Ameliorative Effect of Vitamin C in Lead Poisoning on Sex Hormones in Male Wistar Rats

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Abstract

The World Health Organization (WHO) estimates that, about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution, and that most of these environment-related diseases are however, not easily detected and may be acquired during childhood and manifested later in adulthood. The aim of this work was to evaluate ameliorative effects of Vitamin C following lead poisoning on sex hormones, in Wistar Rats. Forty-five male (45) months old male Wistar rats, were divided into experimental (lead fed), Vitamin C (lead and Vit C fed) and control (distilled water) groups. Sex hormones levels were measured using ELISA immunoassays, while blood lead concentration was determined using the method of Atomic Absorption Spectrophotometer. The study showed that Lead increases LH levels and significantly reduces FSH, Testosterone levels in the experimental group, while Vitamin C ameliorates lead effects on Testosterone.

Keywords: Sex Hormones, Lead acetate, Wistar Rats.

INTRODUCTION

The health of 200 million people in low-income countries is at risk from toxins such as lead or mercury, more than from AIDS, tuberculosis and malaria combined (C-Dynamics, 2014); nearly a quarter of deaths in developing countries, including Nigeria and Ghana are linked to pollution (MSN News, 2013).
Lead poisoning has been documented from ancient Rome, Greece, and China. Lead metal may be found in dirt, dust, household utensils, dishes, furniture, leaded petrol, paints, ceramics, food cans, make-ups, traditional remedies, batteries, soil, and water in varying degrees of concentration; poisoning usually occurs from repeated exposure to small amounts. Each of these media can act as a route of human exposure, through ingestion or inhalation and, to a lesser extent, dermal absorption (Agency for Toxic Substances and Disease Registry (ATSDR, 1999). Human exposure can be estimated directly through body burden measurements (in blood, teeth or bone) or indirectly, by measuring its levels in the environment (air, dust, food or water).

Lead is a chemical element in the carbon group with atomic number 82. The metal is a soft, malleable, and heavy post-translation; it has a bluish-white color after being freshly cut but it soon tarnishes to a dull grayish color when exposed to air. Lead has a shiny chrome-silver luster when it is melted into a liquid. It is also the heaviest non-radioactive element (Olade, 1987).

Lead inhibits or mimics the action of calcium, interacts with proteins and binds with virtually every available functional group, including sulfhydryl, amine, phosphate, and carboxyl groups, with sulfhydryl having the highest affinity (Guidotti, and Ragain, 2007). Recent studies indicate that transition metals act as catalysts in biological macromolecule reactions, and their toxicities might be due to oxidative tissue damage. Redox-active metals such as iron, copper and chromium undergo redox cycling, while redox-inactive metals such as lead, mercury, cadmium, and others deplete cell major anti-oxidants, especially thiol-containing anti-oxidants and enzymes. Such metals cause increase in reactive oxygen species (ROS) production, such as hydroxyl radical (HO\(^-\)), superoxide radical (O\(_2\)\(^-\)) or hydrogen peroxide (H\(_2\)O\(_2\)) leading to oxidative stress, consequently causing protein, lipid, and DNA lesions (Nuran et al., 2001). Fatty acids containing up to 2 double bonds are more resistant to oxidative stress than are the polyunsaturated fatty acids (Bradberry and Vale, 2009).

Countries in Africa, with the exception of the Republic of South Africa, have not implemented lead reduction programmes. Lead pollution has continued to pose health hazards in animal and man in Nigeria and many other parts of the world (Ajayi, 2009). A study on some physico-chemical characteristics and heavy metal profiles of 72 Southern Nigerian rivers, streams, and waterways, conducted in their water samples showed that, about 34 (47%) of them contained some levels of lead (Asonye et al., 2007). The major sources of heavy metal pollution in urban areas of Africa are anthropogenic, while contamination from natural sources predominate in the rural areas. The anthropogenic sources include those associated with fossil fuel and coal combustion, industrial effluents, solid waste disposal, fertilizers, mining and metal processing. At present, the impact of these pollutants is confined mostly to the urban centres with large populations, high traffic density and consumer-oriented industries. Natural sources of pollution include weathering of mineral deposits, bush burning and windblown dusts (Olade, 1987). Other sources include emissions from electric generators used in both homes and industries.

Many anti-oxidants including Vitamin C have been used in the treatment of lead toxicity. Vitamin C is important in maintaining the physiological integrity of the testes (Eltohamy, 2010). The use of
chelating agents and few antioxidants such as vitamin C and E (Mehta et al., 2001) can enhance lead excretion in lead poisoning (Flora and Tandon, 1990).

At the same time, there are reports on the mitigating effects of Vitamin C in lead poisoning on various body systems, organs, and blood parameters by co-administration of Pb and Vitamin C, but studies are limited on its ameliorative effects following the heavy metal exposure and such effects on sex hormones. The purpose of this study is to evaluate the ameliorative effect of Vitamin C following sub-chronic lead poisoning on male sex hormones.

MATERIALS AND METHOD

Fourty five (45) Male Wistar Rats were devided into three groups of Experimental, Vit C and Control groups respectively.

- experimental animals (n=15), were fed orally with aqueous lead acetate solution at 250mg/kg body weight per day (Ambali et al., 2011) for 22 days.
- Vit C group animals (n=15), were fed orally with aqueous lead acetate solution at 250mg/kg body weight per day (Ambali et al., 2011) for 22 days, fed orally with vitamin C in distilled water at 100 mg/kg body weight (Ambali et al., 2011) for another 10 days.
- The control group animals (n=15) were placed on distilled water only.

