Original Research Article

Study on the Therapeutic Benefits of Vitamin E after paraquat toxicity

ABSTRACT

The Paraquat (1, 1-dimethyl-4,4-bipyridlium dichloride) is a non-selective nitrogen herbicide that is used for broadleaf control of weed. It has been shown that paraquat is toxic to human and animal. Vitamin E (α-tocopherol) is a lipid soluble antioxidant found in all cellular membranes. It helps in protecting the cell against lipid peroxidation. It has been documented that vitamin E has a protective effect against PQ-induced hematological toxicity in albino rats. This study was carried out to evaluate the hematological effect of Vitamin E therapy on the chronic toxicity of paraquat in Albino rats. A total of 200 male albino rats were used for the study. The 200 rats were divided into four main groups (A, B, C, D) and each group had 50 rats and was further sub-grouped into two, having 25 rats 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 "Ave" which represented the sub-group not treated with Vit E and the subgroup treated with Vit E (500mg) respectively. This design also applied to group "B", "C" and "D". Paraquat induction frequency was done every forth night for three month followed by weekly treatment for three months. The result showed a significant decrease in PCV, Hb, and TWBC count for group in the Ao, Bo, Co and Do at $p<0.05$ but no significant difference in the neutrophil and lymphocyte count. It also showed a significant increase in the Hb, and PCV of subgroups Ave, Bve, Cve and Dve at $p<0.05$ but no significant difference in the TWBC count and differentials. This suggest that Vitamin E supplementation had an ameliorative effect on the PCV and Hb values but no effect on the TWBC count and differentials on paraquat toxicity.

Keyword: *Vitamin E, paraquat, rat, antioxidant, hematological parameters.*

Introduction

The poor regulation of toxicants present in domestic and industrial product has led to increase in toxicant in the environment especially in under-developing and developing countries (Onwuli *et al*., 2014; Fyneface *et al*., 2018). Herbicides or weed killers are phytotoxic chemicals that are used to destroy weeds or reduce their growth. (Gupta, 2018). These herbicides are mostly used in developing countries because, lack of hand weeding labour and also to promote crop production. (Hossain, 2015). Paraquat (PQ) is one of these herbicides which used globally because of its high efficiency, low pollution and low residues in crops (Ren *et al.*, 2014). The paraquat (1, 1-dimethyl-4,4-bipyridlium dichloride) is a non-selective nitrogen herbicide; that is used for broadleaf control of weed (Guo *et al.*, 2015). Globally, it ranked as the second highest selling herbicide that is available at the rate of 20 percent solution form (Banday *et al.*, 2013). To human and animal, paraquat is highly toxic (Suntres, 2002), its toxicity can lead to Acute Respiratory Distress Syndrome (ARDS)

(Huang *et al.*, 2005). The mechanisms of Paraquat toxicity is yet to be understood fully, but it is presumed that the toxicity is as a result of Reactive Oxygen Specie (ROS) generated through redox-cycling process, which consequently lead to oxidative-stress damage to cellular organelles, proteins, nucleic acids and lipids (Adam *et al.*, 1990; Bonneh-Barkey *et al.*, 2005 & Castello *et al.*, 2007). When paraquat toxicity becomes severe; it can lead to multiple organ failure especially lungs, kidneys and liver (Tavakol *et al.*, 2015).

PQ gain access into the body and is excreted in the form of a prototype in the kidney, where the concentration is highest, which eventually leads to altered kidney function. Since paraquat cannot be excreted normally, it further accumulates in the body. Thus, involving other organs such as the liver, heart and lung, which eventually develops the symptoms of multiple organ failure (Cochemé *et al*., 2008). Creatinine is a product derived from the constant degradation of creatine, large amounts of creatinine get accumulated once kidney function impairs; it ends up producing 1-methylhydantoin and 5-hydroxy-1-methylhydantoin (HMH) (Ienaga *et al*., 2011).

Although the mechanism of paraquat toxicity is still not explained thoroughly. It has shown widely that PQ-induced toxicity as a result of prolonged redox-cycling and generation of reactive oxygen species (ROS) which consequently induce inflammation and oxidative stress (Castello *et al*., 2007). An increase in leukocytes and neutrophil counts, and a marked decline in lymphocyte counts, has observed from several studies when the complete blood count (CBC) during acute inflammatory response due to oxidative stress is analyzed. (Alonso *et al*., 2002). Recently, neutrophil-to-lymphocyte ratio (NLR) has also been shown to be a viable inflammatory marker. NLR has shown to be a very sensitive inflammatory and prognostic indicator in many diseases including sepsis, stroke, cardiac disorders, and cancer etc. (Zahorec, 2001). There is a similar inflammatory response in paraquat toxicity and NLR, therefore the NLR may also be used as prognostic indicator to predict mortality in patients with PQ poisoning. It alterations and changes in the chemical composition and hematological indices were used to provide diagnosis, and the type of toxicants and degree of pollution present in the body (Akil *et al*., 2014).

