-Inflicted Liver Damage

Abstract

Paraquat poisoning causes serious herbicide intoxication commonly and usually as a result of deliberate or accidental ingestion. Vitamins C and E are very good antioxidants that reacts rapidly with radicals and alters lipid peroxidation. This study was done to evaluate the effect of Vitamin E and C combination therapy on the chronic toxicity of paraquat on liver markers in Wistar rats. A total of 200 male rats were used for the study. The rats were divided into four groups of 50 rats in each group (A, B, C, D) and was further subgrouped into two (0 and VEC), having 25rats per subgroup. All "0" subgroups (A0, B0, C0 and D0) except A_0 were treated with paraquat in 0.02g, 0.04g and 0.06g respectively every two weeks for three months. All "VEC" subgroups (A_{VEC}, B_{VEC}, C_{VEC} and D_{VEC}) except A_{VEC} were treated with paraquat similarly, after which they were treated with vitamin E (500mg) and C (2000mg/L) weekly for one month. AVEC was only treated with vitamin E and C. Blood samples were collected and analysed for liver function test (total and direct bilirubin, total protein, albumin and globulin). There was a significant difference in the level of all the parameters among the "Ao", "Bo", "Co" and "Do", p-value<0.05 and also among the "A_{VEC}", "B_{VEC}", "C_{VEC}" and "D_{VEC}", p-value<0.05. The result also showed that there were significant differences in intra-group comparison in all the liver biochemical parameters, p-value<0.05There was no significant difference in the concentration of globulin among the subgroup. This study has confirmed that vitamin E and C combination has a therapeutic effect in male Wistar rats on two month of weekly treatment. Therefore, a weekly treatment with Vit E and C can ameliorate liver toxicity in rats.

Keyword: *Vitamin E and C, paraquat, rat, antioxidant, liver markers.*

1.0 Introduction

Paraquat poisoning causes serious herbicide intoxication commonly and usually as a result of deliberate ingestion. The main cause of death in paraquat poisoning is respiratory failure due to an oxidative alteration to the alveolar epithelium which consequently lead to obliterating fibrosis [1]. There are currently no pharmacological therapy against paraquat and no agent to chelate the poison in blood or other tissues, this has made the management of paraquat poisoning to remain mostly supportive and the aim being to decrease its absorption or enhance its elimination [1].

Paraquat has a very high mortality rate despite its therapeutic intervention, no matter the amount of dosage ingested. It should be noted that the toxic effects of paraquat is induced through oxidative stress-mediated mechanisms [2], and future management should be aimed towards reducing the acute alveolitis and pulmonary fibrosis by the use of antioxidants [2] as have been shown in some previous studies. Two studies carried out previously on the evaluation of the effect of melatonin as an antioxidant on the cell damage and outcome of paraquat-intoxicated rats observed that melatonin increases survival time and provides beneficial effects against paraquat toxicity [3,4]. The same effect was seen in another study using the antioxidant, N-acetylcysteine [5].

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The liver has an important role in the metabolism of xenobiotic compounds with biochemical changes that take place in some toxic conditions [13]. Cytochrome P450 (CYP) and its forms especially CYP1A1, CYP1A2 and CYP2E1 have been observed to facilitate formation of Reactive Oxygen Species (ROS) during xenobiotic metabolism consequently contributing to oxidative stress induced damage [14]. Direct involvement of CYP-mediated free radical generation has been observed in pesticides by several authors [15]. However the metabolism of paraquat is very poor causing it to be excreted almost unchanged in the urine. The metabolism of paraquat has been shown to occur via methylation (monomethyl dipyridone ion) or oxidation (Paraquat pyridine ion and Paraquat dipyridone ion) [16]. It should be noted also that CYP2E1 mediated production of superoxide radicals and hydrogen peroxide in vitro and in transected cultured cells has also been reported in previous studies [16].

Vitamins C and E are very good antioxidants that reacts rapidly with radicals and alters lipid peroxidation. The fluid in lung contain high concentration of Vitamin C to protect against free radicals that are generated by toxic chemicals in air. It has been assumed accordingly that that both vitamins C and E are consumed during radical reactions [6,7]. A study documented that Vitamin C concentration decreases significantly after 48 hours of intraperitoneal administration of paraquat to rats, this suggests that vitamin C directly showed the oxidative stress in the lungs [8]. Another study [9] reported that when the total antioxidant status is increased by high doses of vitamin C could be useful as a free radical scavenger for paraquat-poisoned patients. This is because vitamin C has the ability to quench radicals generated by redox cycling of paraquat [9].

