

Original Research Article

AMELIORATIVE EFFECT OF VITAMIN E ON PARAQUAT INDUCED HAEMATOLOGICAL DISORDER IN MALE ALBINO RATS

Abstract

Paraquat is a controversial herbicide that can increase reactive oxygen species levels by undergoing redox cycling and producing reactive oxygen species such superoxide anion. Vitamin E is a fat soluble vitamin that modulates oxidation processes in the body due to its particular antioxidant activity. It is a powerful chain-breaking antioxidant that limits the synthesis of reactive oxygen species molecules. The goal of the study was to see if vitamin E had a short-term therapeutic impact on paraquat-induced male albino rats. For the experiment, 200 male albino rats were employed. The 200 rats were separated into four primary groups (A, B, C, and D), each of which included 50 rats and was then subdivided into two subgroups, each with 25 rats. The "A" group was not induced paraquat, but the "B," "C," and "D" groups were induced 0.02g, 0.04g, and 0.06g of paraquat, respectively. The "A" group was divided into two subgroups: "A₀" and "A_{VE}," which represented the subgroups that were not given Vit E and those who were given Vit E (500mg) respectively. This design was similar for groups "B," "C," and "D." The frequency of paraquat introduction was fortnightly for three months, then followed by vitamin E treatment weekly for two month. After the treatment with Vit E, the rats were sacrificed and blood was taken to analyze several hematological parameters (Hemoglobin concentration [Hb], Packed Cell volume [PCV], Total White blood cell count [T-WBC], Neutrophils and Lymphocytes). These hematological parameters differed significantly across the "A₀," "B₀," "C₀," and "D₀" groups, at p-value < 0.05. This was equally true for the "A_{VE}," "B_{VE}," "C_{VE}," and "D_{VE}," groups at p-value < 0.05. The findings also revealed that intra-group comparisons of Hb, PCV levels between B₀ vs B_{VE}, C₀ vs C_{VE}, and D₀ vs D_{VE} were significant different, p-value<0.05 whereas WBC, neutrophils and lymphocytes were not p-value>0.05. This study has shown that PQ insult on anaemia indicators in rats can be corrected by a weekly administration of the vitamin.

Keyword: Paraquat, Vitamin E, rat, antioxidant, hematological parameters

1.0 INTRODUCTION

In the course of improving quality of life by way of industrialization and food security, man has introduced toxicants into the environment. [1,2]. PQ, a problematic herbicide, is one of the most widely used total contact herbicide in the world. It is used to manage broad-leaved and grassy weeds in orchards and between crop rows [3]. Clark was the first to describe its harmful effects in rats [4]. PQ poisoning affects the lungs, liver, brain, kidneys, and other organs in several mammalian species [5, 6]. PQ has also been shown to be neurotoxic in people [7,8], rats [9,10], and mice [11]. It is an example of a chemical that can increase reactive oxygen species levels by undergoing redox cycling and producing reactive oxygen species such as hydroxal radical and

superoxide anion [12]. Several significant clinical disorders, including rheumatoid arthritis, myocardial infarction, emphysema, and Parkinson's disease, have been linked to oxidative stress. Although the specific mechanism of PQ toxicity is yet to be understood, it has been suggested that PQ-induced toxicity is caused by persistent redox-cycling and the consequent formation of reactive oxygen species (ROS), which results in oxidative stress and systemic inflammation [13]. Toxic substances, such as PQ, cause hemolysis (the destruction of red blood cells),[14] production failure (by attacking stem cells),[15] transportation failure (by chelating iron, other metals, and proteins involved in cellular functions),[16] regulatory and protective failure (by chelating iron, other metals, and proteins involved in cellular functions by affecting leucocytes and platelet production). Hematological indicators such as hemoglobin (Hb), packed cell volume (PCV), and total white blood cells count (T-WBC) are used to assess blood functionality in both healthy and sick states [17]. Its key tasks include detecting anemia, polycythemia, leucocytosis, and leucocytopenia, amongst others. They also aid in the evaluation of body fluid transit, distribution, control, and protection [18].

