The Effect of Penicillamine in Reducing the Toxic Effects of Lead Acetate on Some Blood Parameters, Liver Functions and Testicular Tissue in Male Rats

**Keywords:** Lead acetate toxicity, D-penicillamine, male rats, blood parameters, liver function, testicular tissue

**ABSTRACT**

Lead is one of the most important environmental pollutants which is toxic to many organ systems. D-penicillamine (D-P) is a chelator drug which is used for treatment of lead toxicity for several years. This study was conducted in order to evaluate the efficacy of D-P in reducing the effects of lead on hematological indices, liver functions and testicular tissue. This study was done on 25 adult male wistar albino rats (14-16 weeks of age) in animal house of Karbala university / pharmacy college. The rats were divided into 5 groups, the first group represented the health control animals, the second and third groups were drenched orally with doses of lead acetate (1/10 and 1/20) of oral LD$_{50}$ respectively, the fourth and fifth groups were drenched orally with doses of lead acetate (1/10 and 1/20) of oral LD$_{50}$ respectively plus 250 mg/kg penicillamine. The experiment lasted for 3 weeks. The blood was collected for analysis and the testes were excised for histological examination. The results showed that lead induced significant elevation in SAT, ALT activity and plasma bilirubin. Also, total soluble proteins were significantly decreased in case of blood picture, lead ingestion reduced the concentration of hemoglobin, RBC, and PCV while WBC count were increased. In case of histological examination of testes, lead ingestion caused vascular congestion and degenerative changes in seminiferous tubules. In our study, d-penicillamine reduced the effects of lead acetate on some blood parameters, liver function and testicular tissue by reducing its concentration.
1. INTRODUCTION

Lead is a ubiquitous environmental and industrial pollutant that has been detected in every facet of environmental and industrial pollutant that has biological systems. Lead is a heavy metal, occurs in nature as an oxide or salts. Lead can be found in water pipes, insecticides, lining of equipment where corrosion resistance and pliability are required, in petroleum refining (1). There are evidences, which show that lead is a toxic agent with multiple target organs such as hematopoietic system, immune system, kidneys, and nervous system (2). The toxicity of lead is closely related to age, sex, route of exposure level intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate, mechanisms and efficiency of excretion (3). Lead is absorbed through digestive and respiratory tracts, and skin. After absorption into the blood, 99% of lead is bound to erythrocytes and the remaining 1 percentage stay in plasma to be carried to other tissues. Serum lead half-life is around 25 days (4). Lead does not remain in tissues for long periods, except in bones where it is deposited in an inert form, but from which it can be liberated at a later date in sufficient quantity to cause chronic lead poisoning (5).

D-Penicillamine (D-P) is a chelator drug which is used for treatment of lead toxicity for several years (6). The efficacy of D-P in reducing blood lead level (BLL) has made it a good choice for treatment of chronic lead poisoning in adults. D-P administration can increase the urinary excretion of lead because of complexes which it forms with this heavy metal (7). In many cases, BLL fell down to acceptable range after D-P treatment (8). However, long period of administration and side effects of D-P are complicated its use in the treatment of lead poisoning (9).

1.1. Lead acetate

Lead acetate is a chemical compound. It is a white crystalline substance with a sweetish taste. Like other lead compounds, it is very poisonous. Lead acetate is used as a mordant in textile printing and dyeing, as a drier in paints and varnishes, and in preparing other lead compounds. It is an environmental and occupational toxicant which has been known to damage vital organs and suppress cellular processes. It is hazardous when swallowed, inhaled or absorbed through the skin (10). There is a risk of cancer depending on the level and duration of exposure. It causes
irritation to skin, eyes and the respiratory tract. It also has effects on the gum tissue, central nervous system, kidneys, blood and reproductive system\(^{(11)}\).

