

Full Length Research Paper

Lead induced dyslipidemia: The comparative effects of ascorbate and chelation therapy

Regina Ngozi Ugbaja*, Beno Okechukwu Onunkwor and Demilade Akinbola Omoniyi

Department of Biochemistry, College of Natural Sciences, Federal University of Agriculture, Abeokuta, Nigeria.

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To investigate the comparative effects of ascorbate and chelating agents on some markers of lipid metabolism in lead exposed rats, 35 male Wistar rats were used. They were grouped randomly into five (n=7); 28 of which were administered 75 mg/kg body weight lead acetate (PbAc) orally for 14 days after which their blood samples were assayed for lead. Three of the groups were further administered 30 mg/kg body weight D-penicillamine (D-pen), 30 mg/kg body weight succimer (DMSA) and 500 mg/kg body weight ascorbate (Asc) daily orally, respectively. The control group was however administered normal saline. The blood lipid profiles were determined spectrophotometrically. Lead exposure resulted in significant dyslipidemia ($p < 0.05$), characterized by 50% hypercholesterolemia and hypertriglyceridemia and 132% hyperphospholipidemia (plasma) while in the red blood cells, hypocholesterolemia and hypophospholipidemia were observed. During the therapeutic doses, the groups administered chelating agents and Asc showed a significant amelioration in the plasma and red blood cell levels of total cholesterol, triacylglycerols and phospholipids in the order, DMSA > Asc > D-pen. Decrease in blood lead levels after therapy indicated that the chelating agents have an advantage over Asc. The study indicates that administration of the antioxidant, Asc may not be more efficacious than the chelating agents but could be a cheaper and more convenient therapy for lead toxicity.

Key words: Ascorbate, chelating agents, dyslipidemia, lead exposure, plumbism.

INTRODUCTION

Metals are elements found in nature usually in the form of their respective compounds. They have been found to have much relevance in many industries; but as economic as most of them are, a couple of them also have adverse effects on man (Patil et al., 2006; Ponce-Canchihuaman et al., 2010; Rao et al., 2007). Most notably, are the heavy metals which have specific density greater than 5 g/cm³ such as mercury (Hg), cadmium (Cd), arsenic (As) and lead (Pb). Most of these heavy metals persist in the environment and produce a variety of adverse effects because they are generally not biodegradable (Alissa and Ferns, 2011).

One of the leading metallic xenobiotic is lead (Pb). It is a stable heavy metal found in the air, water, soil and food

as a contaminant (Allouche et al., 2011; Ait Hamadouche et al., 2009; Ibrahim et al., 2012). Occupational exposure has the highest account of lead poisoning. Environmental sources of lead include inhalation of automobile exhaust from gasoline containing alkyl lead additives, ingestion of dust contaminated with lead, lead-based paints and drinking water that had passed through lead piping (Verma and Dubey, 2003; Flora, 2009; Ait Hamadouche et al., 2009; Kumar et al., 2011; Alissa and Ferns, 2011). Other significant sources include cosmetic products, food-can soldering, toys, ceramic glazes and folk remedies (Mohammed et al., 2008; Sajitha et al., 2010).

Lead is relatively poorly absorbed into the body, but once absorbed it is slowly excreted and so accumulate in the body, especially in the bone, causing several tissue and organ damage (Alissa and Ferns, 2011; Zhang et al., 2012). The severity of cases of plumbism has been of serious concern to international health organizations such as the Centres for Disease Control and Prevention (CDC)

*Corresponding author. E-mail: regiugbaja@yahoo.com. Tel: +2347066050043.

and World Health Organisation Occupational Safety and Health Administration (WHO-OSHA). The CDC redefined elevated blood lead levels (BLL) as that ≥ 10 $\mu\text{g}/\text{dl}$ in children and 40 $\mu\text{g}/\text{dl}$ in adults. Even at these safety levels, cases of lead poisoning have occurred (Mohammed et al., 2008; Flora, 2009; Olympio et al., 2009; Miranda et al., 2011).