Vitamin E plays an important role in paraquat toxicity as has been shown in several studies where deficiency of vitamin E brought about the development of acute paraquat toxicity in animals. Two different studies proved that vitamin E deficiency potentiated a decrease in survival and brought about histological lung damage in rats [10], it also reduced the LD50 in mice that are exposed to paraquat [11]. Studies also show that the administration of vitamin C reversed the potentiation of acute paraquat toxicity by vitamin E deficiency [10].

Despite the number of previous studies carried out on the role of Vitamin C and E combination against paraquat intoxication, there are very rare studies done on this role in the liver. It is therefore important to evaluate the ameliorative effect of Vitamin E and C combination on paraquat induced toxicity on liver markers.

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2.0 Material and Methods

2.1 Study Area/Population

This study was done in the Department of Medical Laboratory Science, Rivers State University. It was a biological trial with Wistar rats because of their availability, cost, genetic makeup, handling technique and nature of the study. Two hundred (200) healthy mature male albino rats with a mean weight of 0.2±0.02kg were used for this study. The rats were obtained from Animal House, Department of Biology, Rivers State University. The rats were transported to the study site and allowed to acclimatize for two week before proceeding with the study. The rats were housed in conventional wire mesh cages under standard laboratory conditions and were allowed free access to water and feed throughout the experiment.

2.2 Grouping and Treatment of Animals

Two hundred (200) male Wistar Rats were used for this research and were divided into 4 groups (A, B, C and D) with each group containing fifty (50) Rats each. Group A was taken as the control group; they were not induced with paraquat. Group B was induced every two weeks with 0.02g of paraquat per kg of rat for three months. Group C was induced every two weeks with 0.04g of paraquat per kg of rat for three months. And Group D was induced every two weeks with 0.06g per kg of paraquat for three months.

Each of the main groups had subgroups. "A" group had "Ao" and "Bvec" subgroups; "B" group had "Bo" and "Bvec" subgroups; "C" group had "Co" and "Cvec" subgroups; "D" group had "Do" and "Dvec". "Ao", "Bo", "Co" and "Do" subgroups were not treated with vitamin E and C. "Avec", "Bvec", "Cvec" and "Dvec" subgroups were treated orally with 500mg of vitamin E and 2000mg/dl of Vitamin C every week for one month.

After one month of weekly treatment with Vit E and C, the rats were sacrificed and their blood samples were analyzed for liver markers.

2.3 Procedures for Administration of Toxicant

Administration of toxicant was via oral gavage route. 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 contents of the syringe were then emptied into the mouth of the rat gradually [17].

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2.4 Sample Collection

cardiac puncture method was used for blood sample collection from the animals and sacrificed under 70% chloroform anesthesia into the lithium heparin specimen bottle and used for analysis of liver parameters [17].

2.5 Laboratory analysis

2.5.1: Bilirubin method (Mallor, *et al.*, 1937; Martinek, 1966 and Young, 1997).

Principle: Conversion of bilirubin to coloured azobilirubin by diazotized sulphanilic acid and measured photometrically. Of the two fractions present in serum (bilirubin-glucuronide and free bilirubin) only the bound bilirubin reacts directly in aqueous solution (Direct bilirubin), while free bilirubin requires solubilization with dimethylsulphoxide (DMSO) to react (Indirect bilirubin). In the determination of indirect bilirubin the direct is also determined, the results corresponds to Total bilirubin. The intensity of the colour formed is proportional to the bilirubin concentration in the sample.

Procedure:

Total Bilirubin: 1.5mls of reagent-1 (Sulphanilic acid, HCl and Dimethylsulphoxide) was added to two glass-tubes labeled 'Blank' and 'Test' respectively. 50μL of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently 100μL of sample was added to the 'Blank' and 'Test' tubes, mixed and incubated for exactly 5 minutes at room temperature. After which the absorbance were read spectrophotometrically at 530 – 580nm and 15 – 25^0C, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 19.1 = Result in (mg/dL). Conversion factor: mg/dL X 17.1 = Result (μL/L).

Direct Bilirubin: 1.5mls of reagent-2 (Sulphanilic acid and HCl) was added to two glass-tubes labeled 'Blank' and 'Test' respectively. 50μL of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently 100μL of sample was added to the 'Blank' and 'Test' tubes, mixed and incubated for exactly 5 minutes at room temperature. After which the absorbance were read spectrophotometrically at 530 – 580nm and 15 – 25^0C, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 14 = Result in (mg/dL). Conversion factor: mg/dL X 17.1 = Result (μL/L).

