PROTECTIVE EFFECTS OF SELENIUM ON HEPATOTOXICITY CAUSED BY SUBACUTE EXPERIMENTAL COMBINED EXPOSURE TO CADMIUM AND LEAD IN RATS

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Abstract

Cadmium (Cd) and lead (Pb) toxicity is mediated by multiple mechanisms. The purpose of this study was to assess the potential protective effects of selenium (Se) in the case of experimental Cd, Pb or combined Cd and Pb exposure through drinking water in rats. Male Wistar rats were used (8 experimental groups, n = 6). The experiment lasted for 56 days. The groups were: Control (distilled water), Se (Se 0.2 mg/L), Cd (Cd - 150 mg/L), Pb (Pb - 300 mg/L), Cd+Pb (Cd - 150 mg/L; Pb - 300 mg/L; Se - 0.2 mg/L), Cd+Se (Cd - 150 mg/L; Se - 0.2 mg/L), Pb+Se (Pb - 300 mg/L; Se - 0.2 mg/L). Cd exposure resulted in an increase in transaminases activities and a modification in the serum protein fractions ratios, which was alleviated by Se. Cd or/and Pb exposure altered the normal hepatic histology, which was improved by Se. Cd exhibited potential protective action by improving some biochemical parameters and the hepatic histological architecture and reducing the intensity of p53 and caspase-3 immunostaining.

Keywords: selenium, cadmium, lead, hepatotoxicity

Introduction

Cadmium (Cd) and lead (Pb) are ubiquitous environmental pollutants, with toxic effects in humans, animals and plants. The general human population is exposed to cadmium through food and water intake, cigarette smoking and inhalation of ambient air [63]. Diet is the main source of Cd exposure in non-smokers. Occupational exposure to Cd occurs mainly through inhalation, in the course of industrial processes in which Cd-containing materials are heated, such as smelting and electroplating [2]. People are exposed to lead by eating food or drinking water that contains lead and by cigarette smoking [1, 52]. Occupational exposure to lead occurs especially through inhalation, in case of the lead-emitting industries, such as lead smelters or lead acid battery recycling plants [52]. International and national institutions have established guidelines regarding the maximum allowable cadmium or lead levels in food products, medicinal plants, drinking water and environment [1, 2, 33, 63]. Cadmium has several toxic effects in animals and humans, including nephrotoxicity, hepatotoxicity, carcinogenicity, teratogenicity, endocrine disruption, reproductive toxicity and undesired effects on bones [43, 48]. The distribution of Cd between various tissues depends on many endogenous and exogenous factors and acute exposure results in a different distribution pattern throughout the body than the chronic exposure does. Cadmium accumulates mainly in the kidneys and in the liver [58]. It generally has a very low excretion rate, thus it has a long biological half-life. In mice and rats the half-life
can range between 200 and 700 days, depending on the level of exposure and other factors [43]. Cadmium is not known to undergo direct metabolic conversions [2]. The interaction between Cd and metallothionein plays a critical role in its toxicokinetics and toxicodynamics [2, 27, 43]. It is retained in the kidneys and in the liver mainly bound to metallothionein [2, 27]. Generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals is the main mechanism of Cd toxicity in the case of acute poisoning, but in the case of chronic poisoning acquired Cd tolerance occurs, with aberrant gene expression [32]. Cadmium may replace some essential metals such as copper, iron, manganese, zinc in their biological systems [40]. Lead exposure produces various harmful effects at hematopoietic, renal, nervous, bone and gastrointestinal tract level [46, 52]. After exposure, Pb accumulates in blood, soft tissues and bones. In blood, approximately 99% of Pb is found in erythrocytes while in soft tissues it accumulates mainly in the liver and in the kidneys [1, 46, 52]. While the half-life for Pb from blood generally does not exceed one month, Pb from bones can have a half-life of decades in humans [1, 52]. Pb has the ability to substitute polyvalent cations of essential metals (especially calcium - Ca\(^{2+}\) and zinc - Zn\(^{2+}\)) in their biological systems and subsequently perturb significant processes, including metal transport, energy metabolism, apoptosis, inter- and intracellular signalling, cell adhesion and enzymatic processes [21]. The main mechanism of Pb toxicity, leading to the reduction of mental activity in children, is considered to be related to the alteration of calcium channels functioning and of glutamate and cholinergic systems in the developing brain [1, 21]. Oxidative stress has been reported as another mechanism of Pb toxicity, with two simultaneous pathways: a) generation of ROS such as hydroperoxides, singlet oxygen and hydrogen peroxide and b) depletion of antioxidant reserves, such as the inactivation of glutathione [19, 52]. In the majority of experimental studies with the objective of assessing Cd or Pb toxicity a single metal is usually used, in high doses. Combined Cd and Pb toxicity has been investigated much less. Co-exposure to Cd and Pb generally results in additive toxic effects [20, 34, 67]. It is possible that Cd and Pb could also exert synergistic toxic effects on particular systems that have not been investigated within experimental studies. Selenium (Se) is an essential microelement for animals and humans. It enters in the structure of some proteins and enzymes with multiple physiological roles, of which the most important are the glutathione-peroxidases, the deiodinases of the thyroid hormones and the thioredoxin reductases. In high amounts, selenium can exert toxic effects [49].

