Original Research Article

A BIOLOGICAL TRIAL ON THE EFFECT OF VITAMIN E AND C COMBINATION THERAPY ON CHRONIC PARAQUAT TOXICITY

Abstract

Paraquat (1,1-dimethyl-4-4-bipiridinium dichloride (PQ) is a non-selective contact herbicide that is a major source of free radicals and generates superoxide anion, and reactive oxygen species (ROS) which causes severe oxidative damage. Vitamin C is a water-soluble vitamin that plays a role using its biological characteristic as a suitable antioxidant in cells' defense against oxygen deprivation and increasing tissue protection from oxidative stress. Vitamin E is a free-radical scavenger renowned for its anti-inflammatory and antioxidant properties. The purpose of the study was to determine the short-term therapeutic effect of a vitamin E and C combination treatment on paraquat-induced male albino rats. For the study, 200 male albino rats were used. The rats were divided into four major categories namely A, B, C, and D with 50 rats each. The "A" group received no paraquat, but the "B," "C," and "D" groups received 0.02g, 0.04g, and 0.06g of paraquat respectively every two weeks for three months. These groups were subsequently subgrouped into two with 25 rats per subgroup. The "A" group was divided into two "A_0" and "A_{VE}"; "B" was subgrouped into "B_0" and B_{VEC}"; "C" was subgrouped into "C_0" and "C_{VEC}" and "D" was subgrouped into "D_0" and "D_{VEC}". A_0, B_0, C_0 and D_0 were subgroups without Vit E and C combined treatment while A_{VEC}, B_{VEC}, C_{VEC} and D_{VEC} were subgroups with combined treatment with 500mg of vitamin E and 2000mg/l of C medicated water every week for two months. Blood was drawn for the analysis of hematogical parameters (Hemoglobin concentration [Hb], Packed Cell volume [PCV], Total White blood cell count [T-WBC] , Neutrophils and Lymphocytes). There was a significant variation in the hematological parameters among the "A_0", "B_0", "C_0", and "D_0" at p-value<0.05. There was also significant variation in the hematological parameters between the "A_{VEC}", "B_{VEC}", "C_{VEC}", and "D_{VEC}", p-value < 0.05. Consequently, it was also observed that there were significant variations in intra-group comparisons of Hb and PCV, p-value<0.05, whereas other markers were not statistically different. This study found that combining vitamin E and C has a therapeutic effect on 'blood anemia indicators' but not the immunity of male albino rats after one month of weekly treatment. As a result, a weekly treatment with Vit E and C combination therapy can alleviate PQ toxicity by ameliorating anemia but not affecting the immune system of male albino rats.

Keyword: *Paraquat, Vitamin E, Vitamin C, combined therapy, rat, antioxidant, hematological paramters.*

1.0 INTRODUCTION

There is currently a clear link between environmental toxicants and the genesis of certain diseases, raising serious concerns about human health [1,2]. Paraquat (1,1-dimethyl-4-4-bipiridinium dichloride (PQ) is a non-selective contact herbicide widely employed in agricultural cultivation and conservation tillage systems around the world [3]. PQ is toxic to mammals, according to accumulating evidence [4,5], with lung toxicity being a common form of acute intoxication. Hepatotoxicity [6,7], nephrotoxicity, neurotoxicity [8], immunotoxicity [9], and reproductive toxicity [3] have all been reported as side effects of PQ.

Many nations have outlawed the use of PQ as herbicide due to its severe toxicity and the functional failure of multiple organs. Despite this, PQ is frequently used in underdeveloped nations due to low manufacturing costs and ease of application, resulting in a high intoxication rate even today [7,10,11,12,13]. PQ poisoning is thought to be responsible for up

to a third of all suicides globally [13]. PQ is a major source of free radicals and is converted to PQ mono-cation free radical by nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P-450 reductase once it enters the cells. The electron delivered to PQ quickly travels to oxygen, generating superoxide anion, and reactive oxygen species (ROS) causing severe oxidative damage [15].

