Effect of Lead on Semen Characteristics and Some Enzymatic Activities in Serum and Semen of Rabbits with A Reference to the Protective Effect of Vitamin C

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Abstract: The effect of lead acetate administration on testicular, hepatic and renal functions and the biomarker effect for them were investigated in the present study with a trial of treatment by vitamin C. A total number of 35 rabbit bucks was divided into five groups. One served as the control group and four groups received low and high doses of lead acetate (10.8 and 15 mg/kg. b. wt. respectively) orally. One low and one high group received, in addition, 1 g vitamin C /L in drinking water. Superoxide dismutase (SOD), γ-glutamyl transferase (γ-GT), Aspartate amino transferase (AST), Alanine amino transeferase (ALT), cholinesterase, acid phosphatase and Lactate dehydrogenase (LDH) activities were measured in both serum and semen. Also, semen characteristics were determined. Results revealed that all the investigated enzymes (SOD, LDH, ALT and acid phosphatase activities) in serum and semen were obviously affected by lead. Vitamin C was a good antioxidant that recuperates the normal enzymatic status in both serum and semen. In conclusion, lead led to testicular hypofunction, which is supported by the result of semen picture. The hazardous effect of lead led to disturbance in the activities of enzymes under investigation such as SOD, γ-GT, LDH, AST, ALT, cholinesterase and acid phosphatase. Vitamin C proved its antioxidant effect on recuperating the normal status of enzymes in serum and semen. LDH and prostatic acid phosphatase are shown to be biomarker of testicular dysfunction, while LDH, ALT may be used as biomarkers for hepatic and renal dysfunctions.

Key words: Lead · Vitamin C · Antioxidant · Male · Rabbits

INTRODUCTION

Lead is one of the most toxic metals that induce wide range of behavioral, bio-chemical and physiological dysfunctions in human and animals [1]. A biomarker is a term which is used to include, almost, any measurement reflecting an interaction between a biological system and environmental agent, which may be chemical, physical or biological in nature. The identified biomarkers are three classes; biomarkers of exposure, effect and susceptibility [2].

Lead toxicity is retarding factor for the vitality of reproductive and internal organs of all animals. The effects of lead toxicity on reproductive organs were screened in mature female and male rabbits [3, 4], rats [5] and in human [6, 7, 8]. These effects were recorded to induce leakage of enzymes from other vital organs, as AST, ALT [9], aminoleviulinic acid dehydrogenase (ALA-d) activities [10], ALT and total acid phosphatase [11]. So these enzymes are used as biomarkers for approaching good diagnosis in case of vital organs dysfunction. On the other hand, there is another category of enzymes, which act on superoxide radicals to eliminate its hazardous effects. The inhibition of these enzymes by lead causes the increase of superoxide radicals $O_2^-$ and its accumulation inside the cell leading to its death [12]. Vitamins A, E, D and C were recorded to have antioxidant activities. Their antagonistic effects to superoxide accumulation varied from one vitamin to another. It has been found that levels of lead in serum is decreased due to reduced absorption not due to increase excretion after 1000 mg vitamin C [13].

In this study, vitamin C was used to investigate its antioxidant activity for decreasing the inhibitory effect of lead acetate on serum and semen enzymes in New Zealand rabbits and monitoring the suitable biomarker for its effect on the vitality of the male.
MATERIALS AND METHODS

Experimental Design: A total number of 35 mature male rabbits (2.5 kg average) were kept in the animal house at National Research Centre. Animals were divided into 5 equal numbered groups (n = 7). The first group is adopted as the control group (treated with distilled water). The second and third groups were orally dosed 10.8 mg lead acetate/ kg b. wt and considered as the low dose groups. The fourth and fifth groups were dosed 15 mg lead acetate/kg b. wt. and considered as the high dose groups. All groups received their dosage orally dissolved in distilled water using animal gavage. The four later groups were treated for 5 consequent days /week and the treatment expended for 8 weeks. The third and fourth groups obtained, in addition to the lead acetate dose, vitamin C (1g / liter drinking water every day without disturbance). All lived animals were sacrificed on the day after the last dose [11].

