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THE ANTIOXIDANT EFFECTS OF VITAMIN C ON LIVER ENZYME (SGPT) ACTIVITY IN RATS UNDER PARAQUAT TOXICITY

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Abstract
Recent studies on exposure assessment and health effects have shown paraquat (PQ) to have serious deleterious effects on certain organs. Activities are now geared towards enhancing the antioxidant system to counter the destructive effects of reactive oxygen species (ROS) initiated by paraquat in the body. This study, therefore, is aimed at understudying vitamin C (antioxidant) ability to quench the ROS effects of paraquat on cells, using the enzyme SGPT as an index. A 2mls sub-lethal dose of the toxicant PQ was administered intraperitoneally (ip) to the animals, once a month, over a period of three months. The test subgroups (A_2, B_2, C_2, and D_2) were maintained with vitamin C solution (200mg/l) and the other subgroups (A_1, B_1, C_1 and D_1) were maintained with water during study period. Four animals per subgroup at the end of each month were decapitated and blood samples taken for SGPT enzyme activity determination. The results obtained indicated that the test subgroups without vitamin C administration had a very-significant elevation of SGPT activities as against the subgroups with vitamin C administration, from month 1 ($P \leq 0.01$) to month 3 ($P \leq 0.001$). The control subgroup on vitamin C maintenance (A_2) had a non-significant elevation of SGPT enzyme activities when compared to the subgroup without vitamin C (A_1) all through the study period. The between groups/subgroups comparison showed a dose and time dependent elevations in enzyme activities. The within groups/subgroups indicated that vitamin C treated rats had less elevated enzyme activities when compared to the rats treated with paraquat without vitamin C. With these results it could be concluded that vitamin C when administered at moderate doses and maintained for a long duration could be used as a life saving medication in patient under toxic insult.

Key words: Paraquat, Vitamin c, SGPT, Reactive oxygen species.

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1. INTRODUCTION

Paraquat is one of the most used pesticides globally. It is a total contact herbicide, applied around trees in orchards and between crop rows to control broad-leaved and grassy weeds (WHO, 1984). Paraquat kills all green tissues, but does not harm mature bark. It is use on plantation crops (Banana, Cocoa-palm, Coffee, Oil-palm and Rubber); Citrus plants (Oranges, Apples, Plums, Vines and Tea) and on certain crops (Potatoes, Pineapples, sugarcanes and sunflowers). It is also used as a desiccant and as a cotton defoliant (WHO/FAO, 1978). Outcropped land on industrial sites, railways, roadsides, etc could be cleared off weed by applying high concentrations of paraquat (WHO, 1984).

Recent studies on exposure assessment and health effects have shown that paraquat has serious deleterious effects on certain organs (Lungs, Kidney, Skin, Liver, Brain, etc). These effects are initiated by paraquat ability to produce Reactive Oxygen Species (ROS) through a process called Redox cycling (Punchard, *et al.*, 1996; Sies, 1997; and Thor, *et al.*, 1982). These superoxides formed overwhelm the electron transport system, resulting in damage to cellular components (Punchard, *et al.*, 1996).

Centered on this, activities are geared towards studies on how to enhance the antioxidant system in the body, so that it can counter the destructive effect of ROS initiated by PQ. This study, therefore, is aimed at understanding one of the potent antioxidant (Vitamin C) ability to quench the ROS effects

on cells. Through its ability to improve the activity of the liver enzyme used as an index of assessment – Serum Glutamate Pyruvate Transaminase (SGPT).

2. MATERIALS AND METHODS

2.1 Materials

RATS: 96 male rats, obtained from the animal house of the Department of Pharmacology and Toxicology, College of Health Sciences, University of Port Harcourt, Choba – Rivers State.

SOURCE OF PARAQUAT: The paraquat used was purchased as a liter volume of 20% w/v solution with the trade name Dizmazone, from Dizengoff W. A. Ltd. Sealed in an opaque plastic container. It was kept at room temperature and during use, proper caution were taken to avoid spillage, fire or poisoning.

SOURCE OF VITAMIN C: Four bottles of Mason Natural® pure vitamin C (1000mg) caplet were used for the research.

2.2 Method

A 2mls sub – lethal dose of the toxicant, Paraquat (PQ) was intraperitoneally (ip) administered to the animals, once a month, over a period of three months (this was in simulation of contamination from polluted feed, water or air), while the control animals received 2mls of Normal Saline (0.95%) likewise.

This study was conducted under four groups (A – D) and eight subgroups (A_1, A_2, B_1, B_2, C_1, C_2, D_1 and D_2). All the subgroups had 12 animals with A_1 and A_2 being the control subgroups that received no paraquat.

A_2, B_2, C_2 and D_2 were the subgroups that were made to drink from a water-can containing vitamin C solution (200mg/l) with straw. On emptying, the water-cans were refilled regularly. A_1, B_1, C_1 and D_1 were the subgroups without vitamin C. They were made to drink from water-cans which contained only water.

