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RESEARCH ARTICLE

Lipid changes in male Albino rats exposed to graded doses of Lead

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Abstract: In order to determine the lipid changes in male albino rats exposed to graded doses of lead as lead acetate for periods of 4, 8 and 12 weeks, a total of 60 male albino rats were divided into four groups as, A, B, C and D. Group A served as the control while groups B, C and D were exposed to 200, 300 and 400 ppm, respectively. At the end of exposure period, blood samples were collected for the determination of lead concentration. Total triglyceride, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and phospholipid concentrations were also determined in the plasma. There was a significant ($p < 0.05$) period and dose dependent increase in blood lead concentrations in treated groups compared to the control group while plasma triglyceride and cholesterol concentrations followed similar patterns of alterations. The high density lipoprotein cholesterol and low density cholesterol concentrations show varying degrees of alterations, with a significant increase in high density cholesterol fractions while a significant decrease in the low density cholesterol fractions. The phospholipid concentration showed a dose-dependent decrease. It can be concluded that exposure to varying concentrations of lead over varying periods of exposures show accumulation of lead in the blood together with varying alterations in some of the lipid parameters of male albino rats.

Keywords: Cholesterol, plasma, phospholipid Lead, triglyceride.

INTRODUCTION

Lead is a non-essential trace element with potentially toxic effects for biological systems. It is a protoplasmic poison harmful to varieties of organs and tissues including smooth muscles and the red blood cell membrane (Gots, 1993; Anetor and Adeniyi, 1999). It is one of the most widespread metal pollutants in the environment and may be disseminated via air, industrial pollution, agricultural technology and food processing (Winder, 1993; Ademuyiwa *et al*., 2005b). Multiple industrial, domestic, agricultural, medical and technological applications of lead is a main reason for it wide distribution in the environment, raising concerns over its potential effects on human health and the environment (Tchounwou *et al*., 2012). Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals (Vassallo *et al*., 2011). Symptoms of chronic lead toxicity usually develop over weeks to month of exposure as lead accumulates in the body during chronic exposure (Ademuyiwa *et al*., 2007; Okediran *et al*., 2008; Abam *et al*., 2008).

Several studies have incriminated lead exposure to lipid abnormalities and risk of atherosclerosis (Newairy and Abdou, 2009; Skoczynska *et al*., 1993). Lipid and lipoprotein abnormalities play a key role in the pathogenesis and progression of atherosclerosis and cardiovascular diseases (Chrysohoou, *et al*., 2004; Glew *et al*., 2002). Several reports have shown that both acute and chronic lead poisoning cause impairment of heart and vessel functions (Kopp *et al*., 1988; Wojtczak-Jaroszowa and Kubow, 1989) and that rate of death from cerebrovascular disease are significantly increased in lead-exposed workers compared with the general population (Dingwall-Fordyce and Lane, 1963; Malkolm, 1971; Gerhardsson, 1986).

Lead induces the production of reactive oxygen species (ROS) that resulting in DNA damage, depletion of cell antioxidant defense systems and risk of atherosclerosis (Skoczynska *et al*., 1993; Newairy and Abdou, 2009). *In* vivo and *in* vitro studies suggest that lipid metabolism is altered both in acute and chronic exposure to lead (Ponce-Canchihuamán *et al*., 2010). Therefore, this study was designed to provide information on the plasma lipid profile of male albino rats exposed to graded doses of lead over a period of four, eight and twelve weeks.

MATERIALS AND METHODS

Experimental animals

A total of sixty male albino rats aged three month were used for this investigation. The average weight of the rats was 152 ± 3.5g. 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 2,900 Kcal.kg^{-1}, (TOPFEEDS®, Lagos, Nigeria) and water provided *ad libitum*. Experimental animals were acclimatized to their housing environment one month before starting the experiment.

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Ethical consideration

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.