Vitamin E refers to a collection of fat-soluble substances identified by Evans and Bishop in 1922, each of which has specific antioxidant properties that are important for human health [19]. Vitamin E can be found in fatty meals [20]. Vit E modulates oxidation processes in the body due to its particular antioxidant activity. When fat is oxidized and free radical reactions propagate, it is a powerful chain-breaking antioxidant that limits the synthesis of reactive oxygen species molecules [21]. It protects cell membranes from free radical attack and acts as a first line of defense against lipid peroxidation. It preserves the polyunsaturated fatty acids found in membrane phospholipids and plasma lipoproteins by scavenging peroxy radicals [22].

Earlier research suggested that vitamin E could counteract the negative effects of oxidative stress caused by free radicals by protecting cell membranes and proteins, or by regulating particular proteins involved in signal transduction and gene expression [23].

As a result, vitamin E may aid tissue repair and help to avoid or delay chronic diseases linked to reactive oxygen species molecules.

Due to the potent functionality of vitamin E, this study is geared towards evaluating the ameliorative effect of Vitamin E therapy on haematological parameters of paraquat induced male albino rats.

2.0 MATERIAL AND METHODS

2.1 Study Design

A chronic experimental design of biological trial was used on 200 male albino rats with a mean weight of 0.20 ± 0.02 kg. The 200 rats were divided into four groups (A, B, C, D) of 50 rats each. The "A" group received no paraquat; the "B" group received 0.02g of paraquat per kg of rat every two weeks for three months; the "C" group received 0.04g of paraquat per kg of rat every two weeks for three months; and the "D" group received 0.06g per kg of paraquat every two weeks for three months. There were subgroups within each of the main groups. The "A" group contained "A₀" and "A_{VE}" subgroups; the "B" group contained "B₀" and "B_{VE}" subgroups; the "C" group contained "C₀" and "C_{VE}" subgroups; and the "D" group contained "D₀" and "D_{VE}" subgroups. The "A₀," "B₀," "C₀," and "D₀" subgroups were not given vitamin E, whereas 500mg of Vitamin E was given, every week, orally, to the "A_{VE}," "B_{VE}," "C_{VE}," and "D_{VE}" subgroups for two months. Treatment with vitamin E, began after the three-month paraquat induction period. The rats were sacrificed after two months of Vit E administration, and their samples (blood) were tested for haematological findings.

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 ± 0.02 kg. Before beginning the trial, the rats were brought to the study site and given two weeks to acclimate. The research was carried out at Rivers State University of Science and Technology's Department of Medical Laboratory Science.

2.3 Sample Collection method

Hematological parameters were determined using a blood sample. Two milliliters of blood was taken and dispensed in EDTA bottles using a syringe and needle. A slight inversion movement was used to thoroughly mix the blood. Hemoglobin levels, PCV, T-WBC, Neutrophils, and Lymphocytes were all measured. The animals were thereafter sacrificed under the influence of 70% chloroform anesthesia. To avoid environmental damage, the carcasses that remained were cremated.

2.4 Laboratory analysis

Haemoglobin (Hb.) Cyanmethaemoglobin method [24]

Principle: Iron (II) of the haem in haemoglobin is oxidized to the ferric state by ferricyanide to form methaemoglobin which then is reduced to cyanmethaemoglobin 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:

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

Packed cell volume (PCV) method [24]

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 [24]

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 [24]

White blood cell differential count was determined by microscopic assessment of thin blood film stained with leishman. For every sample collected, thin blood film was made a stained with leishman stain for microscopic differentiation of neutrophils and lymphocytes. The neutrophil and lymphocyte populations were 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 therapy on the Chronic Toxicity of Paraquat in Albino Rats (*Rattus norvegicus*)

Table 1.: Changes in the Haematological data after two months treatment 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 _{VE}	23.25 ± 0.26	69.25 ± 0.48	19.45 ± 2.05	38.8 ± 3.8	61.3 ± 3.9
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 _e	12.03 ± 1.57 ^{a,b}	37.25 ± 4.82 ^{a,b}	9.30 ± 1.12 ^a	40.0 ± 3.5	60.0 ± 3.5
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 _{VE}	11.93 ± 1.34 ^{a,b}	38.50 ± 3.71 ^{a,b}	11.25 ± 0.43 ^a	35.0 ± 2.8	65.0 ± 2.8
D ₀	12.28 ± 1.16 ^a	39.50 ± 3.01 ^a	8.68 ± 1.39 ^c	43.8 ± 3.3	56.3 ± 3.3
D _{VE}	14.00 ± 1.67 ^{a,b}	44.50 ± 4.13 ^{a,b}	13.05 ± 2.24 ^a	30.0 ± 5.2	70.0 ± 6.2

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: B₀ Vs B_{VE}) at each month.

