

Full Length Research Paper

Distribution of lead in selected organs and tissues of albino rats exposed to acute lead toxicity

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The study investigated the distribution of lead in the blood, liver, lung, kidney, heart and brain of albino rats, after oral administration of 1000, 1500 and 2000 ppm of lead solution for 7 consecutive days. The various organs were digested by standard procedures and lead concentrations in these organs were determined by Atomic absorption spectrophotometry. The results showed that although lead concentrations in the blood and other tissues of albino rats in the lead treated groups (Groups 2, 3 and 4) were significantly different from those of the control group (Group 1) $p < 0.05$, these difference were not proportional to the quantity of lead the animals were exposed to. It is therefore probable that factors other than quantity of exposure may be responsible for the distribution of lead in various organs of albino rats.

Key words: Lead, blood, toxicity.

INTRODUCTION

Heavy metals are chemical elements with specific gravity that is at least five times the specific gravity of water (Passow et al., 1961; Hawkes, 1997). Examples of heavy metals commonly found in the environment include lead, cadmium, mercury, zinc, arsenic, bismuth etc. These metals are particularly dangerous because they tend to bio-accumulate in the body tissues and organs (Luckey et al., 1975; Babalola et al., 2005).

Lead is a ubiquitous and versatile metal which has been used by mankind for many years. It ranks as one of the most serious environmental poisons amongst the toxic heavy metals all over the world. Mankind has used it for many years because of its wide variety of applications. Human exposure to lead is from numerous sources and a myriad of pathways including air, food, dust, soil and water (Gilfillan, 1965; Herman et al., 2007).

In the recent past, lead toxicity has emerged as an important global problem with public health consequences, particularly in children, due to its serious impact on brain functions. A higher incidence of acute intoxication among children than adults has been reported and children are exposed to higher levels of lead

than are adults because of behavioral patterns (for example, characteristic mouthing of objects, pica). Also, exposures to lead from sources such as air, food and water are higher as per kilogram of body weight basis for children than for adults (Baltrop, 1968; Lin-Fu, 1973; Kamala and Kumar, 1998).

The important sources of lead exposure include gasoline additives, food can solder, lead-based paints, ceramic glazes, drinking water system, cosmetics and plastic recycling industries. In Nigeria, the main source of lead pollution is through automobile exhaust because of the use of leaded petrol. Tetra ethyl lead is commonly used as an anti-knocking additive to improve the quality of petrol in Nigeria and many other countries. (Dioka et al., 2004; Kamal and Kumar, 1998). Research has also shown that lead is present in tobacco, cigarettes contain 2.4 μg of lead and 5% of this occurs in ash and side stream smoke (Mussalo-Rauhmaa et al., 1986).

Lead is absorbed by ingestion and inhalation. Absorption varies from individuals to individuals and depends on the chemical form of lead and type of exposure. The alimentary and respiratory tracts are the main portals of entry for lead into the body. Approximately 90% of absorbed lead is reported to be stored in the bone with a half life of 600 - 3000 days. The remaining 10% is stored in soft tissues like kidney, liver and brain. The half

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life of lead in these tissues ranges from 40 - 50 days (Garrettson, 1990). Lead passes through the placenta easily and fetal blood has almost the same lead concentration as maternal blood (Lauyers et al., 1978; Carpenter, 1974; Ong and Lee, 1980). 90% of the ingested lead is excreted in the stool and urine whereas the inhaled lead is excreted through the renal pathway. Lead is also eliminated through sweat and mother's milk (Jensen, 1983).

Lead has very high affinity for red blood cells, it has been shown that lead inhibits the enzymes δ -aminolevulinic acid dehydratase (ALAD) and ferrochelatase of the heme synthetic pathway thus preventing conversion of ALA to protoporphyrin and inhibits incorporation of iron into the protoporphyrin ring respectively. This results in reduced heme synthesis and elevated levels of the precursor δ -aminolevulinic acid (ALA), which is a weak gamma-aminobutyric acid (GABA) agonist that decreases GABA release by presynaptic inhibition (Warren et al., 1998; Roh et al., 2000; Murata et al., 2003).