Lead has been proven to produce a series of adverse effects on numerous organs and biochemical indices (Gurer and Ercal, 2000; Chen et al., 2003; Flora et al., 2004; Ademuyiwa et al., 2005; Dosumu et al., 2005; Choudhary et al., 2007). The adverse effects can be hematologic, reproductive, neurobehavioral, nephrotoxic, cytotoxic and cardiovascular (Patra et al., 2000; Moreira et al., 2001; Ademuyiwa et al., 2005; Diamond, 2005; Patil et al., 2006; Ait Hamadouche et al., 2009a; Olympio et al., 2009; Raafat et al., 2009; Ponce-Canchihuaman, 2010; Harishekar and Kiran, 2011; Mrugesh et al., 2011; Patra et al., 2011).

Lead poisoning has also been reported to cause oxidative damage to DNA by interfering with the incision step of DNA repair system, thus inducing carcinogenicity (Koedrith and Young, 2011). Also, a couple of proteins *in vivo* and the plasma membrane have lost their integrity and hence function as a result of plumbism (Okediran et al., 2009; Abam et al., 2008). Cases of lead induced dyslipidemia, hypertension and atherosclerosis have also been reported (Ademuyiwa et al., 2005; Heo et al., 2004). Chronic lead exposure has been demonstrated to alter fatty acid composition of erythrocyte membranes (Donaldson and Knowles, 1993). Acute lead exposure affects the cardiac function while chronic lead exposure affects the electrical and mechanical activities of the heart and alters the vascular smooth muscle function in experimental animals (Howard, 2001; Patrick, 2006; Mohammed et al., 2008; Alissa and Ferns, 2011).

Several therapies had been introduced by health professionals to treat acute and chronic lead poisoning. Noteworthy is the age-long use of chelating agents such as British Anti Lewisite (BAL), though its use has long been withdrawn. The use of chelating agents such as CaNa_2EDTA , meso-2,3-dimercaptosuccinic acid (DMSA or succimer), D-penicillamine is based on their ability to chelate heavy metals such as lead and consequently aid their excretion from the body (Flora et al., 2004; Kalia and Flora, 2005). They have always been used in severe cases of lead poisoning as the first line of treatment. The major problem about the efficacy and safety of these chelators is their non-specificity, which results in the mobilization of the heavy metals and also the essential elements like iron, zinc, calcium and a host of other divalent elements in the biological system. This is due to the rebound effects of the chelators (Gurer and Ercal, 2000). Aside this, the chelation therapy cannot be started where the subjects are still near or exposed to the source of pollution (Staudinger and Roth, 1998).

Recent findings on the use of antioxidants such as

ascorbate (Asc), vitamin E and α -lipoic acid on lead poisoned subjects have been helpful. They have been found to be capable of restoring the levels of oxidative stress markers such as superoxide dismutase (SOD), catalase and reduced glutathione (Flora et al., 2008; Ait Hamadouche et al., 2009; Alissa and Ferns, 2011; Koedrith and Young, 2011). Precisely, the effectiveness of Asc, a water soluble vitamin, has been attributed to its ability to scavenge or quench free radicals or decrease the intestinal absorption of lead by reducing ferric iron to ferrous iron in the duodenum, Asc then increases the availability of iron which competes with lead for intestinal absorption (Garrow et al., 2000; Erdogan et al., 2005; Bashandy, 2006; Patrick, 2006; Baseem et al., 2009). Also, it forms a complex with lead through a covalent bonding with its hydroxyl groups and being water soluble, it could therefore be easily excreted through urine. An increasing number of scientists are now advocating for their use over conventional chelating agents as they generally do not have any side effects (Abam et al., 2008; Koedrith and Young, 2011).