Vitamin E (α-tocopherol) is a lipid soluble antioxidant found in all cellular membranes. It helps in protecting the cell against lipid peroxidation (Machlin, 1980). One of its functions is to act as a chain-breaking antioxidant; it carries out this function by preventing chain formation and propagation of free radicals which causes lipid peroxidation in cellular membrane (Kamal-Eldin & Appelqvist, 1996). It also influences the cellular response by modifying oxidative stress and signal-transduction pathway (Azzi *et al.*, 1992). Also, vitamin E functions as a membrane stabilizer (Truber & Packer, 1995, Clarke *et al*., 2008). It has the ability to neutralize free radicals, that destroy cellular molecules, and it is also capable of preserving the integrity of renal tubules. In addition, it is an anti-toxin agent (Traber & Atkinson, 2007). Vitamin E has been studied widely because of its reported hepatoprotective effect in animals, because of its ability to attenuate the induced oxidative stress in various tissues by reducing Malondialdehyde (MDA) levels, thereby restoring the levels of Glutathione (GSH), and Superoxide dismutase (SOD), and the recovery of impaired hepatic cells (Bharrhan *et al*., 2010). It has been documented that vitamin E has a protective effect against PQ-induced hematological and biochemical toxicity in albino rats (Ambali *et al*., 2010). It must be noted that literature on hematological effect of Vitamin E therapy on the chronic toxicity of paraquat in albino rats is rare; therefore it is imperative to carry out study on this subject.

Material and Methods

Study Area/Population

The study was carried out in the medical laboratory, Department of Science in the Rivers State University. This study was a biological trial with Albino rats which 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 albino rats with a mean weight of 0.2±0.02kg 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.

Grouping and Treatment of Animals

Two hundred (200) male Albino Rats were used for this research and were divided according to their body weight 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 were induced every two weeks with 0.02g of paraquat per kg of rat for three months.

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

Group D: This group were 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 “Bve” subgroups; “B” group had “Bo” and “Bve” subgroups; “C” group had “Co” and “Cve” subgroups; “D” group had “Do” and “Dve”

“Ao”, “Bo”, “Co” and “Do” subgroups: were not treated with vitamin E

“Ave”, “Bve”, “Cve” and “Dve” subgroups were treated orally with 500mg of vitamin E every week for three months.

After three months of weekly treatment with Vit E, the rats were sacrificed and their blood samples were analyzed for hematological study.

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 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.

Sample Collection

The blood samples were collected via cardiac puncture from the animals and sacrificed under 70% chloroform anesthesia into the Ethylenediamine tetra acetic acid (EDTA) specimen bottle and used for analysis of hematological parameters.

Laboratory analysis

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

Principle: Iron (II) of the haem in hemoglobin is oxidized to the ferric state by ferricyanide to form methaemoglobin which reduced to cyanmnethaemoglobin by ionized cyanide. This is red in color and measured spectrophotometrically at 540 nm.

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 allowed to stand at room temperature for 10 min. This is to ensure complete conversion to cyanmethaemoglobin. The absorbance was read at 540 nm wavelength against a blank (5ml of Drabkins reagent only). The absorbance of known standard was read alongside those of the test samples. The result was calculated by this formula:

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

= 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 15 mm 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.

After preparation films 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 that, the films were examined under the microscope with an oil immersion magnification, and the cells counted and identified as Neutrophils and Lymphocytes rated in percentage of 100 Leucocyte.

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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.

Result

Table 1 shows the comparative effects of vitamin E therapy on the Chronic Toxicity of Paraquat in Albino Rats (*Rattusnorvegicus*). The results show that, there was a significant difference in PCV, Hb and WBC levels in rats among A_0, B_0, C_0 and D_0 groups, and Ave, Bve, Cve and Dve, p-value<0.05. There was no significant difference in neutrophil and lymphocytes. Intra-group comparison showed there was significant difference in Hb and PCV levels between subgroups in the same group, p-value<0.05 except A_0 and Ave, p-value>0.05.

Table 1: ***Changes in the Hematological 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_{VE}	21.75 ± 1.15	64.50 ± 3.50	17.75 ± 2.85	35.0 ± 3.0	65.0 ± 3.7
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
BE	10.10 ± 1.10^{a,b}	33.00 ± 3.00^{a,b}	9.90 ± 0.30^{a}	37.5 ± 2.5	62.5 ± 2.5
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_{VE}	11.80 ± 0.20^{a,b}	37.50 ± 0.50^{a,b}	8.40 ± 0.40^{a}	34.5 ±3.5	65.5 ± 3.5
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_{VE}	12.75 ± 0.75^{a,b}	40.00 ± 2.00^{a,b}	13.40 ± 1.50^{a}	29.5 ± 0.5	70.5 ± 0.5

Statistical significance: P ≤ 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.