INTERPRETATION OF RESULT

In the study, the rats were divided into four major groups.

- A₀ – Not induced with paraquat and no treatment with Vitamin E given
- A_{VE} – Not induced with paraquat with Vitamin E treatment given
- B₀ - Induced with 0.02gc of paraquat and no Vitamin E treatment given
- B_{VE} – Induced with 0.02gc of paraquat and Vitamin E treatment given
- C₀ - Induced with 0.04gc of paraquat with no Vitamin E treatment given
- C_{VE} - Induced with 0.04gc of paraquat with Vitamin E treatment given
- D₀ – Induced with 0.06gc of paraquat with no Vitamin E treatment given
- D_{VE} – Induced with 0.06gc of paraquat with Vitamin E treatment given

Thus, from the analysis,

Table 2 : Hb (g/dl) –Extrapolated table illustrating simplified statistical interpretation (Vit E 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 _{VE}	23.25 ± 0.26	
B _{VE}	12.03 ± 1.57 ^{a,b}	-Significant difference
C _{VE}	11.93 ± 1.34 ^{a,b}	-Significant difference
D _{VE}	14.00 ± 1.67 ^{a,b}	-Significant difference
B ₀	11.33 ± 0.77 ^a	} Significant difference
V _S		
BE	12.03 ± 1.57 ^{a,b}	
C ₀	12.10 ± 1.48 ^a	} Significant difference
V _S		
C _{VE}	11.93 ± 1.34 ^{a,b}	
D ₀	12.28 ± 1.16 ^a	} Significant difference
V _S		
D _{VE}	14.00 ± 1.67 ^{a,b}	

Table 3 : PCV(%) - Extrapolated table illustrating simplified statistical interpretation (Vit E therapy) at P ≤ 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 _{VE}	69.25 ± 0.48	
B _{VE}	37.25 ± 4.82 ^{a,b} -Significant difference	
C _{VE}	38.50 ± 3.71 ^{a,b} -Significant difference	
D _{VE}	44.50 ± 4.13 ^{a,b} -Significant difference	
B ₀	36.00 ± 2.58 ^a	} Significant difference
V _S		
BE	37.25 ± 4.82 ^{a,b}	
C ₀	38.75 ± 3.90 ^a	} Significant difference
V _S		
C _{VE}	38.50 ± 3.71 ^{a,b}	
D ₀	39.50 ± 3.01 ^a	} Significant difference
V _S		
D _{VE}	44.50 ± 4.13 ^{a,b}	

Table 4: T-WBC - Extrapolated table illustrating simplified statistical interpretation (Vit E therapy) at P ≤ 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 -Significant difference

A _{VE}	19.45 ± 2.05	
B _{VE}	9.30 ± 1.12 ^a	} Significant difference
C _{VE}	11.25 ± 0.43 ^a	
D _{VE}	14.00 ± 1.67 ^{a,b}	
B ₀	13.68 ± 1.11 ^a	
V _S		} No Significant difference
BE	9.30 ± 1.12 ^a	
C ₀	13.53 ± 3.62 ^a	} No Significant difference
V _S		
C _{VE}	11.93 ± 1.34 ^a	
D ₀	8.68 ± 1.39 ^a	} No Significant difference
V _S		
D _{VE}	13.05 ± 2.24 ^a	

Table 5 : Neutrophils - Extrapolated table illustrating simplified statistical interpretation (Vit E therapy) at P ≤ 0.05.

