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AMELIORATIVE EFFECT OF VITAMIN C ON LEAD INDUCED HEPATOTOXICTY IN RATS

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ABSTRACT
Various biochemical actions such as antioxidant and reducing activities have been ascribed to ascorbic acid (Vitamin C). This study was designed to investigate the deleterious effects of lead on the liver by determining serum alkaline phosphatase (ALP), aspartate transaminase (AST), alanine Transaminase (ALT), total protein, albumin and ameliorative property of vitamin C in male Albino Wistar rats. The rats (110-130g) were grouped into 4 groups consisting of 8 rats per group. Group 1 served as control, given only rat chow and distilled water. Group 2 was given 100 mg/kg body weight of lead; group 3 was given 100 mg/kg body weight of vitamin C, while group 4 was given both 100 mg/kg body weight of lead and 100 mg/kg body weight of vitamin C for 21 days. These rats were also given rat chow and distilled water *ad libitum*. The biochemical analytes were measured spectrophotometrically. Administration of lead induced significant increase ($P < 0.05$) in the activities of ALP, AST, and ALT. Serum concentrations of total protein and albumin were significantly ($P < 0.05$) decreased. Treatment with vitamin C at a dose of 100 mg/kg body weight reduced the adverse effects on the liver enzymes and increased protein concentration. Histological examination of the liver revealed pathophysiological changes in lead treated rats while treatment with vitamin C improved liver histology. The overall results showed that vitamin C ameliorated lead-induced toxicity in the liver.

INTRODUCTION

Lead (Pb) is an environmental contaminant due to its significant role in modern industry[1]. Both occupational and environmental exposures remain a serious problem in many developing and industrializing countries[1]. Lead has been one of the most important heavy metals because of its common usage in various industrial products, and therefore, is considered as a serious occupational hazard throughout the world[2]. It has been shown that exposure to lead enhances intracellular reactive oxygen species (ROS) production and lipid peroxidation, including tissue damage in animal systems[3].

Ionized lead (Pb^{2+}) is one of the most potentially toxic elements in the environment and is known to be toxic to the body system[4]. Various degree of exposure to Pb^{2+} elicits different Pb^{2+} - induced oxidative stress in various target sites such as the liver, kidney, central nervous system and the brain.[5] Studies by Khan *et al.*,[6] have shown that excessive generation of reactive oxygen species (ROS) in vivo upon Pb^{2+} intoxication, resulted in systemic mobilization and

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depletion of intrinsic antioxidant defenses, destabilizing calcium homeostasis by damaging electron transport, adenosine triphosphate (ATP) depletion, and membrane ion channel(s) disruption, ultimately leading to apoptosis.

It has been revealed that lead toxicity leads to free radical damage via two separate mechanisms. These include: the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide and direct depletion of antioxidant reserves[7], and ionic mechanism where lead substitute other bivalent cations like Ca^{2+}, Mg^{2+}, and Fe^{2+}.[8]

Recently, the use of vitamin C for chelating lead has been a novel development in science and health. Vitamin C, a water soluble vitamin, is a strong reducing agent capable of donating electrons to lead and thereby forming soluble complexes which are easily excreted out of the body[9-10].

The aim of this study was to investigate the effect of lead on the liver and the possible ameliorative effect of vitamin C on lead induced liver toxicity.

MATERIALS AND METHODS

Experimental Animals

Thirty-two male albino Wistar rats were used for this study and were obtained from the animal house of the Department of Anatomy, University of Benin, Benin City. The animals were housed in a wooden cage in the animal house of the Department of Biochemistry, University of Benin and were fed on standard rat chow, with water available ad libitum. They were allowed to acclimatize for 14 days at room temperature.

Lead acetate (50g) and Vitamin C (50g) were obtained from a pharmaceutical store in Benin City. They were reconstituted in distilled water, prior to daily administration.

Experimental Protocol

The experimental animals were divided into four groups of eight rats each and the duration of administration was twenty-one (21) days.

Group 1: serve as controls and were given distilled water.

Group 2: were given lead acetate 100mg/kg body weight.