Discussion

The result of this study showed that, there was a significant decline in the Hb and PCV levels among A0, B0, C0 and D0 groups. This indicates that the toxicant brought a decline in Hb levels as the dosage of toxicant increased. The impact on haematological parameters was dose-dependent of paraquat. This result suggests that toxicity increases anemic tendencies in male albino over chronic period of paraquat exposure. The changes in the mean concentration of Hb could be caused by free radicals, induced damage in accordance with erythrocyte membrane and this agrees with a study by Sato *et al*., (1995). The result of this study is in agreement with an earlier study carried out by Lalruatfel *et al*., (2012). Similar trends were also found in PCV levels among the groups mentioned above. There was a significant decrease in PCV level among A0, B0, C0 and D0 group following increase in paraquat dosage. The PCV level in the rats were decreased by way of hemolysis from the lipid peroxidation due to the production of reactive oxygen species caused by the toxicity of paraquat and in accordance with the previous studies by Lalruatfel *et al*., (2012) The findings of this study demonstrate that paraquat exposure results in substantial hematological changes in the adult male albino rats. All stated alterations revealed that, the exposed albino suffered from anemia caused by the herbicide paraquat. This is shown as an indication of the toxic effects of paraquat on tissues responsible for the production of erythrocytes as well as the viability of the cells (Patnaik & Patra, 2006).

The Total White Blood Cell (TWBC) count also showed a significant decrease when the B_0, C_0 and D_0 groups were compared with the A_0 group. As the concentration of the toxicant increased, the TWBC count increased from B_0 to D_0 group. This indicates that the toxicant brought about a significant decrease in the TWBC count. The result further showed that there was no significant difference in the neutrophil and lymphocyte counts when B_0, C_0 and D_0 were compared with A_0. It suggests that toxicity had no effect on the neutrophil and lymphocyte count of male albino rats. The changes in the TWBC count agrees with the view of Olson *et al*., (2000) who proposed that the toxic effect of paraquat on leucopoiesis will lead to decrease TWBC count.

The results of the study also showed that, there was no significant difference when A_{VE} group was compared with A_0 group indicating that the treatment with Vitamin E had no effect on the Hb concentration of male rats not induced with toxicant. The results also showed that there was a significant increase in the Hb concentration when B_{VE} group was compared with B_0 group. This suggests that the treatment with Vitamin E brought an increase in Hb concentration of rats induced with 0.02 tparaquat. This indicates that Vitamin E increases the Hb concentration of rats induced with 0.02 paraquat. The result also showed an increase in the Hb concentration when the C_{VE} group was compared with C_0 group and D_{VE} compared with D_0 group. It suggests that Vitamin E therapy increases the Hb concentration of rats induced with 0.04g and 0.06g paraquat respectively thereby acting as an ameliorative agent against paraquat toxicity. The result also showed a significant increase in the PCV value when B_{VE} was compared with B_0, and when C_{VE} was compared with C_0. This indicates that the Vitamin E therapy increases PCV of rats induced with 0.02 and 0.04 doses of paraquat toxicity. However, there was a significant decrease in PCV of the rats when D_{VE} group was compared with D_0 group. It means that treatment with Vitamin E brings about a decrease in PCV value in rats induced with 0.06 concentration of paraquat toxicant. There was no significant difference in the TWBC, neutrophil and lymphocyte counts when B_{VE} was compared with $B_{0,}$ C_{VE} was Compared with C_0 and D_{VE} was compared with D_0 respectively at $p<0.05$. This indicates that treatment with Vitamin E has no effect on the TWBC, neutrophil and lymphocyte counts of rats induced with different concentration of toxicant. The result from this study suggests that Vitamin E therapy acts to ameliorate the effects of paraquat toxicity on the haematological parameters such as Hb and PCV of male albino rats by increasing the Hb and PCV values that were previously reduced as a result of paraquat toxicity. However, it had no effect on the TWBC, neutrophil and lymphocyte counts. There is dearth literature on the ameliorative effects of Vitamin E on paraquat toxicity on haematological parameters, therefore more studies in this area would be necessary.

Conclusion

It can be drawn from this study that the Vitamin E therapy can ameliorate the effects of paraquat toxicity on the haematological parameters of anaemic indices, such as Hb and PCV on male albino rats by increasing the Hb and PCV values that were previously reduced as a result of the toxicity.

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