Although there are indications that chronic lead exposure may affect systemic lipid metabolism, causing dyslipidemia, much investigation has not been done on the comparative efficiency of the chelating agents and Asc. Dyslipidemia is defined as a deviation from the normal lipid profile levels of a subject (Allouche et al., 2011). In Nigeria, where little attention is given to public health effects of environmental pollution like exposure to heavy metals such as lead, this study is with the view of evaluating the effect of sub-chronic lead exposure on plasma and erythrocyte lipid profiles. It is also aimed at comparing the efficacy of these chelating agents with Asc in reversing any observed effect of sub-chronic lead exposure in the rat.

MATERIALS AND METHODS

Chemicals

Lead acetate (ACS reagents grade >99% pure), diethyl ether, chloroform, isopropanol and all other chemicals used were of analytical grade.

Experimental design

Healthy 35 male Wistar rats purchased from the Department of Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, were used in this investigation. The animals were kept housed under standard conditions of temperature and natural light-dark cycle. All the animals had access to feed and clean water *ad libitum* and all conditions of animal experimentation conformed to the NIH guidelines as outlined in NIH publication 80-23 (revised, 1978). They were allowed two weeks for acclimation prior to experimental treatment.

The animals with body weight of 150-200 g were then randomly and evenly distributed into five groups (n=7) including the control group. Four groups were administered 75 mg/kg body weight lead acetate (PbAc) orally for 14 days and then followed by the various

therapeutic interventions for another ten days (first five days treatment, followed by five days of rest to allow for redistribution of lead and then a second five days treatment). Two groups were administered chelating agents: D-Penicillamine (D-pen) and meso-2,3-dimercaptosuccinic acid (DMSA) (30 mg/kg body weight) respectively while the third group was administered an antioxidant (500 mg/kg body weight Asc). During the therapeutic period, the fourth group was administered normal saline (0.9% NaCl). The control group (not administered lead) was administered normal saline throughout the period of the study; and all administrations were done orally once daily.

At the end of the 14-day lead acetate (PbAc) administration, blood was collected from the four groups by tail incision blood lead level (BLL) before the commencement of therapeutic intervention.

After the 10 days of administering therapeutics, blood was collected into heparinised tubes via cardiac puncture under light ether anesthesia after an overnight fast. Aliquots of the blood samples were for BLL while the remaining blood samples were centrifuged to separate plasma from red blood cells.

Biochemical analyses

Blood lead analysis

For the estimation of BLL before and after therapeutic administration, 1 ml each of the whole blood samples were acid digested with concentrated nitric acid. BLL was then determined in duplicates using a Thermo Scientific S Series Atomic Absorption Spectrophotometer (Model Type S4 AA System).

Plasma lipid profiles

The plasma concentrations of total cholesterol and triacylglycerols were determined by spectrophotometric using Cypress diagnostic kits. HDL cholesterol and triacylglycerols were determined in plasma with the same diagnostic kits for total cholesterol and triacylglycerols after very low density lipoproteins and low density lipoproteins were precipitated using the method described by Gidez et al. (1982). Total phospholipids in plasma were extracted with chloroform-methanol mixture (2:1, v/v) as described by Folch et al. (1957). Phospholipid concentration was then assessed with ammonium ferrothiocyanate by the method of Stewart (1980). An aliquot of the extract (0.1 ml) was evaporated to dryness at 60°C. After cooling, 2 ml of chloroform was added to the dried extract, mixed and 2 ml of ammonium ferrothiocyanate was then added and then mixed for 1 min. The mixture was left for 10 min for separation to occur. The chloroform layer was then taken and the absorbance read at 488 nm. Phospholipid concentrations were determined using a phospholipid standard as reference.

Red blood cell lipid profile

Since the Folch et al. (1957) method of lipid extraction produced highly pigmented extracts, an improved procedure for red blood cell lipid extraction using chloroform - isopropanol (7:11, v/v) described by Rose and Oklander (1965) was used. For cholesterol determination, 0.1 ml of the extract was evaporated to dryness at 60°C and 20 µl of Triton X-100/chloroform mixture (1:1, v/v) was added to the dried extract for resolution. This was evaporated again and then 1 ml of the cholesterol kit reagent was added, mixed and incubated for 30 min before reading the absorbance spectrophotometrically. The triacylglycerol concentration was determined by evaporating to dryness 0.1 ml of the extract and adding 0.1 ml of 97% ethanol to re-suspend the dried lipid. To this, 1 ml of the triacylglycerol kit reagent was added, mixed and

incubated for 30 min before the absorbance reading was taken. Determination of phospholipids in the red blood cells followed the same procedure as described for plasma.

