Original Research Article

Haematological Recovery by Vitamin E and C on Paraquat inflicted Haematological insult in *Rattus norvegicus*

Abstract

Background

Vitamin E (alpha tocopherol) is a fat-soluble vitamin which plays an important role in sustaining healthy metabolism and physiological functions in the body. Earlier studies showed that vitamin E could ameliorate the damaging effect caused by oxidative stress induced by free radicals. Vitamin C (Ascorbic acid) is a water soluble vitamin which is needed for the formation of collagen and intracellular materials. Hence, it is essential in wound healing and recovery tissue recovery.

Aim

This study was done to evaluate the haematological effect of Vitamin E and C combination therapy on the chronic toxicity of paraquat in Wister rats.

Methodology

A total of 200 male Wister rats were used for the study. The rats were divided into four main groups of 50 rats in each group (A, B, C, D) and was further subgrouped , having 25rats per subgroup. "A" group was not induced with paraquat while "B", 'C" and "D" groups were induced in increasing dose of 0.02g, 0.04g and 0.06g respectively. "A" group had two subgroups; "Ao" and "Avec" which represented the sub-group not treated with Vit E and C and the subgroup treated with 500mg vitamin E and 2000mg/l of vitamin C medicated water respectively. This also applied to group "B", "C" and "D". paraquat was induced every forth night for three month followed by weekly treatment for one month.

Result

The result obtained showed a significant decrease in PCV, Hb, and TWBC count among Ao, Bo, Co and Do groups at $p<0.05$ but no significant difference in the neutrophil and lymphocyte count. Also, there was a significant difference among Ave, Bvec, Cvec and Dvec groups. On intra-group comparison, there was a significant increase in the Hb, and PCV of subgroups Avec, Bvec, Cvec and Dvec at $p<0.05$ but no significant difference in the TWBC count and differentials.

Conclusion

This indicates that Vitamin E and C combination supplementation had an ameliorative effect on the PCV and Hb value but no effect on the TWBC count and differentials on paraquat toxicity.

Keyword: *Vitamin E and C, paraquat, rat, antioxidant, haematological parameters.*

1.0 Introduction

When agrochemicals are used extensively and intensively to meet the agricultural requirements of the population, also the indiscriminate use or misuse of these agrochemicals results in the poisoning of the biosystem (Sekhar *et al*., 2011; Onwuli *et al*., 2014; Fyneface *et al*., 2018). A lot of pesticides used to kill weeds have been proven to have an effect on the biosystem (da-Cruz, 2002; Johal *et al*., 2007). Even though the use of these pesticides has increased agricultural productivity, there are reports of increasing mortality of aquatic organisms after exposure to these toxicants, which in most cases are discharged accidentally (Yuan *et al*., 2004). Cases of death have been reported as a result of pesticide toxicity, but recently chronic toxicity has become common. Organisms are subjected to long term effects (stress) resulting from chronic exposure which may also cause death in the organism or as toxicants to consumers of such organism (Yuan *et al*., 2004; Kori-Siakpere *et al*., 2007).

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However, it has been reported that chronic levels of these chemicals can cause changes in behaviour (da-Cruz, 2002), histopathology (Johal *et al*., 2007), enzymes and metabolites (Geneshwade, 2012) feeding habits and feed conversion ratio (Yaji & Auta, 2007) and haematological parameters (Sekhar *et al*., 2011).

Paraquat (1, 1'- dimethyl- 4,4'- bipyridlium dichloride) is a nitrogen based compound that is of use in the controlling of weeds on farms in the tropics. It was first synthesized in 1882, but its pesticidal property was discovered much later in 1959 (Harley, 1979). It is used globally and can lead to severe, acute and chronic toxicity (Harley, 1979), this is because it readily dissolves and dissociates in water or aqueous solutions (Amdur *et al*., 1991). Paraquat is one of the most commonly used pesticides and comes next to glyphosate. It is sold in over 130 countries for use in both big and small farms, plantations and estates and in non-weed control. It acts very quickly; it is a non-selective herbicide, which destroys green plant tissue on contact and by translocation within the plant (Zeneca, 1996). Although the exact mechanism of PQ toxicity has not been clearly understood, it has been widely shown that PQ-induced toxicity is due to a prolonged redox-cycling when reactive oxygen species are generated, which result in general inflammation due to oxidative stress (Castello *et al*., 2007).

Vitamin E (alpha tocopherol) is a fat-soluble vitamin which plays an important role in sustaining healthy metabolism and physiological functions in the body (Torquato *et al*., 2020). Because of its antioxidant activity, vitamin E regulates oxidation processes in the body (Sepidarkish *et al.,* 2019). Earlier studies showed that Vit. E could ameliorate the damaging effect caused by oxidative stress induced by free radicals by preventing cell membranes or proteins damage, or by regulating certain proteins responsible for regulating signal transduction and gene expression (Khallouki *et al*., 2020).