Some studies have found that Se protects animals against toxicity caused by exposure to and/or intake of metals such as cadmium, lead, mercury, arsenic, vanadium, silver [42]. There are multiple mechanisms involved in Se protective effects. Selenium compounds are known for their ability to scavenge ROS [11]. The glutathione-peroxidase catalytic cycle in which glutathione acts as the reducing substrate involves the formation of active selenol, which reduces peroxide [11, 49]. Some Se compounds have been shown to prevent metal-induced ROS generation by binding metals, such as copper or iron, in vivo and in vitro [10, 11, 65].

The primary aim of this study was to assess the effects of selenium exposure in case of experimental exposure to cadmium, lead or a combination of the two metals through drinking water on histopathological changes of the liver and on some blood biochemical parameters.

Materials and Methods

Reagents
All reagents were of analytical grade. Distilled water was used as drinking water for the animals. Standard dry chow for rats was purchased from “Cantacuzino” National Institute for Research and Development in Microbiology and Immunology. Cadmium chloride hemipentahydrate (CdCl\(_2\) * 2.5H\(_2\)O), lead acetate trihydrate (Pb(CH\(_3\)COO)\(_2\) * 3H\(_2\)O) and sodium selenite pentahydrate (Na\(_2\)SeO\(_3\) * 5H\(_2\)O) were used as the sources of Cd, Pb and Se. Ketamine 10%, injectable solution for veterinary use (Kepro VB, Holland) was used as anaesthetic. A Hydragel β1-β2 (Sebia, France) kit was used for the determination of serum proteins; the other blood biochemical parameters were determined using specific assay kits from Cormay (Poland). Novocastra\(^\text{®}\) lyophilized mouse monoclonal antibody CPP32 (Caspase-3) (Leica Biosystems, Germany), p53 protein (DO-7) mouse monoclonal antibody (Vector Laboratories, USA) and Vectastain Elite ABC kit (Vector Laboratories, USA) were used for immunohistochemistry.

Instruments
 Serum was separated on a Hettich Rotina 38R (Andreas Hettich GmBH, Germany) centrifuge. An Accent 200 (Cormay, Poland) automatic biochemical analyser was used to determine biochemical parameters from blood. The gels for serum proteins were generated using a Sebia system, scanned using a Sebia Gelscan system, analysed and quantified with Sebia’s Phoresis software (Sebia, France). The stained liver sections were analysed using an Olympus CX41 microscope with an integrated DP21 camera.

