Original Research Article

THE COMPARATIVE EFFECTS OF VITAMIN E + C ON THE CHRONIC TOXICITY OF PARAQUAT IN ALBINO RATS (*Rattus norvegicus*)

Abstract

Paraquat is a highly toxic chemical used in weed control. Paraquat is known for its ability to generate reactive oxygen species that attack cells and membranes. Vitamin E is a group of vitamins which dissolves in fat and possess antioxidant properties which act as a defense to the cells against oxidative stress arising from reactive oxygen species, whereas Vitamin C is a water soluble vitamin and a very potent antioxidant which protects the cells of the body from free radicals. The study aim was to evaluate the ameliorative effects of a combination therapy of vitamin E and C on biochemical markers of paraquat induced male albino rats. 200 male albino rats with 0.2±0.02kg mean weight were used. The 200 rats were divided into four main groups with 50 rats within a group. The groups were labeled A, B C and D. The "A" group was neutral; "B" group was induced with 0.02g, "C" 0.06g, and "D" 0.06g of paraquat per kg rat every two weeks for three months. The main groups further had subgroups. "A" group had "A_0" and "A_{VEC}" subgroups; "B" group had "B_0" and "B_{VEC}" subgroups; "C" group had "C_0" and "C_{VEC}" subgroups; "D" group had "D_0" and "D_{VEC}" subgroups. "A_0", "B_0", "C_0" and "D_0" subgroups were not administered with vitamin E + C therapy while "A_{VEC}", "B_{VEC}", "C_{VEC}" and "D_{VEC}" were treated orally with 500mg of vitamin E weekly and 2000mg/l of vit C medicated water for one month. After then, the rats were sacrificed, blood samples were collected and analyzed for the liver function; T. bilirubin, D. bilirubin, T. protein, albumin, and globulin. ANOVA intergroup comparison of A_0, B_0, C_0 and D_0 was statistically significant, p-value<0.05 except for globulin. Intergroup comparison of A_{VEC}, B_{VEC}, C_{VEC} and D_{VEC} was statistically significant, p-value<0.05 except for globulin. Intra-group comparison showed significant difference only in total bilirubin and conjugated bilirubin levels, p-value<0.05. This study therefore has shown that weekly treatment with Vit E + C in one month can treat liver toxicity in rats.

Keyword: *Vitamin E, Vitamin C, paraquat, antioxidant, liver*

1.0 INTRODUCTION

Vitamin E is a group of vitamins which dissolves in fat [1,2]. It is known mainly for its antioxidant properties which act as a defense to the cells against oxidative stress arising from reactive oxygen species [3]. Vitamin E plays a role in immune functions and is essential in health maintenance, prevention, and treatment of disease [3]. Studies show it exists in two main forms; tocopherols and tocotrienols with the tocopherol form being the only useable form by the human system [4,5]. Vitamin E is sourced from foods like wheat germ oil, sunflower, almonds, peanuts, peanut butter, pumpkin, red bell pepper, mango, avocado, fruits, and vegetables which are rich in alpha-tocopherol [6,7].

Vitamin C is a water soluble vitamin which also goes by the names L-ascorbic acid or ascorbic acid [8], and a very potent antioxidant which protects the cells of the body from free radicals [9]. Vitamin C plays several important roles such as tissue repairs, injury/scar healing and is required for normal function, growth and development of the cells [10]. Vitamin C is not produced by the body but it is found naturally in several fruits and vegetables [11,12].

Toxicant introduction into the environment and effect on the living components in the environment is a growing public health concern [13,14]. Paraquat is a highly toxic chemical used in weed control [15]. Due to this high toxicity, it is made available to only licensed commercial users. Paraquat is known for its ability to generate reactive oxygen species that attack cells and membranes, as it goes through redox-cycling [16]. Exposure to paraquat can happen by ingestion, contact with the skin, and inhalation from the surrounding air, and outcomes such as heart, liver, kidney, and lung failures may occur due to this exposures [15]. Death may also occur in fatal cases of exposure and where treatment is not administered quickly [16]. According several studies, paraquat has been associated with high mortality which resulted mainly from multiple organ and respiratory failure [17]. Paraquat was identified to grossly affect some biochemical parameters in test subjects according to studies [17,18].

Since vitamin E and C are both essential vitamins with powerful antioxidant qualities [19] and both vitamins play key roles in wound healings, repair of damages within the cells and the disruption of free radicals activities generated within the cells [20,21], this study, focused at evaluating the ameliorative effects of a combination of vitamin E and C therapy on liver of paraquat induced male albino rats.

