Original Research Article

Effects of vitamin E on Chronic Haematoxicity of Paraquat Exposed Male Albino Rats

(*Rattus norvegicus*)

Abstract

Paraquat is an environmental pollutant that is associated with some disorders including alteration of some harmatological indices and is a concern to public health. Vitamin E has antioxidant property and other characteristic roles that ameliorate toxic effect. 200 male albino rats weighing 0.2±0.02kg on the average were divided into four main groups (A, B, C, and D) with 50 rats in one group. The "A" group was not served paraquat solution and was used as the control group. Group "B", "C", "D" being the treatment group were given dose rates of paraquat of 0.02, 0.04, and 0.06g, of paraquat per kg rat respectively every two weeks for three months. The main groups further had subgroups. "A", "A_0" and "A_{VE}"; "B", "B_0" and "B_{VE}"; "C" had "C_0" and "C_{VE}"; and "D", "D_0" and "D_{VE}" subgroups. Vitamin E was not given to subgroups "A_0", "B_0", "C_0" and "D_0" subgroups while "A_{VE}", "B_{VE}", "C_{VE}" and "D_{VE}" were fed orally with 500mg of vitamin E therapy every week. Treatment with vitamin E began three months after paraquat treatment. At the end of the weekly treatment with vitamin E for a month, the rats were sacrificed and their blood samples were obtained and analyzed for PCV, Hb, WBC, neutrophil and lymphocyte. A_0, B_0, C_0 and D_0 intergroup comparison was statistically significant, p-value<0.05 in PCV and Hb levels while their WBC, neutrophil and lymphocyte had no statistical significance. Ave, Be, Cve and Dve intergroup comparison was statistically significant, p-value<0.05 in PCV and Hb levels while WBC, lymphocyte and neutrophils had no statistical significance among the groups. Intra group comparison showed that only PCV and Hb were significant between groups, p-value<0.05. This study confirms that vitamin E is potent in treating paraquat toxicity in male albino rats on one month of weekly treatment.

Keyword: *Paraquat, Vitamin E, toxicity, antioxidant, liver*

1.0 Introduction

The introduction of toxicants into the environment by virtue of human activities has drawn research interest by many researchers to understand the relationship between environmental toxicant and bioaccumulation (Onwuli *et al*., 2014; Fyneface *et al*., 2018). Paraquat known as 1,1-dimethyl-4-4-bipiridinium dichloride, is a non-selective quick-acting contact herbicide which have wide application in agriculture. It is commonly used in crop cultivation and conservation tillage process globally (Li *et al*., 2019). Paraquat is an environmental pollutant that is associated

with some disorders including alteration of some harmatological indices and it a concern to public health (Kim *et al*., 2019). Findings have established toxic effect of Paraquat to health and various types of acute intoxication have been documented from nephrotoxicity, neurotoxicity, pulmonary toxicity, immunotoxicity, toxicity of the reproductive system, haematological derangement manifesting as anemia, leucocytosis and others (Dinis-Oliveira *et al*., 2008; Li *et al*., 2017; Han *et al*., 2014; Liu *et al*., 2018; Kim *et al*., 2009; Rappold *et al*., 2011; Khilji *et al*., 2011; Li *et al*., 2019). Paraquat is one of the leading producers of free radicals. The impact of oxidative stress on the blood indices numerous and some measures have been adopted to mob up these free radicals through the use of antioxidants such as vitamins E (EL-Hak *et al*., 2019).

Notably, majority of the population round the globe are frequent users of vitamin and many depends on vitamin supplementation in addition to food producing vitamins for health care needs according to WHO (2005). Vitamin E is extensively applied for the fields of human and veterinary medicine including livestock production (Betoret *et al*., 2011). Vitamin E is essential for optimal function of the body. It functions in specific areas like reproduction, tissue protection, growth, disease prevention and more with a daily recommended dose of 1000mg per day for human (Kappus & Diplock, 1992; Hardie *et al*., 1990). Sies and Stahl in 1995 posit that vitamin E is an antioxidant involved in breaking chains; specifically, alpha (α)-tocopherol. Alpha (α)-tocopherol according Miller and colleagues (2005) is one of eight types of vitamin E with the characteristics of lipid-solublity that functions as a chain-breaking antioxidant (Sies & Stahl, 1995).

Vitamins E is not water soluble but fat however, safe for the body with antioxidant property and other characteristic roles (Tappel, 1972). Furthermore, there are studies in recent time that have confirmed advantegous effect of vitamin E and its usefulness in the treatment of infertility particularly male infertility (Keskes-Ammar, et al., 2003), heart conditions such as cardiovascular disease as revealed by Lee *et al*. (2005), antilipid agent for high triglyceride (Engelhard *et al*., 2006), diabetes (El-Aal *et al*., 2018; Pavithra *et al*. 2018). The role of vitamin E is huge even in haematology as studies have confirm restoration of blood level seen in packed cell volume, haemoglobin level and improvement of other red cell indices (Steiner, 1991; Mochegiani *et al*., 2014; Chen *et al*., 2005).

