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Original Research Article

Role of vitamin C in mitigating lead-induced hepatotoxicity in male albino rats

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Abstract

Purpose: *To investigate the adaptive role of vitamin C (VC) against lead-induced hepatotoxicity in male albino rats.*
Methods: *Twenty-four male albino rats were randomly assigned to four treatment groups, with each group consisting of six animals, following a completely randomized design. Animals in group I served as the control. Group II was administered 60 mg/kg body weight (BW) of Pb acetate, group III was administered 100 mg/kg BW of VC, while group IV was treated with 60 mg/kg BW of Pb acetate and VC at 100 mg/kg BW. The animals were treated orally for 65 days. Twenty-four hours after treatment, changes in body weight, liver enzymes and histopathological parameters were compared.*
Results: *Results showed a significant reduction in the body weight of Pb-exposed animals when compared with control group ($p < 0.05$). The level of Pb residue was significantly higher in the liver of rats administered solely with Pb when compared with the control animals and other treatment groups ($p < 0.05$). Moreover, Pb-treated animals showed liver histopathological changes such as injured hepatocytes and cytoplasmic swellings which suggest inflammation, in comparison with animals in control group. Notably, vitamin C administration mitigated Pb-induced effects across all evaluated parameters.*
Conclusion: *The results provided evidence of the adaptive potential of VC in mitigating Pb-induced hepatotoxicity in male albino rats.*

Keywords: *Lead, Vitamin C, Hepatotoxicity, Histopathology, Adaptive effect*

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INTRODUCTION

As the global population expands and industrial activities increase, ecosystems face significant strain, causing many organisms to encounter heightened exposure to environmental contaminants, including heavy metals. These pollutants are linked to adverse effects on mammalian organs, with adverse effects on the immune system [1]. Heavy metals, identified by densities exceeding 5 g/cm^3, include elements like Pb and copper (Cu) [1].

Lead is widely distributed in the environment and absorbed into food, aquatic ecosystems, atmosphere, and topsoil, thus permeating the food chain [2]. Once taken up by plants from contaminated soil, it infiltrates the food chain. Known for its neurotoxic, carcinogenic, and immunotoxic properties, Pb also disrupts cellular

processes, induces genetic damage, and fosters oxidative stress by promoting reactive oxygen species (ROS), which exhaust antioxidant defenses and amplify cellular harm [3-5].

The degree of Pb toxicity depends on multiple factors such as age, dose, and method of exposure. The toxic effects of Pb manifest across biological systems, including the liver, nervous system, and cardiovascular system [6]. Additionally, Pb exposure compromises immune functions by altering antibody responses and affecting immune cells such as T-helper cells and macrophages [7]. Lead exposure induces oxidative stress, particularly through its impact on antioxidants like glutathione, further affecting metabolic and cellular processes, as well as reproductive health, particularly in disrupting hormonal balances and cellular functions in reproductive organs [8].

Vitamin C exhibits potent antioxidant properties that counteract Pb-induced oxidative damage across multiple organs, including the liver, kidneys, brain, and reproductive functions [9]. Recent advances in VC delivery through microencapsulation and nanotechnology have enhanced its efficacy, providing additional protection against Pb toxicity probably by limiting intestinal absorption and promoting Pb binding for detoxification [10]. Given the widespread nature of Pb pollution and its well-documented toxicity, there is a need for adaptive strategies against its deleterious effects. This study assessed the adaptive role of VC in mitigating Pb-induced hepatotoxicity.

EXPERIMENTAL

Materials

Lead was procured from Sigma-Aldrich Limited (St Louis, Missouri, USA) while vitamin C was obtained from Emzor Pharmaceutical Industries Limited, Calabar, Nigeria.

Animals

Twenty-four adult male albino rats of reproductive age, 12 weeks old and weighing between 160 – 200 g, were housed at the Department of Genetics and Biotechnology Animal House at the University of Calabar, Nigeria. Animals were accommodated in breathable metal pens, maintained in a controlled laboratory environment with continuous provision of water and a balanced feed from Top Feed Nigeria. Limited. Prior to the treatment phase, the rats were given time to adapt to the laboratory conditions for two weeks.

METHODS

Design and protocol

The 24 rats were assigned to 4 groups of 6 animals each, following a completely randomized design, with randomization performed using GraphPad randomization software. The sample size was chosen based on prior studies and adhered to the 3Rs principle in animal research. The treatment protocol is presented in Table 1.

