



Histopathological and Biochemical Effect of Vitamin C and D on Phosphine-Induced Hepatotoxicity in Wistar Rats

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Abstract

Background: Aluminum phosphide is becoming a very common agent for self-poisoning. The lack of specific antidote for phosphine poisoning has aroused the current interest of research.

Objectives: This study aimed to investigate the possible protective effect of Vitamin C and D in phosphine-induced hepatotoxicity in Wistar rats.

Methods: In this experimental study, 20 Wistar rats were randomly divided into five groups (n = 12). Group I was apparently normal Control Group. Groups II-V were induced with 2.5 mg/kg ALP. Groups II was without further treatment while Groups III-IV received vitamin C at 100mg/kg/bw and vitamin D at 10mg/kg/bw. Group V was co-administered with vitamin C at 100mg/kg/bw and vitamin at 10mg/kg/bw. The treatments lasted for 28 days.

Results: The results showed that Vitamin C and D (P<0.05) increased antioxidant capacity. Combined active constituents of the Vitamin C and Vitamin D raised the GPx antioxidant activities when compared to the phosphine poisoned group treated individually with Vitamin C and D. The combined activities of Vitamin C and Vitamin D showed the highest antioxidant effect as compared with the control

Conclusion: The combined activities of Vitamin C and D supplementation improved the antioxidant defense system and histology of the liver in phosphine poisoning.

Keywords: Metal phosphide, Carotene, Liver cell damage, Poison

INTRODUCTION

The agricultural revolution and the growing use of pesticides in improving agricultural produce have brought their share of downsides in the form of pesticide poisoning¹. About 300,000 deaths worldwide occur annually as a result of pesticide poisoning^{2,3}. Additionally, self-intoxication accounts for more than 33% of suicides worldwide⁴. Aluminum phosphide (ALP) is the most common pesticides used for the safe storage and transportation of grains especially in Asian, Middle Eastern, and African countries^{5,6}. Unfortunately, considering several health outcomes, it is becoming a very common agent for self-poisoning^{7,8,9}. In Nigeria, ALP is sold in the form of a tablet, granules, or powder as phostoxin, justoxin, cejphos, multiphos, force toxin. When it is, metal phosphides generate toxic phosphine gas when ingested or inhaled by humans as a result of the stomach's diluted acid content, causing multisystem toxicity^{10,11}. The easy availability of ALP and its growing use for both agricultural and non-agricultural purposes is a public concern due to increasing number of suicide cases¹².

Vitamin D is essential for a wide range of non-classical functions and its deficiency has been linked to several chronic disorders including diabetes and cardiovascular disease¹³. It has been reported to inhibit iron-dependent lipid peroxidation and its antioxidant properties are compared to that of the anticancer medication Tamoxifen¹⁴. Vitamin D uptake has shown to prevent destruction of hepatocytes secondary to phosphine-induced lipid peroxidation¹⁵. Although there no available report, some experimental studies suggested that vitamin C and carotene could ameliorate the effects of oxidative phosphine^{16,17}.

The lack of specific antidote for aluminum phosphide poisoning has aroused our interest. This study aimed to investigate the possible protective effect of Vitamin C and D in phosphine-induced hepatotoxicity in male Wistar rats.

METHODOLOGY

The study was approved by the Biomedical Research and Ethics Committee of the Ministry of Agriculture and Forestry, Akure, Ondo State, Nigeria (MNR/V384/9). The experimental animals were handled following the International Humane

Animal Care Standards¹⁸. Twenty (20) inbred Wistar rats weighing 180±20g were used and maintained under the standard husbandry condition (between 22-25°C, 12 hours light and 12 hours dark) in the Animal House. Animals were fed *ad libitum*.

Chemicals and reagents

Superoxide peroxide and glutathione peroxide reagents were purchased from the R and D System in Minneapolis, Minnesota, in the United States. Commercial enzyme-linked immunosorbent test (ELISA) kits for tumour necrosis factor-alpha (TNF- α) and interleukin 6 (IL-6) were purchased from Creative Diagnostics in Shirley, New York, 11967, USA. From stock chemicals of analytical grade, Haematoxylin and Eosin (H&E), PBS solution, and 10 percent neutral buffer formalin were prepared.

Drugs

Aluminum phosphide (ARYSTA Life Science South Africa Pty Ltd) was purchased in Lagos Nigeria. PARTAN-C Vitamin C tablet manufactured by Kunimed Pharmachem Limited, Lagos Nigeria, and Health Aid Vitamin D 1000IU produced by Health Aid Plus Limited, Nigeria.

