Effect of lead acetate toxicity on experimental male albino rat

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Abstract

**Objective:** To evaluate the effect of different doses of lead acetate (1/20, 1/40 and 1/60 of LD50) on body weight gain, blood picture, plasma protein profile and the function of liver, kidney and thyroid gland. **Methods:** Male albino rats were divided into four groups, the first group represented the health control animals, while the second, third and fourth groups were ingested orally with sub lethal doses of lead acetate (1/20, 1/40 and 1/60) of the oral LD50, respectively. One dose was ingested every two days during the experimental period (14 weeks) including the adaptation time. Blood was collected and used for all analysis. **Results:** The results showed that, the ingestion of Pb2+ induced significant stimulation in glutamic-pyruvic transaminase (ALT) and glutamic–oxalacetic transaminase (AST) activity. Also, total soluble protein and albumin contents of plasma were significantly decreased, while the content of globulin was changed by the Pb2+ treatments. The cholinesterase activity was inhibited, but the activities of alkaline and acid phosphates and lactate dehydrogenase were stimulated, while plasma glucose level was elevated as a result of lead acetate intoxication. In case of blood picture, Pb2+ ingestion reduced the contents of hemoglobin and RBCs count of intoxicated rat’s blood and the plasma levels of T3, T4 and blood WBCs count were decreased. **Conclusions:** It can be concluded that lead acetate has harmful effect on experimental male albino rats. Therefore, the present work advises people to prevent exposure to the lead compound to avoid injurious hazard risk.

1. Introduction

Environmental pollution is the presence of a pollutant in environment such as air, water, soil and consequently in food which may be poisonous or toxic and will cause harm to living things in the polluted environment[1]. The excessive amount of pollutants such as heavy metals in animal feed and feed stuffs are often due to human actions, resulting from either agricultural or industrial production or accidental or deliberate misuse[2–5]. There are at least 18 elements that characterize one or more inorganic pesticides. Of these elements, eight (barium, cadmium, mercury, thallium, lead, bismuth, antimony and boron) have not been shown to be essential to the growth of animals[6]. In the instances, a series of elements, such as the heavy metal have been considered in the order of their atomic number[7]. The definition of a heavy metal is one that has a specific gravity of more than 5 g/cm3. By definition this would account for 60 metals several of which are biologically essential and many other lack sufficient information regarding toxicity including platinum, silver and gold. This arrangement of the elements helps to explain the chemistry and toxicology of their compound[7].

Many heavy metals, including Pb, are known to induce over production of reactive oxygen species (ROS) and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acid contents of membrane[8]. Also, it has been shown to enhance the production of ROS in a variety of cells resulting oxidative stress[9]. ROS are the byproducts of many degenerative reactions in many tissues, which will affect the regular metabolism by damaging the cellular components[10]. Extensive study on oxidative stress has demonstrated that exposure of cells to adverse environmental conditions can induce the over production of ROS, such as superoxide radical (O2−), H2O2 and hydroxyl radical (OH·) in plant cells[11]. In addition, ROS are highly reactive to membrane lipids, protein and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage[12–17].

Traces of lead occur in many rocks in addition to those that are qualified as over lead, thus it can find its way into soil and water and hence into food, animals and human tissues even in remote places where there is no use of
the metal or its compounds. In spite of its widespread
distribution in tissues, there is no indication of no beneficial
effect, but it causes many problems to the plant, food
industry and animal health. Although various countries have
established legislation regulating their concentration, they
are still sometimes a danger for consumer health[18].

Lead is translocated through the food chain to man
and animals, its toxicity depends on its chemical form
administered to the animal, the route of administration and
the frequency and duration administered to animals[19]. Lead
is one of the toxic metals, it is dangerous to most human
body organ if exposure exceed to lerable levels. Lead can
affect individuals of any age, but it has a disproportionate
effect on children because their behavioral patterns place
them at higher risk for exposure to lead. Their bodies absorb
a larger percentage of the lead that they ingest and they
exhibit lead toxicity at lower level for exposure than adults
do[19]. Accumulation of lead produces damaging effects in
the hematopoietical, hemenal, renal and gastrointestinal
system[20]. The toxicity of lead is closely related to age, sex,
route of exposure level of intake, solubility, metal oxidation
state, retention percentage, duration of exposure, frequency
of intake, absorption rate, mechanisms and efficiency of
excretion. Lead has been associated with various forms of
cancer, nephrotoxicity, central nervous system effects and
cardiovascular diseases in human[21]. The inhalation of lead
could permanently lower intelligence quotient (IQ), damage
emotional stability and cause hyperactivity, poor school
performance and hearing loss. Foods of animals origin do
usually not have excessive lead concentrations. Animal tissues
are still sometimes a danger for consumer health[18]. Food and
water were supplied ad libitum for all groups during the
period of experiment.