Treatments were administered daily for 65 days. Twenty-four hours after the last treatment, the animals were euthanized using cervical dislocation following Isoflurane anesthesia. The liver was surgically removed and processed for histopathological analysis, while other vital organs were weighed and prepared for Pb residue assays. Blood samples were collected for oxidative stress markers assay.

Ethical approval

The study followed the principles outlined in the guide for the care and use of laboratory animals [11]. The Faculty of Biological Sciences Ethical Committee issued approval for this study (approval no. BIOSC22-10). This manuscript adheres to guidelines set by ARRIVE in conducting studies with animals.

Body weight and weight of organ

Body weight and weight of liver were determined using an electronic weighing balance (Scout Pro SPU 601).

Table 1: Treatment protocol using vitamin C

Group	Treatment
Group I	Control group with no treatment
Group II	60 mg/kg body weight (BW) of Pb acetate
Group III	100 mg/kg BW of VC
Group IV	Combined treatment with 60 mg/kg BW of Pb acetate and 100 mg/kg BW of VC

Determination of Pb residue in the liver

The concentration of Pb in the tissues was quantified using a Perkin-Elmer 2380 atomic absorption spectrophotometer (AAS), following the procedure outlined by [12].

Determination of oxidative stress markers

Levels of Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) were determined using Sunlong ELISA kits [4]. Malondialdehyde (MDA) levels were measured using a fluorometric method while Nitric oxide (NO) assay was carried out using Griess assay to assess the level of the metabolites measured by a spectrophotometer (Jenway 6405, Essex, England) at 540 nm [4].

Histology of liver

Histological examination was done utilizing Hematoxylin and Eosin (H & E) staining technique and viewed with the aid of a light microscope according to [3].

Statistical analysis

Data obtained on body weight, liver weight, Pb residue, and oxidative stress markers were analyzed using one-way ANOVA, followed by mean separation using the post hoc Tukey analysis, with 5 % level of significance using SPSS Statistics version 27 (IBM Corporation, Armonk, NY, USA).

RESULTS

Lead residue concentration

Figure 1 shows results obtained on Pb residue concentration. The results demonstrated a significant accumulation of Pb residues in the liver of animals administered Pb only (0.30 ± 0.02), exceeding control and the VC-treated group levels (both at 0.17 ± 0.01 and 0.17 ± 0.04, respectively). However, results revealed the modulatory potential of VC on Pb residue levels in animals in the combined Pb and VC treatment group (0.21 ± 0.01) relative to the Pb - treated group.

Body weight and weight of liver

The results showed a significant reduction in mean body weight in Pb-treated rats (172.55 ± 9.86 g) compared with the control group (229.00 ± 8.82 g), and the VC - treated group (234.50 ± 10.12 g). Animals in the Pb + VC group had an average body weight of 180.90 ± 6.89 g indicating the adaptive effect of VC (Figure 2).

Results on liver weight are presented in Figure 3, showing a numerical increase in the Pb - treated animals (7.78 ± 0.49 g) compared to the control group (7.45 ± 1.02 g), and the Pb + VC group (7.32 ± 1.08 g). The weight of the liver was significantly reduced in the VC group (6.95 ± 0.56 g) when compared to the other groups.

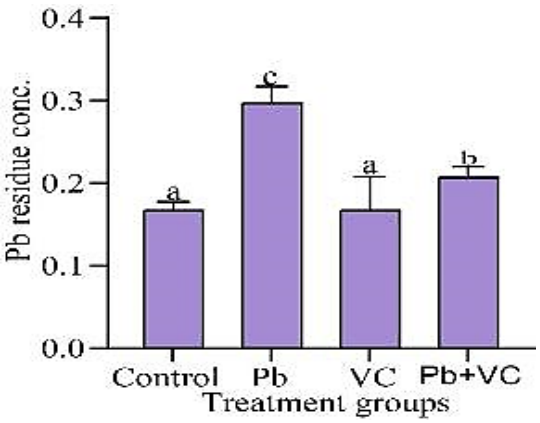

Figure 1: Adaptive effect of VC on Pb residue concentration (Conc.)