Ascorbic acid (vitamin C) is a water-soluble vitamin. It's one of the biological characteristics that plays a role in a cell's defense against oxygen deprivation. Due to its easy, efficient, and safe dietary administration in a wide range of dosages without adverse side effects, it is a suitable antioxidant for increasing tissue protection from oxidative stress in humans.

Vitamin E is a free-radical scavenger found in the lipid compartment of cells and serum. It is renowned for its anti-inflammatory and antioxidant properties [16]. Vitamin E supplementation has been shown to reduce lipid peroxidation, inhibit protein kinase C, 5-lipoxygenase, smooth muscle cell proliferation, platelet aggregation, and neutrophil oxygen burst [17, 18]. Vitamin E pretreatment has been shown to have a neuroprotective impact [19] and has been found to prevent various changes in blood enzymes as well as safeguard increases in hematocrit, leukocyte count, and hemoglobin level, mean osmotic fragility of erythrocytes [20]. To protect and restore normal cellular activities and metabolism, endogenous enzymatic and non-enzymatic antioxidants are required for the conversion of ROS to innocuous metabolites [21]. Antioxidant compounds have previously been shown to protect cells from the damaging effects of a variety of environmental pollutants [22,23].

There is no recognized chelating drug or antidote for paraquat poisoning, according to [24]. However, because PQ's method of action involves the formation of free radicals, which causes oxidative stress, this study was conducted to determine if antioxidants, vitamins E, and C, in combination, may reduce PQ toxicity on haematological parameters in paraquat induced male albino rats.

2.0 MATERIAL AND METHODS

2.1 Study Design

The chronic study was an experimental design involving 200 male albino rats with a mean weight of 0.20±0.02kg. The 200 rats were placed into four groups, each with 50 rats. A, B, C, and D were the four groups. The "A" group was not given paraquat; the "B" group was given 0.02g of paraquat per kg of rat every two weeks for three months; the "C" group was given 0.04g of paraquat per kg of rat every two weeks for three months; and the "D" group was given 0.06g per kg of paraquat every two weeks for three months**.** Subgroups were formed from each of the major groups. "A_0" and "A_{VEC}" subgroups from the "A" group; "B" and "B_{VEC}" subgroups from the "B" group; "C" and "C_{VEC}" subgroups from the "C" group; and "D" subgroups from the "D" group. The "A_0," "B_0," "C_0," and "D_0" subgroups were not given vitamin E or C, whereas the "A_{VEC}," "B_{VEC}," "C_{VEC}," and "D_{VEC}" subgroups were given 500mg of vitamin E and 2000mg/l of vitamin C medicated water orally every week. The

combined treatment regimen began three months following the paraquat induction. The rats were euthanized after the study duration and their blood samples were collected and analyzed.

2.2 Animal source

Animal House, Department of Biology, Rivers State University of Science and Technology provided 200 rats with an average weight of 0.20±0.02kg each. Before beginning the trial, the rats were brought to the research location and given two weeks to acclimate. The research took place at Rivers State University of Science and Technology's Department of Medical Laboratory Science.

2.3 Sample Collection method

Using a 2ml syringe ,blood sample was collected via cardiac puncture into EDTA bottles for the analysis of the hematogical parameters. The blood was mixed properly by a repeated gentle inversion movement. The well mixed blood was analyzed for Hemoglobin concentration, PCV, T-WBC, Neutrophils and Lymphocytes. By means of 70% chloroform anesthesia, the animals were sacrificed. The carcasses remaining were incinerated to avoid environmental pollution.

2.4 Laboratory analysis

Haemoglobin (Hb.) Cyanmethaemoglobin method [25]

Principle: Iron (II) of the haem in haemoglobin is oxidized to the ferric state by ferricyanide to form methaemoglobin which then is reduced to cyanmnethaemoglobin by ionised cyanide. This is red in colour and is measured spectrophotometrically at 540nm.

