Original Research Article

Synergistic Effect of Vitamin E and C Treatment on *Rattus norvegicus* induced with paraquat

Comment [CC1]: Synergistic Effect of Vitamin E and C Treatment on paraquat induced haemotoxicity in Rattus norvegicus

Abstract

Paraquat is a herbicide, commonly used in the agricultural practices to prevent weed infestation. The Environmental Protection Agency in some countries has placed a restriction on paraquat and there is no identified record of chelating agent or antidote for paraquat. Oxidation of paraquat results in the of superoxides formation. Superoxides generation causes cellular damage. Vitamin C (ascorbic acid) is a water soluble vitamin, with antioxidant potential. It is efficient in clearing free radicals, including hydroxyl radicals, peroxyl radicals and superoxide anion. E is a fat soluble vitamin but considered to be relatively safe when compared to other fat-soluble vitamins Empirical evidence has shown therapeutic efficacy of vitamin E hence its common use in therapeutics. Our goal is to determine the potency of combined effect of vit. E + C on paraquat induced toxicity in male albino rats. As much as The 200 rats were obtained and parted into four main groups of 50 rats each. The groups were A, B C and D. The "A" group was not induced with paraquat; groups "B", "C", and "D" was induced every two weeks with 0.02g, 0.04g, and 0.06g of paraquat per kg of rat respectively for three months. Each of the main groups had subgroups. "A" group had "A_0" and "A_{VEC}" subgroups; "B" group had "B_0" and "B_{VEC}" subgroups; "C" group had "C_0" and "C_{VEC}" subgroups; "D" group had "D_0" and "D_{VEC}" subgroups. "A_0", "B_0", "C_0" and "D_0" subgroups were treated with paraquat only, while "A_{VEC}", "B_{VEC}", "C_{VEC}" and "D_{VEC}" were treated orally with 500mg of vitamin E and 2000mg/l of vitamin C medicated water every week. Comparison of intergroups A_0, B_0, C_0 and D_0 statistically was significant, p-value<0.05 in Hb and PCV. There was no statistical difference with respect to T-WBC, neutrophil, and lymphocytes across the groups. A_{VEC}, B_{VEC}, C_{VEC} and D_{VEC} intergroup ~~comparison statistically was significant~~, p-value<0.05 in Hb and PCV, whereas in comparison with T-WBC, neutrophil, and lymphocytes were not statistically significant among the groups. The finding from this study has shown that a combination of vitamin E and C therapy is potent against paraquat toxicity in male albino rats on one month of weekly treatment and can be used to treat the condition on a weekly basis.

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Keyword: *paraquat, vitamin E, vitamin C, male albino rats, haematological parameters*

1.0 Introduction

The introduction of toxicant in the environment due to human, industrial and agricultural chemicals is a growing concern and especially, a public health concern (Onwuli *et al.*, 2014; Fyneface *et al.*, 2018). Paraquat is a herbicide, commonly used in the agricultural practices to prevent weed infestation. Paraquat is usually applied on trees, between crop rows for the control of broad-leaved and grassy weeds. This hercide chemical is broadly used in developing nations with various effects on exposure. The Environmental Protection Agency in some countries have placed a restriction on paraquat (Le Samao, 2001) and there is no identified record of chelating agent or antidote for paraquat (Dinham, 1996). Oxidation of paraquat results in the of superoxides formation. Superoxides generation causes cellular damage (Benjamin *et al*., 2013). In addition, this prominent herbcide called paraquat, have been confirmed to pose threat to lie

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due to its deleterious reported effects (Debe *et al*., 2007). Over 50% fatality rate has been reported due to ingestion of paraquat (Le Samao, 2001; Hutchinson, *et al*., 1999; Booth, 1998; Tinoco *et. al*., 1993). However, there are lots of unreported cases (Hutchinson, et. al., 1999). Similarly, hundreds of ~~report~~ cases of paraquat ~~occurrence~~ (Wesseling *et. al*., 2001). ~~And~~ apart from records of intentional ingestion there ~~are~~ accidental ingestion that have been observed previously Garnier et. al., (2003)

Vitamin C (ascorbic acid) is a water soluble vitamin, with antioxidant potential. It is efficient in clearing free radicals, including hydroxyl radicals, peroxyl radicals and superoxide anion. The mechanism includes its capacity to as bi-electron reducing agent and it confers protection by releasing an electron consequent to the reduction of free radicals, and the neutralization these compounds in extracellular aqueous environment prior to reaction with biological molecules (Evans & Halliwell, 2001; Carr & Frei, 1999). The antioxidant potential of ascorbic acid is not only attributed to its ability to quench ROS, but also to its ability to regenerate other small molecules antioxidants such as α tocopherol, glutathione and beta-carotene (Evans & Halliwell, 2001).

