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# Effect of Exposure and Withdrawal on Lead-induced Toxicity and Oxidative Stress in Cardiac Tissues of Rats

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## Abstract

Lead poisoning continues to pose a serious health challenge and more significantly so in developing countries with ineffective waste disposal systems. Recent efforts at solving lead poisoning issues have seen entire towns being resettled from lead-contaminated areas. This study was designed to investigate whether withdrawal of lead exposure results in a resolution of toxic effects of lead in cardiac tissues. Adult male Wistar rats were exposed orally to lead acetate (PbA) at doses of 0.25, 0.5, and 1.0 mg/ml for 6-week duration, after which one-half was sacrificed and the remaining left for a further 6 weeks without lead treatment. Exposure of rats to PbA produced significant decline ($P < 0.05$) in the activities of antioxidant parameters, including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT), and reduced glutathione (GSH), whereas malondialdehyde (MDA) concentration was significantly elevated. Animals from the withdrawal period exhibited a similar pattern of alterations, with a significant ($P < 0.05$) reduction in GSH, GPx, and SOD and a significant elevation in MDA and $H_2O_2$ concentrations. However, GST activity was elevated, whereas CAT activity remained unaltered in the withdrawal period. The results of this study showed that cardiotoxicity indicated by induction of oxidative stress and reduction in antioxidant parameters failed to resolve upon withdrawal of lead exposure in male rats during the period of study.

**Keywords:** Antioxidants, cardiotoxicity, lead acetate, oxidative stress, rats

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## 1. Introduction

Lead (Latin: *Plumbum*, Mass no. 82, Atomic no. 207.2) is a soft and malleable, bluish-white colored metal which has been industrially used by man for over 6000 years, as far back as the times of the Romans, Egyptians, and Babylonians.[1] As a result of its widespread use in industries, lead is consequently widely distributed in the environment.[2,3] Lead is not known to have any beneficial function in the body rather it exerts a variety of toxic effects on the biological system.[4,5] Neurologic, gastroenteric, cardiovascular, hematological, and renal dysfunctions have been reported with lead intoxication.[6-9] Lead has the potential to induce oxidative stress, and there is ample scientific evidence to corroborate this fact.[4,10,11] In both animal and cultured vascular cell studies, exposure to lead resulted in oxidative stress and nuclear factor kappa B (NFkB) activation, a general transcription factor for numerous cytokines, chemokines, and adhesion molecules.[12] Therefore, the consequence of NFkB activation is inflammation and eventual cardiac remodeling.[13] Exposure to lead may also initiate inflammation through the release of reactive oxygen species (ROS) by activated immune cells thus;

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lead may cause a perpetual cycle of oxidative stress and inflammation.[12,14] Lead-induced oxidative stress is achieved through the generation of ROS as well as the downregulation of the antioxidant cell defense systems through the interference with some essential metals needed for antioxidant enzyme activity, inhibition of sulfhydryl-dependent enzymes, depletion of glutathione, and/or by increasing the susceptibility of the cell to oxidative attack through the alteration of cell's membrane integrity and fatty acid composition.[11,15,16]

Globally, cardiovascular dysfunction is a leading cause of mortality and a contributor to the burden of disease. Recently, the global incidence of lead associated toxicity has risen significantly.[17-19] Studies carried out on hypertensive patients have revealed a positive correlation between exposed people and high blood pressure.[20,21] Myocarditis, arteriosclerosis, altered heart rate activity, and an increased risk for the development of ischemic heart disease have all been reported with lead exposure.[22] Increased bone concentration of lead has been associated with electrocardiographic abnormalities such as prolonged corrected QT interval and QRS complex and conduction defects.[23] Currently approved treatment methods for lead poisoning include the administration of chelating agents such as meso-2, 3-dimercaptosuccinic acid, and monoisoamyl; hence, these chelating agents form insoluble complexes with lead and reduce its ability to exert any effect on biological targets.[24] These drugs, however, are potentially toxic and are sometimes unable to be removed completely from the system.[25] These drugs are hydrophilic and thus cannot cross the cell membrane to the intercellular space.[26,27] Lead is, therefore, an important environmental toxicant that must be given greater attention especially because of the effects on the cardiovascular system.

This study was carried out to evaluate the possible effect of withdrawal on lead-induced cardiotoxicity and oxidative stress in Wistar rats.