Procedure: 2µl of blood was washed into 5ml of Drabkins solution in a test tube. The test tube was covered with a rubber bung, inverted several times and allowed to stand at room temperature for 10min. to ensure complete conversion to cyanmethaemoglobin. The absorbance was read at 540nm wavelength against a blank (5ml of Drabkins reagent only). The absorbance of known standard was read alongside those of the test samples. The result is calculated thus:

$$\frac{\text{Absorbance of Test} \times \text{Standard concentration (mg/dl)}}{\text{Absorbance of Standard}}$$

= The Hb concentration of test (mg/dl)

Packed cell volume (PCV) method [25]

The packed cell volume (PCV) or the haematocrit is a measure of the relative volume of red cells present in a sample of whole blood in percentage.

Well-mixed, anticoagulated, blood was aspirated by capillary action into a microhaematocrit tube, leaving about 15mm unfilled. One end of the tube was sealed with plasticine. The tube was centrifuged at approximately 12,000g (centrifugal force) for 10 minutes using the microhaematocrit centrifuge.

The PCV was subsequently determined by measuring the height of the red cell column and expressing it as a percentage–ratio of the height of the total blood column using a microhaematocrit reader.

Total white blood cell (T-WBC) counts [25]

Quantitative and qualitative alteration in the circulating leucocytes characterizes diverse disease state and is often diagnostically significant. This could also assist us in determining the immune response to the foreign body (paraquat)

Procedure: One in twenty (1:20) dilution of the blood was made using 2% Glacial Acetic Acid tinged with few drops of Gentian violet. The diluted sample was mixed and allowed to stand for 15 minutes for complete destruction of the red cells. A known quantity of the diluted sample was aspirated into the charged chamber (Improved Neubaur Counting Chamber), and the white cells present in the four outer large squares of $1mm^2$ areas were counted.

Calculation:

Number counted X 50 (mf) = T-WBC counted per ml of blood

(mf = multiplication factor).

White blood cells differential count [25]
Each sample collected in the EDTA bottle was well mixed and thin film was made from a drop from the blood using a spreader on a slide. The slide was allowed to air-dry and stained with Leishman stain for microscopic differentiation of neutrophils and lymphocytes. Neutrophil and lymphocytes counts are expressed in percentage.

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

3.0 RESULTS

Table 1 shows the comparative effects of vitamin E and C combination therapy on the Chronic Toxicity of Paraquat in Albino Rats (*Rattus norvegicus*)

Table 1: ***Changes in the Haematological data after two months treament period.***

Sub-group	Hb(g/dL)	PCV (%)	T-WBC	Neutrophil	Lymphocytes
A_0	21.40 ± 1.18	64.25 ± 3.30	19.25 ± 1.23	37.8 ± 6.4	62.3 ± 4.4
A_{VEC}	22.43 ± 1.00	66.75 ± 2.75	18.40 ± 2.16	36.5 ± 3.6	63.5 ± 4.3
B_0	11.33 ± 0.77[a]	36.00 ± 2.58[a]	13.68 ± 1.11[a]	43.0 ± 5.1	57.0 ± 5.1
B_{VEC}	9.13 ± 1.05[a,b]	30.75 ± 3.09[a,b]	10.93 ± 0.60[a]	40.0 ± 4.2	60.0 ± 4.2
C_0	12.10 ± 1.48[a]	38.75 ± 3.90[a]	13.53 ± 3.62[a]	48.5 ± 3.8	49.0 ± 3.3
C_{VEC}	10.18 ± 2.35[a,b]	33.75 ± 6.68[a,b]	13.23 ± 2.46[a]	36.0 ± 3.7	64.0 ± 3.7
D_0	12.28 ± 1.16[a]	39.50 ± 3.01[a]	8.68 ± 1.39[a]	43.8 ± 3.3	56.3 ± 3.3
D_{VEC}	13.43 ± 1.74[a,b]	42.25 ± 4.63[a,b]	9.65 ± 0.91[a]	40.8 ± 2.7	59.25 ± 2.69

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_0 Vs B_{VEC}) at each month.