On monthly intervals, 10mls blood samples were collected from four animals per subgroup and allowed to clot. The clotted samples were separated using centrifugal force and the sera dispensed into plastic sample containers, labeled accordingly and stored frozen, awaiting SGPT enzyme assay later using Kodac Autoanalyser machine.

All through the 3 months study period the animals were fed with pelletized finisher feeds, which contained enough nutrients required for healthy growth and development.

Using the study pattern below, the results were as shown in the tables and figures below.

Procedure for the enzyme (SGPT/ALT) estimation, were based on the principles of the colorimetric method by Reitman and Frankel (1957). The enzymatic activities of different subgroups were as shown below.

Table 1: Treatment pattern for the groups/subgroups

GROUP	SUB-GROUP	METHOD
A Control group given 0.0g/kg PQ	**A_1**	Subsequently received water and feed
	A_2	Subsequently received Vit. C solution (200 mg/l).
B Test group given 0.02g/kg PQ	**B_1**	Subsequently received water and feed
	B_2	Subsequently received Vit. C solution (200mg/l) and feed
C Test group given 0.04g/kg PQ	**C_1**	Subsequently received water and feed,
	C_2	Subsequently received Vit. C solution (200mg/l) and feed
D Test group given 0.06g/kg PQ	**D_1**	Subsequently received water and feed
	D_2	Subsequently received Vit. C solution (200mg/l) and feed

3. RESULTS

Table 2: Values of the enzyme (sgpt) activities within the 3 months

GROUP	SUB-GRP	RAT NO	MONTH 1 (IU/L)	MONTH 2 (IU/L)	MONTH 3 (IU/L)
A (0g/kg)	A1	4	2.25 ± 0.8	4.18 ± 2.7	1.35 ± 0.6
	A2	4	6.50 ± 0.9	6.25 ± 2.4	7.50 ± 1.2
B (0.02g/kg)	B1	4	10.53 ± 2.1	41.15 ± 4.4	99.00 ± 1.0
	B2	4	12.53 ± 2.1	31.13 ± 6.3	46.50 ± 3.5
C (0.04g/kg)	C1	4	134.88 ± 23.4	147.00 ± 15.2	191.00 ± 24.0
	C2	4	83.87 ± 15.9	96.25 ± 11.8	74.50 ± 10.5
D (0.06g/kg)	D1	4	155.67 ± 36.9	258.75 ± 31.98	167.50 ± 12.5
	D2	4	133.23 ± 28.33	142.00 ± 34.3	58.00 ± 6.0