GROUPS	DECISION	
A ₀	37.8 ± 6.4	
B ₀	43.0 ± 5.1	} No significant difference
C ₀	48.5 ± 3.8	
D ₀	43.8 ± 3.3	
A _{VE}	38.8 ± 3.8	
B _{VE}	40.0 ± 3.5	} No significant difference
C _{VE}	35.0 ± 2.8	
D _{VE}	30.0 ± 5.2	
B ₀	43.0 ± 5.1	
V _S		} No Significant difference
BE	40.0 ± 3.5	
C ₀	48.5 ± 3.8	
V _S		} No Significant difference
C _{VE}	35.0 ± 2.8	
D ₀	43.8 ± 3.3	} No Significant difference
V _S		
D _{VE}	30.0 ± 5.2	

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

GROUPS	DECISION	
A ₀	62.3 ± 4.4	
B ₀	57.0 ± 5.1	} No Significant difference
C ₀	49.0 ± 3.3	
D ₀	56.3 ± 3.3	
A _{VE}	61.3 ± 3.9	

B _{VE}	60.0 ± 3.5	No Significant difference
C _{VE}	65.0 ± 2.8	
D _{VE}	70.0 ± 6.2	
B ₀	57.0 ± 5.1	No Significant difference
V _S		
BE	60.0 ± 3.5	
C ₀	49.0 ± 3.3	No Significant difference
V _S		
C _{VE}	65.0 ± 2.8	
D ₀	56.3 ± 3.3	No Significant difference
V _S		
D _{VE}	70.0 ± 6.2	

4.0 DISCUSSION

The goal of this study was to show if vitamin E has a role in the repair of paraquat-induced damage in albino rats' hemograms. Paraquat was given to several subgroups of test subjects, and inter- and intra- comparative study of significant and non-significant repair were studied. Paraquat is a non-selective contact herbicide discovered in 1955 and registered as herbicide by ICI laboratories in 1962. Farmers in developing nations have easy access to it [25]. It is frequently cited as one of the substances that may be employed in suicidal attempts, with the majority of the toxins collecting in the lungs, liver, kidneys, and heart [26].

In the current study, there was a significant decrease in Hb and PCV concentrations when groups that received an induction of paraquat without any treatment (B₀, C₀ and D₀) were compared to that of control (A₀). This in compliance with the earlier studies proposed by [27, 28, 29]. This finding demonstrates the toxicity and subsequently, possibility of paraquat to induce anemia. These alterations (reduction) in the PCV and Hb could be caused by free radical induced damage in accordance with erythrocyte membrane and comparable view had been expressed by [29,30]. Similarly, it was observed that the groups induced with paraquat and treated with the vitamins (B_{VE}, C_{VE}, D_{VE}) when compared to its control (those not induced but treated with the vitamins (A_{VE})), there was a significant difference among the groups.

Furthermore, intra group comparison (B₀ Vs. B_{VE}; C₀ Vs. C_{VE} & D₀ Vs. D_{VE}) was studied to actually assess the therapeutic effect of Vitamin E on single dose and treatment basis. The results

revealed that there were significant increases in Hb and PCV levels between the groups. This means that Vitamin E was able to restore anaemic condition induced by paraquat toxicity on rats. This corresponded to the findings of [29].

Interestingly, the total leucocyte count showed significant decrease in paraquat treatment group when compared with control group. This is consistent with the finding of [27]. These findings were also similar to the observations of [31,32] who reported the reduction in RBC, hemoglobin, PCV, TLC and absolute leucocyte count value in rats due to paraquat toxicity. Intra group comparison of haematological parameters showed that there was a significant increase in PCV, HB and WBC in paraquat induced rats which were treated with vitamin E. This means that vitamin E was capable of restoring haematological disorders induced by paraquat toxicity within two months of treatment.

Furthermore, there appeared to be no statistically significant change in the neutrophil and lymphocyte values cutting across all doses, in all groups. This partly contradicts the findings of [33] who reported an increase in leukocytes and neutrophil counts and a decline in lymphocyte counts, during the acute inflammatory response due to oxidative stress. There has been no much study on the ameliorative effect of vitamin E therapy on neutrophils and lymphocytes.

CONCLUSION

In conclusion, the toxic effects of paraquat dichloride as seen in this study could be useful in educating farmers who regularly use this herbicide and the general public on possible effects of this herbicide to human and animal lives in order to prevent increased mortality rate due to paraquat dichloride toxicity. Subsequently, further studies on the effect of paraquat dichloride on hematological tissues and the ameliorative effect of other vitamins are recommended.

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