Each rat was weighed every week and its daily food
intake was determined. Feed efficiency was calculated as
the following equation (body weight gain / food intake).

3. Results

The results in Table 1 showed that, means that gain in
body weight and feed efficiency were lowered relative to
control which were reduced to 56% and 50%, 58% and 56%,
60% and 67%, respectively under the treatment by ingestion
of lead acetate ingestion highly and significantly
shown in Table 1 and Table 2. In the case of liver function, the parameters including
plasma AST and ALT activities and plasma bilirubin levels are used to check liver function in the intoxicated
animals relative to the health normal rats (Table 3). These results showed that Pb²⁺ ingestion highly and significantly
stimulated the activity of AST and ALT. Data of plasma
bilirubin showed highly significant elevation in bilirubin
value in Pb²⁺ intoxicated rats relative to the control after
the experimental period. The three doses of Pb²⁺ ingestion
exhibited nearly the same levels of plasma bilirubin. Protein
profile of plasma was changed under the ingestion of lead
acetate. The results reported significant reduction in total
soluble protein and albumin, while plasma globulin value
was insignificantly changed. The effects of lead acetate on
enzymes activities were shown in Table 4.
showed marked decrease in its activity as a result of lead acetate treatments. The inhibition was gradually paralleled with the Pb²⁺ ingested doses increased, until it reached the maximum value at 1/20 LD₅₀ of lead acetate treatment (80.21 μg/dL) in comparison with control (110.10 μg/dL). While the results of acid and alkaline phosphatase showed that, activities of both enzymes in intoxicated rats with lead were stimulated relative to non-toxicated control group. Also, lead acetate treatments (1/20 LD₅₀, 1/40 LD₅₀, 1/60 LD₅₀) showed gradually inhibition in lactate dehydrogenase (LDH)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Lead acetate toxicity on the body weight gain, food intake and feed efficiency of the experimental animals (mean±SD).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead acetate treatments</td>
<td>Initial body weight (g)</td>
</tr>
<tr>
<td>Normal control</td>
<td>197 ± 11</td>
</tr>
<tr>
<td>Oral 1/20 LD₅₀</td>
<td>165 ± 9</td>
</tr>
<tr>
<td>Oral 1/40 LD₅₀</td>
<td>175 ± 9</td>
</tr>
<tr>
<td>Oral 1/60 LD₅₀</td>
<td>197 ± 15</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>36</td>
</tr>
</tbody>
</table>

%: relative to control; *: P<0.05 comparing with control.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Lead acetate toxicity on organs weight ratio of the experimental animals (mean±SD).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead acetate treatments</td>
<td>Body weight (g)</td>
</tr>
<tr>
<td></td>
<td>Weight (g)</td>
</tr>
<tr>
<td>Normal control</td>
<td>367 ± 10</td>
</tr>
<tr>
<td>Oral 1/20 LD₅₀</td>
<td>260 ± 11</td>
</tr>
<tr>
<td>Oral 1/40 LD₅₀</td>
<td>273 ± 12</td>
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<tr>
<td>Oral 1/60 LD₅₀</td>
<td>299 ± 13</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>0.51</td>
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%: relative to control; *: P<0.05 comparing with control.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Lead acetate toxicity on plasma total soluble protein profile and liver function of the experimental animals (mean±SD).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead acetate treatments</td>
<td>Total soluble protein</td>
</tr>
<tr>
<td></td>
<td>g/dL</td>
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<tr>
<td>Normal control</td>
<td>7.00 ± 0.41</td>
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<tr>
<td>Oral 1/20 LD₅₀</td>
<td>4.50 ± 0.22*</td>
</tr>
<tr>
<td>Oral 1/40 LD₅₀</td>
<td>4.72 ± 0.29*</td>
</tr>
<tr>
<td>Oral 1/60 LD₅₀</td>
<td>4.59 ± 0.30*</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>1.24</td>
</tr>
</tbody>
</table>

%: relative to control; *: P<0.05 comparing with control.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Lead acetate toxicity on blood glucose, activities of cholinesterase, acid and alkaline phosphatase and lactic dehydrogenase in plasma of the experimental animals (mean±SD).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead acetate treatments</td>
<td>Cholinesterase</td>
</tr>
<tr>
<td></td>
<td>μg/dL</td>
</tr>
<tr>
<td>Normal control</td>
<td>110.10 ± 6.66</td>
</tr>
<tr>
<td>Oral 1/20 LD₅₀</td>
<td>80.21 ± 4.72*</td>
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<tr>
<td>Oral 1/40 LD₅₀</td>
<td>86.61 ± 5.00*</td>
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<tr>
<td>Oral 1/60 LD₅₀</td>
<td>87.77 ± 5.10*</td>
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<tr>
<td>LSD 5%</td>
<td>11.96</td>
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</tbody>
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%: relative to control; *: P<0.05 comparing with control.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Lead acetate toxicity on the blood picture, and thyroid hormones of male albino rats (mean±SD).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead acetate treatments</td>
<td>Total hemoglobin</td>
</tr>
<tr>
<td></td>
<td>g/dL</td>
</tr>
<tr>
<td>Normal control</td>
<td>15.00 ± 1.01</td>
</tr>
<tr>
<td>Oral 1/20 LD₅₀</td>
<td>10.88 ± 0.72*</td>
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<tr>
<td>Oral 1/40 LD₅₀</td>
<td>12.17 ± 0.67*</td>
</tr>
<tr>
<td>Oral 1/60 LD₅₀</td>
<td>12.72 ± 0.71*</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>2.02</td>
</tr>
</tbody>
</table>