Animals and procedures
The animal protocol used in this work was evaluated and approved by the Ethics Committee for Research of “Grigore T. Popa” University of Medicine and Pharmacy, Iași, Romania. All procedures were

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conducted in accordance with the 86/609/EEC directive, with the internal regulations of the university and with other applicable regulations. A number of 48 male Wistar rats, purchased from “Cantacuzino” National Institute for Research and Development in Microbiology and Immunology – Băneasa, Romania, animal facility, having the initial weight: 250 - 400 g and the age approximately 8 months were randomly divided into 8 experimental groups (n = 6) and subsequently marked. The rats were maintained throughout the experiment at 18 - 25°C, with a light/dark cycle of 12/12 h and were housed in collective cages (each experimental group in one collective cage). The animals had free access to food (dry chow) and water (containing the elements of interest), ad libitum. Special water dispensers with stoppers were used and the water consumption for every group was measured on a daily base. The animals were weighed every 7 days. The substances of interest were dissolved in the drinking water.

The experimental groups were: Control group (only distilled water); Se group (Se - 0.2 mg/L); Cd group (Cd - 150 mg/L); Pb group (Pb - 300 mg/L); Cd+Pb group (Cd - 150 mg/L and Pb - 300 mg/L); Cd+Se group (Cd - 150 mg/L and Se - 0.2 mg/L); Pb+Se group (Pb - 300 mg/L and Se - 0.2 mg/L); Cd+Pb+Se group (Cd - 150 mg/L, Pb - 300 mg/L and Se - 0.2 mg/L).

The experiment lasted for 56 days and the animals were subsequently anaesthetised. Ketamine (100 mg/ kg body weight (bw)) was injected intraperitoneally to induce anaesthesia. After the onset of the ketamine effect, the rats were immobilised and the thorax and the abdomen were opened. Blood was collected through cardiac puncture in special vacutainers. The livers were removed, examined and weighed.

### Body weight, liver weight, water consumption and element intake

The evolution of the body weight was calculated as percent of increase/decrease in comparison to the initial body weight, for every rat, at every weighing moment. The mean percent was then calculated for every experimental group. The relative liver weight was expressed for every rat as g of liver/100 g bw at the end of the experiment. Means were then calculated for every experimental group.

Daily approximate water consumption for one rat from a group was estimated by dividing the volume of water consumed by that experimental group during 24 hours to 6 (the number of rats in a group). Means for 7 days intervals were then calculated.

Daily approximate water consumption reported to kg bw for a group was estimated by dividing the volume of water consumed by that experimental group during 24 hours to the total weight of the rats from that group (the previous weighing). Means for 7 days intervals were then obtained.

Based on the daily approximate water consumption reported to kg bw for each group and on the concentrations of Cd, Pb and Se in the drinking water, the daily approximate intake reported to kg bw for each element was obtained. The means for the entire experimental period of 56 days were then calculated. The estimated intake of each element for each experimental group is presented in Table I [53, 54].

### Table I

Estimated intake of cadmium, lead and selenium through the drinking water for the experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Cd (mg/kg bw)</th>
<th>Pb (mg/kg bw)</th>
<th>Se (µg/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Se</td>
<td>-</td>
<td>-</td>
<td>15.48 ± 0.42</td>
</tr>
<tr>
<td>Cd</td>
<td>10.48 ± 0.41</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pb</td>
<td>-</td>
<td>25.34 ± 0.68</td>
<td>-</td>
</tr>
<tr>
<td>Cd+Pb</td>
<td>8.80 ± 0.35</td>
<td>17.60 ± 0.70</td>
<td>-</td>
</tr>
<tr>
<td>Cd+Se</td>
<td>7.36 ± 0.23</td>
<td>-</td>
<td>9.81 ± 0.30</td>
</tr>
<tr>
<td>Pb+Se</td>
<td>-</td>
<td>23.84 ± 0.64</td>
<td>15.90 ± 0.43</td>
</tr>
<tr>
<td>Cd+Pb+Se</td>
<td>7.28 ± 0.24</td>
<td>14.56 ± 0.48</td>
<td>9.71 ± 0.32</td>
</tr>
</tbody>
</table>

Note: Results are expressed as mean ± SEM (n = 6).

### Blood parameters

After clothing, the blood was centrifuged at 3500 rounds per minute for five minutes and serum was separated.