Semen Collection: Semen were collected from bucks using rabbit artificial vagina (AV) and a teaser female [14]. The collection was achieved from the end of the fourth week till the end of the experiment for the routine evaluation of both live sperm and sperm abnormalities percentage using eosin aniline stain and before the slaughter immediately for enzymes determination.

Blood Sampling: While sacrificing animals, blood was collected in sterilized capped tubes and sterilized heparinized tubes for SOD determination. The tubes were incubated at 37° C for 10 minutes in slope position, then centrifuged at 3500 rpm for 10 minutes. Serum was collected and immediately tested for the enzymes.

The whole heparinized blood was treated according to the following method: 0.5 ml of blood was centrifuged for 10 minutes at 3000 rpm and then the plasma was aspirate off. Then the erythrocytes were washed four times with 3 ml of 0.9% NaCl solution and centrifuged for 10 minutes at 3000 rpm after wash. The washed and centrifuged erythrocytes were made up to 2 ml with cold redistilled water, mixed and left to stand at +4 °C for 15 minutes. The lysate was diluted with 0.01 nmol/l phosphate buffer pH 7 (Randox cat. No.SD 124), so that the percentage inhibition falls between 10 and 60%. A 25 fold dilution of lysate is recommended (final dilution factor = 100) [15].

Enzymatic Analysis in Blood and Semen

In Erythrocytes: SOD is analyzed in whole blood using chemical kits obtained from Randox, UK [15].

In Serum and Whole Blood:

- AST, ALT and LDH were analyzed using kits from Stanbio, Texas, USA [16, 17].
- Cholinesterase [18], acid phosphatase[19] and γ-GT [20] were analyzed using kits, Quimica Clinica Aplicada S.A., Spain.

All enzymes were measured using Shimadzu UV 240 Spectrophotometer with different wavelengths specific or each enzyme.

Statistical Analysis: Using one-way ANOVA, data were analyzed to determine whether the effect of lead (Pb) and vitamin C gave a significant difference, or not, as compared with the control group (H0). The results were accepted at a level of 95% confidence.

RESULTS

In Table (1) results of semen analysis revealed that the lead treated groups show lower (P< 0.01) mass motility, individual motility, sperm concentration / ml semen and live sperm percentage, while they show higher (P < 0.01) total primary sperm abnormalities percentage as compared to the control group. Vitamin C was found to improve the hazardous effects of lead in the third and fifth groups.

Inhibition of SOD, in whole blood, increased (P<0.01) in the treated groups (2, 3 and 4) as compared to the control group. At the same time, this activity decreased (P<0.01) in group 5 than in groups 2, 3 and 4. Moreover, γ- GT activity, in serum increased (P<0.01) in group 4 than in the control group, while it increased in groups 2 and 4 than in groups 3 and 5, respectively (Table, 2).

LDH, in serum, showed a decreased (P< 0.01) activity in groups 2 and 4, while it increased (P< 0.01), in semen, in groups 2, 3, 4 and 5 than control group (Tables 2 and 3). Meanwhile, in serum, it is decreased (P< 0.01) in groups 2 and 4 than in groups 3 and 5, respectively and it is increased (P< 0.01), in semen samples, in groups 2 and 4 than in group 3 and 5, respectively (Tables 2, 3).
Table 1: Semen characteristics in rabbits bucks after treatment with lead acetate alone or with the addition of vitamin C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control n=12</td>
<td>Low dose n=36</td>
<td>Low dose + Vit C n=12</td>
<td>High dose n=28</td>
<td>High dose + Vit C n=28</td>
<td></td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>0.48±0.056</td>
<td>0.69±0.55</td>
<td>0.47±0.014</td>
<td>0.39±0.021</td>
<td>0.52±0.063</td>
</tr>
<tr>
<td>Concentration (x10^6/ml)</td>
<td>120.00±6.89</td>
<td>87.50±5.59</td>
<td>77.00±2.71</td>
<td>70.40±3.99</td>
<td>106.86±6.71</td>
</tr>
<tr>
<td>Total concentration (x10^7)</td>
<td>53.80±3.62</td>
<td>58.23±5.24</td>
<td>36.30±2.22</td>
<td>25.83±1.27</td>
<td>62.53±9.33</td>
</tr>
<tr>
<td>Live %</td>
<td>94.33±0.25</td>
<td>82.53±0.32</td>
<td>90.26±0.50</td>
<td>74.21±0.51</td>
<td>818.83±0.46</td>
</tr>
<tr>
<td>Mass motility (score 1-5)</td>
<td>4.5±0.12</td>
<td>3.44±0.13</td>
<td>4.00±0.25</td>
<td>3.57±0.12</td>
<td>3.86±0.22</td>
</tr>
<tr>
<td>Individual motility %</td>
<td>91.25±0.90</td>
<td>76.25±1.33</td>
<td>81.25±3.09</td>
<td>79.64±1.31</td>
<td>80.71±2.32</td>
</tr>
<tr>
<td>Primary sperm abnormalities %</td>
<td>15.33±0.55</td>
<td>25.16±0.25</td>
<td>21.27±0.41</td>
<td>34.31±1.42</td>
<td>19.35±0.78</td>
</tr>
</tbody>
</table>