A_0 – Not induced with paraquat and no treatment with Vitamin E and C given
A_{VEC} – Not induced with paraquat with Vitamin E and C treatment given
B_0 - Induced with 0.02gc of paraquat and no Vitamin E and C treatment given
B_{VEC} – Induced with 0.02gc of paraquat and Vitamin E and C treatment given
C_0 - Induced with 0.04gc of paraquat with no Vitamin E and C treatment given
C_{VEC} - Induced with 0.04gc of paraquat with Vitamin E and C treatment given
D_0 – Induced with 0.06gc of paraquat with no Vitamin E and C treatment given
D_{VEC} – Induced with 0.06gc of paraquat with Vitamin E and C treatment given

Thus, from the analysis,

Table 2 : Hb (g/dl) – Extrapolated table illustrating simplified statistical interpretation (Vit E & C therapy) at $P \leq 0.05$

GROUPS	**DECISION**
A_0	21.40 ± 1.18
B_0	11.33 ± 0.77[a] - Significant difference
C_0	12.10 ± 1.48[a] - Significant difference
D_0	12.28 ± 1.16[a] –Significant difference
A_{VEC}	22.43 ± 1.00
B_{VEC}	9.13 ± 1.05[a] –Significant difference
C_{VEC}	10.18 ± 2.35[a] –Significant difference
D_{VEC}	13.43 ± 1.74[a] –Significant difference
B_0 Vs B_{VEC}	11.33 ± 0.77[a] Vs 9.13 ± 1.05[a,b] – Significant difference
C_0 Vs C_{VEC}	12.10 ± 1.48[a] Vs 10.18 ± 2.35[a,b] – Significant difference

D_0	12.28 ± 1.16^{a}
Vs	Significant difference
D_{VEC}	$13.43 \pm 1.74^{a,b}$

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_0 Vs B_{VEC}) at each month.

Table 3 : PCV(%) - Extrapolated table illustrating simplified statistical interpretation (Vit E & C therapy) at $P \leq 0.05$.

GROUPS	DECISION
A_0	64.25 ± 3.30
B_0	36.00 ± 2.58^{a} - Significant difference
C_0	38.75 ± 3.90^{a} -Significant difference
D_0	39.50 ± 3.01^{a} –Significant difference
A_{VEC}	66.75 ± 2.75
B_{VEC}	30.75 ± 3.09^{a} –Significant difference
C_{VEC}	33.75 ± 6.68^{a} –Significant difference
D_{VEC}	42.25 ± 4.63^{a} –Significant difference
B_0 Vs B_{VEC}	36.00 ± 2.58^{a} / $30.75 \pm 3.09^{a,b}$ — Significant difference
C_0 Vs C_{VEC}	38.75 ± 3.90^{a} / $33.75 \pm 6.68^{a,b}$ — Significant difference
D_0 Vs D_{VEC}	39.50 ± 3.01^{a} / $42.25 \pm 4.63^{a,b}$ — Significant difference

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_0 Vs B_{VEC}) at each month.

Table 4 : T-WBC- Extrapolated table illustrating simplified statistical interpretation (Vit E & C therapy) at $P \leq 0.05$.

GROUPS	DECISION
A_0	19.25 ± 1.23
B_0	13.68 ± 1.11^{a} - Significant difference
C_0	13.53 ± 3.62^{a} - Significant difference
D_0	8.68 ± 1.39^{a} –Significant difference
A_{VEC}	18.40 ± 2.16
B_{VEC}	10.93 ± 0.60^{a} – Significant difference
C_{VEC}	13.23 ± 2.46^{a} –Significant difference
D_{VEC}	9.65 ± 0.91^{a} –Significant difference

B_0 Vs B_{VEC}	13.68 ± 1.11 10.93 ± 0.60	No Significant difference
C_0 Vs C_{VEC}	13.53 ± 3.62 13.23 ± 2.46	No Significant difference
D_0 Vs D_{VEC}	8.68 ± 1.39 9.65 ± 0.91	No Significant difference

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_0 Vs B_{VEC}) at each month.

Table 5 : Neutrophils – Extrapolated table illustrating simplified statistical interpretation (Vit E & C therapy) at $P \leq 0.05$.