Lead is also reported to compete with metals like calcium, zinc, iron and that are essential to our body. Lead's ability to substitute for calcium is a factor common to many of its toxic actions. Picomolar concentrations of lead, competes with micromolar concentration of calcium for binding sites on cerebella phosphokinase C, thereby affecting neuronal signaling (Markovac and Goldstein, 1988).

Lead impairs learning, memory and audio-visual functions in children (Cohn et al., 1993). Toxic effects of lead also include Nephrotoxicity (Nolan and Shaikh, 1992), Hepatotoxicity (Gajawat et al., 2006), Cardiovascular damage (Stofen, 1974). The carcinogenic effect of lead has been receiving increasing attention (IARC, 1993; Silbergeld et al., 2000). Research has shown that lead causes oxidative stress in the body by inducing the generation of free radicals thereby reducing the antioxidant defense system of the cells (Gurer and Ercal, 2000).

Effect of lead on reproductive systems is also well documented. Lead causes sterility in males by damaging the germinal epithelium and also spermatocytes (Goldfrank et al., 1986). In females, menstrual irregularities, preterm deliveries and still births have been reported (WHO, 1986).

This research work was carried out to examine tissue distribution of lead in albino rats exposed to acute lead toxicity. The result will shed more light on lead distribution pattern in human beings exposed to lead

MATERIALS AND METHODS

Animal

Sixteen healthy albino rats weighing between 180 and 200 g were purchased from the Department of Pharmacy, Obafemi Awolowo University, Ile-Ife. The animals were acclimatized for 7 days in the

animal house where they had free access to standard pellets (Guinea Feeds, Benin City, Nigeria) and clean water. The rats were randomly divided into 4 groups and were treated as follow:

Group I (n = 4): The Controls received normal saline (0.9% NaCl) (2 ml per day).

Group II (n = 4): Received 1000 ppm lead acetate (2.0 ml / 100 g body weight)

Group III (n = 4): Received 1500 ppm of lead acetate (2.0 ml / 100 g body weight).

Group IV (n = 4): Received 2000 ppm of lead acetate (2.0 ml / 100 g body weight).

The animals were treated for 7 consecutive days and on the 8th day, they were sacrificed by cervical dislocation and the following were obtained (a) Blood (b) Liver (c) Kidneys (d) Heart (e) Lung (f) Brain

Blood digestion

Whole blood was digested with Concentrated Nitric acid (HNO₃). 1 ml of whole blood was measured into clean test tubes; 1 ml of HNO₃ containing 0.1% triton-100 was added and allowed to mixed thoroughly. The test tubes were plugged with cotton wool and left on the bench overnight. On the second day, the mixture were then heated in a water bath at 100°C for 20 min, thereafter allowed to cool. The digested blood samples were transferred to a measuring cylinder and the volume made up to 25 ml with distilled water.

Tissues and organ digestion

The organs were freeze dried and 1 g of each organ / tissue was grinded to fine powder and 5 ml HNO₃ was added in a clean test tube. The mixture in the test tubes was plugged with cotton wool and it was left on the bench overnight to solubilize. On the second day, the digested sample was heated at 100°C in water bath for 20 min. It was allowed to cool after which 1 ml of hydrogen peroxide was added to each tube to prevent excessive foaming. It was allowed to stand on the bench overnight again.

On the third day, the samples were heated again at 100°C for 1 h and allowed to cool at room temperature. It was thereafter diluted with distilled water to a final volume of 25 ml and stored in a 30 ml polyethylene bottle for later analysis by A.A.S.

Determination of lead concentration

Lead concentration in the digested blood and organs were measured with Alpha 4 Atomic Absorption Spectrophotometer, CHEMTECH 4200 at Obafemi Awolowo University, Ile-Ife Central laboratory. Standard solutions of lead were aspirated to calibrate the AAS before the aspiration of the samples.