2.5.2: Total protein (Biuret colorimetric method by Burtis, *et al.*, 1999)

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Principle: Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant. The intensity of the colour formed is proportional to the total protein concentration in the sample.

Procedure: 1mL of Biuret reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by $25\mu L$ each of Standard (7g/dL) and Sample added to the 'Standard' and 'Test' tubes respectively. The contents were mixed and incubated for 10 minutes at room temperature, after which, the absorbance (A) of the 'Test' and 'Standard' were read against the 'Blank'. The colour produced is stable for at least 30 minutes at room temperature.

Calculation: [A(Test) ÷ A(Standard)] X 7(Standard concentration)

= Result in g/dL

2.5.3: Albumin (Bromocresol green method by Grant, *et al*., 1987)

Principle: The measurement of serum albumin is based on its quantitative binding to the indicator $3,3^{!},5,5^{!}$-tetrabromo-m-cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Procedure: 3mls of Bromocresol green reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by $10\mu L$ each of Water, Standard (7g/dL) and Sample added to the 'Blank', 'Standard' and 'Test' tubes respectively. The contents were mixed and incubated for 10 minutes at 20 – 25^{0}C, after which, the absorbance (A) of the 'Test' and 'Standard' were read against the 'Blank'. The colour produced is stable for at least 30 minutes at room temperature.

Calculation: [A (Test) ÷ A (Standard)] X 7 (Standard concentration)

= Result in g/dL

2.5.4: Globulin calculation method by Grant, *et al*., 1987.

Globulin value are calculated as a difference when albumin value are subtracted from the value of the total protein gotten from the same sample.

Globulin (g/dl) = Total protein (g/dl) – Albumin (unit in g/dl).

2.6 Statistical analysis

The data gotten from the result of this study was analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 for descriptive and inferential statistics (ANOVA) for inter-

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group comparison and T-test for intra-group (sub-group) comparison at test significance, P-value<0.05.

3.0 Result

Table 1 below shows the changes in some biochemical parameters after two months treatment period. The result showed that there was a significant increase in concentrations of total and direct bilirubin among subgroups A_O, B_O, C_O and D_O at $p<0.05$. But there was significant decrease in concentrations of total protein, albumin and globulin among subgroups A_O, B_O, C_O and D_O. The result also shows a significant decrease in concentrations of total protein, albumin and globulin among subgroups A_{VEC}, B_{VEC}, C_{VEC} and D_{VEC} and a significant difference in the levels of total and direct bilirubin among subgroups A_{VEC}, B_{VEC}, C_{VEC} and D_{VEC}. The result also show a significant decrease in concentration of total and direct Bilirubin among B_O and B_{VEC} ,C_O and C_{VEC}, and D_O was compared with D_{VEC}. There was however no significant difference in the concentrations of all the parameters among subgroups A_O and A_{VEC}. It also showed a significant increase in concentrations of total protein and albumin among subgroups B_O and B_{VEC}, C_O and C_{VEC} and when D_O and D_{VEC}. There was also no significant difference in the concentration of globulin among the subgroups.

Table 1: ***Changes in some biochemical parameters after two months treatment period***.

Sub-group	Treatments (4 Rats in each subgroup) Mean ±SEM				
	Tot. Bilirubin (µmol/L)	D. Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_0	2.28 ± 0.84	0.70 ± 0.02	7.24 ± 0.36	3.93 ± 0.01	3.31 ± 0.03
A_{VEC}	4.73 ± 1.41	0.65 ± 0.03	7.27 ± 0.11	3.95 ± 0.01	3.32 ± 0.01
B_0	9.38 ± 1.28^{a}	1.60 ± 0.02^{a}	5.08 ± 0.06^{a}	2.82 ± 0.01^{a}	2.26 ± 0.01^{a}
B_{VEC}	$1.78 \pm 0.62^{a,b}$	$0.60 \pm 0.03^{a,b}$	$5.66 \pm 0.18^{a,b}$	$3.12 \pm 0.01^{a,b}$	2.54 ± 0.02^{a}
C_0	11.45 ± 0.91^{a}	1.38 ± 0.02^{a}	5.00 ± 0.06^{a}	2.33 ± 0.01^{a}	2.68 ± 0.02^{a}
C_{VEC}	$4.18 \pm 0.69^{a,b}$	$0.73 \pm 0.05^{a,b}$	$5.58 \pm 0.21^{a,b}$	$2.98 \pm 0.01^{a,b}$	2.60 ± 0.02^{a}
D_0	16.90 ± 1.31^{a}	1.48 ± 0.07^{a}	4.69 ± 0.22^{a}	2.25 ± 0.01^{a}	2.44 ± 0.02^{a}
D_{VEC}	$7.38 \pm 1.30^{a,b}$	$0.90 \pm 0.04^{a,b}$	$5.54 \pm 0.38^{a,b}$	$2.88 \pm 0.01^{a,b}$	2.66 ± 0.03^{a}