1.2. **Mechanisms of lead toxicity:**

Lead damages cellular material and alters cellular genetics and produces oxidative damage. It causes increased production of free radicals and decreased availability of anti-oxidant reserves to respond to the resultant damage. It also interrupts enzyme activation and competitively inhibits trace mineral absorption. Lead binds to sulphydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis and lowers the levels of available sulphydryl antioxidant reserves in the body. Lead is known to induce overproduction reactive oxygen species (ROS) and consequently enhances lipid peroxidation, decreases the saturated fatty acid, and increase the unsaturated fatty acid contents of membrane\(^{(12)}\). Also, it has been shown to enhance the production of ROS in a variety of cells resulting in oxidative stress\(^{(13)}\). ROS are the by-products of many degenerative reactions in many tissues, which will affect the regular metabolism by damaging the cellular components\(^{(14)}\). Extensive study on oxidative stress has demonstrated that exposure of cells to adverse environmental conditions induces the overproduction of ROS, such as superoxide radical, \(\text{H}_2\text{O}_2\), and hydroxyl radical in plant cells\(^{(15)}\). In addition, ROS are highly reactive to membrane lipids, proteins and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage\(^{(16)}\).

1.3. **Clinical features and symptoms:**

Acute lead poisoning symptoms are metallic taste, dry throat, abdominal pain, nausea, vomiting, peripheral circulatory collapse, paresthesias, depression, coma and death. In children, there may be cerebellar ataxia. Exposure to highly concentrated lead fumes can produce metal fume fever, an influenza-like reaction characterized by an acute self-limited neutrophilic alveolitis\(^{(17)}\). Chronic lead poisoning symptoms are facial pallor especially circumoral which occur due to local vasospasm. A stippled blue line over the gums, known as Burtonian line, is seen due to deposition of lead sulphide granules. Lead sulphide is formed by the action of hydrogen sulphide formed by decomposed food in the mouth. Colic of intestine, ureters and uterus also occur\(^{(18,19)}\).
1.4. Effects on the blood:

The toxic effects of lead on blood indices are well known. Significant decrease in RBC count, hematocrit (Hct) and hemoglobin (Hb) were seen in rats and human with high blood lead levels \(^{(20)}\). Lead directly affects the hematopoietic system through restraining the synthesis of hemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway. It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. The combined aftermath of these two processes leads to anemia \(^{(21)}\). Anemia caused on account of lead poisoning can be of two types: hemolytic anemia, which is associated with acute high level lead exposure, and frank anemia, which is caused only when the blood lead level is significantly elevated for prolonged periods \(^{(22)}\). Lead significantly affects the heme synthesis pathway in a dose-dependent manner by downregulating three key enzymes involved in the synthesis of heme. δ-aminolevulinic acid dehydratase (ALAD), a cytosolic enzyme that catalyzes the formation of porphobilinogen from δ-aminolevulinic acid (ALA), aminolevulinic acid synthetase (ALAS), a mitochondrial enzyme that catalyzes the formation of aminolevulinic acid (ALA), and finally, the mitochondrial enzyme ferrochelatase that catalyzes the insertion of iron into protoporphyrin to form heme \(^{(23)}\). The initial and final steps of heme synthesis take place in the mitochondria. Whereas the intermediate steps take place in the cytoplasm. Lead inhibits the three aforementioned vital enzymes of this pathway but its effect on ALAD is more profound and its inhibition has been used clinically to gauge the degree of lead poisoning. Inhibition of ALAD results in the accumulation of aminolevulinic acid, detectable in the plasma and urine even at blood lead levels of less than 10 μg/dl. Although ALAD inhibition is first noted at blood lead levels of 10–20 μg/dl, heme biosynthesis does not decrease until the activity of ALAD is inhibited by 80–90%, which occurs at a much higher blood lead concentration of about 55 μg/dl \(^{(24)}\). Inhibition of ferrochelatase results in increased excretion of coproporphyrin in urine and accumulation of protoporphyrin in erythrocytes (EP). Moreover, inhibition of this enzyme results in the substitution of iron by zinc in the porphyrin ring forming zinc protoporphyrin (ZPP). The concentration of ZPP thus gets increased, which can also be used as an indicator to monitor the level of lead exposure \(^{(25)}\). Thus, the collective inhibition of these three key enzymes blocks the heme production via the heme synthesis pathway. The mechanism responsible for shortening the life cycle of erythrocytes is not well understood. One of the earliest observed hematological effects of lead revealed basophilic stipplings of red blood cells (presence of dense material in red
blood cells), which is also a potential biomarker for the detection of lead poisoning. These aggregates are degradation products of ribonucleic acid (26).

