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Evaluation of the acute effect of heavy metals exposure on lipid profile of albino wistar rats

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Abstract

Acute effect of heavy metals exposure on lipid profile of male albino wistar rats was evaluated. Sixteen (16) animals weighing 292-298g were randomly assigned four groups of four rats each. Groups 1 and 2 were orally treated ones with 40 percent below the lethal doses (LD_{50}) of Lead and Mercury respectively. Group 3 was treated with both heavy metals (20 percent below the LD_{50} each of the metals). Group 4 was not treated with either of the heavy metals and served as control. Each group of animals was allow free access to commercial rat mash and water for one week that the study lasted. After the 7 days experimentation, the results revealed a non-significant increase ($P > 0.05$) in serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) cholesterol and very low-density lipoprotein (VLDL) cholesterol in group 1 when compared to the control. A significant decrease in serum low-density lipoprotein (LDL) cholesterol ($P < 0.05$) in group 1 was recorded when compared to the control. Groups 2 and 3 animals died 24 hours and 48 hours respectively after treatment. The implications of these results are discussed.

Keywords: Heavy Metals; Acute effect; Lipid profile Albino wistar rats

1. Introduction

Heavy metals are high density elements occurring naturally in the environment in a minute quantity. With the rise in industrialization and anthropogenic activities, heavy metal pollution occurred in different countries especially China [1]. Mining and manufacturing cause these toxic metals to be released into water or the atmosphere. Some of these metals are also used in fuels and coal, and therefore are released during combustion, ore smelting or through waste disposal machineries in the form of metal fumes or suspended particulates. Some trace amounts of heavy metals such as zinc and copper are useful for the body, but other toxic metals can accumulate in large quantities and cause health problems and even death. Multiple heavy metals may simultaneously enter the body through air, food or water. Once in the body, they are not easily metabolized or excreted, but they build up quickly and bioaccumulate in organs and vital tissues, leading to an increase in their concentrations which can result in fatality [1].

Recent studies using fish as bioindicators of trace element pollution revealed high levels of heavy metals in migratory fish [2]. According to the Priority Chemical List (PCL), lead, mercury, organic mercuric compounds, cadmium, hexavalent chromium and nickel are all metals with great public concern [3]. Due to their toxicity and high frequency of occurrence, cadmium, hexavalent chromium compounds and nickel compounds are classified as human carcinogens level 1 and inorganic lead compounds are classified as level 2A by the International Agency for Research on Cancer (IARC). Lead causes damage to the Central Nervous System (CNS), kidneys and affects heme synthesis [4]. Methyl

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mercury is mainly absorbed through the digestive tract and the absorption of inorganic mercury is below 15%, while that of alkyl mercury is above 90% [1].

Over the years, epidemiological studies have identified lipid and lipoprotein abnormalities as dependent risk factors in the pathogenesis and progression of atherosclerosis and cardiovascular diseases [5]. There is also increasing evidence that environmental factors and contaminants (most especially heavy metals) contribute to this dyslipidemia [6]. Thus, this study seeks to evaluate the effect of acute exposure of albino wistar rats on lipid profile in the animals.

2. Material and methods

2.1. Procurement and Preparation of Heavy Metals

The heavy metals [Mercury (Hg) and Lead (Pb)] used for the study were purchased in their salts form (mercuric chloride and lead nitrate) from the Chemistry Unit of the Department of Science Technology, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene, Akwa Ibom State, Nigeria. They were separately stored in air-tight containers protecting them from sunlight. Forty (40) percent below the LD_{50} of each metal was weighed out, dissolved in 4 ml of distilled water to be administered on groups 1 and 2 animals, while 20 percent below the LD_{50} of each metal was weighed out, dissolved in 4 ml of distilled water to be administered on group 3 animals.

2.2. Experimental Design, Grouping and Treatment of the Animals

A total of sixteen healthy adult male albino wistar rats weighing 292-298g were obtained from the disease-free stock of the animal house of the Department of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The animals were housed in a cage with four sizeable compartments of wooden bottom and wire mesh top, randomly assigned four animals per four groups. The rats were maintained under standard conditions of temperature and natural light-dark cycle for 7 days acclimatization in the animal house, Akwa Ibom State Polytechnic, Ikot Osurua. Group 1 animals were treated with 40 percent below LD_{50} of lead nitrate, group 2 with 40 percent below LD_{50} of mercuric chloride based on the average body weights of the groups. Group 3 animals were co-treated with 20 percent below LD_{50} of lead nitrate and mercuric chloride, while group 4 was neither treated with either of the metals nor served as control. All animals were fed with commercial rat mash *ad libitum* and distilled water throughout the duration of one week of experimentation. The treatment was done orally and good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from the cages daily.