Administration of lead acetate

The animals were randomly divided into four groups of fifteen animals per group and treated with lead acetate [$(CH_3.COO)_2Pb.3H_2O$, Assay (ex Pb) 99-103%, maximum limits of Impurities, Chloride (Cl) 0.005%, Copper (Cu) 0.002%] (Cartivalues, England) orally as the source of lead. The treatments included Group A: only distilled water (the control), Group B: 200 ppm of lead as lead acetate, Group C: 300 ppm of lead as lead acetate and Group D: 400 ppm of lead as lead acetate.

Collection of blood samples

At the end of four, eight and twelve weeks of lead acetate treatment, five rats were randomly selected from each group, blood samples were collected via the ocular *median canthus* using heparinized capillary tubes into heparinized tubes. Plasma was separated from whole blood samples as described by Schalm *et al*., (1975).

Blood lead determination

Lead was analyzed in whole blood using atomic absorption spectrometry (Buck Scientific AAS model 200, Connecticut, USA).

Determination of biochemical parameters

The blood samples were centrifuged at 4000 rpm for 10 minutes to separate the plasma from the blood cells. The plasma was then removed and stored in Eppendorf tubes for further analyses at 4°C.

The following biochemical parameters were determined in fresh plasma samples. Plasma cholesterol and triglyceride concentrations were determined spectrophotometrically according to the methods of Allain *et al*., (1974) and Sesin *et al*., (1972) as described in Randox® biochemical kit for cholesterol and triglyceride determination respectively. The high-density lipoprotein (HDL) plasma fraction was isolated by the method of Gidez *et al*., (1982) after precipitating very low density lipoproteins (VLDL) and low density lipoproteins (LDL) with heparin-manganese chloride solution. The high density lipoprotein cholesterol (HDL-cholesterol) concentration was then determined as described by Allain *et al*., (1974) using Randox® biochemical kit for cholesterol. The low-density lipoprotein cholesterol (LDL-cholesterol) and phospholipids were determined as described by Allain *et al*., (1974) and Stewart, (1979) respectively, after plasma lipids were extracted using chloroform-methanol mixture (2:1, v/v) as described by Folch *et al*., (1957).

Statistical analysis

Values were expressed as mean ± Standard Error of mean (SEM). The level of homogeneity among the groups was tested using Analysis of Variance (ANOVA). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). All analyses were done using Statistical Package for Social Sciences (SPSS) version 11.

RESULTS

The mean blood lead concentrations of male albino rats treated with graded doses of lead for a period of four, eight and twelve weeks were given in Table 1. There were significant ($p < 0.05$) and dose dependent increases in blood lead concentrations over varying periods of exposures compared with the control group.

Table 2 shows the plasma cholesterol and triglyceride concentrations of male albino rats treated with graded doses of lead for a period of four, eight and twelve weeks. There were significant increases ($p < 0.05$) in both plasma cholesterol and triglyceride concentrations at higher doses of lead treatment in comparison with the control group.

Table 3 shows the mean plasma high density lipoprotein and low density lipoprotein cholesterol concentrations of male Albino rats treated with graded doses of lead for a period of four, eight and twelve weeks. The high density lipoprotein cholesterol shows a significant ($p < 0.05$) increase in concentrations over the treatment period while plasma low density lipoprotein cholesterol depicts a significant decrease in concentrations.

Table 4 shows the mean plasma phospholipid concentrations of male Albino rats treated with graded doses of lead for a period of four, eight and twelve weeks. There were significant decreases in plasma phospholipid

Table 1: The mean blood lead concentrations of male Albino rats treated with graded doses of lead for a period of four, eight and twelve weeks.

Groups	Blood lead concentration (µg/dl)		
	4 weeks	8 weeks	12 weeks
Control	2.15 ±0.06^a	4.31 ±0.14^a	6.41 ±0.24^a
200 ppm	12.36 ±1.14^b	28.35 ±1.57^b	39.39 ±2.92^b
300 ppm	13.16 ±1.00^b	29.64 ±1.86^b	43.60 ±2.77^b
400 ppm	19.76 ±0.47^c	39.82 ±1.14^c	63.50 ±1.65^c

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

Table 2: The mean plasma cholesterol and triglyceride concentrations of male Albino rats treated with graded doses of lead for a period of four, eight and twelve weeks.