1.5. Effects on the liver:

Continuous environmental and occupational lead exposure can cause several changes in the liver structure. Lead is conjugated in the liver with glutathione were part of it and its conjugate is accumulated in the hepatic tissues, leading to impaired liver functions. Biochemical studies showed that lead induces reduction in lipids, cholesterol and glycogen levels in the liver, but produces an increase in hepatic lipid peroxidation, in addition to alterations in the activity of some hepatic enzymes (27).

1.6. Effects on the calcium function:

Lead interferes with calcium functions. These changes may be mediated through leads effects on intracellular calcium homeostasis, or in the brain, for example, by activation of protein kinase. Lead may interfere with calcium-dependent signal transduction processes, especially those associated with neurotransmitter function. The latter may be reversible if cellular change has not occurred prior to effective intervention. Lead interferes with the formation of active vitamin D, which has an important role in its influence on calcium metabolism. Calcium is under tight homeostatic control in all cells. The active form of Vitamin D is produced, primarily, from activation of Vitamin D by sunlight on the skin. The circulating hormone binds to Vitamin D Receptors (VDRs) in the nucleus of cells in the gastrointestinal tract, kidney and bone. This binding activates a cascade of events to increase calcium absorption. Because of their similar biochemical nature, lead can be absorbed by this mechanism especially in children who have decreased calcium intake. In addition, calbindin-D, the binding protein that aids in calcium transport, binds to lead with high affinity and may increase transport of lead in low calcium states (28,29).

1.7. Diagnosis of lead toxicity:

Diagnosis includes determining clinical signs and medical history with an inquiry into possible routes of exposure. It has been suggested that all children should be screened for blood lead levels on their 1st birthday and if possible at yearly intervals thereafter until they are 6 years old.
If at any time the lead level is more than 20µg/dL, therapeutic intervention is indicated and if it exceeds 70µg/dL, it should be treated as a medical emergency \(^{(30)}\). To investigate the sources of lead in the environment of children with elevated blood lead, a field portable X-Ray fluorescence analyzer can be very helpful. The current pediatric practice in the West is first, measure the free erythrocyte protoporphyrin before carrying out a blood lead quantification. Urine levels of aminolaevulinic acid (ALA) can also serve as a sensitive indicator of lead poisoning. ALA excretion rises only when blood lead concentrations exceed 40 µg/dL \(^{(30)}\).

1.8. Treatment:

Treatment depends on how much lead is in the blood. In cases of mild lead poisoning, the source is identified, then removed or minimized. Appropriate nutrition is advised. In cases of severe lead poisoning, with blood lead levels equal to or above 45 micrograms per deciliter, children are admitted for chelation therapy, which are medications that bind to and remove lead from the body \(^{(31)}\).

1.9. Penicillamine

![Fig. (1) penicillamine](image)

D-Penicillamine (DPA; β-β-dimethylcysteine or 3-mercapto-D-valine) is a sulphydryl containing amino acid and a degradation product of penicillin. Only the D-isomer is used because the L-isomer causes optic neuritis. DPA is used mainly as a chelating agent in heavy metal toxicity viz. lead, mercury and copper poisoning (Wilson’s disease). Its sulphydryl group combines with lead to form ring compounds increasing elimination \(^{(33)}\).