Historically, Vitamins E is a fat soluble vitamin but considered to be relatively safe when compared to other fat-soluble vitamins (Tappel, 1972). Empirical evidence has shown therapeutic efficacy of vitamin E hence its common use in therapeutics. Vitamin E has proven to have protective effect and used for treatment in some clinical conditions way back like muscular dystrophies and some nervous disease according to some age-long studies (Bicknell, 1940; Muller *et al*., 1983). The usefulness of vitamin E has wide application in health. It includes areas of infertility, oxidative stress induced problems, cardiovascular diseases, organ demage, distortion of the haemopeoitic system, metabolic diseases and more (Keskes-Ammar *et al*., 2003; Lee *et al*., 2005; Engelhard *et al*., 2006; El-Aal *et al*., 2018; Pavithra *et al*. 2018). Also, specific protective effect against haematotoxicity has been reported (Mochegiani *et al*., 2014; Chen *et al*., 2005; Steiner, 1991).

This experimental study investigated the ameliorating synergic effect of vitamins C, and vitamin E on the haematological parameters of paraquat exposed laboratory rats. The study compared the mean difference of vitamins C, and vitamin E combination therapy between treatment experienced and treatment naive laboratory rats. Dearth of data exists in this area.

2.0 Material and Methods

2.1 Experimental Design

The study was a chronic experimental design of biological trial on 200 male albino rats with a mean weight of 0.2±0.02kg. The 200 rats were divided into four main groups of 50 rats each. The groups were A, B C and D. The "A" group was not induced with paraquat; "B" group was induced every two weeks with 0.02g of paraquat per kg of rat for three months; "C" group was induced every two weeks with 0.04g of paraquat per kg of rat for three months; "D" group was induced every two weeks with 0.06g per kg of paraquat for three months. Each of the main

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groups had subgroups. “A” group had “A_0” and “A_{VEC}” subgroups; “B” group had “B_0” and “B_{VEC}” subgroups; “C” group had “C_0” and “C_{VEC}” subgroups; “D” group had “D_0” and “D_{VEC}” subgroups. “A_0”, “B_0”, “C_0” and “D_0” subgroups were not treated with vitamin E + C while “A_{VEC}”, “B_{VEC}”, “C_{VEC}” and “D_{VEC}” were treated orally with 500mg of vitamin E and 2000mg/l of C medicated water every week. However, treatment with Vit E + C commenced after the three months paraquat induction. After one month of weekly treatment with Vit E + C, the rats were sacrificed and their blood samples were analyzed haematological assessment.

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2.2 Source of Experimental Animals

The Animal House, Department of Biology, Rivers State university of Science and Technology provided 200 rats with an average weight of 0.2±0.02kg. Before beginning the trial, the rats were brought to the study site and given two weeks to acclimate. The research was carried out in the Medical Laboratory Science and Technology Department, Rivers State University of Science and Technology.

2.3 Sample Collection method

Through heart puncture, 2mLs of blood were obtained and delivered in simple bottles using a syringe and needle. Haemotological parameters; Hb. PCV, T-WBC, Neutrophils, and Lymphocytes were examined.

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2.4 Sacrifice of the Animals

The animals were sacrificed under 70 percent chloroform anesthesia. The carcasses that were left were burned.

2.5 Methods of Sample Analysis

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

Principle: Ferricyanide oxidizes the iron (II) in haemoglobin to the ferric state, forming methaemoglobin, which is then reduced to cyanmnethaemoglobin by ionised cyanide. This has a red color and is spectrophotometrically measured at 540nm.

2 liters of blood were cleaned into a test tube with 5 mL of Drabkins solution. To ensure complete conversion to cyanmethaemoglobin, the test tube was covered with a rubber bung, inverted multiple times, and left at room temperature for 10 minutes. The absorbance was measured against a blank at 540nm wavelength (5ml of Drabkins reagent only). The absorbance of a recognized standard was compared to the absorbance of test samples. The result is calculated thus:

Absorbance of Test X Standard concentration (mg/dl)

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~~Absorbance of Standard~~

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= The Hb concentration of test (mg/dl)

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

The packed cell volume (PCV), often known as the haematocrit is a percentage measurement of the volume of red cells present in a sample of whole blood.

Capillary action was used to aspirate well-mixed, anticoagulated blood into a microhaematocrit tube, leaving about 15mm empty. Plasticine was used to seal one end of the tube. Using the microhaematocrit centrifuge, the tube was centrifuged for 10 minutes at approximately 12,000g (centrifugal force).

Using a microhaematocrit reader, the PCV was calculated by measuring the height of the red cell column and expressing it as a percentage–ratio of the height of the whole blood column.

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

Changes in the amount and quality of circulating leucocytes characterize a variety of illness states and are frequently diagnostically significant. This could also assist in finding out how the immune system reacts to the alien body (paraquat)

Procedure: The blood was diluted one in twenty (1:20) with 2 percent Glacial Acetic Acid tinted with a few drops of Gentian violet. The diluted sample was mixed and left to stand for 15 minutes to ensure that all red cells were destroyed. The white cells contained in the four outer big squares of 1mm2 areas were counted after a known quantity of the diluted sample was inhaled into the charged chamber (Improved Neubaur Counting Chamber).

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)

With a drop of blood, glass slide and spreader, a thin film was made and stained with leishman stain for differential count of neutrophils and lymphocytes. The count was expressed in percentage.