2.0 Materials and Method

2.1 Design of Study

The research was an experimental study on 200 male albino rats with 0.2±0.02kg mean weight. The design grouping was as follows:

Chart 1 : design grouping

Main group	**Sub-group**
A=no paraquat induction	A_0= without Vit E+C treatment A_{ve}= with Vit E+C treatment
B=0.02g of paraquat induction	B_0= without Vit E+C treatment B_{ve}= with Vit E+C treatment
C=0.04g of paraquat induction	C_0= without Vit E+C treatment C_{ve}= with Vit E+C treatment
	D_0= without Vit E+C treatment

D=0.06g of paraquat induction	D_{ve}= without Vit E+C treatment

N=50 rats per main group; N=25 rats per subgroup

According to the group dosage, paraquat was induced every two weeks for three months. After which, it was followed by oral treatment with 500mg of vitamin E weekly and maintained with vitamin 2000mg/l of C medicated water for one month. Then rats were sacrificed and their blood samples were collected and analyzed for liver function

2.2 Source of Animals

200 rats averagely weighing 0.2±0.02kg were obtained from Animal House, Department of Biology, Rivers State University of Science and Technology and were transported to the research location and allowed to acclimatize for two week before proceeding with the study. The experiment was carried out in Department of Medical Laboratory Science, Rivers State University of Science and Technology.

2.3 Method of Sample Collection

Blood sample was obtained for liver function test. With syringe and needle, 2mls of blood was collected by cardiac puncture and dispensed in plain bottles. The blood was allowed to clot. To obtain the serum, the blood was spun at 4000rpm. Analysis for total bilirubin, conjugated bilirubin, total protein, albumin and globulin was done using the serum. The animals were sacrificed under the influence of 70% chloroform anesthesia and carcasses were incinerated to avoid pollution in the environment.

2.4 Laboratory Analysis

Bilirubin method [22]

Procedures:

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^{0}C, 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).

Total protein (Biuret colorimetric method) [23]
Procedure: 1mL of Biuret reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 25μL 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

Albumin (Bromocresol green method) [24]
Procedure: 3mls of Bromocresol green reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 10μL each of Water, Standard (7g/dL) and Sample added to the 'Blank', 'Standard' and 'Test' tubes respectively. The contents were mixed and the mixture 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

Globulin calculation method [24]
Here, the value of globulin is calculated as a difference when albumin value is substituted from the overall figure of protein gotten from the same sample.
Globulin (g/dl) = Total protein (g/dl) – Albumin (unit in g/dl).

2.5 Statistical analysis
The descriptive and inferential statistics (ANOVA) for the comparison of the inter-group and intra-group (sub-group) comparison at test significance, P-value<0.05 was analyzed with SPSS version 23.0, using the data generated from this research.

3.0 RESULT

The comparative potency of vitamin E + C on the Chronic Toxicity of Paraquat in Albino Rats (*Rattus norvegicus*) are represented in **Table 1, 2 and 3**. Comparison of A_0, B_0, C_0 and D_0 intergroup was statistically significant, p-value<0.05 in markers; total bilirubin, conjugated bilirubin, total protein, and albumin whereas there was no statistical significance in globulin levels among the groups. Intergroup comparison of A_{VEC}, B_{VEC}, C_{VEC} and D_{VEC} was statistically significant, p-value<0.05 in total bilirubin, conjugated bilirubin, total protein, and albumin but there globulin levels had no statistical significance among the groups. There was also significant differences in intra-group comparison in total bilirubin and conjugated bilirubin levels, p-value<0.05 while other markers were not significantly different.

Table 1: Inter-group comparison of paraquat liver toxicity in male albino rats

Sub-group	T. Bilirubin (µmol/L)	D. Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_0	0.81 ± 0.35	0.14 ± 0.03	7.52 ±0.25	3.90 ± 0.01	3.62 ± 0.03
B_0	3.53 ± 0.79[a]	1.10 ± 0.03[a]	6.05 ±0.38[a]	3.16 ± 0.01[a]	2.89 ± 0.03
C_0	9.29 ± 2.53[a]	1.08 ± 0.03[a]	6.26 ±0.57[a]	3.40 ± 0.03[a]	2.86 ± 0.03
D_0	13.56 ± 3.14[a]	1.57 ± 0.04[a]	6.54 ±0.51[a]	3.21 ± 0.04[a]	3.32 ± 0.02

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 2: Inter-group comparison of Vit E and C combination therapy on male albino rats

Sub-group	T. Bilirubin (µmol/L)	D. Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_{VEC}	1.44 ± 0.29	0.23 ± 0.04	7.34 ±0.30	4.06 ± 0.03	3.28 ± 0.03
B_{VEC}	3.05 ± 0.62[a]	0.50 ± 0.01[a]	6.73 ±0.25[a]	3.54 ± 0.01[a]	3.19 ± 0.02
C_{VEC}	5.10 ± 0.69[a]	0.55 ± 0.02[a]	6.38 ±0.24[a]	3.32 ± 0.01[a]	3.05 ± 0.01
D_{VEC}	9.15 ± 2.29[a]	0.65 ± 0.02[a]	6.53 ±0.26[a]	3.27 ± 0.02[a]	3.26 ± 0.02

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 3: Inter and Intra groups comparison of liver markers after one month treatment period.