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.

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- Index (b) = represents a statistically significant difference observed within each group (i.e. Group B: B_0Vs B_{VE}) at each month.

4.0 Discussion

The aim in this study was to supply proof of the role played by vitamin E and C combination in the repair of liver injuries caused by paraquat in rats. Different subgroups of the test subjects were treated with paraquat, with inter- and intra- comparative analyses were carried out. However, paraquat is a highly toxic chemical and organic compound control of weed, but toxic when exposed to cells and tissues [18,20].

The result obtained from the study above with paraquat treatment alone at various concentrations, and with vitamin E and C treatment on paraquat induced subjects, confirmed the toxicity of paraquat and also the ameliorative capacity of vitamin E. Comparison on the significance of toxicity among the various subgroups and within the same subgroups was carried out also. Subgroups B_0, C_0, and D_0 were compared against the control group A_0 for the effect of paraquat on biochemical parameters T. bilirubin, D. bilirubin, T. protein, albumin, and globulin as shown in Table 1 above. The result showed that there was a significant increase in concentrations of total and direct bilirubin among subgroups A_O, B_O, C_O and D_O, indicating that paraquat toxicity brought about an increase in the total and direct bilirubin concentrations. This may be due to the reactive oxygen species that are generated by the induction of paraquat. But there was significant decrease in the concentrations of total protein, albumin and globulin among subgroups A_O, B_O, C_O and D_O. This shows that paraquat toxicity brought about a decrease in the total protein, albumin and globulin concentrations. This can be attributed to the damaged caused by the paraquat toxicity on the liver. This result is in agreement with those observed in the study by Rizvi *et al*. (2014) and Howard *et al*. (2011) [21, 22].

Finally, an intra-comparison was made between the control groups A_0 and A_{VEC} and the test subgroups B_0 and B_{VEC}, C_0 and C_{VEC}, D_0 and D_{VEC} to determine the difference in the levels of the parameters under study, between the test groups administered with various doses of paraquat without vitamin E treatment, and the groups given paraquat with vitamin E treatment. The result also showed a significant decrease in concentration of total and direct Bilirubin among the test subgroups treated with vit E and C after paraquat poisoning. This suggest that Vitamin E and C combination decreases the concentrations of total and direct bilirubin indicating the ameliorative effect of the combination of Vitamin E and C. This study agrees with those carried out by Rizvi et al. (2014) and Howard et al. (2011) [20, 21]. There

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was however no significant difference in the concentrations of all the parameters among subgroups A_O and A_{VE}. Showing that at such dose of Vitamin E and E had no effect on the studied parameters. It also showed a significant increase in concentrations of total protein and albumin among subgroups B_{VE}, C_{VE} and D_{VE}. This indicates that treatment with Vitamin E and C combination brought about an increase in the concentrations of protein and albumin. Injury of the liver may affect or impair the synthetic function of the liver in synthesizing albumin. Since albumin makes the greater percentage of total plasma protein, it may be true that the increase in albumin and total protein levels in rats treated with vit E and C were recovery effects of liver albumin synthetic function after a terrible liver toxic insult inflicted by paraquat. There was no significant difference in globulin levels in vitamin E and C treated rats when compared to the paraquat treated rats. The results of globulin in this study support the explanation the increase in total protein was due to the restoration of albumin synthetic function in the liver. This study disagrees with those carried out by Rizvi et al. (2014) and Howard et al. (2011) [20, 21]. In their study they observed a significant decrease in the parameters while this study observed a significant increase.

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Conclusion

The results gotten from this study has proven that Vitamin E and C combination therapy has an ameliorative effect on the liver markers concentration against paraquat and can be used for that purpose.

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