

RESEARCH ARTICLE

Haematological and antioxidant enzyme response to Lead toxicity in male Wistar rats

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Abstract: The study evaluated the haematological and some antioxidant enzymes response to lead toxicity in male Wistar rats. Twenty male Wistar rats were divided into four groups *viz.*, A, B, C and D. Group A served as the control while groups B, C and D were treated with 200, 300 and 400 ppm of lead (Pb) as lead acetate, respectively. Doses were orally administered in divided doses by intubation to ensure that each rat had the specified doses, after which they have access to water and feed. At the end of two weeks of treatment, blood samples were collected via the median canthus into heparinised tubes for blood lead determination and haematological analysis after which the remaining blood was centrifuged to obtain the plasma for determination of malonydialdehyde, catalase, superoxide dismutase and peroxidase levels. There was a significant increase in blood lead concentrations ranging from 2.15 ± 0.10 $\mu\text{g/dl}$ to 9.21 ± 0.05 $\mu\text{g/dl}$ which was dose dependent while decreases in packed cell volume and the red blood cell counts ranging from 32-53% and 15-52%, respectively. At the highest dose of 400 ppm of lead there was significant decrease in the neutrophils and lymphocytes. There was significant ($P < 0.05$) dose dependent increases in malondialdehyde while the activities of catalase, peroxidase and superoxide dismutase were significantly ($P < 0.05$) reduced. In conclusion, lead disrupts the haematological system leading to generation of free radicals that overwhelm the antioxidant enzymes thus leading to oxidative stress.

Keywords: Haematological, malonydialdehyde oxidative stress, free radicals.

INTRODUCTION

Lead poisoning is one of the intoxications most frequently found in the environment, mainly due to anthropogenic activities. Lead is a heavy metal with an evidence of toxicity with widely recognized ubiquitous, long-lived and pervasive

environmental and industrial toxicant throughout the world (Ademuyiwa *et al.*, 2007; Spivey, 1978).

Lead has been found to produce a wide range of toxic-biochemical effects involving several organs, systems and biochemical activities (Ademuyiwa *et al.*, 2002; Sakai, 2000). Neurobehavioral, haematologic, nephrotoxic and reproductive effects of lead have been observed in humans and other animals (Goyer, 1991; Needleman *et al.*, 1990).

The alteration of the haem synthesis is an early effect associated with increased lead concentration in soft tissues. Inhibition of delta aminolevulinic acid dehydratase (ALAD) and elevation of protoporphyrin in erythrocytes are the earliest effects, followed by increased delta aminolevulinic acid (ALA) and coproporphyrin excretion in urine (Makino *et al.*, 2000; Sakai, 2000).

Lipid peroxidation, as evidenced by increased malondialdehyde content as well as alterations in membrane integrity and fatty acid composition, have been observed in red blood cells of animals and humans exposed to lead (Gurer *et al.*, 1999; Osterode and Ulberth, 2000). Lead produces oxidative damage in the liver and kidney as evidenced by enhancing lipid peroxidation (Shafiq-ur-Rehman, 1984; Somashekaraiah *et al.*, 1992). Liver plays a major role in lead metabolism and it is therefore in special risk due to its oxidative action, given the unquestionable evidences that lead-induced lipid peroxidation of cellular membranes play a crucial role in the mechanism of hepatocellular damage (Sivaprasad *et al.*, 2004).

In vivo and *in vitro* studies suggest that lipid metabolism is altered both in acute and chronic exposure to lead (Ademuyiwa *et al.*, 2009). Reports have indicated that antioxidant enzymes play a crucial role in the protection of cells against oxidative stress caused by lead toxicity (Sivaprasad *et al.*, 2004; Ozturk *et al.*, 2001). For these reasons, we assayed for antioxidant enzymes *viz.*, catalase, superoxide dismutase, peroxidase in addition to product of lipid peroxidation, malondialdehyde in male Wistar rats exposed to graded concentrations of lead acetate to elucidate the responses of these scavenging enzymes to the effects of lead toxicity.

MATERIALS AND METHODS

Experimental animals

A total of twenty male Wistar rats were used for this study. The average weight of the rats was 152 ± 3.5 g. They were provided with laboratory animal feed (Fat/oil 6%, Crude fibre 5%, Calcium 1%, Available phosphorus 0.4%, Lysine 0.85%, Methionine 0.35%, Salt 0.3%, Crude protein 18%, Metabolisable Energy 2900 Kcal.kg⁻¹, (TOPFEEDS®, Lagos, Nigeria) and water *ad libitum*. Experimental animals were acclimatized to their housing environment one month before the start of the experiment.