%: relative to control; *: P<0.05 comparing with control.
activity (377.00, 359.70 and 333.10). As shown in Table 4 blood glucose levels significantly increased under the lead acetate intoxication relative to control and reached its maximum (159.00 mg/dL) at 1/20 LD₅₀.

The results in Table 5 showed the effect of lead acetate toxicity on blood picture and thyroid hormones. Total hemoglobin (Hb) levels were reduced by Pb²⁺ ingestion, this trend was observed also for RBCs count, but WBCs count was insignificantly changed relative to control. Also, the effect of Pb²⁺ toxicity on thyroid function was shown in Table 5. The levels of plasma T₄ and T₃ were reduced with the increase of lead acetate ingestion and the effects were paralleled relative with the dose of toxicant ingestion.

4. Discussion

The effect of lead acetate on body weight gain, food intake and feed efficiency was progressively increased during the experimental period of all different four groups. The final body weight of intoxicated rats with lead was significantly lower than that of the health normal group. These results clearly indicated that lead caused a significant decrease in the gain of body weight. This harmful effect of lead on the body weight gain was elevated paralleled with the increase of lead acetate doses. The severe effect was found in the rats ingested with 1/20 LD₅₀ lead acetate. The amount of food intake of the four groups was unchanged significantly. This means that the values of food intake were not paralleled to the rate of growth and feed efficiency. Also, feed efficiency was decreased under the effect of lead acetate relative to the health control which was concurred with the gain in body weight but not with food intake. The harmful effect of lead acetate ingestion in the present results was insignificantly increased with the increasing of its dose. The obtained results are in agreement with the findings in previous study. They found that lead caused decreases in growth rate in rats when ed lead[42]. These results in body weight gain which may be caused by the toxic ions could be associated with several factors, one of which is imbalance metabolism produced by impairing zinc status in zinc-dependent enzymes which are necessary for many metabolic processes. In addition, lead caused lower effects on liver and spleen than those on kidney and heart. These observations of Pb²⁺ ingestion are significantly increased paralleled with the increasing dose. The detected elevation in the organs weight or ratio was thought to be due to the necrosis and apoptosis which could be attributed to the accumulation of the lipids in the four organs. Pb²⁺ treatments produced a significant accumulation of lipids in kidney cells of rats[43]. Also, they reported that there was an increase in the dry weight of the kidney relative to body weight which may be due to a nutritional disturbance caused by pair feedings. A thorough review of tumorigenicity of lead salts in general, revealed that lead acetate is carcinogenic in rats or mice and the kidney is the most important and perhaps the target organ[44]. The dosages necessary to cause the conditions in animals far exceeded the maximal tolerated dosages in human.

The stimulation in ALT and AST levels was gradually paralleled with the increasing Pb²⁺ ingested doses, until it reached the highest value at 1/20 LD₅₀ of lead acetate treatment. That means the stimulations were found to be dose dependent. The effect of Pb²⁺ on AST activity was significantly similar to that of ALT. The lead acetate intoxication produced 10 fold of plasma bilirubin compared with the normal control (non-toxicated rats). The present results of the liver function parameters (ALT, AST and bilirubin) showed the damage in liver cell of Pb²⁺ intoxicated animals. These observations are in agreement with previous study which reported that lead has hepatotoxic effect[45]. The present results showed that effect of lead acetate on the transaminases activity is dose independent. The high plasma ALT and AST activities are accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue histogram. The elevation of plasma bilirubin value under the ingestion of lead acetate may be due to the induction of heme oxygenase. The catabolism of heme from all heme proteins is carried out in the microsomal fraction of cells by a complex enzyme system and heme oxygenase is an enzyme which can convert heme to bilirubin[42,46]. They reported that bilirubin formed in different tissues is transported to liver as a complex with serum bilirubin. Bilirubin is conjugated with glucuronide in the smooth endoplasmic reticulum of liver, but under the effects of lead toxicity, the conjugation of bilirubin with glucuronoid will become inactive. This may be due to the peroxidation of membrane lipids of smooth endoplasmic reticulum. Bilirubin has a protective role against oxidative damage of cell membrane induced by metals[47]. The variation in total protein of plasma was correlated with the changes in albumin value. The reduction in plasma total soluble protein and albumin levels may be due to inhibition of protein biosynthesis through the specific enzymes in cell processes and low significant excretion of hormones (such as T₃ and T₄) in the present study which can regulate protein biosynthesis[46]. Also, lead treatment caused hepatic deficiency in copper and zinc which act as cofactor to antioxidant enzymes. The results of plasma protein profile found decreases in plasma albumin and the total soluble protein, but globulin was insignificantly though exposure to lead toxicity. This means that the alterations in total soluble protein values were correlated with the changed albumin levels. These may be due to inhibition of albumin biosynthesis through specific enzymes in cell processes and low significant excretion of hormones in the present study which can regulate protein biosynthesis. Heavy metals including lead can precipitate soluble protein and albumin in plasma is used as carrier for poison lead. About 9% of inorganic lead is transported mainly in the plasma[48].