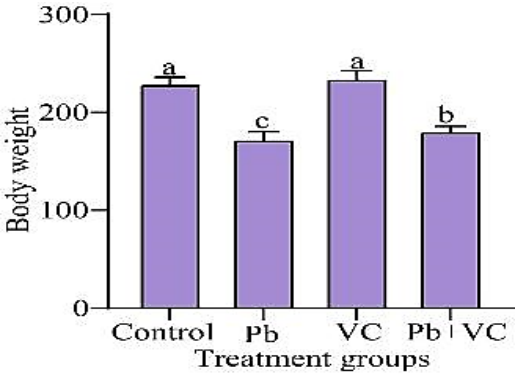

Figure 2: Adaptive effect of VC on body weight of albino rats exposed to Pb

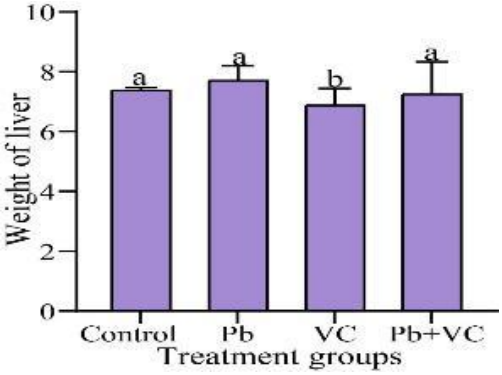

Figure 3: Adaptive effect of VC on weight of liver of albino rats exposed to Pb

Table 2: Effect of vitamin C on lead-induced toxicity on oxidative stress markers

Parameter	Control	Vitamin C	Lead + vit C	Lead
CAT (pg/mL)	445.28±6.99	452.9±5.40	86.77±12.25[b]	20.43±1.28[a]
SOD (pg/mL)	1239.00±15.71	5311.47±55.96[c]	616.13±49.89[b]	135.92±11.38[a]
GPx (IU/mL)	1475.75±45.20	2367.27±40.78[c]	697.65±23.87[b]	138.92±10.21[a]
MDA (µmol/mL)	1.27±0.12	1.15±0.10[c]	1.33±0.07[b]	2.07±0.09[a]
NO (µmol/mL)	4.90±0.24	4.72±0.60	8.07±0.50[b]	11.10±0.57[a]

Values are expressed as mean ± SEM. [a,b,c]$p < 0.05$ vs control. CAT = Catalase; SOD = Superoxide dismutase; GPx = Glutathione peroxidase; MDA = Malondialdehyde; NO = Nitric oxide

Oxidative stress markers

Results obtained indicated significantly reduced levels of SOD, CAT, and GPx in the Pb - treated group compared with the control and VC groups. However, co-administration of Pb and VC reduced the impact of Pb on the antioxidative enzyme activity levels in comparison with animals treated with Pb only (Table 2). In contrast, levels of MDA and NO were markedly higher in the Pb group than in the control and VC groups, but the effect of Pb was mitigated in the combined treatment group (Table 2).

Liver histopathology assessment

The section liver from rats in the control group revealed a preserved architecture, with plates of liver cells extending from the central vein (Figure 4 A). The cytoplasmic mass of the hepatic cells was moderate with prominent nuclear components. The intervening interstitium surrounding the sinusoids was narrowed, and the limiting plate hepatocytes of the portal area remained intact including the bile duct, liver parenchyma, and portal vein. Liver sections of animals exposed to Pb (Figure 4 B) displayed abundant cytoplasmic volume and nuclei that were round to oval in shape, with clearly defined nucleoli. The sinusoidal spaces were dilated, and moderate inflammatory effect was evident, characterized by mononuclear cell infiltration of the sinusoids. The portal area was expanded and contained numerous amounts of mononuclear inflammatory cell infiltrates. Injured hepatocytes and cytoplasmic swellings were also seen. Findings are suggestive of mild portal inflammation.

Results indicated that the liver section of rats administered VC (Figure 4 C) had a preserved architecture with plate of hepatic cells arising from the central vein. The hepatic cells were enlarged and displayed a notable cytoplasmic presence and nuclei that were distinctly prominent. The portal areas show intact limiting plate hepatocytes. The liver section of rats administered with Pb and VC with a preserved architecture showed plate of hepatic cells arising from the central vein. The hepatic cells were enlarged, with reasonable cytoplasmic mass and prominent nuclei (Figure 4 D). Expansion of sinusoidal spaces between the cellular components was evident and contained mononuclear inflammatory infiltrates and showed a well-preserved boundary layer of liver cells. Mild portal inflammatory infiltrates mainly mononuclear cells. Findings suggest reduced portal inflammation.