Animal ethics

All experimental protocols carried out on the animals were in accordance with the internationally accepted principles for laboratory animal use and were approved by the Ethics Committee on Laboratory Animal Use of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria.

Administration of lead acetate

The animals were randomly divided into four groups consisting of five animals each. They were dosed with lead acetate orally for a period of two weeks. The treatments were as follows:

Group A: served as the control and was given distilled water.

Group B: was given 200 ppm (parts per million) of lead as lead acetate.

Group C: was given 300 ppm of lead as lead acetate.

Group D: was given 400 ppm of lead as lead acetate.

Lead acetate, (CH₃COO)₂Pb.3H₂O, Assay (ex Pb) 99-103%, maximum limits of Impurities, Chloride (Cl) 0.005%, Copper (Cu) 0.002% (Cartivalues, England).

Collection of blood samples

At the end of two weeks treatment, blood samples were collected via the ocular *median canthus* using heparinized capillary tubes into heparinized tubes. Packed cell volume, red blood cell and white blood cell count were determined as described by Frankel and Reitman, (1963), while plasma was separated from whole blood samples as described by Schalm *et al.*, (1975).

Plasma preparation

The blood samples were centrifuged at 4000 rpm for 10 minutes to separate the plasma from the red blood cells (erythrocytes). The plasma was then removed and stored in Eppendorf tubes for further analyses.

Blood lead determination

Lead was analyzed in whole blood using Atomic Absorption Spectrometry (Buck Scientific AAS model 200, Connecticut, USA).

Estimation of lipid peroxidation

Product of lipid peroxidation, malondialdehyde (MDA) was estimated according to the method described by Buege and Aust (1978).

Enzymatic assay of antioxidant enzymes

Plasma catalase was assayed as described by Sinha (1972) while superoxide dismutase was assayed as described by Zou *et al.* (1986). Peroxidase was assayed as described by Sharon (1966).

Statistical analysis

Results were expressed as mean \pm SEM. Analysis of the data was done using one-way analysis of variance followed by the Duncan multiple range post hoc test. P value <0.05 were considered significant. All analyses were done using Statistical Package for Social Sciences version 16.

RESULTS

Table 1 shows the blood lead concentrations, packed cell volume, red blood cells and white blood cells counts of male Wistar rats treated with Pb for a period of two weeks. There were significant increases ($P<0.05$) in blood Pb concentrations of treated rats compared to the control group and was dose dependent. Highest blood lead concentration was recorded at 400 ppm treatment which was about 4.3 times of that of the control group. The packed cell volume and red blood cell count revealed a dose dependent significant decrease ($P<0.05$). The decrease in packed cell volume and red blood cell count ranges from 32-53% and 15-52% compared to the control group respectively. Though there was decrease in the white blood cell count, it was however only at 400 ppm treatment that a significant decrease ($P<0.05$) of about 35% was observed.

Table 2 shows the neutrophil, basophil, eosinophil, lymphocyte and monocyte counts of

male Wistar rats treated with Pb for a period of two weeks. There was no significant change in eosinophil, basophil and monocyte counts of treated rats compared to the control group. However, at higher doses of 300 ppm and 400 ppm of Pb a significant decrease ($P<0.05$) was observed in the neutrophil counts while significant increase ($P<0.05$) was observed in the lymphocyte counts.

Table 3 shows the mean concentration of malondialdehyde and activities of catalase, peroxidase and superoxide dismutase of male Wistar rats treated with Pb for a period of two weeks. There was significant increase ($P<0.05$) in the concentration of plasma malondialdehyde which was dose dependent, while there were significant decreases ($P<0.05$) in the activities of catalase, peroxidase and superoxide dismutase in rats treated with lead. These decreases were dose dependent as well.

Table 1: The blood lead (Pb) concentrations, packed cell volume (PCV), red blood cell counts (RBC) and white blood cell counts (WBC) of male Wistar rats treated with lead for a period of two weeks (mean \pm SEM).

