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Effect of Vitamin C on Histopathologic and Antioxidant Status of Lead-Induced Hepatotoxicity in Rats

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Abstract
Lead is a heavy metal that for decades has been known for its adverse effects on various body organs and systems such that functions are compromised. The present study was carried out to evaluate the oxidative status in Wistar rat's liver induced by lead acetate toxicity and also to investigate the ameliorative effects of vitamin C against lead poisoning. The rats (110-130g) were grouped into 4, 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 acetate. 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 acetate and 100 mg/kg of vitamin C for 21 days. These rats were also given rat chow and distilled water ad libitum. The biochemical analytes: malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were measured spectrophotometrically. Administration of lead acetate induced significant increase (P< 0.05) in the concentration of hepatic MDA while hepatic SOD and CAT were significantly decreased (P< 0.05). Histopathological changes were observed in the liver as hepatic vacuolation and moderate activation of Kupffer cells. Treatment with vitamin C reduced the hepatic MDA and increased SOD and CAT activities. These findings lead to the conclusion that vitamin C significantly decreased the adverse harmful effects of lead acetate exposure on the liver and has protective actions against lead induced histopathological changes in tissue tissue.

Keywords: Superoxide dismutase, Catalase, Malondiadehyde, Lead, Vitamin C, Histopathology

Introduction
General population may be exposed to lead through various sources such as dietary contamination (via food chain and lead released from food containers or ceramic glaze), public water supplies contamination, herbal remedies, paint, and manufacturing by-products such as manufacture of batteries, sheet lead, solder, brass, and bronze plumbing, radiation shields, circuit boards, and military equipment.[1]
Lead (Pb) is a toxic heavy metal and harmful even in small amounts[2]. Manifestations of lead poisoning in humans are nonspecific, which may include weight loss, anemia, nephropathy, infertility, liver, testis and heart damages[3] etc. Lead is known to produce oxidative damage in the liver tissues by enhancing peroxidation of membrane lipids, a deleterious process solely carried out by free radicals[4]. Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects[5]. Disruption of pro-oxidant/antioxidant balance might lead to tissue injury. It was reported that lead increased the level of lipid peroxides and altered the antioxidant defense system in the hepatic tissues[6]. A previous study confirmed the possible involvement of reactive oxygen species (ROS) in lead- induced toxicity[7].
The biochemical mechanism of lead toxicity has been proposed to be by oxidative stress induction[8]. Oxidative stress is described as a physiological stage in which antioxidant defense is inadequate to detoxify the reactive oxygen species (ROS). This oxidative process results in the damaging of essential biomolecules such as protein, lipid, and DNA. Overproduction of ROS has been demonstrated in lead-induced oxidative stress as previous experimental studies revealed that lead could promote ROS production in kidney, liver and cardiovascular tissues[9-10]. In addition, lead could also influence cell membrane alterations, such as lipid component, membrane integrity, permeability, and function, and finally leading to lipid peroxidation[11].
Vitamin C (ascorbic acid), a water soluble vitamin is derived from dietary sources such as citrus fruits, berries, cabbage, tomatoes and leafy vegetables. The therapeutic potential of vitamin C is as a result of its antioxidant effect on free-radicals[12]. The aim of the study was to evaluate the ameliorating effect of vitamin C on lead oxidative status of the liver by estimating malondialdehyde (MDA) levels, superoxide dismutase (SOD) and catalase (CAT) activities and histopathological changes in the liver tissue.

Materials and Method
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

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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 and Vitamin C were obtained from a reputable 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 control 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 vitamin C 100mg/kg body weight.
In all the groups, the route of administration of the treatments to the experimental animals was orogastric, ensuring maximum utilization of what was given and the treatments lasted 21 days. After the 21days duration of orogastric administration, the rats were sacrificed and liver tissues collected for oxidative status determination and also histological examination by staining with Haematoxylin and Eosin stain.

Measurement of Oxidative Status

Liver tissue was minced and homogenized (10% w/v) in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15mins. The supernatant was used for the biochemical assays.
Lipid peroxidation was estimated by measuring the malondialdehyde with Guttride and Wilkings[13] method. Superoxide dismutase activity was measured spectrophotometrically using Misra and Fridovich[14] method. Catalase activity was measured spectrophotometrically using the method described by Cohen et al.[15].

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

Malondialdehyde (MDA)

Fig 1 shows hepatic malondialdehyde concentration in control, lead, vitamin c, and lead + vitamin C treated rats. There was a significant increase (P<0.05) in hepatic MDA concentration in the lead-treated group (2.71±0.41) when compared to control (1.25±0.13). There was a significant difference (P<0.05) between lead + vitamin C treated group (2.02±0.24) and control group (1.25±0.13).