Experimental Design

Twenty (20) adult male wistar rats obtained from the Animal Holding of the Department of Anatomical Sciences, University of Medical Sciences, Ondo state were randomly allotted into five groups consisting of four rats each. Each of the test groups was exposed to aluminum phosphide at a standard dosage of 2.5 mg/kg/body weight.

Group I was the apparently normal control group

Group II was induced with 2.5 mg/kg ALP without further treatment

Group III was the ALP-exposed group administered with vitamin C at 100mg/kg/bw

Group IV was the ALP-exposed group administered with vitamin D at 10mg/kg/bw

Group V was the ALP-exposed group co-administered with vitamin C at 100mg/kg/bw and vitamin at 10mg/kg/bw respectively.

The treatments lasted for 28 days.

Induction of hepatotoxicity

Two and a half percent solution of the ALP was prepared and used to induce phosphine toxicity via an orogastric tube.

Necropsy

Upon completion of the experiment, the Wistar rats were euthanized and the liver of the rats was carefully excised and immediately transferred in 10% neutral buffered formalin for adequate fixation followed by histological processing for microscopic studies while the liver samples for biochemical analysis were rinsed in freshly prepared 0.1 mol/L phosphate-buffered saline (PBS, pH 8.0), followed by homogenisation and centrifuged at 3000 revolutions per minute for 20 min at 4°C. The supernatant obtained was preserved at -80°C for the estimation of biochemical analytes.

Histopathological study

Upon completion of fixation in 10% Neutral buffered formalin. The liver was processed for light microscopic study using an automatic tissue processor machine (Thermo Scientific Spin Tissue Processor, STP120, Frankfurt, Germany). The tissues were dehydrated in graded alcohol concentrations, cleared in two changes of xylene, infiltrated in two changes of wax bath, and lastly embedded in paraffin wax. Olympus binocular light research microscope was used to histologically assess the thin sections of 4 microns stained with Haematoxylin and Eosin (H&E). Micrographs were taken with a Kodak Digital Camera (Kodak Easyshare C183).

Biochemical analysis

The activity of superoxide dismutase (SOD) was determined by the method of Misra *et al*¹⁹. While glutathione (GPx) activity was assayed according to Rahman *et al*²⁰. Levels of interleukin-6 (IL-6) and tumour necrosis factor-ALpha (TNF- α) were evaluated using Sandwich ELISA kit protocols described by the manufacturer.

Statistical Analysis

The data were analysed using statistical software for social sciences (SPSS) 23.0 version. Data were presented using mean standard deviation (mean SD) for all quantitative values, and one-way analysis of variance (ANOVA) was used for comparisons between the groups. Quantitative values of $p < 0.05$ were considered statistically significant.

RESULTS

Biochemical Findings

Table 1 Showed the Immunomodulatory effect of Vitamin C and D on IL-6 and TNF- α . Regarding the GPX and SOD activities, IL-6 and TNF- α levels were significantly ($p < 0.00001$) reduced compared to the control group. However, combined active constituents of ALP+Vit.C & Vit D raised the GPx antioxidant activities when compared to the phosphine poisoned group treated individually with Vitamin C and D. Combined activities of Vit.C & Vit D showed the highest antioxidant effect as compared with the control (Table 1).

Table 1: Immunomodulatory effect of IL-6 and TNF- α

Parameter	Normal control	Positive control (ALP-induced animals)	ALP+Vit. C (100 mg/kg/bw)	ALP+Vit. D (10 mg/kg/bw)	ALP+Vit.C & Vit D.
SOD (U/mg)	36.89±0.27	2.90±0.09	2.08±0.02	2.10±0.03	2.58±0.18
GPx (μ moles)	116.92±0.63	39.90±0.36*	39.56±0.42*	34.95±0.22*	43.29±0.88*
IL-6	27.62±0.24	123.24±7.06*	231.29± 3.53*	224.73±9.38*	234.7±2.29*
TNF alpha	217.32±1.07	525.52±3.74*	473.76±8.98*	570.86±19.73*	491.23±2.97*

Values were expressed as mean value \pm standard deviation (SD).*mean values were significantly different compared to the Normal control at $P \leq 0.05$.#mean values were significantly different compared with the control