Sub-group	T. Bilirubin (μmol/L)	D. Bilirubin (μmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_0	0.81 ± 0.35	0.14 ± 0.03	7.52 ±0.25	3.90 ± 0.01	3.62 ± 0.03
A_{VEC}	1.44 ± 0.29	0.23 ± 0.04	7.34 ±0.30	4.06 ± 0.03	3.28 ± 0.03
B_0	3.53 ± 0.79[a]	1.10 ± 0.03[a]	6.05 ±0.38[a]	3.16 ± 0.01[a]	2.89 ± 0.03
B_{VEC}	3.05 ± 0.62[a,b]	0.50 ± 0.01[a,b]	6.73 ±0.25[a]	3.54 ± 0.01[a]	3.19 ± 0.02
C_0	9.29 ± 2.53[a]	1.08 ± 0.03[a]	6.26 ±0.57[a]	3.40 ± 0.03[a]	2.86 ± 0.03
C_{VEC}	5.10 ± 0.69[a,b]	0.55 ± 0.02[a,b]	6.38 ±0.24[a]	3.32 ± 0.01[a]	3.05 ± 0.01
D_0	13.56 ± 3.14[a]	1.57 ± 0.04[a]	6.54 ±0.51[a]	3.21 ± 0.04[a]	3.32 ± 0.02
D_{VEC}	9.15 ± 2.29[a,b]	0.65 ± 0.02[a,b]	6.53 ±0.26[a]	3.27 ± 0.02[a]	3.26 ± 0.02

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_0Vs B_{VE}) at each month.

DISCUSSION

The focus of this research was to identify the potency of a combination of vitamin E and C therapy in ameliorating the short term toxicity of paraquat on liver in male albino rats. The rats were parted into groups and subgroups for both inter and intra-group comparison. The toxicity of paraquat and the ameliorative ability of vitamin E and C therapy are represented in the Table 1.above. This result confirmed the toxicity of paraquat and its role in organ failure. This finding is in support with many studies [17,18]. The result also showed the effect of vitamin E and C therapy on paraquat induced rats, confirming the outcome of the research conducted by Maret *et al*., (2011), Michels *et al*. (2012), and Ebuehi *et al.* (2012) [19,20,21].

The inter-group comparison of A_0, B_0, C_0, and D_0 to determine the effect of paraquat on the biochemical markers T. bilirubin, D. bilirubin, T. protein, albumin, and globulin showed there was a significant difference in the levels of the parameters T. bilirubin, D. bilirubin, T. protein, and albumin but there was no significant difference in globulin level among the groups. The significant rise in total bilirubin was due to the destruction of the liver cells by the toxicant. Especially, in total bilirubin, the consistent rise reported was an indication that the liver was unable to conjugate bilirubin product because of hepatotoxicity of paraquat, thus leading to steady rise in unconjugated bilirubin fraction. The significant drop in total protein and no

significant change in globulin level suggested that the drop was due to the significant drop in albumin level. Since albumin is the most abundant protein in the plasma, any significant fall in albumin would result to significant fall in total protein level. This result is consonance with that obtained by Lalruatfela in 2014 and Onur in 2022, affirming the toxicity of paraquat on biochemical markers [17,18].

Inter-comparison of the ameliorative activity of vitamin E+C combined therapy on paraquat induced rats was done among $A_{VEC,}$ B_{VEC}, C_{VEC} and D_{VEC} groups. A significant difference, expressing antioxidant activity of vitamin E and C treatment was observed in total bilirubin, direct bilirubin and total protein. It was necessary to further study sub-groups on intra-group basis to actually determine the ameliorative activity of the combination therapy of Vit E+C. The result showed that there was significant decrease in total bilirubin and conjugated bilirubin level between A_0 and Ave intra-group, B_0 and Bve intra-group, C_0 and Cve intra-group, and D_0 and Dve intra-group. But there was no significant difference in total protein, albumin and globulin levels among the intra-groups. This means that there was ameliorative success in the combination therapy with vit E+C. The combination therapy was able to achieve reduction in toxicity effect in rats treated with vitamin E and C. This means that the liver was able to regain in a large extent its ability to metabolize bilirubin. The loss of recovery of protein markers, in this case albumin could be due to the short treatment duration of one month reported. A long term treatment may provide significant recovery of liver protein synthetic. This study is in agreement with the studies by Maret in 2011, Michels in 2012 and Ebuehi in. (2012) which reported that both vitamins under study have the ability to treat cell injuries and to counter the activities of free radicals within the cells [19,20,21].

Conclusion

Finally, this study reveals that a combination of Vitamin E + C therapy has ameliorative potency on liver excretory function than protein synthetic function on one month weekly treatment after prolong paraquat induced liver damage in male albino rats..

Recommendation

More studies may be required in the area where vitamin E and C treatment had no ameliorative effect on paraquat induced toxicity of total protein and albumin.

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