Total proteins, total cholesterol, serum triglycerides (TG), total bilirubin, direct bilirubin, serum alanine aminotransferase activity (ALT) and serum aspartate aminotransferase activity (AST) were determined on the automated biochemical analyser. Gel electrophoresis was employed to separate and quantify serum protein fractions. Serum albumin (ALB), serum alpha 1 globulins (α1-GLO), serum alpha 2 globulins (α2-GLO), serum beta 1 globulins (β1-GLO), serum beta 2 globulins (β2-GLO) and gamma globulins (γ-GLO) were expressed as percentage from the total amount of proteins. Albumin/globulin ratio (ALB/GLO) was automatically calculated by the software. Each kit was employed according to the manufacturer’s instructions for use.

### Histology and immunohistochemistry

Liver fragments from each rat were preserved in 10% neutral buffered formalin (approximately 4%
The absorption and digestion of some nutrients [18], as well as the metabolism of cadmium, may contribute to weight gain being influenced by the availability of cadmium and other factors [2, 7, 67]. The explanation could be that weight gain is influenced by the availability and absorption of nutrients and cadmium decreases the absorption and digestion of some nutrients [18].

Results and Discussion

Figure 1A shows that the rate of body weight gain was lower in case of Cd, Pb and Cd+Pb groups when compared to Control; the lowest was observed in case of Cd+Pb group. The rate was slightly higher in case of Se group in comparison to Control. Co-exposure to selenium alleviated the decrease in body weight gain in case of lead and cadmium + lead exposure (Pb+Se > Pb; Cd+Pb+Se > Cd+Pb), while it apparently had no effect on cadmium exposure (Cd+Se < Cd, overall). Cadmium intoxication is known to cause reduction or stagnation in weight gain, depending on dose, exposure time, route of exposure and other factors [2, 7, 67]. The explanation could be that weight gain is influenced by the availability and absorption of nutrients and cadmium decreases the absorption and digestion of some nutrients [18].

Immunostaining was conducted according to the manufacturer’s suggested techniques. Sections of 5 µm were obtained from the paraffin blocks and dewaxed. The epitopes were revealed by heating at 95°C in 10 mmol of citrate acid buffer (pH = 6) for 10 minutes in a microwave oven, after which were left at room temperature for 20 minutes. The sections were washed twice in phosphate buffer solution (PBS) with a pH of 7.5 for 5 minutes, after which were incubated with primary anti-p53 and anti-caspase-3 antibodies and diluted 1:100 at room temperature for 1 h, in a humid chamber. After washing with PBS, the sections were incubated with the secondary antibody (horseradish peroxidase goat anti-mouse IgG) for 1 h, in a humid chamber, at 4°C, then washed with PBS and incubated with 3,3’-diaminobenzidine substrate for 7 minutes, counterstained with Harris haematoxylin, clarified with xylene and mounted. The slides were examined under the microscope and relevant photographs were taken.

Statistical analysis

Results of biochemical blood parameters, water consumption, estimations of elements intake, body weight evolution and liver weight were expressed as mean ± SEM. The normal distribution of the values of biochemical blood parameters was assessed by using the Shapiro-Wilk test, then the values were subjected to one-way ANOVA, followed by Tukey’s post hoc test in order to estimate data variance between experimental groups. The minimum level of significance was set at 0.05. IBM SPSS Statistics 20 and MS Excel 2007 were used for the statistical analysis and graphical representation. GIMP 2.8 was used for editing photographs.

Results of biochemical blood parameters, water consumption, estimations of elements intake, body weight evolution and liver weight were expressed as mean ± SEM. The normal distribution of the values of biochemical blood parameters was assessed by using the Shapiro-Wilk test, then the values were subjected to one-way ANOVA, followed by Tukey’s post hoc test in order to estimate data variance between experimental groups. The minimum level of significance was set at 0.05. IBM SPSS Statistics 20 and MS Excel 2007 were used for the statistical analysis and graphical representation. GIMP 2.8 was used for editing photographs.

