DETERMINATION OF CADMIUM AND LEAD RESIDUES IN TISSUES AND ADMINISTRATION PROTECTIVE TREATMENT

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ABSTRACT

Chronic exposure to heavy metals including lead and cadmium remains a serious problem for humanity. The current study aims to evaluate the impact of exposure to lead (Pb) and cadmium (Cd) on liver and kidney tissues of albino rats. 50 albino rats were divided equally into five groups respectively orally fed with lead acetate and cadmium chloride at 25 mg/Kg body weight, and 5.0 mg/kg. Body weight. The blood of each animal was collected and biochemical assays were conducted. Data were processed with SPSS 15.0. The results showed a significant increase in lead and cadmium residues in groups exposed to lead and cadmium. Not only that but also, the function of oxidative damage Pb and Cd induced changes in the liver and kidney. Administration of thiocytic acid as a treatment for these changes lead to significant improvement for these ratio. In conclusion, this study highlights a real problem of public health, in the light of thousands of patients receiving random therapy after exposure to heavy metals

Keywords: Lead acetate, Cadmium chloride, Biochemical parameters, Heavy metal, Thiocitic acid, Albino rats

1. INTRODUCTION

"Heavy metals" are of a specific gravity that is at least five times the specific gravity of water, which is 1 at 4 °C. For example, the specific gravities of cadmium and lead are 8.7 and 11.3, respectively (CRC, 1992). In recent years,
the level of heavy metals, particularly lead has increased in air, water and soil in both urban and periurban areas (Gupta, 2007). There is a lot of heavy metals in our environment: cadmium, chromium, cobalt, copper, lead, mercury, etc. Interestingly, small amounts of these elements are common in our environment and are actually necessary for good health (Jasim et al., 2012).

Although, humans were exposed to Pb through their environment and diet so, more than 75% of lead-exposure for the general population comes from ingestion (Patrick, 2006). Lead is considered a natural element and widespread in the environment. This heavy metal is still mined and added to many products including paints, eye cosmetics, gasoline, water pipes and health care supplies. The two major routes of lead entry into the body are the alimentary and respiratory tracts (Fischbein, 1992). Lead absorption and ingestion depends on many factors such as the particle size, physical form, gastrointestinal transit time and nutritional status of a person. Lead absorption increases, with the increase of age, making children more vulnerable to lead intoxication (Campbell et al., 2004).

On the other hand, when cadmium is given during lactation, to lactating mothers and newborn pups, cadmium chloride residues were found in the gastrointestinal tract of each (Saillenfait et al., 1992). In experimental animals application of cadmium affected tissue, caused blood vessel hemorrhage and resulted in cellular degeneration (Fréry et al., 1993). The use of antioxidant rich food or antioxidant food supplements became immensely popular since many diseases have been associated with oxidative stress. Therefore, in the last decade, an increasing attention has been focused on free radical scavengers that are able to protect against aberrant effects of free radicals (Marangon et al., 1999). Administration of antioxidants is effective in reducing the toxic effects of some heavy metals (Inkielewicz-Stepniak and Knap, 2013).

Although preliminary studies have indicated possible benefit of thioctic acid in the treatment of alcoholic liver disease, thioctic acid had no significant influence on the course of the disease (Marshall et al., 1982), so in our study we will investigate the protective effect of ALA from increasing of lead and cadmium concentration after intoxication of rats.

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Experimental animals:

Fifty healthy white male albino rats, 8-10 weeks old and weighting 140-180 gm, were used in the experimental investigation of this study. Rats were obtained from the Egyptian company for production of vaccines, sera, and drugs (Vacsera), Helwan branch. Animals were housed at faculty of veterinary
medicine, Mansoura university in separate metal cages, fresh and clean drinking water was supplied ad-libitum.

2.1.2. Chemical and drugs
1- Lead acetate, rats received lead acetate (1/20 of LD$_{50}$) orally and daily at a dose level of (25 mg/Kg b.wt) (Debosree et al., 2012).
2- Cadmium chloride, rats received cadmium chloride (1/20 of LD$_{50}$) orally and daily at a dose level of 5.0 mg/kg. Bodyweight (Van et al., 1981).
3- Thiocetic acid (Alpha lipoic acid), Rats received Thiocetic "Alpha lipoic acid" capsules dissolved in distilled water (freshly prepared) orally and daily at a dose of 54 mg/kg body weight as recommended by (Gruzman et al., 2004).

2.1.3. Experimental design
The rats were divided into five equal groups after accommodation to the laboratory conditions, one control and four experimental groups, each consisting of ten animals placed in individual cages.