Group 3: were given vitamin C 100mg/kg body weight.

Group 4: were given both lead acetate 100mg/kg body weight and 100mg/kg body weight.

Administrations of both lead acetate and vitamin C were orogastric. This helped to ensure very effective treatments of the study animals. After the 21 days duration of oral administration, the animals were scarified and blood samples were collected for liver function tests. Liver tissues were collected and examined histologically by staining with Haematoxylin and Eosin stain.

Measurement of liver function test

Serum transaminases (Enzymatic method for aspartate aminotransaminase and alanine aminotransaminase)[11], total protein (Biuret method)[12] and albumin (Bromocresol blue method) were measured spectrophotometrically using reagent kits from Randox Laboratories Ltd, Ardmore, Diamond Road, Crumlin, Co. Antrim, UK.

Serum alkaline phosphatase[13] was analyzed with reagent kit from Teco Diagnostics, 1268N. Lakeview Ave. Anaheim, CA92807.

Statistical analyses

Statistical analyses were computed and results presented as Mean ± Standard error of mean (SEM). Analysis of variance (ANOVA) was used and a P- value < 0.05 was considered to be significant. Statistical analysis was performed using GraphPad Instat3 software.

RESULTS

Figure 1 shows serum alkaline phosphatase (ALP) activity in control rats, lead, vitamin C and lead+vitamin C treated rats. There was significant increase ($P < 0.05$) for serum alkaline phosphatase activity in lead-treated rats (78 ± 3.86) when compared with control (51 ± 2.02). Increase in vitamin C- treated (53 ± 3.35) subgroup was not significant ($P > 0.05$). Lead+vitamin C treated group (63 ± 1.78) was significantly decreased ($P < 0.05$) when compared with lead-treated group (78 ± 3.86).

Figure 2 shows serum aspartate transaminase (AST) activity in control, lead, vitamin c and lead + vitamin C treated rats. There was a significant increase ($P < 0.05$) in AST activity in the lead-treated group (92 ± 2.67) when compared to control (47 ± 2.51). There was significant difference ($P < 0.05$) between lead+vitamin C treated rats (71 ± 2.95) and vitamin C-treated rats (50 ± 2.58). There was no significant difference ($P > 0.05$) between control (47 ± 2.51) and vitamin C treated group (50 ± 2.58).

Figure 3 shows serum alanine transaminase (ALT) activity in control, lead, vitamin C and lead + vitamin C-treated rats. There was a significant increase ($P < 0.05$) in ALT activity in the lead-treated group (58 ± 2.10) when compared to control (36 ± 2.93), vitamin C (29 ± 1.66) and lead + vitamin C treated (40 ± 1.31) rats. There was no significant difference ($P > 0.05$) between lead+vitamin C treated rats (40 ± 1.31) and control (36 ± 2.93), but there was significant difference ($P < 0.05$) between vitamin C treated rats (29 ± 1.66) and lead+vitamin C treated rats (40 ± 1.31).

Figure 4 shows serum total protein concentration in control, lead, vitamin c and lead+vitamin C treated rats. There was significant decrease ($P < 0.05$) in the serum total protein concentration in the lead-treated rats (6.6 ± 0.16) compared to control (7.3 ± 0.23). There was no significant difference ($P > 0.05$) between vitamin C (6.9 ± 0.13), lead + vitamin C treated rats (7.5 ± 0.14) and control (7.3 ± 0.23).

Figure 5 shows serum albumin concentration in control, lead, vitamin C, and lead + vitamin C treated rats. There was significant decrease ($P < 0.05$) in serum albumin concentration in lead-treated rats (3.2 ± 0.05). There was no significant difference ($P > 0.05$) between control (4.0 ± 0.13), vitamin C (3.9 ± 0.04), and lead + vitamin C treated (4.1 ± 0.06) rats.