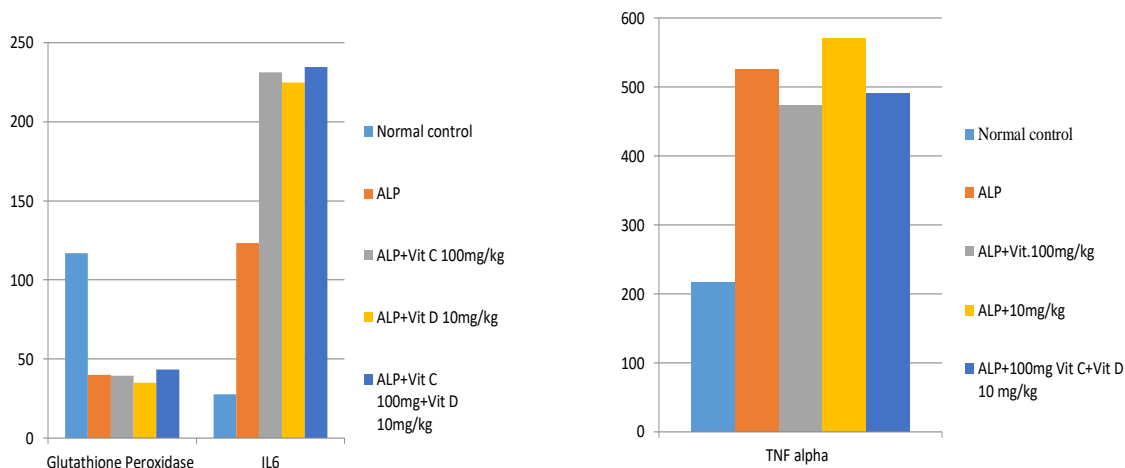


Figure 1: Immunomodulatory effect of Vitamin C and D on ALP poisoning

Histopathological result

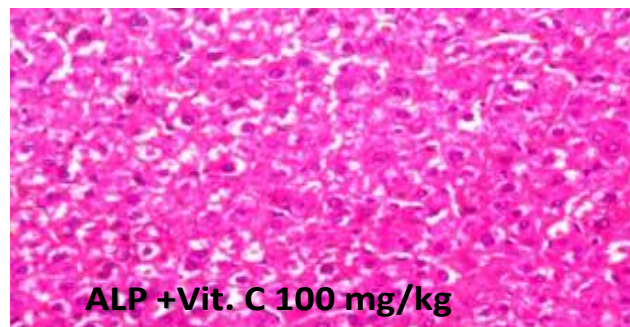
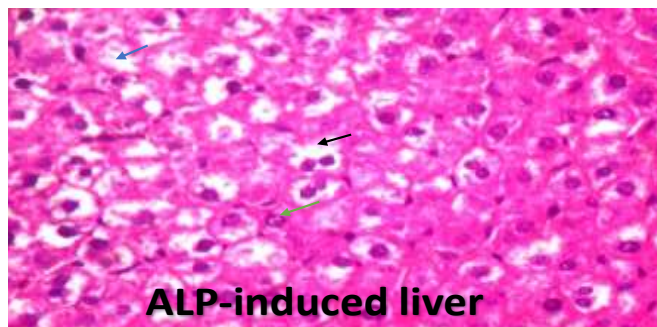
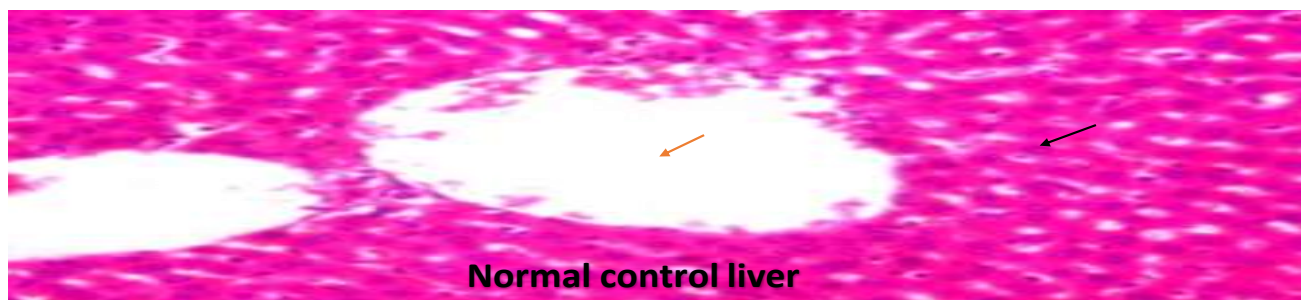


Figure 1: Histological representation of normal liver cells showed a central vein (red arrow) and radiating chords of hepatocytes (black arrow) with sinusoids devoid of congestion. ALP-induced liver exhibited hepatocyte vacuolation (black arrow), sinusoidal congestion (blue arrow), and nuclear fragmentation (green arrow). ALP-induced liver administered with Vit. C 100 mg/kg illustrated regenerative changes (pyknotic nuclei) characterized by reduced congestion in the sinusoidal space and the absence of nuclear fragmentation of hepatocytes. (H&E. X 400).