Estimation of lipid peroxidation

Lipid peroxidation in plasma and red blood cells was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) method of Buege and Aust (1978). In brief, 0.1 ml of test sample (plasma and red blood cell) was treated with 2.0 ml of TBA-TCA-HCl, 1:1:1 reagent (thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA) and incubated in a water bath at 95°C for 15 min. The tube was then placed on ice, centrifuged and the absorbance of clear supernatant was measured against blank at 535 nm. TBARS (malondialdehyde MDA) content was determined using the extinction coefficient of 155 nM⁻¹cm⁻¹.

Statistical analysis

The results obtained are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was used to analyze the results. Values with p<0.05 were regarded as being significant, using the Statistical Package for Social Sciences (SPSS) version 16.0.

RESULTS

Figure 1 shows the blood lead levels of the animals after therapeutics while the concentration of lead in the control remained insignificantly different. Throughout the period of experiment, marked changes were recorded in the BLL of the other four groups. The three groups administered therapeutics showed a significant decrease (p<0.05) in the BLL at the end of the experiment. The decrease ranged from 20.8% in the lead + Asc group to 43.5% in the Lead + D-pen group. The lead only group showed no significant change in the level of concentration of lead in the blood.

Figures 2 and 3 depict the plasma and HDL lipid profiles of animals after therapeutics, respectively. The plasma concentrations of total cholesterol and triacylglycerol of the groups treated with lead only were significantly higher (p<0.05) than the control by two folds while for the phospholipids, it was by 2.4 fold. While in the HDL, the cholesterol and triacylglycerol values of the lead only group were lower and higher respectively, though not significantly different (p>0.05) from the other groups. The plasma concentrations of total cholesterol and triacylglycerol of the animals treated with DMSA and Asc were not significantly different (p>0.05) from the control and they showed a significant reduction (p<0.05) compared with the lead only group. However, there was still a significant difference (p<0.05) in the concentration of phospholipids found in the plasma of groups treated with D-pen and Asc compared with the control.

Figure 4 shows the red blood lipid profile of animals after therapeutics. The red blood cell concentrations of total cholesterol and phospholipids of the groups treated

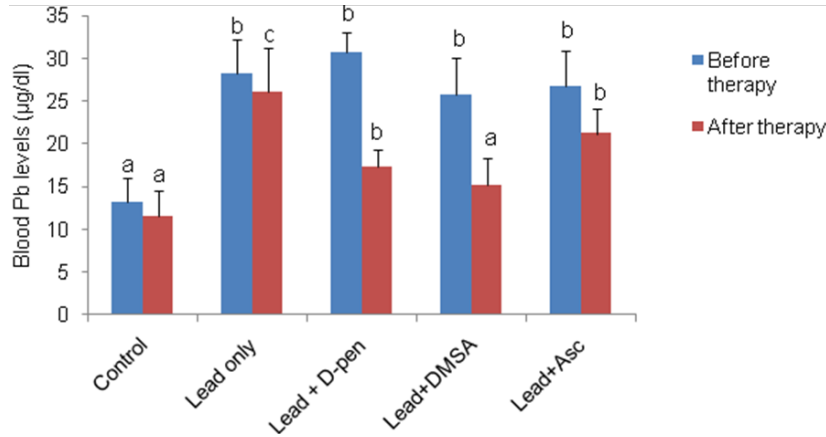


Figure 1. Blood lead levels in male Wistar rats exposed to lead (PbAc) after treatment with the chelating agents D-penicillamine (D-pen), meso-2,3-dimercaptosuccinic acid (DMSA or succimer) and the antioxidant ascorbate (Asc).

