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Research Article

Study on Antidotal Therapy of Vitamins in Paraquat Induced Dyslipidemia in Rats

Benjamin Nnamdi Okolonkwo[1]*, Maureen Ibitoroko George-Opuda[2], Doris Nnenna Ajibo[3], Clement Ugochukwu Nyenke[1], Kingsley Kalawari Odiabara[4]

[1] Department of Medical Laboratory Science, PAMO University of Medical Sciences, Rivers State, Nigeria.
[2] Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
[3] Department of Experimental Pharmacology and Toxicology, University of Port Harcourt, Port Harcourt, Nigeria
[4] Medical Laboratory Services Directorate, Federal Ministry of Health, Abuja, Nigeria.

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Corresponding author:
B N Okolonkwo
bokolonkwo@pums.edu.ng

Abstract: Paraquat (PQ) is a toxic chemical substance used in farming for weed management. It is an herbicide with harmful effect when exposed to animals. This study aimed at assessing the effect of vitamin E and C on fasting lipid profile in paraquat induced toxicity in rats. The experimental study consisted of 180 rats divided equally into 4 groups (A, B, C and D) with each group having 3 subgroups of 15 rats (0, VE and VEC). A group served as the control group while B, C, D were test groups. B0, C0 and D0 were treated with 0.02g, 0.04g and 0.06g of paraquat respectively twice in two weeks for 3 months. A0 had no treatment. BVE, CVE and DVE were similarly treated in increasing doses of paraquat and in addition were treated with 500mg of vitamin E once every week for 3 months. AVE had only vitamin E treatment. BVEC, CVEC and DVEC were similarly treated in increasing doses of paraquat and in addition were treated with both 500mg of vitamin E and 2000mg/dl of vitamin C once every week for 3 months. AVEC had only vitamin E and vitamin C treatments. All treatments were done orally. B0, C0 and D0 subgroups were sacrificed and sample collected via cardiac puncture for fasting lipid profile (FLP) after 3 months of paraquat treatment. A0 was also sacrificed too alongside. Other subgroups; AVE, AVEC, BVE, BVEC, CVE, CVEC, DVE and DVEC were sacrificed and sample analyzed for FLP on monthly basis for 3 months. The results showed that paraquat caused a significant drop in FLP levels from the second month after paraquat treatment and this was ameliorated by the treatment of vitamin E and C treatment. This study has therefore demonstrated that vitamins could restore lipid disorder initiated by paraquat poisoning in rats.

Keywords: lipid, paraquat, rat, vitamins.

1. Introduction

Studies on toxicology of environmental pollutants on human health have gained compelling interest with rising cases of cancer, pesticide production and improper waste disposal, exploitation of environmental resources followed with poor environmental

protection or safety policy and implementation [1-4]. According the Centre for Disease Control and Prevention (CDC) paraquat (PQ) is a toxic herbicide used in crop farming to control weeds [5]. In the United States, it is only used by licensed individuals and as such it is categorized as "restricted used chemical" because of the potential toxicity it poses on lives. However, paraquat still remains one of the leading herbicide in use worldwide by farmers. The main route of paraquat exposure that causes paraquat poisoning is via ingestion; that is, orally taken. However, exposure to skin has also been reported and becomes severe especially when the skin is not intact such as when there exists sores or cuts on the skin. This skin injury creates easy penetration of the toxicant to the circulation. On the other hand, if paraquat is inhaled, it could cause lung damage especially in cases of high dose of exposure and duration of the exposure [5].

The severity of paraquat toxicity is implicated in the dose of exposure and duration of exposure such that people with decreased exposure time and dose will have mild effect compared to those with heavy dose of exposure and exposure frequency [6]. Some studies have reported that paraquat poisoning could lead to the following organ failure; heart, kidney, liver and lungs [6,7]. According to the CDC report in April 4, 2018, there exists no proven antidote for paraquat poisoning [5]. Hence, this study is focused on assessing the effect of paraquat on lipid parameters of various levels of paraquat exposure and the treatment effect of vitamins E and C in rats.