There was no significant difference among groups regarding relative liver weight (Figure 1B). Figure 1C and Figure 1D show that the estimated water consumption was overall lower in case of Cd, Pb and Cd+Pb groups, in comparison to Control; the lowest was observed in the case of Cd+Pb group. Water consumption was lower for the Se group than for the Control group. The water consumption was lower in the case of co-exposure to selenium (Cd+Se < Cd; Pb+Se < Pb; Cd+Pb+Se < Cd+Pb). The lowest water consumption was observed in the case of the Cd+Pb+Se group. Some studies have also reported decreased fluid intake in case of cadmium or lead exposure [8, 39], while other studies did not [41, 59]. One cause for the decrease in fluid intake, when the drinking water contains cadmium and/or lead can be the alteration of taste. It has been demonstrated that cadmium chloride in solutions is aversive to rats and the decrease in drinking water intake occurs rapidly, so the physiological impairment could be excluded as a cause [14]. Another study concluded that Meriones shawi rodents maintained a homeostasis state and presented an adaptation to regulate volume during cadmium exposure by decreasing diuresis and increasing urine osmolality [35]. It is generally known that fluid intake decreases with the increase in the osmolarity of the drinking water, so another less plausible cause for the difference in water consumption among groups in this study could be the increase in the osmolarity of the drinking water. Still, the concentrations of the salts were not high enough in order to lead to hypertonic solutions.
Figure 1.
Evolution of the body weight, relative liver weight and water consumption. (A) Evolution of the body weight, expressed as percent of increase/decrease in comparison to the body weight at the beginning of the experiment; (B) Relative liver weight of the rats from all the experimental groups, expressed as grams of liver per kg bw; (C) Estimated water consumption during 24 hours per kg bw; (D) Estimated water consumption during 24 hours per rat. Error bars are SEM

Table II shows the values determined for total cholesterol, TG, total bilirubin, direct bilirubin ALT and AST.

Selenium co-exposure reduced total cholesterol levels (Cd > Cd+Se; Pb > Pb+Se). As in the case of this study, an attenuation caused by selenium co-administration was also observed by other research groups [44]. Apparently cadmium or lead resulted in a small decrease, statistically insignificant, in total bilirubin
and direct bilirubin exposure, when compared to Control (Control > Cd; Control > Pb). Apart from this change, there was no consistent and coherent influence of exposure to a combination of any of these elements on total bilirubin and direct bilirubin levels. Total bilirubin level is a marker of liver damage [55]. Bilirubin is a product of haemoglobin degradation and is a marker of hepatobiliary damage, especially cholestasis and biliary effects [45]. Some researchers reported elevated total bilirubin levels in the case of cadmium [29] or lead [5, 24] exposure. Another study reported no marked difference between groups of control rats, rats exposed to cadmium and rats exposed to cadmium and selenium [3].

Exposure to cadmium determined an increase in ALT and AST activity, in comparison to Control (Cd > Control) while co-exposure to selenium had a decreasing effect (Cd > Cd+Se). An increase in serum trans-amaminas usually accompanies damage to the liver through a necrotic mechanism [55]. Serum ALT activity is usually a more specific biomarker of liver function than AST activity, because AST is released from damaged myocytes as well as hepatocytes [45]. Injured hepatocytes release enzymes into the blood stream, resulting in ALT and AST increase. The high ALT and AST activities are accompanied by high liver microsomal membrane fluidity, alteration in the liver tissue histogram and ROS generation [24]. Although the kidney is generally the main target for cadmium toxicity because it accumulates preferably in high quantities in this organ, cadmium also accumulates in the liver and exerts hepatotoxic effects [2, 30, 63]. Lead is also accumulated in the liver and manifests its toxicity in this organ [36, 61]. Our results are in agreement with those observed within other studies, which showed an increase in ALT and AST in case of cadmium [16, 29] intoxication. In this study, combined exposure to cadmium and lead did not result in a significant increase in transaminases when compared to individual exposure, which is in accordance with the results obtained by other researchers on mice, during a 60 day exposure time through the drinking water [13]. This can be due to the fact that water and the subsequent elements intake were lower for the combined exposure groups than for the individual exposure groups. Other studies have reported a decrease in AST and ALT in the case of selenium co-exposure or co-administration with cadmium [15, 28] or lead [4, 44].