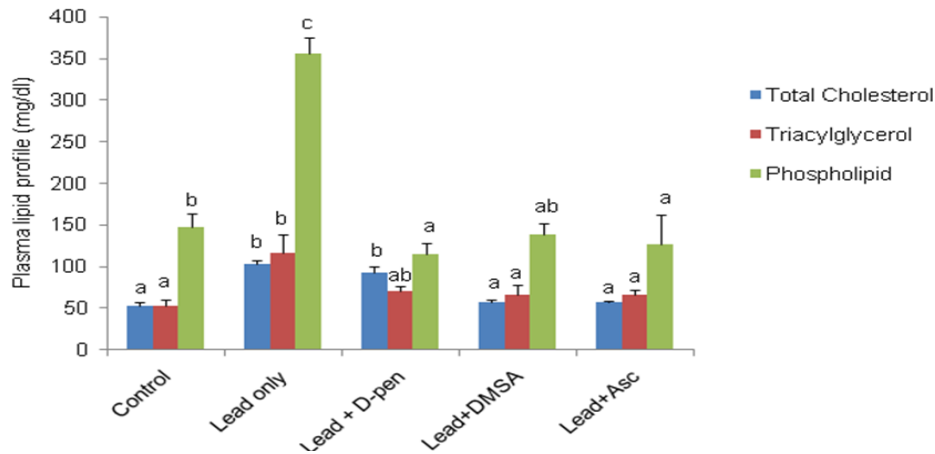


Figure 2. Plasma lipid profile in male Wistar rats exposed to lead (PbAc) after treatment with the chelating agents D-penicillamine (D-pen) meso-2,3-dimercaptosuccinic acid (DMSA or succimer), and the antioxidant ascorbate (Asc).

with lead only were significantly reduced ($p < 0.05$) compared with the control. The triacylglycerol concentration of the lead only group was however not significantly different ($p > 0.05$) from the other groups. The red blood cell concentrations of total cholesterol and phospholipids of the animals treated with the chelating agents (D-pen and DMSA) and Asc were not significantly different ($p > 0.05$) from the control and they showed a significant increase ($p < 0.05$) compared with the lead only group.

Figure 5 depicts the lipid peroxidation in the plasma and red blood cells. Lead exposure resulted in a significant increase ($p < 0.05$) of lipid peroxidation both in the plasma and red blood cells. While the increase in plasma lipid peroxidation was by 2.65 fold, it was by 1.23 fold in the red blood cells compared with the control. The therapeutics ameliorated the levels in the two compartments to near normal. Comparing the effects of

the therapeutics, the DMSA was observed to be most effective in the plasma while Asc was most effective in the red blood cells.

DISCUSSION

Lead exposures in humans and animals have been reported to produce a series of adverse effects in the biological systems. One of these is the perturbations in lipid metabolism in different compartments of organisms (Ademuyiwa et al., 2008). These perturbations were reported as up-/down- regulation of the concentrations of the lipids. Lead exposure was able to cause these perturbations through the activities of some of the enzymes involved in the metabolism of these lipids being up-/down- regulated. The effects of a sub-chronic lead exposure on the lipid profiles of rats along with the