2. Materials and Method

Study Design

The experimental study consisted of 180 rats weighing an average of 200g ± 20g which were grouped and treated accordingly as stated in the table below:

Table 1. Rat grouping

Main groups (45 rats per group)	Sub-groups (15 rats per sub-group)
A	A_0 = without any treatment A_{VE} = treated only with 500mg of vitamin E weekly for 3 months A_{VEC} = treated with both 500mg of vitamin E and 2000mg/dl vitamin C weekly for 3 months
B	B_0 = treated with 0.02g of paraquat every 2 weeks for 3 months B_{VE} = treated with 0.02g of paraquat every 2 weeks for 3 months and then treated with 500mg of vitamin E weekly for 3months B_{VEC} = treated with 0.02g of paraquat every 2 weeks for 3 months and then treated with both 500mg of vitamin E and 2000mg/dl vitamin C weekly for 3 months
C	C_0 = treated with 0.04g of paraquat every 2 weeks for 3 months C_{VE} = treated with 0.04g of paraquat every 2 weeks for 3 months and then treated with 500mg of vitamin E weekly

	C_{VEC} = treated with 0.04g of paraquat every 2 weeks for 3 months and then treated with both 500mg of vitamin E and 2000mg/dl vitamin C weekly for 3 months
D	D_0 = treated with 0.06g of paraquat every 2 weeks for 3 months D_{VE} = treated with 0.06g of paraquat every 2 weeks for 3 months and then treated with 500mg of vitamin E weekly D_{VEC} = treated with 0.06g of paraquat every 2 weeks for 3 months and then treated with both 500mg of vitamin E and 2000mg/dl vitamin C weekly for 3 months

The rats were obtained from Animal House, Department of Biology, Rivers State University and transported to the Department of Chemical Pathology where the study was conducted. The rats were allowed to acclimatize for 2 weeks prior to the commencement of the study.

Paraquat treatment was administered via oral gavage route on the test groups according to the dose-dependent group classification as indicated on the table above. Vitamins E and C were also administered orally [6,7].

In the A group, after 3 months, the rats were anesthetized using chloroform and then sacrificed. The blood was collected via cardiac puncture and assayed for fasting lipid profile (FLP).

In the B group, subgroup B0 was anesthetized and sacrificed after paraquat intoxication but in the vitamin treatment subgroups, a month after treatment, 5 rats were anesthetized and sacrificed from each subgroup and samples collected were analyzed for FLP. On the second month of vitamin, another 5 rats were anesthetized from each subgroup and sample analyzed for FLP. Similarly, on the third month of treatment, the last 5 rats were anesthetized from each subgroup and samples were analyzed for FLP as well.

Laboratory analysis of FLP include total cholesterol, triglyceride, HDL and LDL. The methods used for the determination of FLP parameters are methods described by Oladapo-Akinfolarin et al. [8].

Statistical analysis.

The obtained data were analyzed descriptively as mean ± SD and inferential statistics were analyzed as Two-Way ANOVA using SPSS version 23.0. The test was considered significant at p-value < 0.05.

3. Results

Table 2 shows the effect of paraquat on lipid parameters and the effect of vitamins C and E single and combined treatment in restoring lipid levels in the first month of treatment. The results showed in the first month that there was no significant effect of paraquat on lipid levels except in LDL subgroups BVE and BVEC, however, there were significant changes in lipid levels after administration of vitamins E and EC in the test groups.

Table 2. Changes in Lipid biochemical data after one-month treatment period.

Sub-groups	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-Cholesterol (mmol/L)	LDL-Cholesterol (mmol/L)
A_0	4.27 ± 0.03	2.16 ± 0.02	2.10 ± 0.02	1.20 ± 0.01
A_{VE}	3.27 ± 0.05	1.69 ± 0.03	1.60 ± 0.04	0.82 ± 0.02
A_{VEC}	3.72 ± 0.05	2.08 ± 0.04	1.68 ± 0.03	1.10 ± 0.02
B_0	4.66 ± 0.02	1.70 ± 0.03	2.21 ± 0.02	1.69 ± 0.01
BE	4.00 ± 0.03[b]	1.58 ± 0.03[b]	2.44 ± 0.04	0.85 ± 0.02[a]
B_{VEC}	3.27 ± 0.06[b]	1.93 ± 0.05[b]	1.42 ± 0.03[b]	0.97 ± 0.02[a]
C_0	3.63 ± 0.02	1.63 ± 0.04	1.34 ± 0.01	1.55 ± 0.01
C_{VE}	3.94 ± 0.05[b]	1.94 ± 0.03[b]	1.45 ± 0.02[b]	1.61 ± 0.03
C_{VEC}	3.77 ± 0.05[b]	1.57 ± 0.02[b]	1.51 ± 0.03[b]	1.55 ± 0.02
D_0	2.37 ± 0.03	1.04 ± 0.01	0.70 ± 0.02	1.20 ± 0.01
D_{VE}	3.28 ± 0.05[b]	1.33 ± 0.03[b]	1.44 ± 0.04[b]	1.24 ± 0.01
D_{VEC}	3.54 ± 0.05[b]	1.69 ± 0.03[b]	1.32 ± 0.03[b]	1.46 ± 0.02

a = means significant difference between the test group and the control group
b = means significant difference within the group