Table III shows the values determined for total serum proteins and for serum protein fractions. No significant differences in total serum proteins were observed between groups, with the exception of the Cd+Pb group, for which the level was significantly reduced when compared to Control (Cd+Pb < Control). Exposure to cadmium and lead resulted in a significant decrease in ALB and ALB/GLO (Control > Cd+Pb), while the co-exposure to selenium apparently had no significant or marked effect when compared with the Cd+Pb group. Exposure to cadmium or lead increased β2-GLO. Albumin, the most abundant individual serum protein is responsible for maintaining osmotic pressure and serves as a transport protein [23]. Among the globulins, α1-GLO and α2-GLO include many clinically important acute-phase proteins [68]. α1-GLO include, among other proteins, α1-antitrypsin, α1-antichymotrypsin and α1-lipoprotein while α2-GLO include α2-globulins, haptoglobin, α2-macroglobulin, ceruloplasmin and α2-antiplasmin [60]. Beta globulins include other acute-phase proteins in addition to complement and various proteins important in coagulation [60, 68]. The γ-GLO include the immunoglobulins (IgA, IgM, IgE and IgG) but some immunoglobulins can migrate in the β area [68]. Other studies observed that cadmium or lead caused a decrease in albumin levels while selenium partially alleviated the cadmium-induced changes [15, 16, 51]. The reduction in total proteins and ALB may be caused by an inhibition of protein bio-synthesis through the specific enzymes involved in cell processes and a low excretion of some hormones involved in protein biosynthesis [24].

<table>
<thead>
<tr>
<th>Group</th>
<th>Total proteins (g/dL)</th>
<th>ALB (%)</th>
<th>α1-GLO (%)</th>
<th>α2-GLO (%)</th>
<th>β1-GLO (%)</th>
<th>β2-GLO (%)</th>
<th>γ-GLO (%)</th>
<th>ALB/GLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.03 ± 0.12</td>
<td>61.18 ± 0.78</td>
<td>19.78 ± 0.68</td>
<td>4.91 ± 0.24</td>
<td>2.83 ± 0.45</td>
<td>1.38 ± 0.27</td>
<td>8.39 ± 1.39</td>
<td>1.50 ± 0.09</td>
</tr>
<tr>
<td>e</td>
<td>5.61 ± 0.21</td>
<td>60.16 ± 1.19</td>
<td>18.55 ± 1.11</td>
<td>3.93 ± 0.70</td>
<td>4.08 ± 0.29</td>
<td>1.93 ± 0.60</td>
<td>11.33 ± 0.29</td>
<td>1.52 ± 0.07</td>
</tr>
<tr>
<td>d</td>
<td>6.04 ± 0.14</td>
<td>56.88 ± 0.48</td>
<td>15.20 ± 0.62</td>
<td>4.96 ± 0.48</td>
<td>5.41 ± 0.27</td>
<td>4.43 ± 0.47</td>
<td>13.10 ± 0.27</td>
<td>1.32 ± 0.02</td>
</tr>
<tr>
<td>b</td>
<td>5.81 ± 0.13</td>
<td>54.45 ± 1.10</td>
<td>17.71 ± 0.82</td>
<td>5.16 ± 0.47</td>
<td>5.50 ± 0.64</td>
<td>4.68 ± 0.18</td>
<td>12.48 ± 0.50</td>
<td>1.20 ± 0.05</td>
</tr>
<tr>
<td>d+Pb</td>
<td>4.74 ± 0.22</td>
<td>51.80 ± 0.76</td>
<td>18.25 ± 0.55</td>
<td>4.28 ± 0.51</td>
<td>6.58 ± 0.91</td>
<td>3.68 ± 0.61</td>
<td>15.16 ± 0.55</td>
<td>1.08 ± 0.03</td>
</tr>
<tr>
<td>d+Se</td>
<td>6.28 ± 0.14</td>
<td>57.61 ± 1.25</td>
<td>13.88 ± 0.35</td>
<td>5.68 ± 0.51</td>
<td>5.70 ± 0.38</td>
<td>3.96 ± 0.27</td>
<td>13.15 ± 0.54</td>
<td>1.36 ± 0.07</td>
</tr>
<tr>
<td>b+Se</td>
<td>5.98 ± 0.18</td>
<td>53.38 ± 3.54</td>
<td>16.41 ± 2.59</td>
<td>7.35 ± 0.26</td>
<td>5.50 ± 0.38</td>
<td>3.75 ± 0.31</td>
<td>13.60 ± 1.09</td>
<td>1.19 ± 0.14</td>
</tr>
<tr>
<td>d+Pb+Se</td>
<td>6.30 ± 0.13</td>
<td>52.75 ± 2.18</td>
<td>14.93 ± 1.02</td>
<td>8.23 ± 0.96</td>
<td>6.48 ± 1.92</td>
<td>7.38 ± 1.28</td>
<td>10.21 ± 0.50</td>
<td>1.14 ± 0.10</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n = 6); statistical analysis consisted in one-way ANOVA followed by Tukey’s test; a value of p < 0.05 was considered significant; * – significant difference when compared to Control; **– significant difference when compared to Se; ***– significant difference when compared to Cd; **** – significant difference when compared to Pb; ***** – significant difference when compared to Cd+Pb
Liver sections of rats from the experimental groups. 