2.3. Collection of Blood Sample and Preservation of Serum

At the end of 7 days experimental period, the animals were fasted for 12 hours and were anaesthetized under chloroform vapour and were sacrificed by dissecting medioventrically and blood was collected via cardiac puncture by means of syringe and needle into a sterile EDTA sample bottles and then centrifuged at 3,000rpm for 15minutes to separate serum from the plasma. The serum was used to determine the levels of Total Cholesterol (TC), Triglyceride (TG), High-density lipoprotein (HDL), Low-density lipoprotein (LDL) and Very low-density lipoprotein (VLDL) in the animals.

2.4. Methods

2.4.1. Determination of High-Density Lipoprotein (HDL)

The procedure for the determination of HLD was put forward by NHANES [7]. About 200μl of standard and 200μl sample was measured out into test tubes and labeled as standard and test respectively. About 500μl of distilled precipitate reagent was added to each of the content in the tubes, mixed and allowed to stand for 10 minutes at room temperature, then centrifuged for 10 minutes at 4000rpm. The clear supernatant was separated within 24 hours to be used to determine the cholesterol content by CHOP PAP method. About 100μl each of distilled water, standard supernatant and sample supernatant were measured into sample tubes labeled blank, standard and test respectively. To each tube, 100μl of cholesterol reagent was added, mixed and incubated at 37^0C for 15 minutes. The absorbances of the samples (A sample) and standard (A standard) were read against the reagent blank using spectrophotometer at 500nm wavelength. The concentration of HDL was calculated thus;

$$\text{Concentration of HDL (mmol/l)} = \frac{\text{Ab sample}}{\text{Ab standard}} \times \frac{\text{concentration of standard}}{1}$$

Where Ab = Absorbance.

2.5. Determination of Low-Density Lipoprotein (LDL)

Low Density Lipoprotein (LDL) is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship;

$$\text{Concentration of LDL-Cholesterol (mg/dl)} = TC - HDL - (TG/5)$$

Where (TG/5) is an estimate of VLDL- cholesterol in mg/dl [9].

2.6. Determination of Triglyceride

About 10µl of serum sample was measured into test tube labeled sample, and to another test tube, labeled standard, 10µl of each standard was measured. Later, 1000µl each of reagents was measured into the two labeled tubes (sample and standard) and another test tube labeled blank. They were mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample (A Sample) and standard (A standard) were measured against the reagent blank with a spectrophotometer at 500nm wavelength. The concentration of the TG was calculated thus;

$$\text{Concentration of TG (mmol/l)} = \frac{\text{Ab sample}}{\text{Ab standard}} \times \frac{\text{concentration of standard}}{1}$$

2.7. Determination of Total Cholesterol (TC)

Serum total cholesterol (TC) concentration was assayed by the method described by Sharul et al. [10]. To 3 sample tubes labeled reagent blank, standard and sample, 10µl of sample, distilled water, standard and serum sample were added respectively. Later, 1000µl reagent was added to each tube, mixed and incubated at 37^0C for 5 minutes. The absorbance of the sample (A sample) and standard (A standard) were read against the reagent blank within 60 minutes with spectrophotometer at 500nm. The concentration of TC was calculated thus;

$$\text{Concentration of TC (mmol/l)} = \frac{\text{Ab sample}}{\text{Ab standard}} \times \frac{\text{concentration of standard}}{1}$$

2.8. Determination of Very Low-Density Lipoprotein (VLDL)

The concentration of VLDL cholesterol in the serum samples was determined by calculation using the formula:

Concentration of VLDL (mmol/l) = Triglyceride

2.9. Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA). Results were expressed as mean ± standard error of mean (SEM). Significant differences were obtained at $P. < 0.05$. This was estimated using statistical package for social sciences (SPSS) version 23.

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3. Results

Table 1 Mean Lipid Profile in Albino Wistar Rats exposed to acute toxicity of Heavy Metals

Lipid Profile	Group 1	Group 2	Group 3	Group 4 (Control)
HDL (mmol/l)	0.35± 0.55	Dead	Dead	0.34± 0.27
LDL (mmol/l)	0.79± 0.25	Dead	Dead	0.41± 0.76
TG (mmol/l)	0.96± 0.90	Dead	Dead	0.95± 0.81
TC (mmol/l)	1.58± 0.18	Dead	Dead	1.39± 0.15
VLDL (mmol/l)	0.43± 0.04	Dead	Dead	0.42± 0.04

Result expressed as mean ± SEM (N = 4). Significant difference was accepted at P < 0.05.

4. Discussion

Heavy metals are very toxic substances and are great concern to public health. Heavy metal toxicity is the harmful effects of certain metals in certain forms and doses on life. Some metals have no biological role, that is, they are not essential minerals but are toxic in certain forms. These metals are ubiquitous in various forms in the environment primarily, human beings and animals are intoxicated with heavy metals by means of consumption of contaminated foods, water, and air or through mining, battery recycling and plastics. Exposure to metals like mercury, cadmium, lead and arsenic even at low level, may contribute much towards causing impaired functioning in the biological system among which perturbations are caused in lipid metabolism.