Table 3 shows the effect of paraquat on lipid parameters and the effect of vitamins C and E single and combined treatment in restoring lipid levels in the second month of treatment. The results showed that in the second month there were significant drops in lipid levels after paraquat poisoning and there were significant improvements in lipid levels after administration of vitamins E and EC in the test groups.

Table 3. Changes in Lipid biochemical data after two months' treatment period.

Sub-groups	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-Cholesterol (mmol/L)	LDL-Cholesterol (mmol/L)
A_0	5.33 ± 0.05	2.73 ± 0.04	2.23 ± 0.03	1.87 ± 0.01
A_{VE}	3.44 ± 0.02	1.92 ± 0.03	1.48 ± 0.01	1.09 ± 0.11
A_{VEC}	5.18 ± 0.16	3.51 ± 0.14	2.17 ± 0.06	1.42 ± 0.04
B_0	1.65 ± 0.05[a]	0.61 ± 0.03[a]	0.57 ± 0.02[a]	0.81 ± 0.02[a]
BE	2.44 ± 0.10[a,b]	1.61 ± 0.08[a,b]	1.01 ± 0.05[a,b]	0.70 ± 0.02[a,b]
B_{VEC}	1.63 ± 0.03[a,b]	1.21 ± 0.02[a,b]	0.40 ± 0.01[a,b]	0.68 ± 0.01[a,b]

C_0	2.51 ± 0.03[a]	0.86 ± 0.02[a]	1.06 ± 0.02[a]	1.06 ± 0.01[a]
C_{VE}	1.84 ± 0.05[a,a]	0.90 ± 0.03[a,b]	0.70 ± 0.02[a,b]	0.72 ± 0.02[a,b]
C_{VEC}	2.35 ± 0.04[a,b]	1.48 ± 0.03[a,b]	0.92 ± 0.03[a,b]	0.67 ± 0.02[a,b]
D_0	0.65 ± 0.04[a]	0.29 ± 0.01[a]	0.14 ± 0.01[a]	0.37 ± 0.02[a]
D_{VE}	0.88 ± 0.03[a,b]	0.59 ± 0.03[a,b]	0.19 ± 0.01[a,b]	0.42 ± 0.01[a,b]
D_{VEC}	1.73 ± 0.04[a,b]	1.14 ± 0.03[a,b]	0.68 ± 0.02[a,b]	0.60 ± 0.01[a,b]

a = means significant difference between the test group and the control group
b = means significant difference within the group

Table 4 shows the effect of paraquat on lipid parameters and the effect of vitamins C and E single and combined treatment in restoring lipid levels in the third month of treatment. The results showed that in the third month there were significant drops in lipid levels after paraquat poisoning and there were significant improvements in lipid levels after administration of vitamins E and EC in the test groups.

Table 4. Changes in Lipid biochemical data after three months' treatment period.