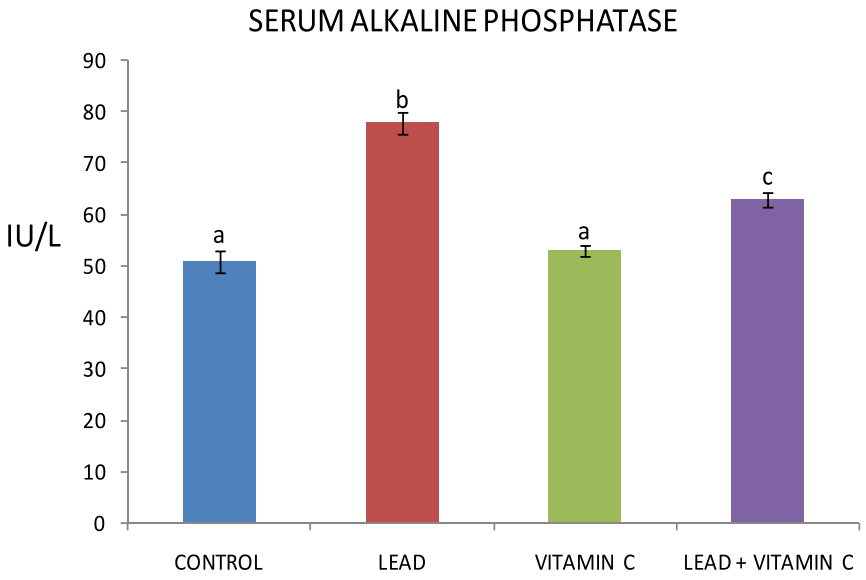

Figure 1: Serum alkaline phosphatase (ALP) activity in treated rats.

Serum alkaline phosphatase activity for control rats, lead, vitamin C and lead +vitamin C treated rats. Different superscript letters differ significantly from each other at P<0.05.

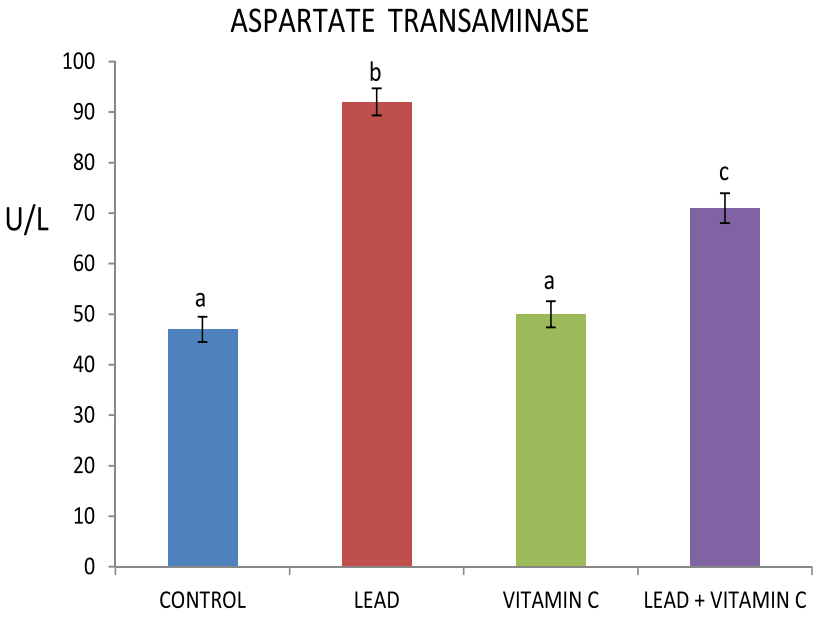

Figure 2: Serum aspartate transaminase (AST) activity in treated rats.

Serum aspartate aminotransferase activity for control rats, lead, vitamin C and lead + vitamin C treated rats. Different superscript letters differ significantly from each other at P<0.05.