(A) Control group (p53 staining); normal structure with no pathological modifications observed; (B) Cd group (caspase-3 staining); disorganization in the architecture of hepatic lobules, focal areas of necrosis near the portobiliary veins, caspase-3 positive marked cells both in the portobiliary space and around the centrolobular vein; (C) Cd group (caspase-3 staining); caspase-3 overexpression in the hepatocytes around the centrolobular vein, dilated centrolobular vein, a large number of Kupffer cells at sinusoidal capillaries level; (D) Cd group (p53 staining); positive p53 nuclei, in large numbers; (E) Pb group (p53 staining); positive p53 nuclei; (F) Pb group (caspase-3 staining); overexpression of caspase-3 in hepatocytes around the veins from the portobiliary space and around the centrolobular vein; (G) Pb group (caspase-3 staining); hepatocytes positively marked for caspase-3 and visible Kupffer cells at sinusoidal capillaries level, dilated sinusoidal capillaries; (H) Cd+Pb group (caspase-3 staining); conjunctive proliferation and large portobiliary space with newly formed biliary ducts; (I) Cd+Pb group (p53 staining); p53 positive nuclei in large number, modified architecture of the hepatic lobules; (J, K) Cd+Se group (caspase-3 staining); reduced focal areas of necrosis, reduced intensity of caspase-3 marking, typically organized hepatic lobules, sinusoidal capillaries dilated in some areas, visible Kupffer cells; (L, M) Pb+Se group (caspase-3 staining); reduced focal areas of necrosis, smaller number of cells positively marked for caspase-3, reduced intensity of caspase-3 marking, the cytoplasm has a vacuolar aspect; (N, O) Cd+Pb+Se group (caspase-3 staining); focal areas positively marked for caspase-3, reduced intensity of caspase-3 marking, reduced focal areas of necrosis.
The livers of the rats from the Control and Se groups had normal specific histological structure, without modifications. Portobiliary spaces, hepatocyte plates arranged radially from the centrolobular vein towards periphery and separated by sinusoidal capillaries can be observed in Figure 2A (Control). Areas of necrosis were observed in the livers of the rats from the Cd group, especially around the vein from the portobiliary space and in some cases around the centrolobular vein. The hepatocytes around the centrolobular vein were dense and swollen, with signs of granular and hydropic degeneration. The hepatocyte cytoplasm was positively marked for caspase-3 (Figure 2B; Figure 2C) and the nuclei were positively marked for p53 (Figure 2D). An intense degeneration in the normal architecture of the hepatic lobule and in the radially disposition of the hepatocytes around the centrolobular vein were observed in the Pb (Figure 2F; Figure 2G) and Cd+Pb groups (Figure 2H; Figure 2I). Fibroblastic proliferation in the portobiliary space, diffuse leucocytary infiltration, periductal fibrosis and focal necrosis in the portobiliary space were observed in the Cd+Pb group (Figure 2H; Figure 2I). The biliary ducts proliferated, forming new biliary ducts (Figure 2H). Positive p53 nuclei were observed in the Pb group (Figure 2E); in the case of Cd+Pb group the positive p53 nuclei were more numerous (Figure 2I). Positive caspase-3 hepatocytes were observed both in the case of Pb group (Figure 2F; Figure 2G) and in the case of Cd+Pb group (Figure 2H). The intensity of necrosis was reduced and the hepatic sinusoidal capillaries were less dilated in the Cd+Se group compared to Cd group (Figure 2J; Figure 2K). Pb+Se group compared to Pb group (Figure 2L; Figure 2M) and Cd+Pb+Se group compared to Cd+Pb group (Figure 2N; Figure 2O).