Same superscript are non-significantly different within row (P< 0.05) - Duncan test

Table 2: Some enzymes bio markers in blood of rabbits bucks after treatment with lead acetate alone or with the addition of vitamin C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control n=5</td>
<td>Low dose n=7</td>
<td>Low dose + Vit C n=6</td>
<td>High dose n=7</td>
<td>High dose + Vit C n=4</td>
<td></td>
</tr>
<tr>
<td>SOD units/ml (whole blood)</td>
<td>115.43±18.27</td>
<td>210.64±32.08</td>
<td>187.58±7.82</td>
<td>262.81±25.89</td>
<td>109.78±10.53</td>
</tr>
<tr>
<td>γ- GT (U/L)</td>
<td>9.63±0.51</td>
<td>11.39±1.95</td>
<td>7.78±0.80</td>
<td>14.09±0.47</td>
<td>10.26±0.76</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>518.66±22.87</td>
<td>349.19±13.30</td>
<td>559.71±21.04</td>
<td>355.32±7.68</td>
<td>532.6±20.60</td>
</tr>
<tr>
<td>AST (UL)</td>
<td>81.33±3.55</td>
<td>95.37±2.95</td>
<td>80.82±3.07</td>
<td>81.86±4.47</td>
<td>72.79±1.44</td>
</tr>
<tr>
<td>ALT (UL)</td>
<td>88.04±2.84</td>
<td>100.09±11.50</td>
<td>78.30±2.60</td>
<td>133.78±9.06</td>
<td>61.46±4.25</td>
</tr>
<tr>
<td>Choline esterase (UL)</td>
<td>1049.44±109.82</td>
<td>838.04±162.24</td>
<td>1051.23±128.86</td>
<td>479.25±67.69</td>
<td>1090.89±166.16</td>
</tr>
<tr>
<td>Total acid Phosphatase (U/L)</td>
<td>59.51±2.40</td>
<td>66.72±1.97</td>
<td>84.91±2.16</td>
<td>66.68±1.26</td>
<td>76.84±2.83</td>
</tr>
<tr>
<td>Prostatic acid Phosphatase (U/L)</td>
<td>1.82±0.33</td>
<td>3.31±0.43</td>
<td>8.00±0.56</td>
<td>3.00±0.43</td>
<td>7.45±1.56</td>
</tr>
</tbody>
</table>

Same superscript are non-significantly different within row (P< 0.05) - Duncan test