Group I: (control group):
Rats of this group received drinking water without any chemical drugs, served as control for all experimental groups.

Group II: (Lead only exposed group)
Rats of this group received lead acetate 1/20 of LD$_{50}$ (25mg/Kg b.wt) orally once per day over a period of 10 weeks as applied by (Debosree et al., 2012).

Group III: (Cadmium only exposed group):
Rats of this group received cadmium chloride 1/20 of LD$_{50}$ (5.0 mg/kg, body weight) orally and once per day over a period of 10 weeks as recommended by (Van et al., 1981).

Group IV: (lead acetate with thiocetic acid "ALA" treated group)
Rats of this group received lead acetate orally and daily (25mg/Kg b.wt) and treated with Thiocetic acid "Alpha-lipoic acid" at a dose of (54 mg/kg body weight orally /day) (Gruzman et al., 2004).

Group V: (Cadmium Chloride with thiocetic acid "ALA" treated group):
Rats of this group received cadmium chloride (5.0 mg/kg, body weight) and treated daily with Thiocetic acid "Alpha-lipoic acid" at a dose level of (54 mg/kg body weight orally /day) (Gruzman et al., 2004).

2.1.4. Sampling for Tissues:
Tissue (liver and kidney) samples were collected from all animals groups, control and four experimental groups two times along the duration of experiment at five and ten weeks from the beginning of rats exposure to lead, cadmium and antioxidant administrated.

2.2. Methods and Instrumentation:
2.2.1. Varian Atomic Absorption spectrometry
Samples were analyzed by Varian atomic absorption spectrometry (AAS) for the determination of lead and cadmium concentrations in liver and kidney
"residue" at wavelength of 217 and 228 nm respectively (Varian®, 2010) and (Skoog, 1992).

2.2.2. Statistical analyses
All statistical analyses were done by statistical software package “SPSS 15.0 for windows, SPSS Inc. Chicago, Illinois” and the GraphPad Prism package; v.5.0 (GraphPad Software, San Diego, CA). Animal’s baseline characteristics were descriptively summarized and reported as mean ± standard error of mean (SEM). Student’s test was used to compare continuous variables. All tests were two-tailed. The result of the t-values was then checked on student's-t-table to find out the significance level (P value) as reported by (Pearson and Hartley, 1951).

3. RESULTS

3.1. Lead and cadmium residues concentration in liver and kidney:
Effect of thiocytic acid treatment on lead and cadmium residues concentration in normal and intoxicated rats with lead and cadmium is illustrated in figures (1 and 2), respectively.

3.1.1. Lead residue in liver
Rats which exposed to lead, showed a significant increase in liver lead residues concentration after five and ten weeks from beginning the experiment comparing with normal control group where P value was 0.0001 as presented in table (1). After administration of thiocytic acid for lead intoxicated male rats after five and ten weeks from the beginning of experiment comparing with normal control group, there are a significant decrease in liver lead residues concentration where P value was 0.0025 after five weeks and 0.0028 after ten weeks as presented in table (2).

3.1.2. Lead residue in kidney
Rats which exposed to lead, showed a significant increase in renal lead residues concentration after five and ten weeks from the beginning of the experiment comparing with normal control group where P value was 0.0001 as presented in table (1). After administration of thiocytic acid as in case of liver, there are a significant decrease in renal lead residues concentration where P value was 0.0001 after five and ten weeks as presented in table (2).

3.1.3. Cadmium residue in liver
Rats which exposed to cadmium, showed a significant increase in liver cadmium residues concentration after five and ten weeks from the beginning of the experiment comparing with normal control group where P value was 0.0001 as presented in table (3). After administration of thiocytic acid for cadmium intoxicated as in lead case, there are a significant decrease in liver cadmium residues concentration where P value was 0.0001 after five weeks and 0.0002 after ten weeks as presented in table (4).

3.1.4. Cadmium residue in kidney
Rats which exposed to cadmium, showed a significant increase in renal cadmium residues concentration after five and ten weeks from the beginning of the experiment comparing with normal control group where $P$ value was 0.0001 as presented in table (3). After administration of thioctic acid as in case of liver, there are a significant decrease in renal cadmium residues concentration where $p$ was 0.0001 after five and ten weeks as presented in table (4).