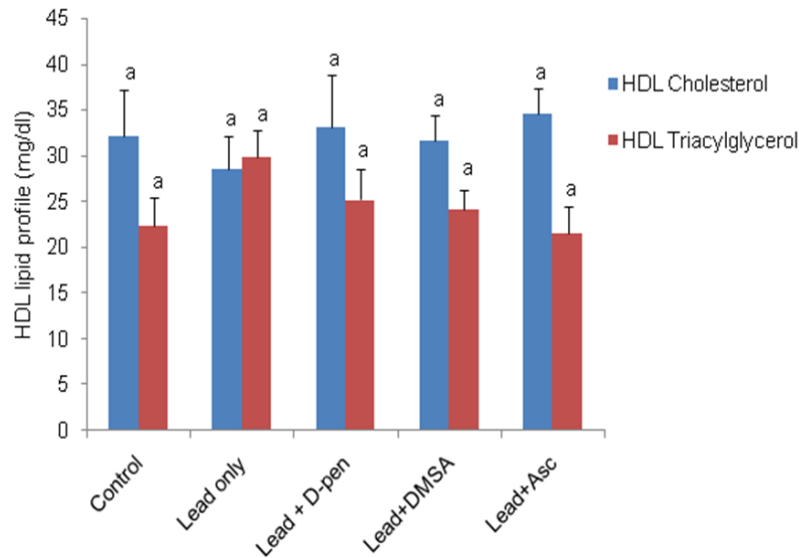


Figure 3. HDL lipid profile in male Wistar rats exposed to lead (PbAc) after treatment with the chelating agents D-penicillamine (D-pen), meso-2,3-dimercaptosuccinic acid (DMSA or succimer) and the antioxidant ascorbate (Asc).

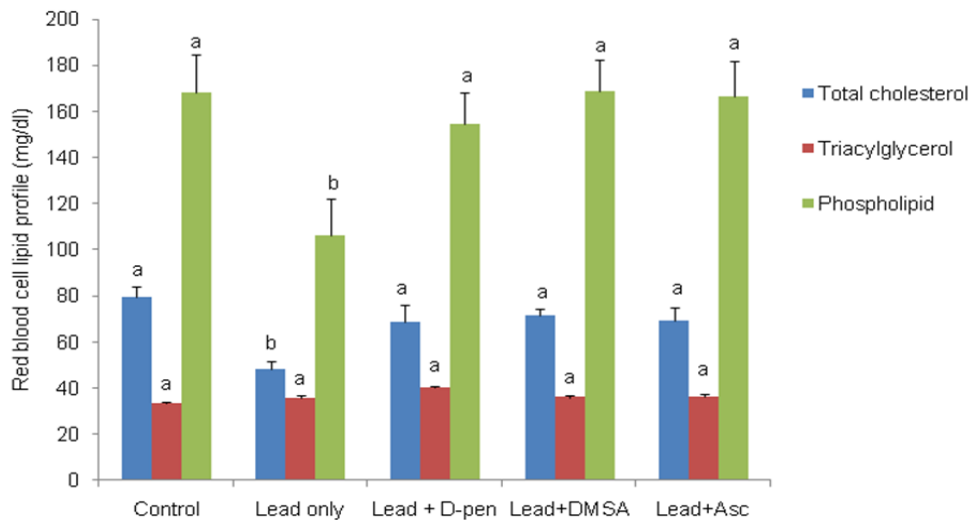


Figure 4. Red blood cells lipid profile in male Wistar rats exposed to lead (PbAc) after treatment with the chelating agents D-penicillamine (D-pen), meso-2,3-dimercaptosuccinic acid (DMSA or succimer) and the antioxidant ascorbate (Asc).

efficacy of chelating agent in comparison with an antioxidant vitamin (Asc) were examined in this study. The exposure of lead acetate was observed to induce plumbism (an elevation in blood lead levels) in the animals (Figure 1). This may be an indication of the extent of risk humans in an environment where such compound of lead exists. Lead still finds an active use in paints and as a fuel additive in developing countries like Nigeria. A higher risk of atherogenesis and

cardiovascular diseases has been observed in those who are occupationally exposed to this, such as auto mechanics and painters to mention but a few (Ademuyiwa et al., 2005; Nriagu et al., 1997).