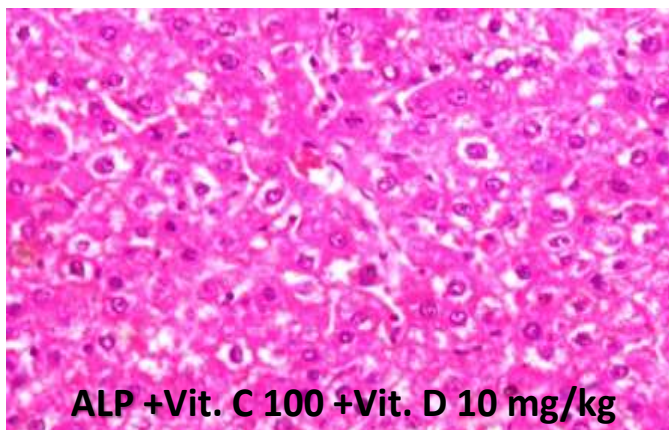
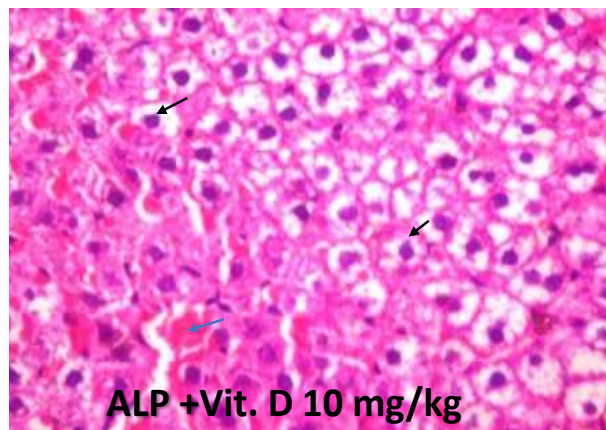


Figure 3: Histological representation of ALP-induced liver administered with Vit. D 10 mg/kg showed interstitial hemorrhage (green), and pyknotic nuclei (blue) which is suggestive of reparation changes. ALP-induced liver treated with Vit. C +Vit. D showed apparently normal liver cells with a significant reduction in sinusoidal congestion. (H&E. X 400).

DISCUSSION

Hepatic damage is a major cause of death due to phosphine poisoning²¹. To date, there is no substantial treatment for the several cases reported²². This study aimed to investigate the immunohistopathological effect of Vitamin C and D in Aluminium phosphide poisoning. The present study showed that ALP-induced liver exhibited hepatocyte vacuolation, sinusoidal congestion, and nuclear fragmentation. Consistent with our result, previous studies has reported hepatocyte vacuolation, sinusoidal congestion, fatty liver changes, centrilobular necrosis, and destruction of nucleolus of hepatocytes in phosphine- poisoning^{22,23}. Haghi *et al*²⁴ concisely discussed the mechanisms of hepatocyte phosphine-induced cytotoxicity, highlighting the production of reactive hydroxyl radicals, induction of cellular hypoxia, and free radical-mediated injury in addition to the inhibition of cytochrome C-oxidase and other essential cellular enzymes^{25,26,27}. Vitamin C administration at a dose of 100 mg/kg exhibits a recovery in the ALP-induced hepatotoxicity with reparation features suggestive of regenerative changes. In addition, the sinusoidal congestion subsided with no evidence of nuclear fragmentation in the group. Interstitial haemorrhage and the presence of pyknotic nuclei were seen in the liver of the ALP-induced toxicity in rats administered Vitamin D at a dose of 10mg/kg. The liver of the ALP-exposed rats co-administered with vitamin C and D appeared apparently normal with a significant reduction in the sinusoidal congestion.

According to the present study, phosphine decreases SOD and GPX activities in the liver tissue and this effect is slightly inhibited by the concurrent administration of Vitamins C and D. Documented evidence abound, antioxidant supplements especially vitamin C and vitamin D relieve the body of stress associated with phosphine poisoning²⁸. Studies have also revealed that vitamin C enhance the strength of the antioxidant defense system by increasing the antioxidant capacity and controlling reactive oxygen species²⁹. Vitamin D reduces ROS and pro-inflammatory cytokines possibly by improving cellular Glutathione levels^{30,31}.

CONCLUSION

In conclusion, our finding showed that the combination of Vit C and Vit D supplementation improved the antioxidant defense system and improved the histopathology of the liver in phosphine poisoning and reduces oxidative stress.

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