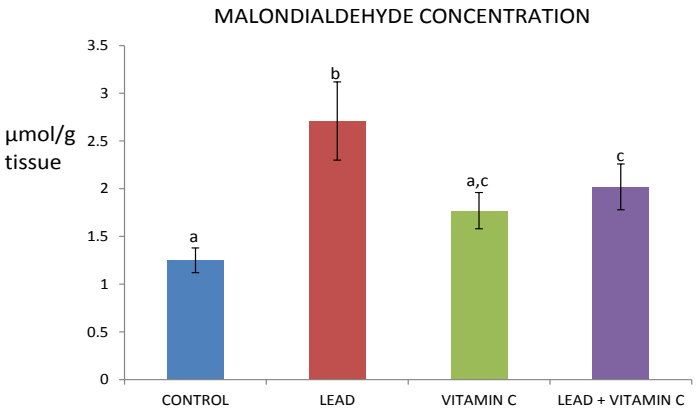

Fig 1: Hepatic malondialdehyde (MDA) concentration in treated rats.Hepatic malondialdehyde concentration for control rats, lead, vitamin C and lead + vitamin C treated rats. Different superscript letters differ significantly from each other at P<0.05.

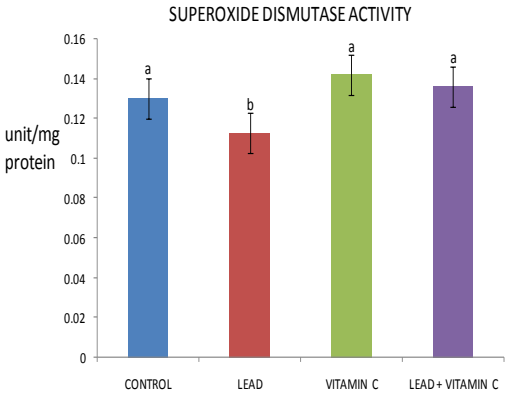

Fig 2: Hepatic superoxide dismutase (SOD) activity in treated rats. Hepatic SOD activity for control rats, lead, vitamin C and lead + vitamin C treated rats. Different superscript letters differ significantly from each other at P<0.05.

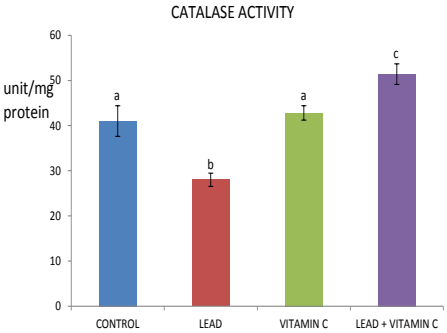

Fig 3: Hepatic catalase (CAT) activity in treated rats. Hepatic CAT activity for control rats, lead, vitamin C and lead + vitamin C treated rats. Different superscript letters differ significantly from each other at P<0.05.

Superoxide Dismutase (SOD) Activity

Fig 2 shows hepatic superoxide dismutase (SOD) activity in control, lead, vitamin C and lead + vitamin C treated rats. There was a significant decrease (P<0.05) in hepatic SOD activity in the lead-treated group (0.113±0.01) when compared to control (0.130±0.01), vitamin C (0.136±0.01) and lead + vitamin C treated (0.150±0.01) groups. There was no significant change (P>0.05) between the control group (0.130±0.01) and vitamin C (0.136±0.01) or lead + vitamin C treated (0.150±0.01) groups.

Hepatic Catalase Activity

Fig 3 shows hepatic catalase (CAT) activity in control, lead, vitamin C and lead + vitamin C treated rats. There was significant decrease (P<0.05) between lead (28.0±1.48) and control (41.0±3.39) or vitamin C treated (42.8±1.60) group. There was significant difference (P<0.05) between lead + vitamin C (51.4±2.29) treated group and control (41.0±3.39) and lead-treated (28.0±1.48) group.

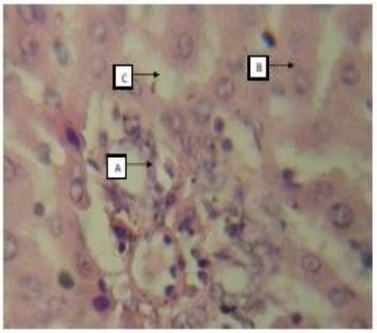

Plate 1: Control: Rat Liver showing portal triad A, hepatocytes B and sinusoids C (H&E x 40)

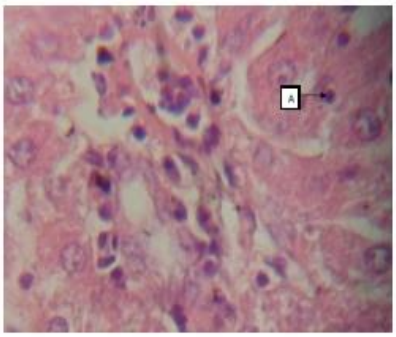

Plate 2: Rat Liver treated with 100mg/kg Vitamin C for 21 days showing moderate activation of kupffer cells A (H&E x 40)