The control group also had minor quantities of lead in the blood. This may be as a result of contamination from the air they inhaled, or the feed and water ingested. This further affirms the ubiquity of lead poisoning observed in early reports (Zhang et al., 2012; Hassan and Jassim,

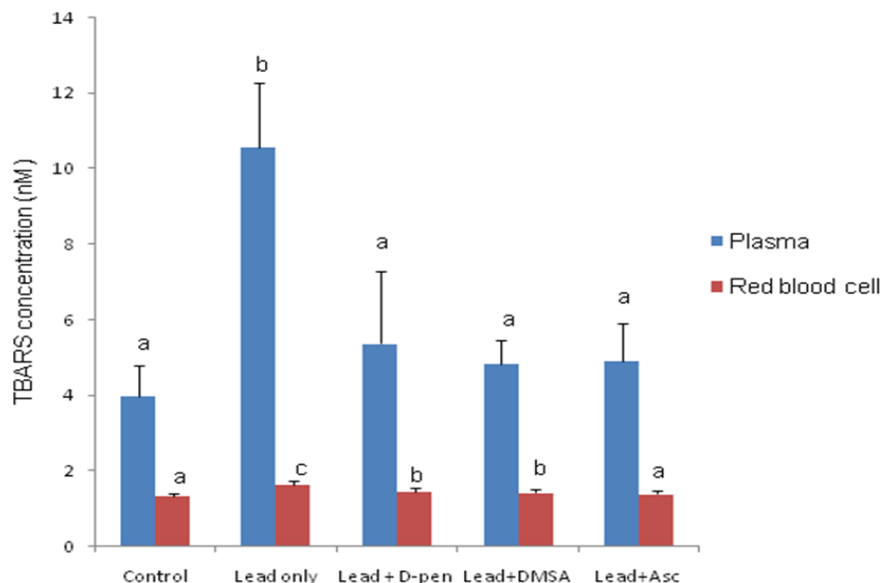


Figure 5. TBARS concentrations in the plasma and red blood cells of male Wistar rats exposed to lead (PbAc) after treatment with the chelating agents D-penicillamine (D-pen), meso-2,3-dimercaptosuccinic acid (DMSA or succimer), and the antioxidant ascorbate (Asc).

2010).

Lead was found to up-regulate the plasma concentrations of cholesterol, triacylglycerol and phospholipids and down-regulate their concentrations in the red blood cells significantly. These were in agreement with the findings of other researchers (Kristal-Boneh et al., 1999; Ait Hamadouche et al., 2009a). The disruption of the pro-oxidant/antioxidant balance that induces tissue injury via oxidative damage to biomolecules may be responsible (Erdogan et al., 2005; Bashandy, 2006; Flora, 2009; Sajitha et al., 2010). The high level of the lipids in the plasma could be attributed to changes in the rate of a number of processes. For cholesterol: a) altered distribution between the plasma and the tissues; b) enhanced absorption of exogenous cholesterol from diet; c) enhanced synthesis of endogenous cholesterol; d) decreased cholesterol excretion in the form of neutral sterols and e) decreased cholesterol transformation to bile acids (Kilic, 1993). It is also possible that the cholesterol synthesis and transport pathways may be adversely affected as observed in the significant decrease in the cholesterol: phospholipids ratio of the group administered lead only (data not shown). This may also imply an alteration in major cation fluxes of the cell membrane. It could also be as a result of the overproduction of very low density lipoprotein (VLDL), thus increasing the burden of triacylglycerol-rich lipoproteins on the common lipolytic pathway (Hassan and Jassim, 2010). Lead has also been known to bind directly to phosphatidylcholine in the red blood cell membrane, leading to a decrease in phospholipids levels (Ademuyiwa et al., 2005; Patrick, 2006; Abam et al.,

2008). Lead exposure has been reported to cause a cytotoxic effect on the red blood cells due to its high potential in changing the osmotic fragility, interaction with membrane proteins and some essential trace metals in the blood (Gurer and Ercal, 2000; Mrugesh et al., 2011). From results, there were decreases in the red blood cell lipid concentration, indicating an efflux of the lipids from the intracellular to the extracellular fluid. This may be arising from the fact that the function of the plasma membrane may have been compromised.