Vitamin C (Ascorbic acid) is a water soluble vitamin which is needed for the formation of collagen and intracellular materials and hence for the development of cartilage, bone and for wound healing (Sato *et al*., 1990). It has been shown by several studies that ascorbic acid blocks the formation of carcinogenic compounds formed by chemical reaction of food in human stomach. It also plays a role in detoxification of insecticides by decreasing their mutagenic effects on testis and bone marrow of mice (Chakrabrty *et al*., 1973).

This study was focused on assessing the therapeutic effect of vitamin E and C combination therapy on male Wister rats exposed to paraquat.

2.0 Material and Methods

2.1 Study Area/Population

The study was carried out in the Medical Laboratory Science Departmental Laboratory of Rivers State University. Wister rats were considered the choicest animals for this experiment because of their availability, cost, genetic makeup, handling technique and nature of the study. Two hundred (200) healthy mature male Wister rats with a mean weight of 0.2±0.02kg

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were used in 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 weeks 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 Wister Rats were used for this research and were divided into 4 groups with each group containing fifty (50) Rats each.

Group A: This was the control group. They were not induced with paraquat

Group B: This group was induced every two weeks with 0.02g of paraquat per kg of rat for three months.

Group C: This group was induced every two weeks with 0.04g of paraquat per kg of rat for three months.

Group D: This group 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 "Avec" subgroups; "B" group had "Bo" and "Bvev" subgroups; "C" group had "Co" and "Cvev" subgroups; "D" group had "Do" and "Dvev"

"Ao", "Bo", "Co" and "Do" subgroups: were not treated with vitamin E and C combination

"Avec", "Bvec", "Cvec" and "Dvec" subgroups: were treated orally with 500mg of vitamin E and 2000mg/l of medicated C every week.

The treatment with Vit E and C commenced after the three months paraquat induction. After three month of weekly treatment with Vit E and C, the rats were sacrificed and their blood samples were analyzed for haematological parameters.

2.3 Procedures for Administration of Toxicant

Toxicant was administered 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 content in the syringe was then emptied into the mouth of the rat gradually.

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2.4 Sample Collection

Two milliliters of blood samples were collected via cardiac puncture from the animals and sacrificed under
70% chloroform anesthesia into the Ethylenediaminetetra acetic acid (EDTA) specimen bottle and used for analysis of haematological parameters.

2.5 Laboratory analysis

2.5.1 Haemoglobin (Hb.) estimation by 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, was inverted severally and then allowed to stand at room temperature for 10min. This is to ensure complete conversion to cyanmethaemoglobin. The absorbance was then 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)

2.5.2. 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.

2.5.3. Total white blood cell (T-WBC) counts (Baker, *et al*., 1985):

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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).

2.5.4. 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° 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 neutrophils and lymphocytes cells were counted and rated in percentage of 100 Leucocyte.

2.6 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 Result

Table 1 showed the comparative effects of vitamin E and C combination therapy on the Chronic Toxicity of Paraquat in Wister Rats (*Rattusnorvegicus*). There was a significant difference among A0, B0, C0 and D0 subgroups, P-value<0.05 but there was no significant difference in neutrophil and lymphocyte level among the groups, p-value>0.05. The table also showed that there was a significant difference in Hb level, PCV level and WBC among

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Ave, Bve, Cve and Dve subgroups, P-value<0.05 but there was no significant difference in neutrophil and lymphocyte level among the groups, p-value>0.05. Intra-group comparison showed there was a significant increase in Hb and PCV in Bvec, Cvec and Dvec when respectively compared with B_0, C_0 and D_0, p-value<0.05. But there was no significant difference in WBC, neutrophil and lymphocytes between the subgroups.

Table 1: ***Changes in the Haematological data after three months treatment period.***

Sub-group	Hb(g/dL)	PCV (%)	T-WBC	Neutrophil	Lymphocytes
A_0	22.95 ± 0.35	68.00 ± 1.00	16.70 ± 1.40	53.0 ± 4.0	47.0 ± 2.5
A_{VEC}	21.80 ± 2.50	64.00 ± 6.00	17.65 ± 0.35	55.0 ± 5.0	45.0 ± 2.5
B_0	9.25 ± 0.65[a]	31.00 ± 2.00[a]	9.40 ± 0.30[a]	38.5 ± 2.5	61.5 ± 2.5
B_{VEC}	14.20 ± 0.00[a,b]	44.00 ± 0.00[a,b]	8.80 ± 2.40[a]	45.0 ± 2.0	55.0 ± 2.0
C_0	10.80 ± 1.20[a]	35.00 ± 3.00[a]	10.25 ± 0.35[a]	45.5 ± 3.5	54.5 ± 3.5
C_{VEC}	14.55 ± 2.95[a,b]	45.00 ± 8.00[a,b]	8.80 ± 0.60[a]	39.5 ± 1.5	60.5 ± 1.5
D_0	10.80 ± 1.10[a]	43.00 ± 3.00[a]	12.15 ± 1.95[a]	34.5 ± 2.5	65.5 ± 2.5
D_{VEC}	13.45 ± 1.15[a,b]	34.00 ± 3.00[a,b]	12.00 ± 0.80[a]	29.0 ± 3.0	71.00 ± 3.00