Inhibition in plasma cholinesterase activity by lead acetate is usually used as an indicator exposure to pesticides[49]. The stimulations of acid and alkaline phosphatases were increased with the increasing lead acetate dose. Acid and alkaline phosphatase can be considered as markers of the possible neuro–toxicity of lead. Intoxication with lead was associated with alterations which caused renal toxicity and damage[50]. The effect of lead on renal function could be attributed to alterations in the antioxidant defensive system, resulting in kidney injury. LDH activity of intoxicated rats with lead acetate was stimulated compared with normal control. The effect was increased with the increasing Pb²⁺ dose. Lead acetate ingestion induced alteration in redox status as indicated by a decrease in glutathione levels, an increase in lipid peroxidation end product–4–hydroxynonenal levels which may be produced by damage
in RBCs membrane and increased LDH in plasma[42]. The elevations in blood glucose levels may be due to the increases in the rate of glucose transport from the tissues to blood, glycogenolysis and gluconeogenesis or decreased rate removal of glucose from the blood to tissues. Present results which found a disorder in thyroid function (T4 and T3) in lead intoxicated rats are confirmed by the elevation of blood glucose levels and lead induced hepatotoxicity by activation. Therefore, its selectivity causes toxicity in liver cells marinating semi-normalmetabolic function[51]. Although many enzymes are inhibited by Pb2+, no specific inhibition has been identified as the biochemical lesion. Antioxidant enzymes were affected by higher doses of Pb2+[18,52]. Heavy metals induced hepatotoxicity through the depletion of glutathione and protein, resulting in enhanced production of reactive oxygen species such as peroxide ion, hydroxl radical and H2O2. These reactive oxygen species increased lipids peroxidation and cell membrane damage. These alterations caused the leakage of liver enzymes into the blood. Delta-aminolevulinc acid was accumulated in blood by acute lead intoxication which caused a marked elevation in lipid peroxidation, reduced glutathione levels and inhibited the activity of many enzymes including antioxidative ones[51].

The reduction of Hb confirmed the decreases in RBCs which may be attributed to the toxicity of lead acetate. It is in agreement with the elevation of plasma bilirubin level by Pb2+ ingestion which could be due to the induction of heme oxygenase. The blood pressure was significantly increased compared with the control group. It is possible that Pb2+ can cause anemia and growth retardation. It is thought that the action of lead is particularly marked in the blood vessel and some effects are secondary to this injury[54]. In addition, lead acetate ingestion caused insignificantly reduction of levels of plasma T4 and T3. These are in agreement with previous study, they found that Pb2+ decreased the thyroxin (T4) and the 3, 5-triiodothyronine (T3) levels with the concomitant rise in thyroid stimulator hormone (TSH) levels. This indicates that animals exposed to Pb2+ may be at a risk of thyroid damage[51]. It is established that Pb2+ induced hepatotoxicity may be due to its selectivity in causing toxicity in the semi-normal metabolic function of the liver cells[51]. Lead treatment provoked increased lipid peroxidation, catalase activity, glutathione level but reduced superoxide dismutase activity as compared with normal rats. These results suggest the involvement of free radicals in the pathogenesis of Pb2+ poisoning[56,57]. Lead is a protoplasmic poison lead which can cause damages in many organic bodies. There is little doubt that the nervous system and kidney especially the tubules are affected directly[54]. Finally one of our interests in this investigation was to clearly show that acute intoxication with Pb2+ caused disturbance in the body metabolism as well as oxidative antioxidative balance in the different tissues and plasma. These results suggest that the harmful effect in the metabolism was produced by the Pb2+ toxicant which was increased by increasing the heavy metals (Pb2+) dose. The present results advise people to prevent any exposure to Pb2+ compounds to avoid injurious hazard risk.

Conflict of interest statement

We declare that we have no conflict of interest.

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References