Statistical analysis

The results of each of the test groups in Table 1 namely, Groups 2, 3 and 4 were each compared with that of the control (Group 1) using Student t test. Data in Table 2 were analyzed by one way analysis of variance (ANOVA) follow by Duncan's multiple range test (DMRT) using statistical software package (SPSS for Windows). The results were presented as Mean \pm SD. P < 0.05 were regarded as statistically significant.

RESULTS

Table 1 shows the mean concentration of blood lead in

Table 1. Mean concentration of lead in the blood of albino rats.

Group	Lead conc. ($\mu\text{g}/\text{dl}$)
Group I (Control)	9.06 \pm 0.34
Group II (1000 ppm treated rats)	17.22 \pm 0.77*
Group III (1500 ppm treated rats)	13.24 \pm 0.46 *
Group IV (2000 ppm treated rats)	17.89 \pm 0.33*

*P < 0.05.

Each value represents Mean \pm SD (Standard Deviation).**Table 2.** Mean concentration of lead in organs of albino rat ($\mu\text{g}/\text{g}$).

Groups	Liver	Kidneys	Lungs	Heart	Brains
Group I (Control)	3.0 \pm 1.0 ^b	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	2.0 \pm 1.0 ^b
Group II (1000 ppm treated rats)	14.0 \pm 3.0 ^a	24.0 \pm 4.0 ^b	13.0 \pm 4.0 ^a	0.0 \pm 0.0 ^c	3.0 \pm 1.0 ^c
Group III (1500 ppm treated rats)	6.0 \pm 2.0 ^a	20.0 \pm 3.0 ^c	8.0 \pm 2.0 ^a	2.0 \pm 1.0 ^b	6.0 \pm 4.0 ^a
Group IV (2000 ppm treated rats)	8.0 \pm 1.0 ^a	11.0 \pm 3.0 ^b	5.0 \pm 4.0 ^c	0.0 \pm 0.0 ^d	8.0 \pm 3.0 ^a

Values not sharing a common superscript within each row differ significantly at P < 0.05.

Each value represents Mean \pm SD (Standard Deviation).

the four groups of albino rats while Table 2 shows the mean concentration of lead in the blood, liver, lung, kidney, heart and brain of albino rats in the four groups.

DISCUSSION

Lead concentration in the blood is transient and only represents recent exposure of some days (Baltrop, 1969; Maugh, 1978; Laker, 1978). As blood flows through the soft tissues, lead is deposited and bio-accumulated. The half life of lead in the some tissues is about 3 months (Baltrop, 1969; Maugh, 1978; Laker, 1978). Lead accumulates permanently in the bones, consequently, estimates of lead in the teeth can provide a good index of the body burden (De la Burde and Shapiro, 1975; Shapiro et al., 1972). However, teeth and bones are not readily available for such study. Hair tissue has been found to provide an excellent alternative, as hair fixes easily elements such as lead and thus provides an accurate and permanent record of exposure of some minutes' duration (Ajayi et al., 2001; Nnorom et al., 2005; Babalola et al., 2005).

In this study, lead is expected to accumulate in the soft tissues within 7 days of exposure. In addition to the quantity that is distributed to the organs from the blood it is also probable that some quantity of this metal was present in the food given to the animals. The calcium supplement in the feed contains traces of lead hence animals that graze on these contaminated feed will contain substantial amount of lead.

The presence of lead in the blood and tissues of the control rats that were not given lead can also be attributed to the factor mentioned above. Lead

concentration in the blood of experimental rats was also expected to be proportional to the quantity of lead they were exposed to however the results obtained in this study did not agree with this view.

The same mean concentrations of blood lead were obtained for groups II and IV (17 $\mu\text{g}/\text{dl}$) despite the fact they were given different concentrations. This may be partly due to a common problem usually encountered in oral exposure method. It is almost impossible to determine accurately the quantity of materials ingested by the animal, spillage and regurgitation can not be overruled in many cases.

Quantity of food and water taken by the animals also affect the amount of the material that will be absorbed by the animal. This observed discrepancy was also true for the concentration of lead in the various organs of the animal. It is therefore probable that factors other than quantity of exposure may be responsible for the distribution of lead in albino rat. A future study is expected to identify some of these other factors.

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