1.10. Pharmacokinetics:

Penicillamine is absorbed rapidly, but has an oral bioavailability of 40 to70%. It is not dose dependent \(^{(34)}\). Food, antacids, and iron decrease absorption. The peak occurs within 1 to 3 hours.
regardless of the dose. Penicillamine forms disulphide bonds with many proteins in the blood and tissues, creating potential slow release reservoirs of the drug\(^{(35)}\). Only a small portion of the parent compound is metabolized in the liver to S-methylpenicillamine. Fecal elimination does occur but accounts for a small portion of the total. The primary route of elimination is through the kidneys. The elimination half-life of unchanged penicillamine after single dosing ranges from 1.6 to 3.2 hours\(^{(36)}\). After a steady state concentration is obtained, the elimination is prolonged (4 to 6 days) suggesting a slow release from deep tissues and skin\(^{(37)}\).

## 2. MATERIALS AND METHODS

### 2.1. Chemicals:

Penicillamine 250 mg tablet was obtained from the IBN Hayyan Pharmaceuticals, R. Faysal & Co. Homs- Syria, and given orally at a dose of 250 mg/kg body weight, we used two doses of powdered lead acetate. The first dose is 1/10 of LD\(_{50}\) (LD\(_{50}\) of lead acetate = 600 mg/kg) which is 60 mg/kg of body weight and the second dose is 1/20 of LD\(_{50}\) which is equal to 30 mg/kg of body weight\(^{(38)}\), these doses dissolved in 0.5 ml of water. Each dose gave orally daily for three weeks.

### 2.2. Animals:

In this study, we used 25 adult male Wister albino (230-250 g) rats obtained from animal house of Karbala university/pharmacy college which were housed in groups in plastic cages, standard diet, tap water and placed on a 12-hour light/dark cycle for 21 days (the experimental period).

### 2.3. The experimental design:

The animals were randomly divided into five groups, each experimental group consisted of 5 animals:

**Control group**, they were fed with only standard diet, glucose water and tap water for 21 days.

**Lead acetate group 1**, rats were treated with lead acetate with a dose of 1/10 of lethal dose mg/kg daily orally via needle gavage started from the first day to last day of the experiment.
Lead acetate group 2, rats were treated with lead acetate with a dose of1/20 of lethal does mg/kg daily orally started from the first day to last day of the experiment.

Lead acetate (1/10) + penicillamine group, rats were treated with lead acetate (1/10) and penicillamine 250 mg/kg were given orally via gavage daily started from the first day to last day of the experiment.

Lead acetate (1/20) + penicillamine group, rats were treated with lead acetate (1/20) and penicillamine 250 mg/kg were given orally via gavage daily started from the first day to last day of the experiment, then 5 ml of blood samples were collected from each rat in centrifuge tubes. Serum was separated from coagulant blood by centrifugation and then frozen for biochemical analysis.

2.4. Biochemical assay methods: (AST, ALT, Bilirubin, total protein)

2.5. Blood parameter assay methods: (RBC, WBC, PCV, Hb)

2.6. Tissue preparation:

The organs were carefully made free from surrounding fat and connective tissue, washed briefly with tap water and immediately placed in 10% normal saline. The organs from one side of each animal were tested for histopathological changes.

2.7. Statistical analysis:-

Results were expressed as mean ± SE. Statistical significance was calculated by using one way analysis of variance (ANOVA) by Graph pad prism software (p< 0.05) was considered as significant. Values bearing different letters as superscripts showed significant differences (p< 0.05).
3. RESULTS

Table (3.1.1) show the effect of lead acetate and penicillamine on blood parameters of male rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RBC count (10⁶ cells/microliter)</th>
<th>WBC count (cells/mm³)</th>
<th>PCV %</th>
<th>Hb (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.37± 0.28492</td>
<td>6.18± 0.25884</td>
<td>38.06± 0.76681</td>
<td>12.01± 0.35777</td>
</tr>
<tr>
<td>Lead1/10</td>
<td>5.73± 0.14926</td>
<td>6.8± 0.18708</td>
<td>30.48± 0.92032</td>
<td>10.58± 0.40865</td>
</tr>
<tr>
<td>Lead1/20</td>
<td>6.18± 0.33466</td>
<td>6.44± 0.15165</td>
<td>33.72± 1.06864</td>
<td>11.02± 0.19235</td>
</tr>
<tr>
<td>Lead1/10+pen250</td>
<td>6.3± 0.31622</td>
<td>6.02± 0.19235</td>
<td>34.2± 0.39370</td>
<td>10.54± 0.29495</td>
</tr>
<tr>
<td>Lead1/20+pen250</td>
<td>7.02± 0.13038</td>
<td>6.02± 0.10954</td>
<td>34.82± 0.31144</td>
<td>11.3± 0.25495</td>
</tr>
</tbody>
</table>