DISCUSSION

This study revealed a significant increase in Pb residue concentrations in the liver tissues of Pb-exposed rats compared to the control group. This observation aligns with existing studies on bioaccumulation of Pb in critical organs. Pb is widely distributed throughout the body, particularly in vital organs such as the liver, kidneys, brain, and reproductive systems. Its strong affinity for sulfhydryl groups in proteins underpins its toxic potential, with severe implications for physiological functions in these organs [13]. Specifically, the liver, a crucial organ for detoxification, accumulates Pb, disrupting metabolic activities and contributing to hepatotoxicity [14]. Findings from this study emphasize the systemic impact of Pb toxicity, with organ-specific accumulation triggering cascading negative health outcomes. The differences in Pb residues between the Pb-exposed and control groups underscore the severe health risks associated with environmental Pb exposure and the necessity for preventive interventions. Vitamin C administration significantly reduced Pb residues in tissues. The capacity of VC to bind and facilitate the excretion of Pb ions appears crucial to reducing tissue accumulation. Moreover, the antioxidant properties of VC help correct the oxidative imbalance induced by Pb exposure, thereby mitigating the retention of Pb [10].

The effectiveness of VC in chelating Pb and inhibiting its absorption in the gastrointestinal tract has been reported by Somparn *et al* [10].

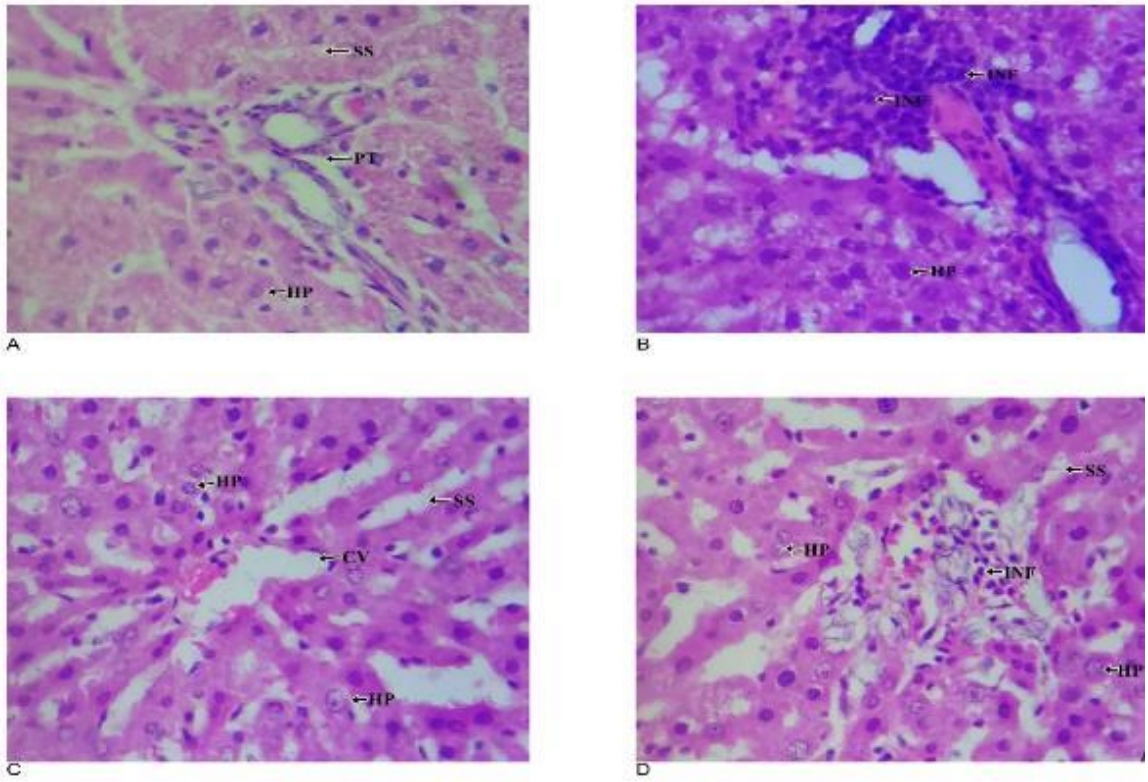

Figure 4: The effect of VC on Pb-induced liver toxicity of rats. (H&E x400). (SS = sinusoidal spaces; INF = inflammation; HP = hepatocytes and PT = portal triad. 1A: Control 1B: Pb group. 1C: VC. 1D: Pb + VC)