Both Experimental, Vit C and Control animals were acclimatized for 7days prior to commencement of experiment; animals were housed in metallic cages and given free access to laboratory chow and water. After intervention, rats were lulled to sleep by intravenous injection of 0.5cm$^3$ of 0.4% solution of sodium thiopental (Greene, 2002) and afterwards decapitated. Blood samples were collected and separated into two: first sample collected portion was centrifuged to get plasma which was used for the estimation of plasma FSH, LH, and testosterone concentrations using ELISA immunoassay; second sample was collected in heparinised test-tubes was used for spectrophotometric analysis of blood lead concentration in blood samples, using Atomic Absorption Spectrophotometer (BUCK Scientific; model: 210 VGP, USA).

Data was collected, Mean and P-Value were presented in tables. Data was analyzed using ANOVA, and Bonferroni (Post-hoc) to compare significant difference between experimental, Vit C and control groups.

RESULTS

Table 1: Weight (g) Change after Experiment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Vit C</th>
<th>Experimental</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight</td>
<td></td>
<td>4.06**</td>
<td>-24.25*</td>
<td>31.37</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* indicates significant difference.
Table 2: Sex Hormones Concentration

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Group</th>
<th>Vit C</th>
<th>Experimental</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td></td>
<td>0.6273**</td>
<td>3.6491</td>
<td>3.2877</td>
<td>0.046</td>
</tr>
<tr>
<td>FSH</td>
<td></td>
<td>1.1982</td>
<td>1.4864</td>
<td>2.7231</td>
<td>0.507</td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
<td>1.0782*</td>
<td>0.7627*</td>
<td>2.8477</td>
<td>0.042</td>
</tr>
</tbody>
</table>

*P ≥ 0.05 compared to control animals
**P ≥ 0.05 compared to Experimental animals

DISCUSSION

Result of the experiment showed that, sub-chronic lead poisoning led to significant loss of weight in all lead-treated animals. Significant changes in weights were recorded in all age groups, weight gain of 31.37g and 4.06g in control and Vit C groups respectively, while experimental had mean weight loss of 24.25g.

This study supports other workers (Cezard and Haguenoer, 1992) who suggested that the weight loss might be attributed to loss of appetite and gastrointestinal disturbances; as well as to interruption in absorption and overall metabolism of feed nutrients (Marchlewicz et al., 2006).

Such reduction of body weight has been reported by other workers (Aseth et al., 1995; Teijon et al., 2006; Jegede, Ajadi and Akinloye 2013). Such loss of body weight might also be due to interruption of absorption and metabolism of essential feed nutrients by lead acetate (Marija et al., 2004).

There was no significant difference in LH levels between the, Experimental and control animals. This is in accordance to Sokol et al., (1987), and Fatima et al., (2011) that lead showed no significant effect on LH level, but contrary to findings of Camoratto(1990); Mukherjee and Mukhopadhyay, (2009) and Taiwo et al., (2010) who reported that lead decreases LH concentration in experimental animals. On comparism Vitamin C significantly reduced LH level below control group level.

Studies in rats have demonstrated direct effect of lead on the testes and interference in the hypothalamic-pituitary axis (Saxena, 1986); disruption of the hypothalamic control of pituitary hormone secretion and spermatogenesis (Sokol, 1987); also gonadotoxic (Taiwo, et al. 2010).

Lead caused insignificant reduction in FSH level in this study. This is in conformity with Sokolet al.,(1987), but contrary to Raule, (1952) and Petrusz, (1979) who reported that lead increased FSH concentration. Vitamin C also revealed no ameliorative effect on the FSH level.

Even though unchanged FSH concentrations in lead acetate treated mice were also reported (Pinon-Lataillade et al., 1995), such differences in FSH secretion levels might relate to differing lead levels and/or the duration of exposure (Mohsen et al., 2011).
Lead poisoning led to reduction of testosterone level, while Vitamin C showed insignificant ameliorative role to the effect of lead. Lead significant reduction in Testosterone Levels\((P \leq 0.05)\) is also in conformity with reports by Cullen \textit{et al.},(1983); Sokol \textit{et al.},(1987); Camoratto, (1990); Mukherjee and Mukhopadhyay, (2009); Fatima \textit{et al.}, (2011); Taiwo \textit{et al.}, (2010) who reported similar effects. The study by Fatima, \textit{et al.}, (2011) supports the hypothesis that lead exerts its toxic effects on the hypothalamic or supra-hypothalamic sites, leaving pituitary-testicular axis intact.

In this study lead showed no significant effect on the LH levels, but significant decrease in testosterone. This suggests that lead exerts its effect more on the testicular level. This study also supports the finding of Sokol, (1987), Wadi, (1999) and Taiwo \textit{et al.}, (2010) who ascerted that lead targets the spermatogenesis and sperm within the epididymis by producing reproductive toxicity rather than acting within the hypothalamic-pituitary-testicular axis.

The role of vitamins (particularly B, C and E) has been found to be extremely significant and competitive in fighting toxicological manifestations of lead poisoning (Naidu, 2003). Vitamins C and E reduce the production of reactive oxygen species (ROS) through their scavenging, quenching (Das and Saha, 2010; Tariq, 2007) and mopping up of ROS (Valko \textit{et al.}, 2006). Such vitamins also significantly reduce blood lead concentration, ameliorate hepatic damage and significantly reduce the oxidative stress as indicated by decreased MDA and NO levels, while at the same time increased glutathione levels, activities of SOD and catalase in the brain of rats (Ebuehi, 2012); erythrocytes (Ganesh, 2016).

**CONCLUSIONS**

Lead increases LH levels and significantly reduces FSH, Testosterone levels in the experimental group, while Vitamin C ameliorates lead effects on Testosterone.
REFERENCE
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