2.6 Statistical analysis

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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

The comparative efficacy of vitamin E in combination with vitamin C on paraquat induced toxicity in Albino Rats (*Rattus norvegicus*) is shown in Table 1 below. Comparison of intergroups A_0, B_0, C_0 and D_0 statistically was significant, p-value<0.05 in Hb and PCV. There was no statistical difference with respect to T-WBC, neutrophil, and lymphocytes across the groups. A_{VEC}, B_{VEC}, C_{VEC} and D_{VEC} intergroup comparison statistically was significant, p-value<0.05 in Hb and PCV, whereas in comparison with T-WBC, neutrophil, and lymphocytes were not statistically significant among the groups.

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

Table 1: ***Changes in the Haematological data after one month treament period.***

Sub-group	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_{VEC}	21.70 ± 1.86	64.67 ± 5.19	9.70 ± 0.72	37.2 ± 2.6	62.8 ± 2.6
B_0	16.78 ± 2.71[a]	51.17 ± 7.37[a]	10.32 ± 0.69	39.3 ± 1.6	60.7 ± 1.6
B_{VEC}	18.92 ± 1.71[a,b]	57.50 ± 4.33[a,b]	8.65 ± 0.51	40.2 ± 1.9	59.8 ± 1.9
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_{VEC}	19.68 ± 1.26[a,b]	59.00 ± 3.52[a,b]	9.80 ± 0.56	43.7 ± 2.5	56.3 ± 2.5
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_{VEC}	17.43 ± 1.99[a,b]	53.33 ± 5.28[a,b]	8.02 ± 0.87	33.8 ± 2.6	66.2 ± 2.6

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_{VEC}) at each month.

4.0 Discussion

Studies have shown that red blood cells are more sensitive to changes in the antioxidant/pro-oxidant equilibrium due to limited defense system. Paraquat exposure introduces a haematotoxic

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effect and red blood cells might probability be destroyed leading to paraquat induced anaemia with low levels of packed cell volume and decreased concentration of haemoglobin. In addition, ~~hematogen~~ bone marrow is highly affected by paraquat toxicity due to oxidative stress with likely chromosomal aberration in the hematopoietic cells (Tan *et al*., 1994; Pieniążek *et al*., 2004; Prasad *et al*., 2009; Lalruatfela *et al*., 2014)

However, improvements in the packed cell volume and haemoglobin indices were observed after treatment with vitamins C and E combination therapy. Vitamins C and E are known for their antioxidant properties. By implication, the paraquat induced oxidative stress on the red blood cell line was countered through the antioxidant action of two vitamins (C and E) with antioxidant potential effect. This observed effect is associated to this unque property in addition to immune stimulation effect on the myeloid tissue. Also, it could be seen that Vitamins C and E crosses all morpho-physiological barriers, such as; the blood–brain barrier (Koc *et al*., 2002; Singh & Haldar, 2007; Srinivasan *et al*., 2011; Kara *et al*., 2012)

Study findings demonstrated alteration of the erythrocyte cell line parameters specifically packed cell volume and haemoglobin due to exposure to paraquat chemical and further ameiolorating function of vitamins C and E combination. This observed effect of vitamins C and E confers an advantage that protects the erythrocyte cell line for toxicity thereby reducing haemtotoxicity due to paraquat. This antioxidant potential and synergic effect of vitamins C and E was observed by Akinloye et al. (2011). The finding from this study confirms the report of an earlier study by Evans and Halliwell (2001) which reported protective merit of these antioxidants. The study result shows a packed cell volume and haemoglobin that was improved following administration of vitamins C and E combination. This means it is a useful tool to avert anaemia if present by helping the red cells fight off oxidative stress. Oxidative stress is seen as depleted GSH on the surface of the erythrocyte; this predisposes the red cells to oxidative lysis causing aniaemia with low packed cell volume and low haemoglobin (Akinloye et al., 2011). This was averted in paraquat exposed subjects due vitamin C and vitamins E antioxidant combined effect.

Vitamins C and E are known antioxidants which help to combat the menace of free radicals released in the body. The underlying mechanism of action of paraquat is based on formation of free radicals consequent to oxidative stress. This study has supported the report of previous research showing the antioxidant potential of vitamins C and E thereby ameiolorating the altered effect on the haematological indices of erythrocyte population emphatically, packed cell volume and haemoglobin concentration from the empirical findings obtained here.

The outcome of the research on the white blood cell line showed no statistical significant difference as observed in other studies. This study is in disagreement with the work of Akinloye et al. (2011) which reported increased white cell count. The lack of discrepancy in the white cell count and differentials (neutrophils and lymphocyte) is an evidence of null alteration of the immune system with no inflammatory trigger to bring about a visible reaction as noted in other studies.

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Remarkably, the ample pragmatic observations obtained in this study show haematotoxicity of the erythrocyte cell line with an ameiolorating effect of vitamins C and E therapeutic combination in the improvement of the red blood cell parameters specifically increased packed cell volume and high haemoglobin concentration. This was however not observed for white cell line.

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Conclusion

The findings from this study has shown that a combination of vitamin E and C therapy is potent against paraquat toxicity in male albino rats on one month of weekly treatment and can be used to treat the condition on a weekly basis effectively.

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