Table 3: Some enzymes bio markers in semen of rabbits bucks after treatment with lead acetate alone or with the addition of vitamin C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control n=5</td>
<td>Low dose n=7</td>
<td>Low dose + Vit C n=6</td>
<td>High dose n=7</td>
<td>High dose + Vit C n=4</td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>954.75±52.98</td>
<td>3850.83±39.45</td>
<td>1646.90±30.45</td>
<td>4817.65±390.60</td>
<td>1788.63±5.07</td>
</tr>
<tr>
<td>AST (UL)</td>
<td>420.79±62.50</td>
<td>1614.77±104.17</td>
<td>247.13±26.13</td>
<td>773.99±1.49</td>
<td>330.81±8.23</td>
</tr>
<tr>
<td>ALT (UL)</td>
<td>43.32±6.12</td>
<td>44.20±7.56</td>
<td>38.60±1.15</td>
<td>23.18±4.87</td>
<td>16.50±0.93</td>
</tr>
<tr>
<td>Choline esterase (UL)</td>
<td>195.50±25.60</td>
<td>119.9±4.93</td>
<td>156.40±3.41</td>
<td>106.87±7.88</td>
<td>119.9±4.93</td>
</tr>
<tr>
<td>Total acid Phosphatase (U/L)</td>
<td>124.74±5.40</td>
<td>113.86±12.24</td>
<td>107.01±9.53</td>
<td>171.20±14.70</td>
<td>99.76±10.84</td>
</tr>
<tr>
<td>Prostatic acid Phosphatase (U/L)</td>
<td>12.63±0.95</td>
<td>16.57±2.74</td>
<td>16.06±1.19</td>
<td>24.88±2.37</td>
<td>15.48±3.28</td>
</tr>
</tbody>
</table>

Same superscript are non-significantly different within row (P< 0.05) - Duncan test

AST in serum showed increase activity (P< 0.01) in group 2 than control group (Table 2). At the same time, its activity in semen is increased (P< 0.01) in both groups 2 and 4 than control group (Table, 3). Also, the activity of AST in serum is increased (P< 0.01) in group 2 than group 3; and in groups 2 and 4 than group 3 in the semen sample.

ALT activity in serum and semen are higher (P< 0.01) in groups 4 and 2 and lower (P< 0.01) in group 5 in both as compared to the control group. On the other hand, its activity in serum, is higher (P< 0.01) in groups 2 and 4 than in groups 3 and 5, respectively. While, in the semen, it showed a lower (P< 0.01) results in groups 4 and 5 than in other groups (Tables 2 and 3).

Regarding Cholinesterase activity, in serum, it is decreased (P< 0.01) in group 4 than the control group, third and fifth groups (Table, 2) and, in semen, in groups 2, 3, 4 and 5 than the control group (Table, 3). Meanwhile, this activity is high (P< 0.01) in group 3 than other treated groups (2, 4 and 5) (Table, 3).
Total prostatic acid phosphatase activities increased ($P<0.01$) in serum in all treated groups as compared to the control group. Moreover, the increment in both groups 3 and 5 was clear ($p<0.01$) than in groups 2 and 4 (Table, 2). But, in semen it is increased ($P<0.01$) in group 4 only than all other groups and control group (Table, 3).

**DISCUSSION**

In this study, results showed that lead has a significant effect on semen characteristics in rabbit bucks in both low and high dose groups of lead acetate, while these adverse effects have been corrected in groups treated with vitamin C (Table, 1). These results are in agreement with the findings of Lähdetie [6] and El-Nattat [4].

Most of the enzyme markers contain sulfhydryl (-SH) group at the site of action [21], such group is a target for metals, while others like some metallo-enzymes have a prosthetic group that may be replaced by heavy metals leading to inhibition of the enzymatic activity [2]. This suppression masks their physiological actions, especially those enzymes related to superoxide radicals $O_2^-$ that accumulate inside the cells leading to the deterioration and death of the cells. SOD is one of those enzymes [22]. The results indicated that lead acetate induced an exaggerated inhibitory effect for SOD. As lead mimics Cd in replacing Zn in its site, this suggested the interaction of lead with the Cu, Zn and Mn moieties. Such interaction has been demonstrated where lead replaced Zn to form Cu-Pb-SOD [23]. Vitamin C ameliorates the inhibitory action of lead either by reducing its absorption [13] or by removing ROS (reactive oxygen system) once formed, thus prevent the radical chain reaction. This observation supports the hypothesis that SOD activity is stimulated by an increased superoxide radical generation associated with the decline of SOD and GSH-Px[24] generated by inhibitory action of lead, while, the anti-oxidant refreshes the enzyme activity and antagonizes the inhibitory effect of lead.