**Table 1.** Lead levels (ppm) in renal and liver tissues after 5 and 10 weeks of rat’s treatment with lead.

<table>
<thead>
<tr>
<th>Type of tissues</th>
<th>After 5 weeks of treatment</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Pb treated animals</td>
</tr>
<tr>
<td>Renal tissues</td>
<td>14.9±1.3</td>
<td>133.8±0.3</td>
</tr>
<tr>
<td>Liver tissues</td>
<td>17.6±1.1</td>
<td>46.8±1.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of tissues</th>
<th>After 10 weeks of treatment</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Pb treated animals</td>
</tr>
<tr>
<td>Renal tissues</td>
<td>35.5±1.2</td>
<td>176.4±2.7</td>
</tr>
<tr>
<td>Liver tissues</td>
<td>41.0±1.5</td>
<td>69.3±1.9</td>
</tr>
</tbody>
</table>

Continuous variables were expressed as mean ± SEM. Pb= lead; $P>0.05$ is considered not significant, $P<0.05$ considered significant

**Table 2.** Role of thioctic acid on reduction of lead levels (ppm) in renal and liver tissues of lead intoxicated rats after 5 and 10 weeks of treatment.

<table>
<thead>
<tr>
<th>Type of tissues</th>
<th>After 5 weeks of treatment</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pb treated animals</td>
<td>Pb+TA cotreated animals</td>
</tr>
<tr>
<td>Renal tissues</td>
<td>133.8±0.3</td>
<td>36.5±1.3</td>
</tr>
<tr>
<td>Liver tissues</td>
<td>46.8±1.7</td>
<td>31.2±1.6</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of tissues</th>
<th>After 10 weeks of treatment</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pb treated animals</td>
<td>Pb+TA cotreated animals</td>
</tr>
<tr>
<td>Renal tissues</td>
<td>176.4±2.7</td>
<td>67±1.1</td>
</tr>
<tr>
<td>Liver tissues</td>
<td>69.3±1.9</td>
<td>56.9±1.9</td>
</tr>
</tbody>
</table>

Continuous variables were expressed as mean ± SEM. Pb= lead; TA= thioctic acid; $P>0.05$ is considered not significant, $P<0.05$ considered significant.
Table 3. Cadmium levels (ppm) in renal and liver tissues after 5 and 10 weeks of rat’s treatment with cadmium.

<table>
<thead>
<tr>
<th>Type of tissues</th>
<th>After 5 weeks of treatment</th>
<th></th>
<th>After 10 weeks of treatment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Cd treated animals</td>
<td>Controls</td>
<td>Cd treated animals</td>
</tr>
<tr>
<td>Renal tissues</td>
<td>19.5±1.4</td>
<td>511.0±14.6</td>
<td>19.1±0.8</td>
<td>601.0±10.3</td>
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<tr>
<td>Liver tissues</td>
<td>16.8±1.1</td>
<td>243.0±8.0</td>
<td>17.0±1.4</td>
<td>298.3±7.6</td>
</tr>
</tbody>
</table>

Table 4. Role of thioctic acid on reduction of cadmium levels (ppm) in renal and liver tissues of cadmium intoxicated rats after 5 and 10 weeks.

<table>
<thead>
<tr>
<th>Type of tissues</th>
<th>After 5 weeks of treatment</th>
<th></th>
<th>After 10 weeks of treatment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cd treated animals</td>
<td>Cd+TA cotreated animals</td>
<td>Cd treated animals</td>
<td>Cd+TA cotreated animals</td>
</tr>
<tr>
<td>Renal tissues</td>
<td>511.0±14.6</td>
<td>234.3±9.7</td>
<td>601.0±10.3</td>
<td>319.0±10.7</td>
</tr>
<tr>
<td>Liver tissues</td>
<td>243.0±8.0</td>
<td>101.0±5.5</td>
<td>298.3±7.6</td>
<td>228.8±4.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of tissues</th>
<th>After 10 weeks of treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P value</th>
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<tr>
<td></td>
<td>Cd treated animals</td>
<td>Cd+TA cotreated animals</td>
<td>Cd treated animals</td>
<td>Cd+TA cotreated animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal tissues</td>
<td>601.0±10.3</td>
<td>319.0±10.7</td>
<td>601.0±10.3</td>
<td>319.0±10.7</td>
<td>&lt;0.0001</td>
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<tr>
<td>Liver tissues</td>
<td>298.3±7.6</td>
<td>228.8±4.5</td>
<td>298.3±7.6</td>
<td>228.8±4.5</td>
<td>0.0002</td>
<td></td>
</tr>
</tbody>
</table>
4. DISCUSSION

From the above results presented in tables (1-4) and figures (1,2), it was concluded that, a significant increase in Kidney and liver residues concentration from lead and cadmium was observed in lead and cadmium
intoxicated male rats after five and ten weeks of the experiment when compared with control group. In case of lead intoxicated rats, these results came in accordance with the recorded data of (Liu et al., 2012) who reported that, the lead levels in blood and kidney of lead-treated rats are significantly higher than those of control rats. Also, these results came in accordance with the recorded data of (Gaurav et al., 2011) who reported that, a significant increase in the toxic metal level in the liver, kidney and blood. Not only that but also, the function of oxidative damage in Pb and Cd induced changes in steroidogenesis in the liver and kidney (Dai et al., 2013).