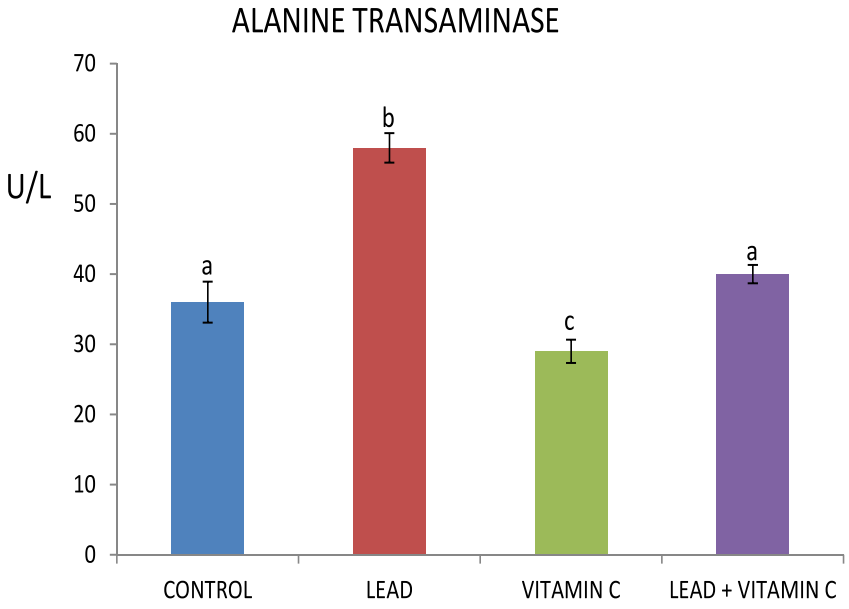

Figure 3: Serum alanine transaminase (ALT) activity in treated rats.

Serum alanine transaminase activity for control rats, lead, vitamin C and lead + vitamin C treated rats. Different superscript letters differ significantly from each other at $P<0.05$.

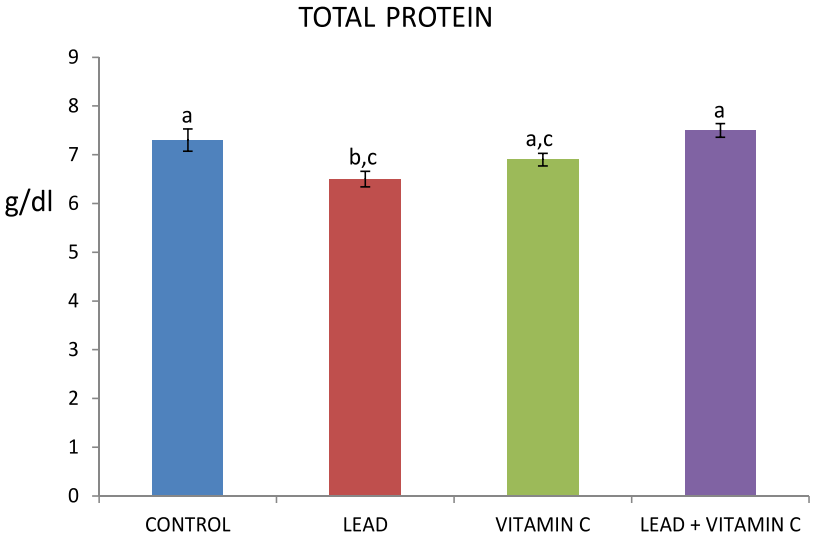

Figure 4: Serum total protein concentration in treated rats.

Serum total protein concentration for control rats, lead, vitamin C and lead + vitamin C treated rats. Different superscript letters differ significantly from each other at $P<0.05$.