There are some plasma enzymes which have unknown physiological function in blood, but only provide a valuable diagnostic and prognostic clinical evidence in case of dysfunction or diseases. Prostatic acid phosphatase is one of these plasma enzymes [25].

The $\gamma$-GT, an enzyme that supports the transfer of certain amino acids into the GSH, which reduces the accumulation into the RBCs and other cells. It is present in the plasma membrane of renal tubular cells and in endoplasmic reticulum of the hepatocytes [26]. In the present study, the results showed a significant increase in the enzyme activity in case of high dose of lead acetate (14.09 U/l) than the control group. This indicates that there is a destruction of the enzyme in the store cells and released in the blood [26]. Therefore, the activity in the store cells like hepatocytes and renal tubular cells is decreased [27]. As reported by Murray [26] that the cells which are under deteriorating stress factors including heavy metals, showed lower activities of SOD and GSH-Px. Moreover, this author added that a powerful antioxidant can reverse the oxidative damage by bringing about an improvement in the reductive status of the cell. The present study proved that vitamin C has stored the integrity of cells and reduced the leakage of enzymes outside the damaged cells, in addition to its antioxidant effect via an indirect way. Moreover, vitamin C may help in decreasing the superoxide radicals’ accumulation inside the cells although the SOD activity is inhibited by lead, thence, it conserves the soundness of the cells in return, no leakage of enzymes and no increased enzymes activity in the serum than normal. This finding is in agreement with Sivaprasad et al. [27]. In addition, vitamin C had a significant anti-oxidant activity thereby protecting the organs from the lead-induced toxicity [28].

LDH is a cytosolic enzyme which is released into systemic circulation in case of damage of the liver, lung, muscle, kidney, testicles or heart [29]. In the present study, the decrease in activity of LDH in serum due to lead toxicity and recuperation of activity again after treatment with vitamin C; found to be in agreement with the findings of Yagminas et al. [30]. Moreover, the leakage of the enzyme in the semen from the testicular and glandular tissues was, approximately twice to four times than the treated groups with vitamin C and triple the control group. This goes with Gulvic [31] who recorded a reduced activity of LDH in the testicular tissue of the rat. The results indicated that LDH is a sensitive and convenient biosensor for detection of heavy metal salts; and this is in agreement with Fennouh et al. [32].

The effect of lead on AST and ALT in the serum have been discussed [11, 33]. Moreover, LC$_{50}$ of lead acetate (100 micromole) caused significant leakage of AST and ALT in the medium of the liver cell culture [34]. So, the leakage of cytoplasmic enzymes appear to be a sensitive indicator of cell injury produced by heavy metals. The results of this study indicated that lead acetate led to increase the activity of the enzymes in both serum and semen; while treatment with vitamin C removed the effect of leakage. This was clear as the ALT activity in serum and semen have been decreased.
Cholinesterase is classified to true cholinesterase; which is found in nerve tissue and in the red blood cells; and pseudo cholinesterase which is present in various tissues and in plasma or serum [35]. Since, the heavy metal ions (mercury and lead) and their organic compounds belong to non-competitive inhibitors of enzymes; they may block the –SH groups that make part of the catalytic site of the enzyme [36]. The significant decrease of cholinesterase in both serum and semen, in the large dose treated group, indicates that the enzyme may behave the same behavior in both of them.

Regarding the significant increase of both total and prostatic acid phosphatase activities in serum of the lead and lead + vitamin C treated groups which may indicates presence of prostatic carcinoma; this is in agreement with Krupp et al. [37]; who reported an increase of acid phosphatase in serum due to carcinoma in prostate.

In conclusion, lead had led to testicular hypofunction, which is supported by the semen picture and the disturbances in the enzymes under investigation. Vitamin C, as antioxidant, counteract the oxidant activity of lead and improved its effects in both serum and semen. LDH and ALT and acid phosphatase are good biomarkers.

REFERENCES