Experimental studies have shown devastating toxic effect of paraquat to haematological indicies according to a report by El-Hak et al. (2019). The herbicidal agent paraquat, posses a toxic effect which affects the haematopoietic system; thereby causing alteration of the haematological indices. Majority of the published articles have centred on biochemical indices and organs specific toxicological effect of paraquat and many on antioxidant parameters as well as the protecting effect of vitamin E but only few focused on ameliorating effect of vitamin E on paraquat induced toxicity on haematological indices hence, this study.

2.0 Material and Methods

2.1 Experimental Design

The study was aimed at determining the effect of vitamin E on paraquat induced toxicity in rats. 200 male albino rats weighing 0.2±0.02kg on the average were divided into four main groups (A, B, C, and D) with 50 rats in one group. The "A" group was not served paraquat solution and was used as the control group. Group "B", "C", "D" being the treatment group were given dose rates of paraquat of 0.02, 0.04, and 0.06g, of paraquat per kg rat respectively every two weeks for three months. The main groups further had subgroups. "A", "A_0" and "A_{VE}"; "B", "B_0" and "B_{VE}"; "C" had "C_0" and "C_{VE}"; and "D", "D_0" and "D_{VE}" subgroups. Vit. E was not given to subgroups "A_0", "B_0", "C_0" and "D_0" subgroups while "A_{VE}", "B_{VE}", "C_{VE}" and "D_{VE}" were fed orally with 500mg of vitamin E therapy every week. Treatment with Vit. E began three months after paraquat treatment. At the end of weekly treatment with Vit. E for a month, the rats were sacrificed and their blood samples were obtained and analyzed. The table below provides a detailed summary of the experimental design:

Table 1 Details of experimental design

Groups	No of Rats (kg)	Paraquat Treatment (g)	Vitamin E Treatment (mg)
A_0	25	Nil	Nil
A_{VE}	25	Nil	500
B_0	25	0.02	Nil
BE	25	0.02	500
C_0	25	0.04	Nil
C_{VE}	25	0.04	500
D_0	25	0.06	Nil
D_{VE}	25	0.06	500

Table showing the summary of the experimental design. A_0 = neutral group; A_{VE}= neutral group fed with Vit. E only, B_0, C_0, D_0= subgroups treated with paraquat alone; B_{VE}, C_{VE}, D_{VE} = subgroups treated with paraquat and Vit. E therapy

2.2 Experimental Animals

The animals were obtained from Animal House, Department of Biology, Rivers State University of Science and Technology and the guiding principles in the handling and use of laboratory animals were applied. A total of 200 rats weighing 0.2±0.02kg on the average were transported in cages to the research location and kept for two weeks in a controlled environment before continuing with the study. The experiment was conducted in Department of Medical Laboratory Science, Rivers State University of Science and Technology.

2.3 Method of Sample Collection

Blood sample was obtained for full blood count. 2mls of blood was collected by cardiac puncture with syringe and needle and dispensed in EDTA bottles. The animals were sacrificed using 70% chloroform anesthesia. The remains were properly discarded via incineration.

2.4 Laboratory Analysis

Haemoglobin (Hb.) Cyanmethaemoglobin method (Baker, et al., 1985)

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:

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

= The Hb concentration of test (mg/dl)

Packed cell volume (PCV) method (Baker, *et al*., 1985):

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 (Baker, *et al*., 1985):

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 (Baker, *et al*., 1985)

A drop of the anticoagulated blood sample on a clean, grease free slide was spread with a glass spreader at angle of 45^{o} to the slide. With a swift, forward movement, the drop of blood is spread on the slide, making a uniform film of equal distribution of cells.

The films after preparation were air dried, fixed in alcohol (methanol), air dried again, and stained with field stain 'A' and 'B'. It is first stained in field stain 'B' within two seconds, brought out and rinsed in distilled water; followed with field stain 'A' within the same time interval, rinsed in distilled water, and air dried. After which the films were examined under the microscope with an oil immersion magnification, and the cells counted and identified as neutrophils and lymphocytes; and rated in percentage of 100 Leucocyte.

2.5 Statistical analysis

Statistical analysis was carried out with SPSS version 23.0, using the data generated from this research. 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.

3.0 RESULTS

Table 2 is the tabular representation of the comparative theraputic effects of vitamin E on the chronic toxicity of paraquat induced albino rats (*Rattus norvegicus*). A_0, B_0, C_0 and D_0 intergroup comparison was statistically significant, p-value<0.05 in Hb and PCV levels while WBC, neutrophil and lymphocytes had no statistical significance, p-value>0.05. Ave, Be, Cve and Dve intergroup comparison was statistically significant, p-value<0.05 in Hb and PCV levels while WBC, neutrophil and lymphocytes had no significant difference while WBC, neutrophil and lymphocytes had no statistical significance among the groups p-value>0.05. There was significant difference, p-value<0.05 in Hb and PCV levels between B_0 and Bve, C_0 and Cve, and D_0 and Dve.

Table 2 : ***Changes in the Haematological data after one month treatment period***.