GROUPS	DECISION	
A_0	37.8 ± 6.4	No significant difference
B_0	43.0 ± 5.1	
C_0	48.5 ± 3.8	
D_0	43.8 ± 3.3	
A_{VEC}	36.5 ± 3.6	No significant difference
B_{VEC}	40.0 ± 4.2	
C_{VEC}	36.0 ± 3.7	
D_{VEC}	40.8 ± 2.7	
B_0 Vs B_{VEC}	43.0 ± 5.1 40.0 ± 4.2	No Significant difference
C_0 Vs C_{VEC}	48.5 ± 3.8 36.0 ± 3.7	No Significant difference
D_0 Vs D_{VEC}	43.8 ± 3.3 40.8 ± 2.7	No Significant difference

Table 6 : Lymphocytes - Extrapolated table illustrating simplified statistical interpretation (Vit E & C therapy) at P ≤0.05.

GROUPS	DECISION	
A_0	62.3 ± 4.4	No Significant difference
B_0	57.0 ± 5.1	
C_0	49.0 ± 3.3	
D_0	56.3 ± 3.3	
A_{VEC}	63.5 ± 4.3	No Significant difference
B_{VEC}	60.0 ± 4.2	
C_{VEC}	64.0 ± 3.7	
D_{VEC}	59.25 ± 2.69	
B_0 Vs B_{VEC}	57.0 ± 5.1 / 60.0 ± 4.2	No Significant difference
C_0 Vs C_{VEC}	49.0 ± 3.3 / 64.0 ± 3.7	No Significant difference
D_0 Vs D_{VEC}	56.3 ± 3.3 / 59.25 ± 2.69	No Significant difference

4.0 DISCUSSION

A statistically significant drop in Hb, PCV and WBC levels were seen in this study when groups that received a paraquat induction without any treatment (B_0, C_0, and D_0) were compared to control rats (A_0). This conclusion is in line with [24, 26, 27], which demonstrates paraquat's toxicity and, as a result, the risk of anemia induction. PQ-induced GSH depletion on the red blood surface, exposing the cell to oxidative lysis, could explain the lower amounts of PCV and Hb in the PQ alone group [28].

When those induced with paraquat and treated with vitamins (B_{VEC}, C_{VEC}, D_{VEC}) were compared to those not induced but treated with vitamins (A_{VEC}), the same was discovered. There was a significant difference in Hb, PCV and WBC levels among the subgroups. This trend was observed to be consistent with those induced with paraquat along. An intra-group comparison was studied (B_0 vs. B_{VEC}; C_0 vs. C_{VEC} & D_0 vs. D_{VEC}). The result showed a significant increase in PCV and Hb level in rats treated with Vit E and C combination. This implies that Vit E and C combined therapy was able to restore haematological function of anaemic indices after chronic exposure to paraquat. This work corresponded to the findings reported in another study [24].

Surprisingly, the total leucocyte count of paraquat treatment group was much lower than the control group. This is in direct opposition to [24]. The total leukocyte count in different treatment groups (intra-group) showed no significant differences when compared to the control group. This corresponds to [26]. Paraquat poisoning lowered RBC, hemoglobin, PCV, TLC levels in rats, according to [29,30].

Furthermore, across all doses and groups, no statistically significant variations in neutrophil and lymphocyte levels were observed. This is in contrast to the findings of [31], who discovered that leukocytes and neutrophil numbers increased while lymphocyte counts fell during the acute inflammatory response caused by oxidative stress. The effects of vitamin E and C combined therapy on neutrophils and lymphocytes have not been well studied. However, in this study, it could be observed that the vitamin combination therapy had no significant effect on the immune state of the rats but ameliorated reductions in red blood cells. It is worth noting that decreased availability of vitamin C during oxidative stress as a result of its shunting for vitamin E recycling in converting peroxytocopherol to tocopherol may result in a combined therapy regimen with a low ameliorative effect of both vitamins [32].

CONCLUSION

Finally, it is a well-known fact that biocide herbicides have always had hazardous effects on animals. PQ, as a biocide herbicide, has the most potent and notable toxic effect. Because its pathogenic pathway and therapeutic target are similar to those of other herbicides like diquat, understanding its toxicity and therapy can help with the treatment of disorders caused by other biocidal herbicides.

Ethical Approval:

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

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