Treatment with thiocitic acid (α-lipoic acid) to lead intoxicated rats, significantly reduced elevated Kidney and liver lead residues concentration and Liver lead residues concentration in lead intoxicated male rats after eight and ten weeks from the onset of treatment with α-lipoic acid. These results came in accordance with the recorded data of (Osfor et al., 2010) who reported that, alpha lipoic acid decrease lead levels in serum and kidney tissue of lead intoxicated rats compared to the control rats. The liver and the kidneys are also known to play a major role in the elimination of lead (Goyer and Chirian, 1979) and hence, account for the toxic actions (Lockitch, 1993).

In case of cadmium intoxicated rats, a significant increase in liver cadmium residues concentration was observed in cadmium intoxicated male rats after five and ten weeks of the experiment when compared with control group. These results came in accordance with the recorded data of (Gaurav et al., 2011), who reported that, a significant increase in the toxic metal level in the liver, kidney and blood with higher amount in the kidney which was evident from the data showing maximum accumulation of cadmium after 21 days. Also, (Ji et al., 2010) reported that, the absolute amounts of Cd in serum, livers, kidneys and testes were significantly increased in Cd treated mice as compared with controls. In addition, pubertal exposure to Cd also obviously increased the relative contents of Cd in mouse livers, kidneys against serum. Treatment with thiocitic acid (α-lipoic acid) to cadmium intoxicated rats, significantly reduced elevated Kidney Cadmium residues concentration and Liver Cadmium residues concentration in cadmium intoxicated male rats after four and ten weeks from the onset of treatment with thiocitic acid. These results came in accordance with the recorded data of (Packer et al., 1995) and (Biewenga et al., 1997) who reported that, LA has the ability to generate endogenous antioxidants, such as GSH.

REFERENCES


تعيين نسب الكادميوم والرصاص المتبقية في الأنسجة
وتقديم العلاج الواقي لها

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مدير جودة الصرف الصحي - شركة مياه الشرب والصرف الصحي بالدقهلية
مدير معمل بلقاس - الدقهلية، وباحث دكتوراه في الكيمياء الحيوية.

أجرت الدراسة الحالية على عدد 50 فأر من ذكور الفئران البيضاء، وتم تقسيم هذه الفئران إلى خمسة مجموعات كل مجموعة تحتوي 10 فئران، المجموعة الأولى "الضابطة" لم تعطي أي من المواد الدخيلة، والمجموعة الثانية "المسممة بالرصاص" حيث تم تجريعهم بالرصاص يومياً عن طريق الفم بجرعة 25 ملليجرام لكل كيلو جرام من وزن الجسم. والمجموعة الثالثة "المسممة بالكادميوم" تم تجريعهم بالكادميوم يومياً عن طريق الفم بجرعة 5 ملليجرام لكل كيلو جرام من وزن الجسم والمجموعة الرابعة "المسممة بالرصاص مع العلاج بمضاد التأكسد" وتم تجريعهم الرصاص بنفس الجرعة وتقديم مضاد الأكسدة "حمض الثايوكتك" بجرعة قدرها 54 ملليجرام لكل كيلو جرام من وزن الجسم لمدة عشر أسابيع. والمجموعة الخامسة "المسممة بالكادميوم مع العلاج بمضاد التأكسد" وتم تجريعهم الرصاص بنفس الجرعة وتقديم مضاد الأكسدة "حمض الثايوكتك" أيضاً بنفس الجرعة السابقة لمدة عشر أسابيع.

واخذ عينات الأنسجة بعد ذبح الفئران بعد خمس وعشر أسابيع لتخضع للتحليل.

وقد أظهرت النتائج زيادة ملحوظة في تركيز نسب الرصاص والكادميوم في انسيجة الكبد والكلى مما يؤدي إلى تلف هذه الخلايا، وتقديم حمض الثايوكتك كعلاج للتسمم ومعرفة مدى تغير هذه النسب المتبقية في خلايا الكبد والكلى، وآسفرت النتائج عن انخفاض ملحوظ في هذه النسب مقارنة بالمجموعات الضابطة، لذلك نوصي باستخدام حمض الثايوكتك في علاج سمية تراكم العناصر الثقيلة في كبد وكلى الحيوانات.