Sub-group	Treatments (4 Rats in each subgroup) Mean ±SEM

	Hb(g/dL)	PCV (%)	T-WBC	Neutrophil	Lymphocytes
A_0	22.42 ± 0.46	66.50 ± 1.36	9.05 ± 0.35	40.0 ± 2.1	60.0 ± 2.1
A_{VE}	21.00 ± 1.30	62.67 ± 3.61	9.17 ± 1.00	37.3 ± 2.0	62.7 ± 2.0
B_0	16.78 ± 2.71[a]	51.17 ± 7.37[a]	10.32 ± 0.69	39.3 ± 1.6	60.7 ± 1.6
BE	19.13 ± 1.32[a,b]	57.50 ± 3.54[a,b]	7.70 ± 0.89	40.0 ± 3.0	60.0 ± 3.0
C_0	15.12 ± 2.21[a]	47.33 ± 5.78[a]	10.10 ± 0.66	46.3 ± 2.4	53.7 ± 2.4
C_{VE}	19.05 ± 1.18[a,b]	57.33 ± 3.22[a,b]	9.73 ± 0.67	45.2 ± 1.6	54.8 ± 1.6
D_0	14.07 ± 2.23[a]	44.00 ± 5.87[a]	9.50 ± 1.01	38.8 ± 2.3	61.2 ± 2.3
D_{VE}	16.18 ± 1.42[a,b]	50.00 ± 3.61[a,b]	9.73 ± 1.67	36.5 ± 4.4	63.5 ± 4.4

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.

4.0 Discussion

Vitamin E is required in preventing or minimizing free-radical damage associated with specific diseases and lifestyle patterns and processes, including cancer, aging, circulatory conditions, arthritis, cataract, pollution, and strenuous exercise (Packer, 1991).

The hematological parameters can be used to find the blood relating functions of the supplement used according to Davis et al. (2008). The hemopoietic system is one of the most sensitive targets of toxic compounds and it is a key indicator of physiological and pathological states of the body both in humans and animals as described by Tchounwou et al. (2012). The human haematopoietic system have shown to be susceptible to paraquat toxicity as seen from review of literatures and ample observation from this experiment as well as pragmatic confirmation of the ameiloriting effect of vitamin E on paraquat induced haematoxicity found on the haematological indices evaluated.

The comparative effects of vitamin E on the Chronic Toxicity of Paraquat in Albino Rats (*Rattusnorvegicus*) showed changes in the haematological indices. Study findings revealed evidence of statistical significance differences between test subgroups of B, C and D and control subgroups per treatment duration of one month while test subgroup A demonstrated null disparity with the control group. Remarkably, the observed differences were found in the

haematological indices of erythrocyte origin specifically, the packed cell volume and haemoglobin concentration. These two indices are indicators of anemia. Experimental findings showed changes in the haematological indices in an increamental level. This implies that the administration of vitamin E following parquet exposure causes an increase in the packed cell volume as well as the haemoglobin concentration at varying degrees. This elevated levels of packed cell volume and haemoglobin is dissimilar with the finding of El-Hak et al. (2019).

This study is in support of previous research which proved a protective advantage of vitamin E against paraquat induced damages mainly among vitamin E deficient subjects whereas, little or no benefit is derived from extra therapeutic supplementation of vitamin E (Evans & Halliwell, 2001).The considerable increase in the haemoglobin level reported in this study is opposition with Jilani and colleagues (2008) study which found marked decrease in the hemoglobin concentrations in vitamin E treated rats at same duration of 30 days supplementation. This means that in this study, the study subjects had no red blood cell reduction and by implication the haemopeoitic system suffered no suppression as assumed by other study (El-Hak *et al*. (2019; Jilian *et al*., 2008). This current study outcome supports the fact that Vitamin E supplementation improves blood level (packed cell volume), Hemoglobin and erythropoietin levels.

This study observed no statistically significant difference in the leucocyte cell line as there was no indication found in the total white blood cell count, neutrophil and lymphocytes. This finding of no variation in the white cell line after administration of vitamin E to paraquat exposed subjects is in contrary with older study by El-Hak et al. (2019) who in a study demonstrated a significant increase in total leukocyte count with the vitamin E treatment and this was attributed to response to liver and kidney tissue damages. Furthermore, this study is in disagreement with a study (Ambali *et al*., 2011) which found decreased leucocytes as opposed to this present study.

The assessment of hematologic parameters tells the physiological state as an indicator of oxidative stress in the body (Yousef *et al*., 1995; Jasper *et al*., 2012). This study has supported the alteration of haematological indices by paraquat and protective function of vitamin E through improvement of the blood level. The variation between this study and other studies are due to many factors such as geographic location, exposure rate, mode of administration, study design, protocol, procedure used including analytical and laboratory methods used. These and other

factors like physical, some environmental and biological factors might be responsible for any inter-study disparity.

Conclusion

This work has confirmed that vitamin E is potent in treating haematological disorder due to paraquat toxicity in male albino rats on one month of periodic weekly treatment.

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