- a,b: mean there are significant differences between the control group and lead 1/10 group.
- c: mean there are significant differences between lead 1/20 with control group and lead 1/10 group.
- d: mean there are significant differences between (lead 1/10 + penicillamine), control, lead 1/10 and lead 1/20 groups.
- e: mean there are significant differences between (lead 1/20 + penicillamine), control, lead 1/10, lead 1/20 and (lead 1/10 + penicillamine) groups.
- Significant differences (p<0.05).

Table (3.1.2) show the effect of lead acetate and penicillamine on liver function of male rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AST</th>
<th>ALT</th>
<th>Total protein</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.58 ± 0.91214</td>
<td>31.8 ± 0.59581</td>
<td>7.006 ± 0.04827</td>
<td>0.424 ± 0.02966</td>
</tr>
<tr>
<td>Lead 1/10</td>
<td>118.2 ± 4.76445</td>
<td>68.12 ± 0.68702</td>
<td>5.854 ± 0.22700</td>
<td>3.848 ± 0.04969</td>
</tr>
<tr>
<td>Lead 1/20</td>
<td>109.6 ± 4.97995</td>
<td>59.5 ± 0.54313</td>
<td>6.008 ± 0.28577</td>
<td>3.002 ± 0.03492</td>
</tr>
<tr>
<td>Lead 1/10 + pen 250</td>
<td>72.4 ± 3.64691</td>
<td>58.18 ± 0.50695</td>
<td>6.19 ± 0.25855</td>
<td>2.66 ± 0.32093</td>
</tr>
<tr>
<td>Lead 1/20 + pen 250</td>
<td>67.6 ± 2.07364</td>
<td>56.04 ± 0.69137</td>
<td>5.998 ± 0.07791</td>
<td>1.298 ± 0.42663</td>
</tr>
</tbody>
</table>

-a, b: mean there are significant differences between the control group and lead 1/10 group.

- c: mean there are significant differences between lead 1/20 with control and lead 1/10 groups.

- d: mean there are significant differences between (lead 1/10 + penicillamine), control, lead 1/10 and lead 1/20 groups.

- e: mean there are significant differences between (lead 1/20 + penicillamine), control, lead 1/10, lead 1/20 and (lead 1/10 + penicillamine) groups.

- Significant differences (p<0.05)
Figure (3.1.3). Effect of lead acetate and penicillamine on ALT conc. in male rats

Figure (3.1.4). Effect of lead acetate and penicillamine on AST conc. in male rats

Figure (3.1.5). The effect of lead acetate and penicillamine on bilirubin concentration in male rats

Figure (3.1.6). The effect of lead acetate and penicillamine on total proteins conc. in male rats
3.2. Histological results:

Image (3.2.1): Transverse section of control group seminiferous tubules show normal cellularity and normal spermatogenic action of the seminiferous tubules (X20, H & E stain).