## 2. Materials and Methods

### 2.1 Animal treatment

In this study, 56 adult male rats weighing between 200 and 290 g were used. They were obtained and housed in the Experimental Animal facility of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. After a period of acclimatization for 2 weeks, they were randomly divided into four groups of 14 animals per group. The animals were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow *ad libitum* and liberally supplied with water.

The control group (Group I) received normal saline while Groups II, III, and IV received 0.25, 0.5, and 1.0 mg/ml of lead acetate (PbA), respectively, in drinking water for 6 weeks as described in our previous studies.[7,8] At the end of 6 weeks of treatment, half of the population of rats was sacrificed, and PbA was withdrawn from the remaining rats for another 6 weeks. All of the animals received humane care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments.[28]

### 2.2 Preparation of postmicrosomal fractions

Rats were starved overnight and sacrificed by cervical dislocation. The hearts were removed, rinsed in 1.15% KCl and homogenized in aqueous potassium phosphate buffer (0.1 M, pH 7.4) and homogenates were centrifuged at 10,000 g for 10 min to obtain the postmicrosomal fractions.

### 2.3 Biochemical assays

The activity of catalase (CAT) in cardiac tissue was determined spectrophotometrically as earlier described.[29] Superoxide dismutase (SOD) was also determined spectrophotometrically as described with modification from our laboratory.[7,8,30] Glutathione-S-transferase (GST) was estimated by the method of Habig *et al.*[31] Protein concentration was determined by the method of Lowry *et al.*[32] reduced glutathione (GSH) was determined at 412 nm wavelength using the method described by Jollow *et al.*[33] Hydrogen peroxide generation was determined as described by Woff.[34] The malondialdehyde (MDA) level was measured according to the method of Varshney and Kale.[35] Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5$/M/Cm. Glutathione peroxidase (GPx) activity was measured as described by Buetler *et al.*[36]

# 3. Results

## 3.1 Effect of lead on markers of oxidative stress

The effects of lead exposure for 6 weeks and subsequent withdrawal on the myocardial content of markers of oxidative stress are presented in Figure 1. There was a significant increase ($P < 0.05$) in the myocardial content of GSH of rats in Groups III and IV (0.5 and 1.0 mg/ml of PbA while Group II (0.25 mg/ml of PbA) rats had a significant decrease in myocardial GSH content. A statistically significant ($P < 0.05$) increase in MDA and hydrogen peroxide $H_2O_2$ content was observed in all the treatment Groups (0.25, 0.5, and 1.0 mg/ml of PbA, respectively.

## 3.2 Effect of lead on antioxidant enzymes following exposure and withdrawal

Antioxidant enzymes CAT and GPx were significantly ($P < 0.05$) reduced in Group II (0.25 mg/ml of PbA) rats while there was a significant ($P < 0.05$) increase in the activities of CAT, SOD, GPx, and GST in Groups II and III (0.25 and 0.5 mg/ml of PbA) as shown in Figure 2. The increase in the activities of these antioxidant enzymes indicated an adaptive response to oxidative stress induced by administration of lead. These findings were not ameliorated after 6 weeks of withdrawal.

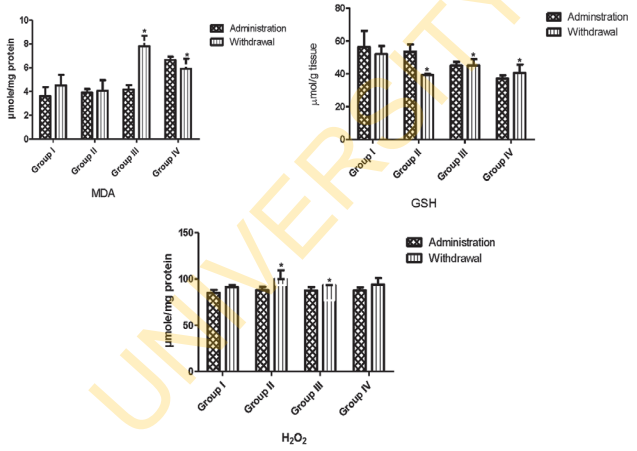


**Figure 1.** Myocardial content of oxidative stress markers after administration and subsequent withdrawal of lead acetate exposure. Values are presented as mean ± standard deviation, *indicates significant difference at P < 0.05 $H_2O_2$ (hydrogen peroxide generation. MDA: Malondialdehyde, GSH: Reduced glutathione.