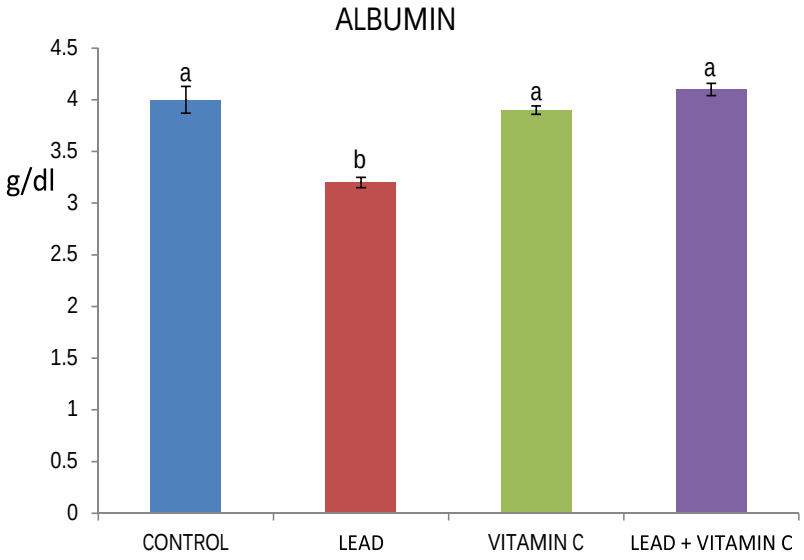

Figure 5: Serum albumin concentration in treated rats.

Serum albumin concentration for control rats, lead, vitamin C and lead + vitamin C treated rats. Different superscript letters differ significantly from each other at $P<0.05$.

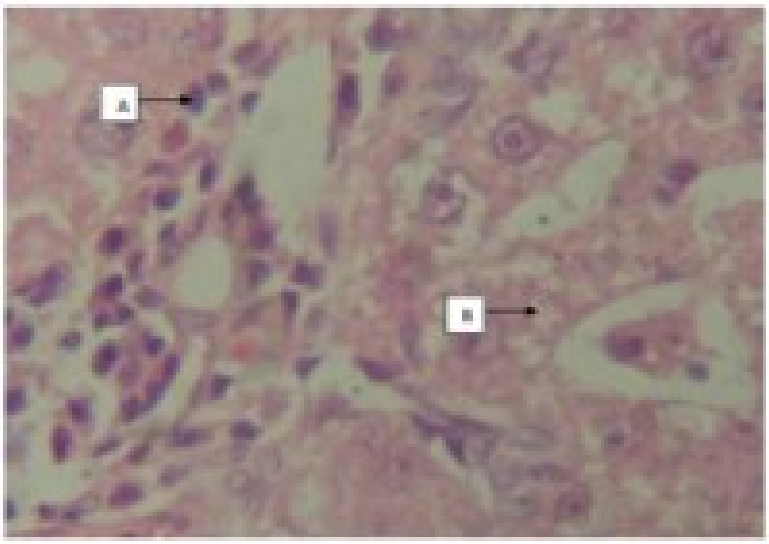

Plate 1: Photomicrograph of rat liver treated with 100mg/kg Lead for 21 days.

The treated liver showed moderate activation of Kupffer cells A and mild hepatocytes vacuolation B (H&E x 40).

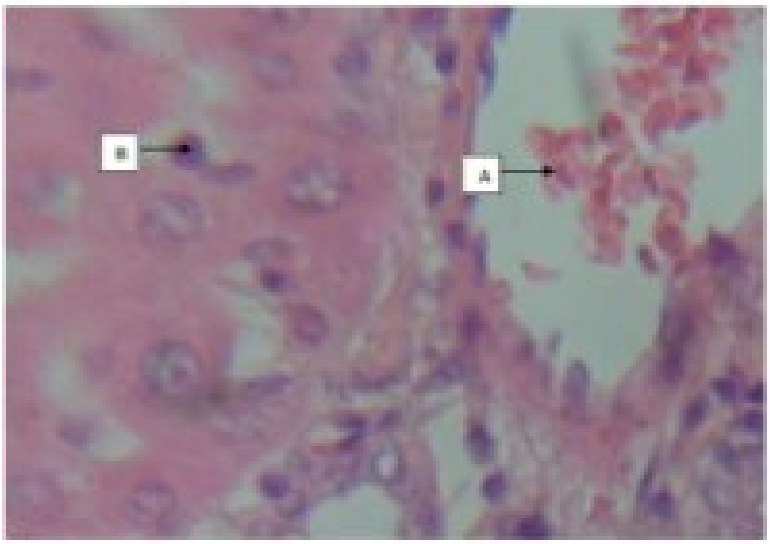

Plate 2: Photomicrograph of rat liver treated with 100mg/kg lead and 100mg/kg Vitamin C for 21 days.