Image (3.2.2): Transverse section in lead acetate 1/10 of LD₅₀ treated group seminiferous tubules show degenerative changes and high space in some tubules.(X200, H & E stain).
Image (3.2.3): transverse section in lead acetate 1/20 of LD$_{50}$ treated group testis show vascular congestion and slightly degeneration. (X20, H & E stain)

Image (3.2.4): Transverse section of (lead acetate 1/10 of LD$_{50}$ + penicillamine 250 mg/Kg) treated group testis show significant reduction in space (X20, H & E stain).
Image (3.2.5): transverse section of (lead acetate 1/20 of LD₅₀+penicillamine 250 mg/Kg) treated group testis show significant reduction in space and low congestion. (X200, H & E stain)

4. DISCUSSION

In our study, there are reduction in RBCs, PCV and Hb was observed in table (3.1.1) which might be due to effect of lead on activity of δ-aminolevulinic acid dehydratase, key enzyme of heme synthesis. Moreover, lead also inhibit the conversion of coproporphyrinogen III to protoporphyrin IX leading to reduction in haemoglobin production and shortened life span of erythrocytes (39). Progressive destruction of RBCs due to binding of lead with RBCs, leading to increasing fragility and destruction; could be another reason for decrease in haematological values (40). Similarly, significant decrease in Hb and PCV were observed following exposure of rats to lead acetate (41). Total leukocyte count had increased due to lead –induced inflammation (42). While 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 healthy rats. These results in table (3.1.2) showed that Pb²⁺ ingestion highly stimulated the activity of AST and ALT. The stimulation was gradually paralleled with the increasing of Pb²⁺ ingested doses until it reached the highest value at 1/10 LD₅₀ of lead acetate treatment. That means that the stimulations were found to be dose dependent. The effect of Pb²⁺ on AST

activity was significantly similar to that of ALT. Data of plasma bilirubin showed highly significant elevation of bilirubin value in Pb2+-intoxicated rats relative to the control after the experimental period. The present results of the liver function parameter (ALT, AST, and bilirubin) resulted in damaging in the liver cell of Pb2+-intoxicated animals. In addition, it was reported that lead has a hepatotoxic effect. The high plasma ALT and AST activity was accompanied with high liver microsomal membrane fluidity, free radical generation, and alteration in the liver tissue histogram. The evaluation of plasma bilirubin value under the ingestion of lead acetate may be due to the induction of hemeoxygenase, the catabolism of heme from all heme proteins appears to be carried out in the microsomal fraction of cells by a complex enzyme system, hemeoxygenase, which converted heme to bilirubin. Also, bilirubin formed in the different tissues is transported to the liver as a complex with serum bilirubin, that bilirubin is conjugated with glucouronoid in the smooth endoplasmic reticulum of the liver, but under the effects of lead toxicity, the conjugation of bilirubin with glucouronoid was not active; this may be due the peroxidation of membrane lipids of smooth endoplasmic reticulum. Bilirubin has a protective role against oxidative damage of cell membrane induced by metals.

Protein profile of plasma was changed under ingestion of lead acetate. The results reported significant reduction in total soluble protein. The reduction in plasma total soluble protein levels may be due to inhibition of protein biosynthesis through the specific enzymes in cell processes and low significant excretion of hormones (such as triiodothyronine (T3) and T4) in the present study which regulated protein biosynthesis.

While the histological results were compatible with Al-Omar, et al. who reported that the lead causes decrease in seminiferous tubules diameter in adult rats. And also with Corpas, et al. who showed that the lead acetate causes decrease in the diameter and epithelial thickness of rat seminiferous tubules. Lead exposure produced pronounced testicular damages evidenced by histological alternations in testis include degeneration of seminiferous tubules, germ cell necrosis and vacuolar degeneration especially in secondary spermatocytes. These findings correspond with the observations that showed lead acts as a spermicidal agent in the case of high exposure, leading to a dose-related suppression of spermatogenesis.

In our study, in case of (lead acetate + d-penicillamine) treated groups, the results of RBCs count, WBCs count, Hb, PCV%, GOT, GPT, serum bilirubin, total protein were not significantly

changed from the control group. Also, the histological examination of the rat’s testis shows that there is significant reduction in space of the seminiferous tubules and low congestion. These results were because that penicillamine reduced the effects of lead acetate on the blood parameters, liver function and the testicular tissue by increasing the elimination of lead (acted as chelator).

6. REFERENCES