Furthermore, Biser and Berger [1] reported that VC treatment lowers Pb levels in organs such as the liver, primarily through its antioxidant capabilities, which stabilize cellular structures and mitigate oxidative damage. Additionally, its interaction with glutathione, a critical antioxidant for detoxification, further explains its efficacy. Adalikwu *et al* [3] highlighted the centrality of oxidative stress in Pb toxicity and underscored the importance of antioxidants like VC. Additional studies by Sharma *et al* [15] support this assertion, demonstrating that VC not only reduces oxidative stress induced by Pb but also restores the function of antioxidant enzymes suppressed by Pb exposure. This enzyme activity restoration is pivotal for decreasing overall Pb toxicity.

The significant reduction in body and liver weights in Pb-exposed animals is consistent with previous studies which reported that Pb interferes with metabolic and endocrine processes, disrupting nutrient absorption and organ function, which contributes to weight loss and reduced organ mass [16]. Specifically, liver weight reduction is likely linked to oxidative damage, inhibiting protein synthesis and hepatocellular function, as documented by [3].

The results revealed a significant decrease in serum levels of CAT, SOD, and GPx in animals treated with Pb, in comparison to the control group, indicating the presence of oxidative stress. Oxidative stress results from an increase in reactive oxygen species (ROS) and free radicals produced during normal physiological processes but becomes harmful when not adequately neutralized by the antioxidant defense system [15]. Antioxidants such as SOD, GPx, and CAT play a critical role in scavenging ROS [4]. Furthermore, Pb treatment resulted in significantly higher MDA and NO concentrations, consistent with Offiong *et al* [5], who reported a decline in the activities of these antioxidants and an increase in lipid peroxidation markers such as MDA due to exposure to toxicants. Elevated MDA indicates lipid peroxidation, a hallmark of oxidative cellular damage, reinforcing the presence of oxidative stress [13].

Conversely, VC treatment significantly increased the serum levels of SOD, CAT, and GPx while reducing MDA and NO levels, demonstrating its protective effect against Pb – induced oxidative stress. This is attributed to the ability of VC to enhance the antioxidant defense system and neutralize free radicals generated by lead exposure, thereby preserving the bioavailability of critical antioxidants. These findings underscore the adaptive potential of VC in mitigating oxidative stress caused by lead toxicity [3].

The histological alterations observed in the Pb-treated animals were relatively mild compared to those reported in other studies [17]. Previous studies have demonstrated that hepatic effects of Pb exposure are characterized by elevated serum enzyme levels and mild hepatitis, indicating liver damage [8]. The Pb exposure – induced inflammatory cell infiltration in hepatic tissue suggests a potential mechanism involving Pb-protein/enzyme interactions within the hepatic interstitial space, leading to oxidative stress and reactive oxygen species-mediated inflammation [18].

The formation of cytoplasmic inclusions in hepatocytes following Pb exposure, as observed agrees with the findings reported by Del-Monte [19]. These inclusions may indicate Pb–induced hepatocyte injury, resulting from the inability of the cells to manage accumulated residues stemming from metabolic and structural disruptions caused by Pb toxicity. Furthermore, the observed cytoplasmic swelling and hydropic degeneration in Pb-treated animals may be alongside the disruption of lysosomal membranes, leading to enzyme leakage, leading to cytoplasmic degeneration and interference of macromolecular movement and interaction [19]. The distortions in the histological integrity of the liver induced by Pb exposure were mitigated in animals co-treated with VC, suggesting an adaptive effect of VC. This protective effect may be attributed to antioxidant properties, which mitigate Pb-induced liver dysfunction and damage [3,5].

CONCLUSION

The present study demonstrates the adaptive protective role of VC against Pb-induced hepatotoxicity in a mammalian model using male albino rats. This result points to the fact that VC has the potential to serve as a valuable therapeutic or prophylactic agent in mitigating Pb toxicity, particularly in environments where Pb contamination is prevalent and poses a significant public health risk.

DECLARATIONS

Acknowledgement

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Ethical approval

The Faculty of Biological Sciences Ethical Committee issued approval for this study (approval no. BIOSC22-10).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ukam Uno and Utip Ekaluo designed the study and conducted the experiments. Reagan Agbor and Ekerette Ekerette analyzed and interpreted the data, while Dominic Offiong, Idara Esua and Uduak Edem prepared the manuscript, with contributions from all the coauthors.

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