Sub-group	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-Cholesterol (mmol/L)	LDL-Cholesterol (mmol/L)
A_0	5.58 ± 0.14	3.84 ± 0.02	2.52 ± 0.05	1.32 ± 0.01
A_{VE}	6.75 ± 0.10	4.04 ± 0.09	3.24 ± 0.07	2.34 ± 0.06
A_{VEC}	5.99 ± 0.11	4.01 ± 0.18	2.43 ± 0.03	1.74 ± 0.01
B_0	3.31 ± 0.01[a]	1.32 ± 0.02[a]	1.00 ± 0.03[a]	1.72 ± 0.02[a]
BE	2.83 ± 0.01[a,b]	1.59 ± 0.01[a,b]	1.54 ± 0.03[a,b]	0.58 ± 0.03[a,b]
B_{VEC}	3.72 ± 0.07[a,b]	1.91 ± 0.01[a,b]	1.97 ± 0.06[a,b]	0.88 ± 0.00[a,b]
C_0	1.79 ± 0.03[a]	1.00 ± 0.04[a]	0.57 ± 0.01[a]	0.77 ± 0.01[a]
C_{VE}	1.74 ± 0.01[a,b]	0.91 ± 0.02[a,b]	1.00 ± 0.02[a,b]	0.32 ± 0.02[a,b]
C_{VEC}	1.97 ± 0.02[,ba]	1.64 ± 0.02[a,b]	1.10 ± 0.03[a,b]	0.18 ± 0.01[a,b]
D_0	1.90 ± 0.03[a]	0.72 ± 0.03[a]	0.40 ± 0.01[a]	1.17 ± 0.02[a]
D_{VE}	1.20 ± 0.00[a,b]	0.97 ± 0.00[a,b]	0.44 ± 0.01[a,b]	0.33 ± 0.00[a,b]
D_{VEC}	2.09 ± 0.03[a,b]	1.31 ± 0.06[a,b]	1.01 ± 0.00[a,b]	0.48 ± 0.01[a,b]

a = means significant difference between the test group and the control group
b = means significant difference within the group

4. Discussion

The changes observed in the lipids of rats treated with PQ were quite pronounced. PQ toxicity produced no changes in the mean values of total cholesterol and triglycerides

(TG) at month 1, but by month 2, a dose dependent reduction in the mean values of total cholesterol and TG of PQ treated rats occurred ($P < 0.05$), and the reductions were sustained more at month 3 ($P < 0.05$). The High-density lipoproteins (HDL) fraction had mean values that were dose and exposure time significantly reduced at months 2 and 3. Low density lipoproteins (LDL) fraction differed from the others in that PQ toxicity raised the mean values of LDL at month 1 and later reversed to lower the mean values significantly at month 2.

Lipid's concentrations, especially cholesterol, are determined by metabolic functions which are influenced by the integrity of vital organs such as liver and kidney [9], once the functional integrity of these vital organs is compromised, the tendency is that most of the macronutrients and micronutrients will be lost. El-Hennawy, *et al*. [10] reported that administration of low dose of some herbicidal agents on rats resulted in a decrease of serum cholesterol probably as a hepatotoxicity effect. Similar results were also seen in rabbits [11]. Also, Shakoori, *et al*. (1988) and Saleh (1990) [12,13] have shown that different insecticides and herbicides produced decreases cholesterol content. Thus, it appears that PQ administration caused disturbances in lipid metabolism as a result of hepatic and renal dysfunctions. These results differed with that of other researchers who stated that a significant increase in total cholesterol and LDL cholesterol levels that were observed in PQ treated group compared to other groups could predispose an animal to the risk of coronary heart disease. Also, the work of Wershana (2001) [14] differed with the results, when he demonstrated that PQ administration resulted in significant hyperlipidaemia and hypertriglyceridaemia. He emphasized that hyperlipidaemia is a complication arising from parenchymal liver disease and nephrotic syndrome. The primary mechanism of hyperlipidaemia appears to be increased hepatic synthesis with reduced catabolism of lipids [15]. But in PQ toxicity, the reverse is the case; the primary mechanism of hypolipidaemia appears to be increased hepatocellular damage and leakage, increased catabolism of lipids, and increased renal loss of macro- and micro-nutrients [10], giving support to the finding of this present study. The administration of antioxidant, vitamins C and E alone or in combination, to the PQ treated rats reversed most of these findings. It raised the levels of total cholesterol at month 2 ($P < 0.05$), TG at months 2 and 3 ($P < 0.05$) and HDL at months 2 and 3 ($P < 0.05$), but it did not have any significant change on the LDL values and the values of total cholesterol, TG and HDL at month 1. In support of these findings, Regoli and Winstom (1999) singled out vitamin C in its effectiveness to protect lipids from peroxidative damage and have shown that it is a potential scavenger of ROS [16]. Wershana [14] also presented data in support which revealed that the co-administration of selenium (Se) with PQ succeeded partially in preventing the disturbances in the number of blood platelets, transaminases activities, total lipids and TG, cholesterol and Fe^{3+} concentrations. The improvements of lipid parameters of PQ oxidatively stressed animals by vitamin E were reported by authors [17-20]. This works also found out that the antioxidant of most affect is the combined (E + vitamin C) treatments and it gained support from the works of other researchers [21-23].

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