Lipids and lipoprotein abnormalities play a major role in the development and progression of coronary artery diseases. Among the cardiovascular complications, atherosclerosis is responsible for approximately 50 percent of death in Western countries [10]. Cardiovascular disease is characterized by the elevation of serum TG, TC and LDL with a decrease in HDL values [10]. However, negative alterations in these lipid profile fraction provides useful information concerning the status of lipid metabolism as well as predisposition to atherosclerosis and its corresponding implications [11]. Under this condition, lipids and other related substances accumulate on the arterial wall, forming plague, which occlude the vascular lumen ad obstruct the flow of blood to vital organs such as the heart, brain, liver etc. Therefore, this study evaluated the acute effect of heavy metal exposure on lipid profile of albino wistar rats. Exposure to these metals come from different routes and sources, but for this study, they were administered orally after being dissolved in water. The results indicated that oral administration of lead nitrate has significant effect on the lipid profile of the rats. However, oral administration of mercuric chloride and co-administration of mercuric chloride and lead nitrate also had significant effects on the lives of the rats and this was witnessed by their mortality. Group 2, which was treated with mercuric chloride ($HgCl_2$), all died within 24 hours of treatment and group 3 animals treated with combination of $HgCl_2$ and $PbNO_3$ died after 48 hours. These might have been as a result of toxic effect and dose of the administered heavy metals, resulting in oxidative stress.

Numerous studies revealed that mercury generates oxygen free radicals mainly by activation of NADH oxidase. According to Vassallo et al. [12] and Moreira et al. [13], acute inorganic mercury exposure in vivo promotes reduction of myocardial force development and inhibits myosin ATPase activity. However, the mechanism involved seems to be dependent on both the dose and time of exposure. Raymond and Ralston [14] studied the hemodynamic effects of an intravenous injection of $HgCl_2$ (5mg/kg) in rats and observed that mercury produced cardiac diastolic failure and pulmonary hypertension. Moreso, Naganama et al. [15], reported that acute exposure of $HgCl_2$ (680mg/kg) increased blood pressure, heart rate, vascular reactivity to phenolephine and even death in rats. The increased reactivity seems to depend on an increased generation of free radicals.

Furthermore, the results of this study showed a non-significant increase in TC in group 1 when compared to the control. Mean serum TC level in group 1 animals was within the normal range for rats (0.37-2.11mmol/l) and below that of human (3.1-6.8mmol/l). This could have been as a result of oxidative stress from lead poisoning caused by the imbalance between the production of free radicals and the generation of antioxidants to detoxify or to repair the resulting damage [12]. It could also be possible that the cholesterol synthesis and transport pathways were adversely affected resulting in increased mean serum TC level in group 1 animals. This observation aligns with a report by Ahyayauch et al..[16], which showed that high levels of lead and lead induced changes in red blood membrane in both human and rats include the changes in lipid and protein profiles of some membrane-associated enzymes.

High-density Lipoprotein (HDL)- cholesterol is a good cholesterol and plays cardioprotective role. It contributes to the reverse cholesterol transport and removal of cholesterol from the peripheral tissues. Furthermore, HDL plays an antioxidant role due to the enzymes paraoxonase activity and prevents LDL from getting oxidized [17]. Hence, an increase in HDL level is considered advantageous. In this study, HDL concentrations were within the normal range of 0.30-1.55mmol/l for human and 0.25-1.09 mmol/l for rats. On the other hand, LDL plays atherogenic role as it transports cholesterol from the liver to other tissues, and increase in its level is considered as one of the predisposing factors for the development of cardiovascular diseases. LDL level was below the physiological range of 1.50-3.50 mmol/l and 0.53-1.29 mmol/l for human and rats respectively in this work, except the value of VLDL that fell within the normal range in rats (0.31-0.67mmol/l) and below that of human (0.62-1.03 mmol/l). The result also indicated TG concentrations to be within the normal physiologic range of 0.4-2.0mmol/l for human and 0.03-1.75 mmol/l for rats.

5. Conclusion

Regulation of lipid metabolism by lipid metabolizing agents is of great importance in the wellbeing of an individual with regards to cardiovascular diseases (CVDs). Heavy metals perturb lipid metabolism amongst its other effect on the biological system, thus increasing the risk of CVD and even death when exposed to them for a prolonged period. This study revealed that exposure of male albino wistar rats to mercury (mercuric chloride) and a combination of lead (lead nitrate) and mercury resulted in mortality. However, though lead nitrate alone did not cause fatality, it resulted in the alteration in the lipid profile of the group of animals exposed to. These results implied that human beings should not be exposed to any heavy metals to avoid mortality.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

The authors declared that there is no conflict of interest

Statement of ethical approval

The ethical approval for the use of experimental animal was obtained from appropriate body.

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