Treatments	Pb ($\mu\text{g}/\text{dl}$)	PCV (%)	RBC Count ($10^{12}/\text{L}$)	WBC Count ($10^9/\text{L}$)
Control	2.15 \pm 0.10 ^a	43.00 \pm 3.22 ^b	5.94 \pm 0.23 ^c	10.30 \pm 0.26 ^b
200 ppm	4.01 \pm 0.21 ^b	29.33 \pm 2.33 ^a	5.05 \pm 0.79 ^c	9.32 \pm 0.29 ^b
300 ppm	7.11 \pm 0.27 ^c	26.33 \pm 1.84 ^a	4.26 \pm 0.13 ^b	8.88 \pm 0.55 ^b
400 ppm	9.21 \pm 0.05 ^c	20.00 \pm 2.71 ^a	2.84 \pm 0.50 ^a	6.65 \pm 0.44 ^a

Values within the same column with different superscripts are significantly different at $p<0.05$

Table 2: The mean differential leucocyte counts of male Wistar rats treated with lead for a period of two weeks (mean \pm SEM).

Treatments	Neutrophil (%)	Eosinophil (%)	Basophil (%)	Monocyte (%)	Lymphocyte (%)
Control	56.67 \pm 3.18 ^b	1.33 \pm 0.33 ^a	1.00 \pm 0.58 ^a	1.00 \pm 0.58 ^a	40.00 \pm 3.06 ^a
200 ppm	54.00 \pm 3.06 ^b	1.00 \pm 0.58 ^a	0.67 \pm 0.33 ^a	0.67 \pm 0.23 ^a	38.66 \pm 4.38 ^a
300 ppm	44.50 \pm 4.18 ^a	1.00 \pm 0.58 ^a	0.67 \pm 0.33 ^a	0.33 \pm 0.01 ^a	24.50 \pm 0.88 ^b
400 ppm	40.00 \pm 1.16 ^a	0.67 \pm 0.21 ^a	0.67 \pm 0.33 ^a	0.33 \pm 0.01 ^a	22.33 \pm 1.16 ^b

Values within the same column with different superscripts are significantly different at $p<0.05$.

Table 3: The mean concentration of malondialdehyde and activities of antioxidant enzymes of male Wistar rats treated with lead for a period of two weeks (mean±SEM).

Treatments	Malondialdehyde (nmol/ml)	Catalase (µmol/min)	Peroxidase (units/ml enzyme)	Superoxide dismutase (units)
Control	13.20±2.87 ^a	0.83±0.16 ^b	0.52±0.07 ^b	33.12±7.61 ^a
200 ppm	15.04±2.01 ^b	0.49±0.18 ^a	0.50±0.09 ^b	28.04±7.56 ^b
300 ppm	19.22±2.11 ^c	0.43±0.15 ^a	0.25±0.11 ^a	22.83±5.61 ^b
400 ppm	21.54±3.12 ^c	0.31±0.16 ^a	0.22±0.08 ^a	12.96±2.48 ^b

Values within the same column with different superscripts are significantly different at $p < 0.05$.

DISCUSSION

Lead is not known to serve any necessary biological function within the body and its presence in the body may lead to toxic effects. It has been recognized as a major heavy metal pollutant in some areas, because of its wide distribution in the environment. It is one of the most pervasive heavy metals contaminant with wide spread industrial and domestic applications. It is a poison to living organisms, with negative effects on general health, reproduction and behavior which could result in death (Finkel *et al.*, 1983). Blood or blood constituents are the best indicators of internal exposure of an individual to lead. The result in table 1 shows an increase in the blood lead concentrations which was consistent with earlier findings (Okedirani *et al.*, 2010; Moussa and Bashandy, 2008) where they showed that absorbed lead following oral ingestion is carried via blood to soft tissues and 95% of blood lead is transported on the erythrocyte as lead diphosphate. Accumulation of lead produces damaging effects in the haematological, haematic, renal and gastrointestinal systems (Correia *et al.*, 2000). The toxicity depends on its chemical form, the route, frequency and duration of administration. The lower packed cell volume coupled with reduction in red blood cell count observed in this study revealed the presence of anaemia. Lead-induced anaemia could be due to the interference of lead with haem biosynthesis through inhibition of δ -aminolevulinic acid dehydratase and ferrochelatase activities and by also decreasing erythrocyte survival (Suleiman *et al.*, 2010). The interference of lead with the development of haematopoietic progenitor and alteration of production of renal erythropoietin are increasingly linked with anaemia in lead

poisoning (Osterode *et al.*, 1999). Similarly, increased plasma malondialdehyde (MDA) concentration indicating membrane lipoperoxidation which was recorded in the present study suggest an increased oxidative damage to the erythrocyte membranes, which has been associated with increased red blood cell fragility (Suleiman *et al.*, 2010).