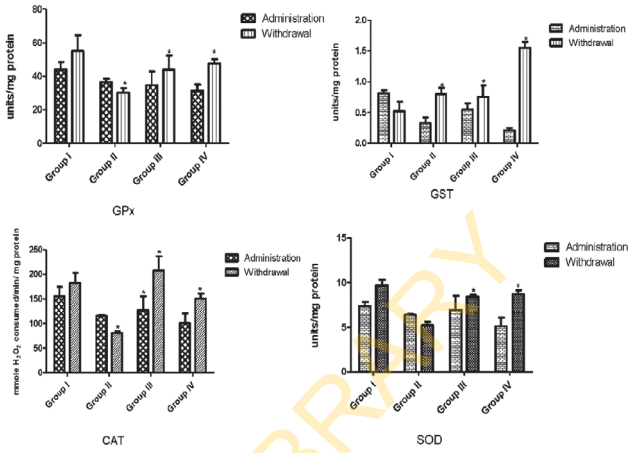


**Figure 2.** Myocardial enzymic antioxidant activity after administration withdrawal of lead acetate exposure. Values are presented as mean ± standard deviation, *indicates significant difference at P < 0.05. GPx: Glutathione peroxidase (units/mg protein), catalase: mmole $H_2O_2$ consumed/min/mg protein), SOD: Superoxide dismutase; units/mg protein), GST: Glutathione S-transferase; mmole CDNB-GSH complex formed/min/mg protein).

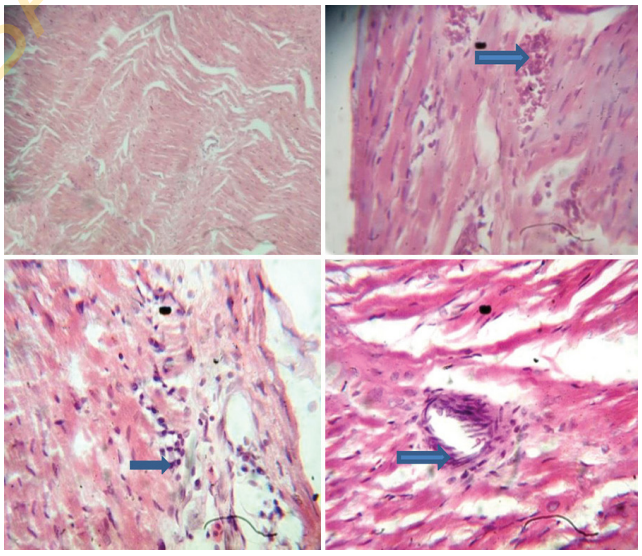

**Figure 3.** Photomicrograph of hematoxylin and eosin stain of cardiac tissue section of rats exposed to lead acetate. Control group (a) no visible lesion. Group (b-d) treated with lead showed cardiomyocytes with focal areas of moderate congestion of vessels (arrows) as well as infiltration by inflammatory cells, focal area of necrosis, following 6 weeks of lead acetate exposure (H and E, ×100).

## 3.3 Histological evaluation following exposure and withdrawal of lead

Histological evaluation showed cardiomyocytes with focal

areas of moderate congestion of vessels, infiltration by inflammatory cells, and focal area of necrosis, following 6 weeks of PbA exposure. A similar picture was observed after 6 weeks of withdrawal of lead [Figures 3 and 4].

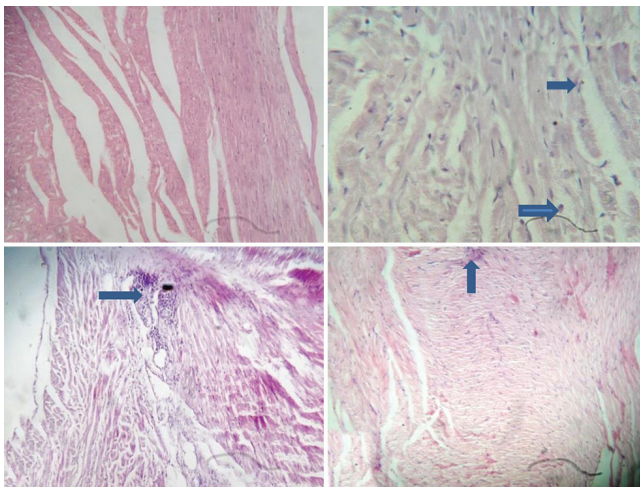

**Figure 4.** Photomicrograph of haematoxylin and eosin stain of cardiac tissue section of rats recovering from lead acetate. Control group (a) no visible lesion. Group (b-d) treated with lead showed cardiomyocytes with focal areas of moderate congestion of vessels (arrows) as well as infiltration by inflammatory cells, focal area of necrosis, following 6 weeks of lead acetate exposure (H and E, ×100).