Groups	Plasma cholesterol concentrations (mg/dl)			Plasma triglyceride concentrations(mg/dl)		
	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks
Control	68.88±2.56[a]	65.58±3.40[a]	64.19±7.14[a]	34.55±2.17[a]	32.23±1.90[a]	30.46±1.35[a]
200 ppm	73.06±12.87[a]	77.56±5.47[a]	82.25±4.21[b]	43.30±5.19[a]	52.74±1.32[b]	62.58±1.24[b]
300 ppm	78.68±16.65[a]	83.68±4.64[b]	98.02±5.33[b]	54.12±4.62[b]	62.72±0.89[b]	77.91±1.90[b]
400 ppm	84.69±13.03[b]	88.20±1.89[b]	98.19±4.47[b]	68.94±2.53[b]	73.84±1.07[b]	88.02±1.30[b]

Values are mean ±SEM. Values within the same column with different superscripts are significantly different at p<0.05

Table 3: The mean plasma high density lipoprotein and low density lipoprotein cholesterol concentrations of male Albino rats treated with graded doses of lead for a period of four, eight and twelve weeks.

Groups	High density lipoprotein cholesterol concentrations (mg/dl)			Low density lipoprotein cholesterol concentrations (mg/dl)		
	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks
Control	27.80±2.32[a]	30.67±3.10[a]	32.21±1.20[a]	20.83±1.37[b]	22.72±1.12[b]	21.68±1.15[b]
200ppm	34.69±2.79[a]	44.23±3.04[b]	54.57±13.09[b]	14.43±2.10[a]	12.37±1.01[a]	11.30±1.01[a]
300ppm	44.06±1.02[b]	47.05±1.63[b]	59.60±2.47[b]	11.26±3.03[a]	10.33±1.02[a]	10.45±1.03[a]
400ppm	56.58±1.24[b]	53.54±2.95[b]	62.35±2.58[b]	10.35±2.03[a]	9.42±1.03[a]	10.25±1.01[a]

Values are mean±SEM. Values within the same column with different superscripts are significantly different at p<0.05

Table 4: The mean plasma phospholipid concentrations of male Albino rats treated with graded doses of lead for a period of four, eight and twelve weeks.

Groups	Plasma phospholipid concentrations (mg/dl)		
	4 weeks	8 weeks	12 weeks
Control	69.85 ±3.31[a]	66.85 ±10.38[a]	62.09 ±10.27[a]
200ppm	43.50 ±4.22[b]	55.37 ±13.19[b]	35.62 ±11.49[b]
300ppm	35.64 ±4.72[b]	45.49 ±6.00[b]	30.22 ±12.54[b]
400ppm	29.08 ±4.91[b]	40.83 ±8.20[b]	29.79 ±5.19v

Values are mean ±SEM. Values within the same column with different superscripts are significantly different at p<0.05

concentrations compared with the control groups.

DISCUSSION

Lead is a persistent metal commonly present in our living environment. Multiple industrial, domestic, agricultural, medical and technological applications have led to the wide distribution of lead residues in the environment, raising concerns over their potential effects on human health and the environment. Lead toxicity depends on several factors including the dose, route of exposure and chemical species as well as the age, gender, genetics and nutritional status of the exposed individuals (Vassallo *et al*., 2011). 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 of Moussa and Bashandy (2008), Okediran *et al*., (2017) who reported that absorbed lead following oral ingestion is carried *via* blood to soft tissues and 95 % of blood lead is transported in erythrocyte as lead diphosphate. Therefore, accumulation of lead in the body could lead to destructive impacts in haematic, gastrointestinal and renal system (Correia *et al*., 2000).