At the end of the ten days of therapeutics, a marked change was observed in the blood lead levels. This confirms the findings of other reports which indicate the effectiveness of chelating agents and the recent use of antioxidant vitamins in aiding the excretion of absorbed lead (Gurer and Ercal, 2000; Onunkwor et al., 2004; Bashandy, 2006; Flora, 2009). This result however reveals that chelating agents remain a more rapid treatment for acute lead poisoning since they were more able to mobilize the removal of lead from the blood. Normally, after an ingestion or exposure to lead, it is primarily found in the red blood cells as it has a very high affinity for the cells. However, as blood flows through the soft tissues, it is deposited and bio accumulated (Babalola et al., 2010). During chelation therapy, lead in the blood is cleared giving rise to a redistribution of the lead from the tissues into the blood again for further clearance. The rate of mobilization from our results may be depicted as: DMSA > D-pen > Asc. The higher rate of reversal observed for DMSA may be as a result of its greater ability to mobilise lead even from soft tissues (Flora, 2009; Kalia and Flora, 2005). The lesser efficacy

of Asc may be attributed to the fact that it is a water-soluble vitamin, only protective enough to intercept oxidants in the aqueous phase before they attack or cause any detectable damage to the lipids (Hassan and Jassim, 2010).

Also noteworthy is the result of the BLL obtained from the group administered lead only. There was no significant difference ($p < 0.05$) in the BLL before and after, but there was a numerical decrease. It may be predicted that there is a gradual mobilisation of the accumulated lead from the blood into soft tissues such as the liver and kidney and hard tissues such as the bones (Flora, 2009).

The chelators and as well Asc were observed to decrease the accumulated lipids in the plasma (Figure 2). For total cholesterol concentration, the rate of reversal to normal level may be depicted as Asc > DMSA > D-pen while for phospholipids, it can be stated as DMSA > Asc > D-pen. For the rate of reversal of triacylglycerol concentration, however, it may be depicted as DMSA > Asc > D-pen. Virtually the reverse is observed for the red blood cells of the animals. The mechanisms by which Asc, a water soluble vitamin could be able to reduce the plasma concentration of the lipids especially cholesterol can be as follows: (i) it has been reported that the oxidation of cholesterol to bile acids is dependent on Asc status (Kilic, 1993), so having administered 500 mg/kg body weight of Asc to the animals in the first five days treatment, it could be said that the animals were already on an adequate intake of the vitamin. This adequacy is actually necessary for the transformation of cholesterol to bile acids at the rate limiting steps of bile acid biosynthesis; (ii) for the hydroxylation of carbon 7 (C7) of the cholesterol nucleus, Asc is important in the catalytic reaction by 7- α hydroxylase. In an Asc deficient condition, this reaction is inhibited, leading to a high plasma cholesterol concentration (Holloway and Rivers, 1984; Hemila, 1992). Although D-pen was able to reduce the BLL of subjects significantly, it was ineffective in restoring normal total cholesterol and triacylglycerol levels. This may be due to the fact that D-pen can penetrate the cell membranes and get metabolized easily before being able to accomplish the needed cholesterol and triacylglycerol reversal (Flora, 2009). In general, the chelating agents and Asc could have bound to the lead in the system thereby releasing the bound enzymes involved in the metabolism of these lipids so that their normal homeostasis can be maintained.

In conclusion, the results of this study have again indicated that lead poisoning is capable of inducing dyslipidemia, therefore capable of predisposing subjects to other risks such as atherosclerosis. The therapeutic interventions have also proved effective, although the DMSA has proved to be most effective of the three, in ameliorating the perturbations observed in lipid metabolism in this study. The chelating capacity of Asc is limited in sub-chronic dosage, so possibly a combination therapy could be more desirable. The therapeutic use of

Asc would however be more advantageous than the chelating agents since the occupationally exposed subjects undergoing treatment would not need to be removed from their means of livelihood, it is very cheap and its use does not require the expertise of a physician. Further study may be carried out at lower doses of lead for a longer period as it is often the case practically, perhaps Asc may be more effective in chronic lead exposure due to the reasons above.

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