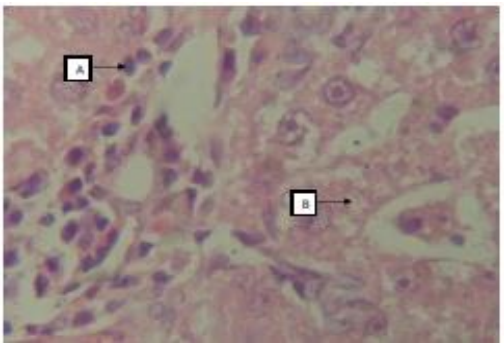

Plate 3: Rat Liver treated with 100mg/kg Lead for 21 days showing moderate activation of kupffer cells A and mild hepatocytes vacuolation B (H&E x 40)

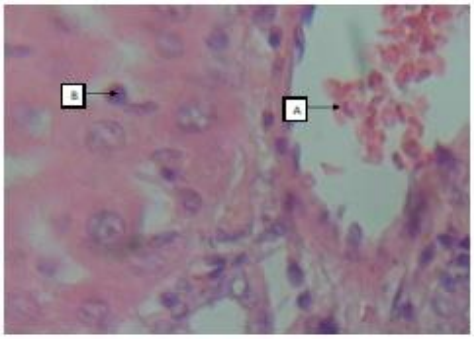

Plate 4: Rat Liver treated with 100mg/kg lead and 100mg/kg Vitamin C for 21 days showing mild portal vascular congestion and dilatation A and mild kupffer cell activation B (H&E x 40).

Discussion

Lead (Pb) has been widely known as a major environmental pollutant and many people that are exposed to it have suffered a lot of health problems such as nephropathy, neurological problems, coronary heart disease, gout, hepatotoxicity and anaemia[16]. It has been revealed that lead toxicity could lead to free radical damage via two separate mechanisms. These include: the generation of reactive oxygen species (ROS), including hydroperoxides and singlet oxygen and direct depletion of antioxidant reserves[17]. The effects of lead on liver of Wistar rats and the ameliorative effects of vitamin C were biochemically and histologically investigated.

The results obtained show decrease in the endogenous antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) caused by lead intoxication. Usually, the deleterious effects of oxidative stress are counteracted by SOD and CAT by mopping up the free radicals generated by oxidative process. Superoxide dismutase and catalase are metalloproteins accomplishing their antioxidant functions by enzymatically detoxifying superoxide and hydroxyl radicals. These antioxidant enzymes depend on various essential trace elements and prosthetic groups for proper molecular structure and enzymatic activity[18]. The pathogenesis of lead toxicity is multifactorial as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption and binds to sulfhydryl proteins (interrupting structural protein synthesis)[18]. Thus, activities of SOD and CAT antioxidants were reduced by lead administration thereby exposing the tissues to peroxidative damage. Administration of vitamin C however increases the activities of SOD and CAT and thus protects tissues from damage by oxidative stress. This study corroborates earlier reports by Shalan *et al.*[19] and Mohammad *et al.*[20]. The primary role of vitamin C is to neutralize free radicals, both inside and outside the cells. A free radical will seek out an electron to regain their stability. Vitamin C being an excellent source of electrons can donate electrons to free radicals such as hydroxyl and superoxide radicals and thus quench their reactivity[21].

Data obtained from the present study showed that administration of lead caused an increase in liver malondialdehyde (MDA) concentration. MDA is a biomarker of lipid oxidative damage and is a major product of peroxidized polyunsaturated fatty acids[22]. Lead has been shown to cause oxidative damage to tissues by the generation of reactive oxygen species such as superoxide, peroxide, and singlet oxygen[23-24]. The consequence of oxidative damage to the cell is the alteration of the cellular integrity and functions. These may have been partly responsible for the altered biochemical parameters and histology recorded in the lead-treated rats in the present study.

Treatment with vitamin C significantly decreased MDA concentration in the liver indicating its mitigation of the oxidative damage induced by lead. In line with other findings[25] vitamin C, a potent hydrophilic antioxidant has been shown to ameliorate the ravaging effect of free radicals induced by lead. Vitamin C is also known to complex with lead, thereby mitigating the toxicity induced by the heavy metal[26].

Histologically, the liver of rats treated with lead acetate showed hepatocyte vacuolation and moderate activation of Kupffer cells. The Kupffer cells activation could be due to inflammation of the liver by lead exposure. Treatment with vitamin C improved most of the histological alterations induced in lead acetate group. These findings corroborate the protective effect of vitamin C against the histological changes in lead acetate hepatotoxicity as reported by Shalan *et al.*[19].

Conclusion

The study has shown that administration of lead acetate causes an alteration in the oxidative status of the liver indicating damages due to peroxidation. Vitamin C administration ameliorates the biochemical and histological alterations caused by lead due to its antioxidative ability.

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