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

This study evaluated haematological parameters such as Haemoglobin (Hb), Packed Cell Volume (PCV), Total White Blood Cell (TWBC) count, Neutrophil and Lymphocyte counts of rats induced with different doses of paraquat toxicant and then treated with Vitamin E and C combination.

The result of the study carried out showed that there was a significant decline in the Hb concentration among A_0, B_0, C_0 and D_0 groups. That meant that there was a significant decrease in the Haemoglobin concentration across the groups from A_0 to D_0 indicating that the toxicant (Paraquat) brought about a decline in Hb concentration. The result also showed a similar decline in the PCV value among A_0, B_0, C_0 and D_0 groups. These significant fall in Hb and PCV levels suggest that paraquat has effect on red blood cells; either by way of red cell destruction or interfering with the process of red blood cell production. There was a decrease in PCV, and Hb, observed in other studies when rats were exposed to toxicants (Omoniyi et al., 2002) which conformed to the ones observed in this study. The decrease in the above named parameters suggests that paraquat induction could lead to anaemia in the rats at different concentrations (Seth & Saxena, 2003). According to Sampath et al. (1993), decrease in these blood parameters can be as a result of haemolysis which leads to haemodilution in order to reduce the effect of the toxicant in the tissue system. This can also

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influence the increased rate of the Hb destruction or decrease in its production or synthesis (Reddy *et al*., 1989). Decrease in PCV might be due to the shrinkage of cell size and or decrease in the number of cells (Ahmad *et al*., 1995). The value of Hb in the blood is an indication of the amount of oxygen available to the tissues of the organism, therefore decrease in Hb levels will consequently impair oxygen supply to the various tissues resulting in low energy production and slow metabolic rate (Atamanalp & Yanik, 2003). When this is continued for a long time in the presence of the toxicant, the rats will develop blood dyscrasis and degeneration of erythrocytes.

The Total White Blood Cell (TWBC) Count also showed a significant difference among A_0, B_0, C_0 and D_0 groups although these differences were not regular; either increased or decreased. There was no steady fall or rise in WBC following increasing paraquat dosage. This means that paraquat could significantly alter white blood cell count but increasing the dose may or may not cause further decrease. Contrarily, there was no significant difference in the neutrophil and lymphocyte level among A_0, B_0, C_0 and D_0 subgroups. This suggests that paraquat had no effect on the neutrophil and lymphocyte count in male Wister rat even with toxicity level of 0.06g/kg.

The results also showed that there was a significant increase in the Hb concentration when B_{VEC} group was compared with B_0 group, C_{VEC} group compared with C_0 group and D_{VEC} group was compared with D_0 group respectively. This suggests that the treatment with Vitamin E and C combination brought about an increase in Hb concentration of rats induced with different concentration of paraquat toxicant. The result also showed a significant increase in the PCV value when B_{VEC} was compared with B_0, and when C_{VEC} was compared with C_0. This indicates that Vitamin E and C therapy increases the PCV of rats induced with 0.02g and 0.04g doses of paraquat toxicant. However, there was a significant decrease in PCV of the rats when D_{VEC} group was compared with D_0 group. This suggests that treatment with Vitamin E and C combination brought about a decrease in PCV value of rats induced with 0.06g concentration of paraquat toxicant. There was no significant difference in the TWBC, neutrophil and lymphocyte counts when B_{VEC} was compared with B_0, C_{VEC} Compared with C_0 and D_{VEC} compared with D_0. This indicates that treatment with Vitamin E and C combination has no effect on the TWBC, neutrophil and lymphocyte counts in rats induced with different concentration of toxicant.

The results from this study suggests that Vitamin E and C combination therapy acts to ameliorate the effects of paraquat toxicity on the haematological parameters such as Hb and PCV of male Wister rats by increasing the Hb and PCV values that were previously reduced as a result of the toxicity. However, it had no effect on the TWBC, neutophil and lymphocyte counts. There are limited studies on the ameliorative effects of Vitamin E and C combination on paraquat toxicity on haematological parameters.

Conclusion

Generally, this study has shown that treatment with Vitamin E and C combination did not change haematological outcomes or offer haematological benefits in normal rats (rats not

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exposed to paraquat) but rather, rats with haematotoxicity will benefit more from the therapeutic benefit of vitamin E and C combination therapy.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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