One group of researchers found that cadmium exposure resulted in various degenerative changes in the liver of rats: enlargement of cell sizes, condensed chromatin, necrosis of single cells, pyecnotic nuclei, the vicinity of some sinusoids infiltrated by mononuclear cells [26]. Co-exposure to selenium resulted in the absence of nucleus fragmentation and a decrease in the necrosis of single hepatocytes and in the mononuclear cell infiltrations [26], which is in accordance to the results of our study. Another group of researchers observed that lead exposure in rats resulted in hydropic degeneration, anisokaryosis, nuclear vesiculation, binucleation, cytoplasmic inclusions, cytoplasmic swelling and necrosis [25].

Apoptosis is a complex process through which an organism kills and removes unwanted cells. Caspases are crucial in the mediation of apoptosis. Caspase-3 is a frequently activated death protease and acts in a tissue selective manner [47]. Caspase-3 is considered a marker of DNA damage [22]. Increased immunoreactivity to or expression of caspase-3 in kidneys, prostate and other organs, in case of cadmium exposure, was previously reported [6, 22]. It is also known that lead exposure results in increased immunoreactivity to caspase-3 in rat testes [17] and leads to caspase-3 mediated cell death in vitro when employing N2a neuroblastoma cells [31]. In an in vitro experiment using human myeloid HL-60 cells, selenium, at low concentrations, activated multiple survival mechanisms, including caspase-3 expression that counteracted oxidative stress-induced cell apoptosis [62].

p53 activates caspase by releasing apoptogenic factors from the mitochondria [50]. p53 is crucial in the cellular response to DNA damage and it is primarily involved in defence mechanisms by transcriptional activation of genes responsible of growth arrest and apoptosis for the elimination of severely damaged cells [64]. It is known that cadmium interferes with the structure and function of p53, but opposite effects have been reported; some authors reported the induction of p53-mediated stress response while others reported inactivation of p53 via structural changes, in vitro [12, 37, 64]. Some researchers found that lead exposure resulted in an increase in p53 expression in mouse liver [66]. Some selenium compounds are known to modulate p53, by various mechanisms, which can differ among compounds. In vitro, sodium selenite causes phosphorylation of some key cysteine residues in p53, leading to its activation [56].

Conclusions

According to our results, selenium exhibited a potential protective action against hepatotoxicity induced by cadmium, lead or a combination of lead and cadmium by improving some biochemical parameters and the histological architecture of the liver and reducing the intensity of p53 and caspase-3 immunostaining. In order to extrapolate the findings of the study to humans, additional clinical studies are required, in order to establish the safety and efficacy of selenium for reducing Cd and/or Pb toxicity in humans. Selenium has very narrow safety limits that need to be carefully considered if one would intend to use it in humans for reducing Cd and/or Pb deleterious effects.

References