Analysis of total leucocyte and differential leucocyte counts revealed dose dependent leucopenia and lymphopenia in higher dose groups of 300 ppm and 400 ppm. This might be due to direct toxic action of Pb on leucopoiesis in lymphoid organs. Decrease in total leucocyte count is directly related with either decreased production from germinal center of lymphoid organs or increased lysis due to presence of Pb in the body (Avadheshkumar, 1998). Repeated exposure to lead has been demonstrated to induce lymphopenic leucopenia (Hashem and El-Sharkawy, 2009). The leucopenia observed in this study also shows the level of stress being experienced by the rats as a result of exposure to the Pb thus inducing oxidative damage to the leucocytes. Malondialdehyde is a well established biomarker of cellular injury and is used as an indicator of oxidative stress in cells and tissues (Kihc *et al.*, 2003). Oxidative stress and enhanced lipid peroxidation have been associated with several models of liver and tissue injury (Panazzo *et al.*, 1995)

Dose dependent increase in plasma malondialdehyde (MDA) concentration was observed in this study. This is an indication of increased lipid peroxidation in the lead treated groups compared to the control. The increased malondialdehyde was produced at a higher rate than what the antioxidant enzymes could cope with thus overwhelming the defense system. This

increased lipoperoxidative changes compromises the structural integrity of the cellular membranes. Levels of MDA were significantly increased in lead treated groups, this could be due to decrease activity of the defense system protecting tissues from free radicals damage. The presence of unsaturated fatty acids in cell membrane makes the membrane susceptible to peroxidation. The peroxidation of cell membrane leads to the impairment in its semi-permeability and triggers the series of reactions that may result in cell death (Comporti, 1993; Gutteridge, 1993). Many heavy metals including lead are known to induce over production of reactive oxygen species (ROS) or free radicals and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acids content of membranes (Maleeka et al., 2001). Lead has been reported to enhance the production of ROS in a variety of cells resulting in oxidative stress (Xienna et al., 2000). ROS are the byproducts of many degenerative reactions in many tissues, which affect regular metabolism by damaging the cellular components (Foyer and Noctor, 2002). Extensive study on oxidative stress has demonstrated that exposure of cells to adverse environmental conditions can induce the over production of ROS, such as superoxide radicals, (O_2^-), H_2O_2 and hydroxyl radicals (OH^\cdot) in cells (Wise and Neylor, 1987). In addition, reactive oxygen species are highly reactive to membrane lipids, proteins and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage (Aly and El-Beltagi, 2010; El-Beltagi et al., 2011). There are several intracellular defense mechanisms to prevent the potential oxidative damage. These defense systems are classified as enzymatic and non-enzymatic. The enzymatic defense systems include the antioxidant enzymes. These enzymes protect the cell membrane against peroxidation by converting the reactive compounds to less harmful or harmless metabolites. The determinations of MDA concentrations and antioxidant enzymes activities are the major criteria concerning the severity of possible peroxidation, which takes place in cell membrane (Aydemir et al., 2000; Gokhan et al., 2004; Ozturk-Ureket et al., 2001). The decrease in activities of these antioxidant enzymes proved that lead indeed induced generation of reactive oxygen species/free radicals leading to increase production of MDA which was much higher than the level which could be compensated by the cellular defense

systems, thus these compounds may not be converted to less harmful or ineffective metabolites at the sufficient levels. The increase in MDA which was dose dependent could be attributed to lead acetate ingestion causing alteration in redox status as indicated by a decrease in enzymatic antioxidant status which may be produced by damage in red blood cells (Seddik et al., 2010). Antioxidant enzymes were affected by higher doses of lead, heavy metals induced toxicity through the depletion of antioxidant enzymes resulting in enhanced production of reactive oxygen species such as peroxide ion, hydroxyl radicals and hydrogen peroxide, and these reactive oxygen species increased lipids peroxidation and cell membrane damage (El-Beltagi and Mohammed, 2010; Zhang et al., 2007).

These results further suggest or confirm earlier findings, the involvement of free radicals in the pathogenesis of lead poisoning. Lead is a protoplasmic poison which can cause damages to many organic bodies. There is little doubt that haematological parameters are seriously affected due to free radicals generated as a result of lead treatment thus reducing the activities of the antioxidant enzymes. Lead also caused disturbances in the body metabolism as well as oxidative-antioxidative balance in different tissues and plasma. These results suggest that the harmful effects observed in this study were produced by a dose dependent lead toxicant.

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