## 4. Discussion

Lead exposure has been associated with heart disease, and positive correlation occurs between lead exposure and cardiovascular disease.[37] Some of these adverse cardiovascular events have been reported even in low blood lead concentrations.[12] There is ample scientific evidence linking oxidative stress with the pathophysiologic mechanism of lead-induced toxicity.[4,9,10,11] Predominantly, lead toxicity manifests in oxidative damage as a result of the generation of ROS as well as the direct depletion of the antioxidant defense.[38-40]

In our study, exposure to lead caused a statistically significant ($P < 0.05$) increase in markers of oxidative stress. Exposure of rats to PbA in drinking water for a period of 6 weeks produced a significant decline ($P < 0.05$) in the activities of antioxidant markers studied such as GPx, GST, CAT, SOD, and GSH whereas MDA concentration as a marker of oxidative stress was significantly elevated. With the withdrawal of PbA for a period of 6 weeks, a similar pattern of alterations was observed, with a significant ($P < 0.05$) reduction in GSH, GPx, and SOD and a significant elevation in MDA and $H_2O_2$ concentrations. However, GST activity was elevated, while CAT activity remained unchanged in the withdrawal period. These findings clearly indicate an induction of oxidative stress during the period of lead exposure in myocardial tissues. The elevation of MDA levels pointed to enhanced lipid peroxidation which may be due to the production of superoxide, peroxyl, and hydroxyl radicals.[41] This imbalance can cause damage to biomolecules such as DNA, proteins and lipids hence exposure to lead can cause changes in antioxidant enzyme levels in a dose-response relationship.[42] The elevation of MDA in myocardial tissues may lead to significant pathology and subsequent cardiovascular dysfunction. The heart is believed to be highly susceptible to oxidative damage because of its low total reactive antioxidant potential.[43] In this study, exposure to PbA caused a significant ($P < 0.05$) reduction in the levels of SOD. This finding is in agreement with some earlier reports on the effect of lead-induced oxidative stress.[4,7,8] SOD, like CAT, is a metalloprotein which enzymatically accomplishes its antioxidant activities by the detoxification of $H_2O_2$. Lead can interact with copper and zinc, which are both cofactors required for the activity of SOD. This interaction results in a reduced activity of SOD.[4] In this study, there was an increase in $H_2O_2$ generation which is another pointer to the occurrence of oxidative stress. This increase in $H_2O_2$ could have been due to a reduction in antioxidant enzymes CAT, GPx, and GST observed in this study.

The administration of PbA caused a statistically significant reduction in the activity of GSH, a tripeptide which plays a vital role in cytoprotection against oxidative stress. Lead interferes and decreases the antioxidant activity of GSH by binding exclusively to its-SH group.[44] In this study, exposure to lead caused histological abnormalities such as focal areas of moderate congestion of vessels as well as infiltration by inflammatory cells and focal area of necrosis. This pattern was observed both at the exposure and withdrawal phases of this study. Hence, withdrawal of lead for a period of 6 weeks did not result in a reversal of abnormalities recorded.

Failure to achieve a recovery in the parameters studied after 6 weeks of exposure and eventual withdrawal might have been due to the fact that lead is stored in bone where it persists for a long time with the half-life ranging from years to decades.[45-47] Other cardiovascular complications which

may arise from the underlying oxidative stress initiated by exposure to lead include hypertension, atherosclerosis, reduced coronary blood flow, thrombosis, and vascular smooth muscle cell proliferation.[12] The exposure to PbA for 6 weeks resulted in histomorphological abnormalities as well as a significant dose-dependent disruption of the antioxidant defence system in the cardiac tissues of rats. This abnormality was not reversed by withdrawal for another 6 weeks.

## 5. Acknowledgments

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