



Haematological Recovery by Vitamin E and C on Paraquat Inflicted Haematological Insult in *Rattus norvegicus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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group "B", "C" and "D". paraquat was induced every fourth night for three months followed by weekly treatment for one month.

Results: 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.

Keywords: Vitamin E and C; paraquat; rat; antioxidant; haematological parameters.

1. 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 [1,2]. A lot of pesticides used to kill weeds have been proven to have an effect on the biosystem [3,4]. 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 [5]. 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 [5, 6]. However, it has been reported that chronic levels of these chemicals can cause changes in behaviour [3], histopathology [4], enzymes and metabolites [7] feeding habits and feed conversion ratio [8] and haematological parameters [1].

Paraquat (1, 1'- dimethyl- 4,4'- bipyridium 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 [9]. It is used globally and can lead to severe, acute and chronic toxicity [9], this is because it readily dissolves and dissociates in water or aqueous solutions [10]. 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 [11]. 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 [12].

Vitamin E (alpha tocopherol) is a fat-soluble vitamin which plays an important role in sustaining healthy metabolism and physiological functions in the body [13]. Because of its antioxidant activity, vitamin E regulates oxidation processes in the body [14]. 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 [15].

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 [16]. 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 [17].

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

2. MATERIALS 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 ± 0.02 kg 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 "Bvec" subgroups; "C" group had "Co" and "Cvec" subgroups; "D" group had "Do" and "Dvec"

"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.

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

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: 2 μ l 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

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

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 Neubauer Counting Chamber), and the white cells present in the four outer large squares of 1mm² 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

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. RESULTS

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 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 B₀, C₀ and D₀, p-value<0.05. But there was no significant difference in WBC, neutrophil and lymphocytes between the subgroups.

- Index (a) = represents a statistically significant difference among inter-groups such as (A₀, B₀, C₀ and D₀) and (Ave, Bve, Cve and Dve)
- Index (b) = represents a statistically significant difference observed within each group (i.e. Group B: B₀Vs B_{VE})

4. 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.

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: $P \leq 0.05$

The result of the study carried out showed that there was a significant decline in the Hb concentration among A₀, B₀, C₀ and D₀ groups. That meant that there was a significant decrease in the Haemoglobin concentration across the groups from A₀ to D₀ indicating that the toxicant (Paraquat) brought about a decline in Hb concentration. The result also showed a similar decline in the PCV value among A₀, B₀, C₀ and D₀ 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 [18] 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 [19]. According to Sampath et al. [20], 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 influence the increased rate of the Hb destruction or decrease in its production or synthesis [21]. Decrease in PCV might be due to the shrinkage of cell size and or decrease in the number of cells [22]. 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 [23]. When this is continued for a long time in the presence of the toxicant, the rats will develop blood dyscrasia and degeneration of erythrocytes [24,25].

The Total White Blood Cell (TWBC) Count also showed a significant difference among A₀, B₀, C₀ and D₀ 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 [26,27]. Contrarily, there was no significant difference in the neutrophil and lymphocyte level among A₀, B₀, C₀ and D₀ 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 B₀ group, C_{VEC} group compared with C₀ group and D_{VEC} group was compared with D₀ 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 B₀, and when C_{VEC} was compared with C₀. 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 D₀ 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 B₀, C_{VEC} Compared with C₀ and D_{VEC} compared with D₀. 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 [28,29]. However, it had no effect on the TWBC, neutrophil and lymphocyte counts. There are limited studies on the ameliorative effects of Vitamin E and C combination on paraquat toxicity on haematological parameters.

5. CONCLUSION

Vitamin E is a fat-soluble vitamin that is essential for maintaining a healthy metabolism and physiological functioning. This research has discovered that treatment with Vitamin E and C combination did not change haematological outcomes or offer haematological benefits in normal rats (rats not exposed to paraquat) but rather, rats with haematotoxicity will benefit more from the therapeutic benefit of vitamin E and C combination therapy.