The liver showed mild portal vascular congestion and dilation A and mild Kupffer cell activation B (H&E x 40)

DISCUSSION

The present study was carried out to investigate the ameliorative effects of vitamin C on hepatotoxicity and biochemical alterations in Wistar rats exposed to lead.

The results of the present study indicated that the enzymes of the liver function: serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were all elevated in the lead intoxicated animals. Previous study by Suleiman *et al.*,[14] also reported increased liver enzyme activities in lead toxicity in Wistar rats. The increase in liver enzymes by lead exposure could be due to the generation of reactive oxygen species (ROS), which caused oxidative damage to the cell membrane of the liver cells. Serum AST and ALT are sensitive markers in the diagnosis of hepatic damage as they are cytoplasmic enzymes released into circulation upon hepatic cellular damage[17].

However, serum ALT activity is more liver specific than AST[15]. Thus, the significant increase in serum alanine aminotransferase (ALT) activity observed in the rats administered lead was an indication of hepatic damage. The elevated AST and ALT activities indicated that prolonged exposure to lead might have deleterious effect on the hepatocyte.

The increase in serum alkaline phosphatase (ALP) activity observed in the study showed that administration of lead to the animals could also cause extrahepatic damage. There could have been a biliary cell membrane damage caused by lead toxicity thereby eliciting elevated serum ALP activity. Thus, increased ALP activity observed in the lead-treated group of rats corroborates with the previous findings which reported an increase in ALP activity in lead treated rats[14]. However, the high level of serum ALP activity could be due to increased permeability, damage, and/or necrosis of cells,[17] which; in the present study, might have been caused by lead toxicity.

Treatment with vitamin C caused a decrease in the activities of AST and ALT suggesting amelioration of hepatic damage. This showed that vitamin C a powerful reducing agent, protects the organs from damage caused by administration of lead by neutralizing free radicals generated. Vitamin C might have donated electrons to free radicals such as hydroxyl and superoxide radicals generated by lead toxicity[17] and thus, quenched their reactivities.

Administration of vitamin C to the study animals also decreased ALP activity thus mitigating the effect of lead in hepatotoxicity. This observation agrees with the work of Ajayi *et al.*,[18] which stated that vitamin C decrease ALP activity in lead treated rats. This can be as a result of the antioxidative action of vitamin C in the inactivation of reactive oxygen species by electron transfer and thus ameliorating hepatotoxicity.

Serum total protein and albumin levels of lead-treated rats were decreased. The decrease in serum total protein concentration was apparently due to hypoalbuminemia. Besides, albumin as an antioxidant[19] might have been used in the process of combating oxidative stress evoked by lead, thereby contributing to the low albumin concentration in the lead-treated group of animals.

Treatment with vitamin C has been shown by the present study to mitigate hypoproteinemia and hypoalbuminemia associated with exposure to lead. This amelioration of the hypoalbuminemia by vitamin C might be due to protection of the liver from oxidative damage induced by lead thereby encouraging albumin synthesis by the liver. Vitamin C, being an antioxidant, effectively neutralized the free radicals generated by lead.

Histologically, the liver of rats treated with lead showed hepatocyte vacuolation and moderate activation of Kupffer cells. The Kupffer cells activation was due to inflammation of the liver by lead exposure. Administration of vitamin C showed mild portal vascular congestion and dilatation, as well as reduced Kupffer cell activation corroborating with the report of Shalan *et al.*,[20] that treatment of lead–exposed animals with Vitamin C showed marked improvement of the biochemical, molecular and histopathological findings.

CONCLUSION

The present study indicated that vitamin C treatment ameliorated lead acetate-induced changes in hepato-chemical parameters. This could be due to its antioxidant nature, by donating electrons to free radicals and thus stabilizing them. The ameliorative effect of vitamin C was also confirmed by histological observations, which suggests that vitamin C can be effective in bringing about functional improvement of hepatocyte. Vitamin C may be given as a dietary supplement to human populations exposed to environmental toxicants like lead as it could provide protection against its toxic effects.

Conflict of interest

The authors state that there is no conflict of interest.

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