Several studies have incriminated lead exposure to lipid abnormalities and risk of atherosclerosis (Skoczynska *et al*., 1993; Newairy and Abdou, 2009). Dyslipidaemia is defined as elevated total cholesterol, low density lipoprotein cholesterol (LDL-cholesterol), or triglycerides; decreased high-density lipoprotein cholesterol (HDL-cholesterol), or combination of these abnormalities (Well *et al*., 2009). Cholesterol is an essential part of every cell in the body, it is necessary for formation of new cells and for older cells to repair themselves after injury. It is also used by the adrenal glands in the synthesis of some hormones, such as cortisol, by the testicles to form testosterone, and by the ovaries to form estrogen and progesterone (Jefcoate *et al*., 1992). The high cholesterol level in plasma may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism. Lead induced development of hypercholesterolemia involves the activation of cholesterol biosynthetic enzymes (i.e3-

hydroxy-3-methylglutaryl-CoA reductase, farnesyl diphosphate synthase and squalene synthase, CYP51) and the simultaneous suppression of cholesterol catabolic enzymes such as 7-alpha hydroxylase (Kojima *et al*., 2002).

The result of this study shows an increase in both total plasma cholesterol and triglycerides. Similar findings have been reported for lead exposure by Peters *et al*. (2012) whereas Cocco *et al*., (1991) indicated that lead induced a remarkable decrease in total cholesterol. However, another study of occupational lead exposure found a positive correlation between blood lead and total cholesterol (Ademuyiwa *et al*., 2005), also low doses of lead were associated with a decrease in total cholesterol and an increase in triglyceride concentration (Skoczynska *et al*., 1993). The findings of Shyam *et al*., (2012) indicated that exposure to lead alters the metabolism of cholesterol and thus increases the risk of cardiovascular disease and atherosclerosis in lead exposed subjects with an increase in total cholesterol, LDL- cholesterol and ratio of LDL to HDL- cholesterol and decrease in HDL. The association between lead exposure and high plasma lipid levels is biologically plausible and could be due to either increased synthesis or to impaired feedback inhibition (Newairy and Abdou, 2009).

Our results show an increase in high density lipoprotein cholesterol (HDL-cholesterol) and decrease in low density lipoprotein cholesterol (LDL-cholesterol). Several studies have shown that lead exposure induces alterationa (Gatagonova, 1994; Kirkby and Gyntelberg, 1995) or no changes (Cocco *et al*., 1995; Osterode, 1996) in serum lipid profiles. The elevation of HDL-cholesterol in the lead exposed group compared to the control group demonstrated either increase in the hepatic cell biosynthesis of cholesterol or decrease in the hepatic reuptake of the molecules from the circulation by a receptor mediated endocytosis (Tietz, 1999). There are some evidences about the positive effects of lead on the activity of enzymes regulating the biosynthesis of cholesterol in lead exposure also resulted in enhanced hepatic cholesterogenesis and hypertriglyceridemia (Ademuyiwa *et al*.,2009). Serum lead level is positively associated with the levels of serum cholesterol, HDL-cholesterol and LDL-cholesterol, the oxidative stress generated by the exposure to lead acetate may cause a defect in the receptor and decrease the reuptake of these molecules causing a high level of them. HDL molecules which indicate the cholesterol transport from the peripheral tissues to the liver for more metabolism and excretion as bile acids (Kwiterovich, 2000), the reduction of the biosynthesis of this molecule by hepatocytes is triggered by low intracellular cholesterol level (Tietz, 1999). In the present study the increased HDL-cholesterol in lead exposed group may be attributed to a defect in the intrahepatic cholesterol metabolism while reduced LDL-cholesterol could be a result of activation of endogenous antioxidant enzymes overwhelming the reactive oxygen species that might have caused lipid peroxidation (Shalan *et al.,* 2005).

Lead has being reported to induce lipid peroxidation most especially when the antioxidants were overwhelmed. Also, binding of lead to phosphatidylcholine in the cell membrane of red blood cells, lead to reduction of phospholipid levels (Patrick, 2006). Decreased phospholipid concentrations were observed in the lead exposed groups compared to the control. This may be due to ability of lead acting as a prooxidant thereby generating free radicals leading to peroxidation of plasma and membrane lipids.

CONCLUSIONS

Our findings revealed that the exposure to lead resulted in accumulation in blood, thus predisposing varying degrees of alterations in the lipid metabolism in male albino rats.

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