MEAN ± SEM; WHERE n=4

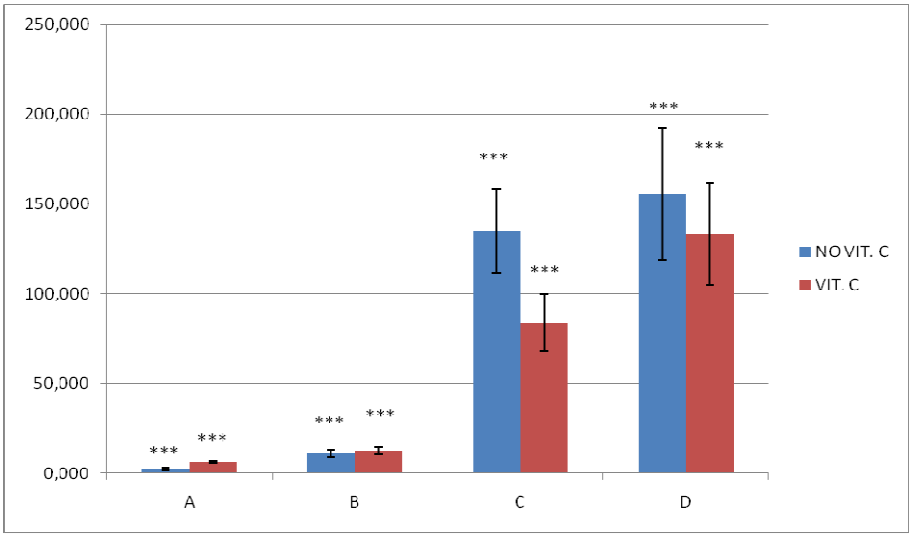

Figure 1: SGPT enzyme activity values for month 1

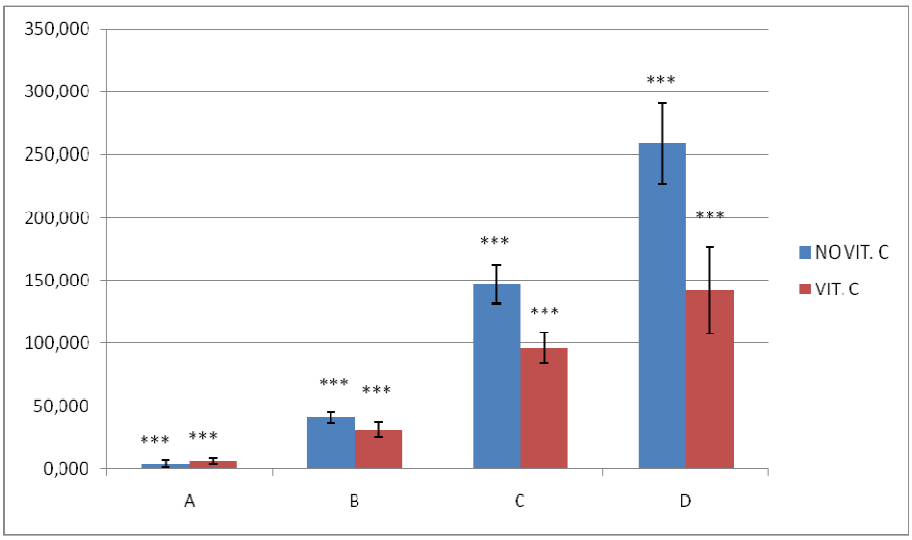

Figure 2: SGPT enzyme activity values for month 2

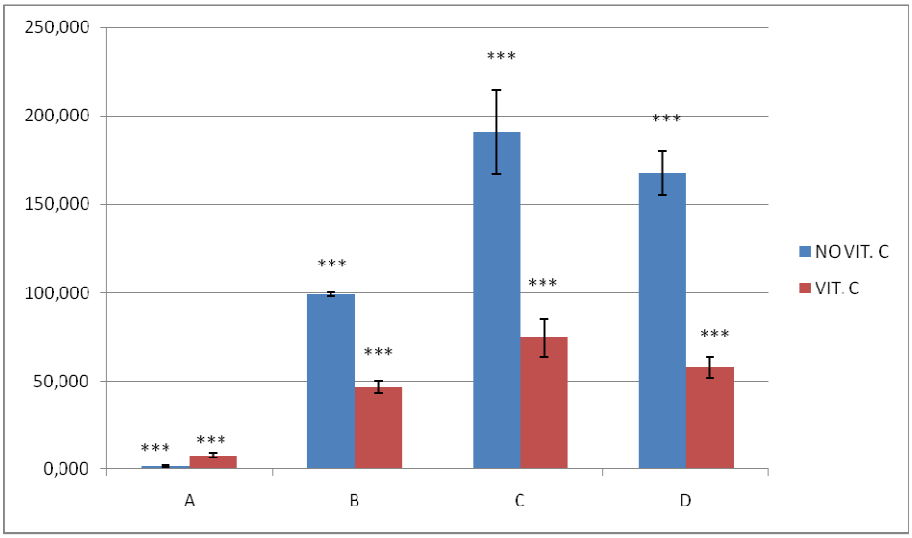

Figure 3: SGPT enzyme activity values for month 3

4. DISCUSSION

Paraquat, a bipyridyl compound, has been shown to be highly hepatotoxic (Dede, *et al*. 2007), and the mechanism of its toxicity on the liver cells have been extensively reported (Bus, *et al*.1976; Clark, *et al*. 1966 and Murray and Gibson, 1972).

The liver enzyme SGPT in this study had values that were highly elevated ($P \leq 0.001$) in both within and between subgroups (Table 1 and Figs 1 – 3), and these increases in enzyme activity were both dose and time dependent. The activities of the enzyme on rats that received only paraquat (PQ) insult were almost two- to three-folds that of the subgroups that received both PQ and vitamin C, except in the control subgroups were the results were reversed. The slight increases in enzyme activity of the control subgroup on vitamin C could be due to the high dose of the antioxidant given, but they were within reference level. The between groups comparison of the enzyme activities of the test groups to that of the control group (B A; C A and D A) had values that were between two- to ten-folds that of the control ($P \leq 0.001$), with the subgroups on paraquat only having values that were near hundred-folds that of the control. This was corroborated by studies by Dede, *et al*. (2007); Bainova and Vulcheva (1974) and Conning, *et al*. (1969), were they found out that the oxygen radicals produced as a result of paraquat insult directly or indirectly cause cell death leading to the release of intracellular enzymes that resulted in high values. Also, Wershana (2001) in his study on the influence of vitamin C or selenium on paraquat – induced toxicity in Guinea pigs, corroborated the findings in this study, that the administration of vitamin C, reduced the activities of the ROS which led to the reduction in intracellular enzyme release and an improvement in liver enzyme activity, as shown in the values of B_2, C_2 and D_2 of months 1 – 3 in table 2 and figures 1 – 3. This confirmed that vitamin C confers some level of antioxidant protection against toxic insult.

5. CONCLUSION

Successful antioxidant treatment of the so-called "free radical diseases" has been reported by Tantcheva, *et al*. 2003. This study therefore corroborated the findings that vitamin C (a potent antioxidant) improved the liver intracellular enzyme activity, thereby maintaining better liver cell integrity and a more improved health status of the animal under toxic insult.

Recommendation

Vitamin C, a potent water soluble antioxidant, should be one of the first line

treatments (emergency procedure) for paraquat accidental, recreational and occupational insult. Vitamin C's continuous administration in PQ toxicity should not be ignored and it should be extended even after patient's recovery, to completely repair the affected cells, tissues or organs.

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