DISCLAIMER

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sekhar P, Prakash DJ, Lessley K, Sounderraj SF. Haematological changes in freshwater catfish *Mystus vittatus* exposed to sublethal concentrations of monocrotophos. *International Journal of Pharmaceutical and biological Archives*. 2011;2(4):1215-1217.
2. Onwuli D, Ajuru G, Holy B, Fyeface CA. The concentration of lead in periwinkle (*Tympanotonos fuscatus*) and river sediment in Eagle Island River, Port Harcourt, Rivers state, Nigeria. *American Journal of Environmental Protection*. 2014;2(2):37-40
3. da-Cruz AL. Sublethal concentrations of monocrotophos affect aggressive behaviour of the fishes *Astyanax altiparanae* Garutti and Britski (*Teleostei Characidae*) and *Oreochromis niloticus* (Linnaeus) (*Teleostei Cichlidae*). *Revta Braz. Zoology*. 2002;19(4):1131-1138.
4. Johal MS, Sharma ML, Ravneet J. Impact of low dose of organophosphate, monocrotophos on the epithelial cells of gills of *Cyprinus carpio communis* Linn. *Journal of Environmental Biology*. 2007;28(3):663-667.
5. Yuan VC, Chen HC, Yuan YK. Sublethal effects of paraquat and malathion on the fresh water shrimp, *Macrobrachium nipponense*. I, *Acta Zoologica Taiwanica*. 2004;14(2):87-95.
6. Kori-Siakpee O, Adamu K, Achakpokiri JA. Sublethal effects of paraquat on plasma glucose levels and glycogen reserves in liver and muscle tissues of African catfish (*Clarias gariepinus*) under laboratory conditions. *Journal of Fisheries and Aquatic Sciences*. 2007;2(3):242-247.
7. Geneshwade RM. Effect of dimethoate on the level of cholesterol in fresh water *Puntius ticto* (Ham). *Science Research Reporter*. 2012;2(1):26-29.
8. Yaji AJ, Auta J. Sublethal effect of monocrotophos on growth and food utilization of the African catfish *Clarias gariepinus* (Teugels). *Journal of Fisheries International*. 2007;2(2):127-129.
9. Harley TJ. Review of the toxicology of paraquat (1,1- dimethyl-4,4- bipyridinium chloride). *Clinical Toxicology*. 1979;14:1-46.
10. Amdur MO, Doull J, Klaassen CD. *Toxicology the basic science of poisons*. 4th edition, Pergamon Press, New York; 1991.
11. Zeneca K. To boost herbicide plant. *Financial Times*; 1996.
12. Castello PR, Drechsel DA, Patel M. Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain. *The Journal of biological chemistry*. 2007;282:14186–14193.
13. Torquato P, Marinelli R, Bartolini D, Galli F. Vitamin E: nutritional aspects. In: Patel, V. B. ed. *Mol. ecular Nutrition*. Academic Press. 2020;447-48.

14. Sepidarkish M, Akbari-Fakhrabadi M, Daneshzad E, Yavari M, Rezaeinejad M, Morvaridzadeh M, Heshmati J. Effect of omega-3 fatty acid plus vitamin E Co-Supplementation on oxidative stress parameters: A systematic review and meta-analysis. *Clinical Nutrition*. 2019;39(4):1019-1025.
15. Khallouki F, Wyn-Owen R, Akdad M, El-Bouhali B, Silvente-Poirot S, Poirot M. Vitamin E: an overview. In: Patel; 2020.
16. Sato KE, Niki E, Shimasaki A. Free radical mediated oxidation of low-density lipoprotein and its synergistic inhibition by Vitamin C. *Archives of Biochemistry and Biophysics*. 1990;279:402-405.
17. Chakrabarty D, Bhattacharyya A, Mujumdar K, Chatterjee K, Chatterjee S, Sen A, Chatterjee GC. Studies of L-ascorbic acid metabolism in rats under chronic toxicity due to organophorous insecticides. *Journal of Nutrition*. 1976;108:973-980.
18. Omoniyi I, Agbon AO, Sodunke SA. Effect of lethal and sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and haematological changes in *Claris gariepinus* (Burchell). *Journal of Applied Sciences and Environmental Management*. 2002;6(2):37-41.
19. Seth N, Saxena KK. Haematological response in freshwater fish *Cyprinus carpio* exposed to Chlorpyrifos. *International Journal of Integrated Biology*. 2003;3(1):80-83.
20. Sampath K, Velamnia S, Kennedy IJ, James R. Haematological changes and their recovery in *Oreochromis mossambicus* as a function of exposure period and sublethal levels of ekalus. *Acta Hydrobiologica*. 1993;35:73-83.
21. Reddy PM, Bashamohideen M. Fenvalerate and cypermethrin induced changes in haematological parameters of *Cyprinus carpio*. *Acta Hydrochim. Hydrobiologia*. 1989;17:101-107.
22. Ahmad F, Ali SS, Shakoori AR. Sublethal effects of danitol (fenprothrin), a synthetic pyrethroid on freshwater Chinese grass carp, *Ctenopharyngodon idella*. *Biol. (Krakow)*. 1995;43:151-159.
23. Atamanalp M, Yanik T. Alterations in haematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb. *Turkish Journal of Veterinary Animal Science*. 2003;27:1213-1217.
24. Amaefule KU, Obioha FC. Performance and nutrient utilization of starter chicks fed diets containing raw, boiled or dehulled pigeon pea seeds (*Cajanus cajan*). *Nigerian Journal of Animal Production*. 2001;28:31-39.
25. Andersson T, Forlin L, Hardig J, Larsson A. Physiological disturbances in fish living in coastal water polluted with bleached kraft mill effluents. *Canadian Journal of Fisheries and Aquatic Science*. 1988; 45:1525-1535.
26. Elskus AA, Stegeman JJ. Induced cytochrome P-450 in *Fundulus heteroclitus* associated with environmental contamination by polychlorinated biphenyls and polynuclear aromatic hydrocarbons. *Marine Environmental Research*. 1989;2: 31-50.
27. Gabriel UU, Egobueze EC, Edori OS. Haematotoxicity, condition and organ indices of *Heterobranchius bidorsalis* treated with cymbush under laboratory conditions. *Nigerian Journal of Fisheries*. 2011;8(2): 250-258.
28. Jenkins JA. Fish bioindicators of ecosystem condition at the Calcasieu Estuary, Louisiana. National Wetlands Research Center, USGS, Lafayette; 2004.
29. Ramaneswari K, Rao LM. Influence of endosulphan and monocrotophos exposure on the activity of NADPH cytochrome C reductase (NCCR) of *Labeo rohita* (Ham). *Journal of Environmental Biology*. 2008;29(2):183-185.

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