

## Investigating the Effect of Lead Poisoning on All Related Organs Biochemically

### Abstract

Lead is considered one of the most significant poisons and poisonings in farm animals. Young cows, sheep, and horses are affected by this poisoning. However, their sensitivity is lesser than cows. The present study investigates the effect of lead poisoning on all related organs biochemically.

In the present study, fourteen New Zealand white male rabbits were divided into experimental (N=7) and control (N=7) groups. They were kept in metal cages with the same food and water and the laboratory conditions with an average weight of 2700 gr to 3500 gr. The experimental group was injected with 6.5 mg/kg of lead acetate and 3 ml of distilled water intraperitoneally every other day for 7 weeks. The control group was injected with physiological serum every other day for 7 weeks intraperitoneally. Subsequently, the biochemical parameters of ALT, AST, glucose, creatine, and urea enzymes were determined by the photometric method.

In the experimental group, there is no protective response in the eyes, and the rabbits have movement disorders and were pale. Also, the biochemical results of the study showed a significant difference between the experimental group and the control group in terms of aspartate aminotransferase ( $P < 0.05$ ).

**Keywords:** Lead poisoning, Biochemical changes, Aspartate aminotransferase, Alanine aminotransferase.

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### Introduction

The presence of small amounts of lead in the blood may result in disorders in the production of red blood cells, delay in the physical and brain development of children, learning disorders in children, and increased blood pressure in adults. However, the American Pediatric Association considers a concentration above 10  $\mu\text{g}/\text{dL}$  dangerous for infants, children, and women of reproductive age. In the industry, blood lead above 30  $\mu\text{g}/\text{dL}$  is considered unsafe (Patrick, 2006). The diagnosis of lead poisoning is usually based on an increase in the blood level of lead and its management is based on the diagnosis of the contamination source and the method of contact (Beigmohammadi et al., 2008). Lead is a divalent cation and has a high binding capacity with sulfhydryl groups. Accordingly, it can disrupt the function of enzymes. The most well-known of them is the disorder in the Delta-aminolevulinic acid dehydratase (ALAD), which causes disorders in the production of heme and anemia. ALAD activity is inhibited by about 50% if the blood lead concentration exceeds 20  $\mu\text{g}/\text{dL}$ . The increase in urinary excretion of aminolevulinic acid (ALA), due to a decrease in ALAD activity, was used as an indicator of lead poisoning in the past. Consequently, fatigue and anemia are signs of chronic lead poisoning (Patrick, 2006). The dangerous effects of lead are created gradually over a long period. It means that the resistance to this metal is different in different animal tissues and appears based on time. When tissues are exposed to large amounts of lead for long periods, it can cause rapid and irreversible damage. Extensive studies have been conducted on the different effects of lead in different tissues of the body (Perottoni et al., 2005). Lead can be

absorbed through inhalation and ingestion. Lead inhibits heme synthesis and causes anemia by reducing the circulating hemoglobin level. Red blood cells become small and pale. Basophilic Stippling of red blood cells is often seen (Beigmohammadi et al., 2008). Many of the symptoms of lead are non-specific and it may cause a delay in diagnosis and even non-diagnosis of poisoning. Ambiguous symptoms such as headache, fatigue, nausea, and abdominal pain, commonly seen in emergency room visitors, may be caused by lead poisoning (Farrell et al., 1999).

Also, chronic poisoning with low amounts may cause glomerular and tubulointerstitial disorders and symptoms such as glycosuria, proteinuria, chronic renal failure, and hypertension (Sanborn et al., 2002). The neurological effects of lead poisoning have also been investigated. Lead can destroy the blood-brain barrier structure, especially in the cerebral cortex in the frontal lobe, hippocampus, and cerebellum. It also may cause symptoms such as memory and attention disorders (Sanborn et al., 2002).

Alwaleedi (2016) examined the biochemical changes of experimental lead poisoning in albino rats. Karima et al. (2012) examined the long-term effect of lead proximity in male rabbits on chromosomes and testicular tissue (Ahmed et al., 2012). Poisoning with this heavy metal can disrupt a wide range of physiological and biochemical functions of the body, including the kidney (Frantz et al., 2012). When kidney function is progressively endangered, the creatinine concentration may increase slightly. However, approximately two-thirds to three-quarters of the nephrons may lose their function before the creatinine concentration reaches the upper limit of the normal

range. Both acute and chronic renal failures are usually associated with elevated serum creatinine.

Acute renal failure, especially in animals with a lack of or reduced urine secretion, is primarily associated with extensive changes in blood parameters. In contrast, the blood parameters of animals suffering from chronic renal failure remain almost constant (Smith, 2010). Also, urea is excreted by the kidneys, primarily through glomerular filtration. Urea is produced almost exclusively in the liver, and liver failure is usually associated with reduced BUN. Also, BUN in baby animals is naturally lower than in adults. The rare causes of reduced blood urea nitrogen are a low-protein diet and anabolic steroid administration.

The common causes of increased BUN are prerenal azotemia, reduced renal perfusion, reduced blood volume, congestive heart failure, renal azotemia, acute renal failure, chronic renal failure, post-renal azotemia, urinary stones, kidney stones, and bladder rupture. Rare causes of increased BUN are abscesses around the kidney, kidney carcinoma, bladder carcinoma, etc. (Smith, 2001). Also, alanine aminotransferase, which is glutamic pyruvic transaminase or GPT, is present in plasma and some cells. In acute liver diseases that cause membrane damage or cell necrosis, this enzyme activity increases significantly in the blood serum. It should be clean and not stained with cleaning materials, and the blood serum should be separated as soon as possible after blood collection (Mojabi et al., 2011).

Aspartate aminotransferase, also known as glutamic-oxaloacetic transaminase or GOT, is found in high concentrations in various tissues, including cardiac and skeletal muscles, red blood cells, kidneys, and the liver. This enzyme is a non-specific indicator of tissue necrosis. It is less sensitive than tissue-specific enzymes such as SDH and CPK in mild attacks. The half-life of AST in the bloodstream is relatively long. Its increase after the necrotic attack of muscle tissue or liver damage may last up to 10 days. In general, extensive muscle necrosis increases AST to a greater extent than severe liver necrosis (Smith, 2001).

Koohi et al. (2016) examined the possible changes in the onset of pain sensation and the intensity of response to it in mice after long-term lead exposure. They showed that animals exposed to lead showed a delay in responding to chemical pain by up to 68% and a reduction in the pain intensity by up to 80%, none of which was statistically significant. Blood cortisol level was almost unchanged in the groups. It suggests a delay in the response to painful stimuli, which is seen after long-term exposure to Acetate. It may be considered a disorder leading to the weakening of the warning role of pain (Koohi et al., 2016). Perottoni et al. (2005) showed that lead dangerous effects are created in the long term and gradually.

It means that the resistance to this metal is different in different animal tissues and appears based on time. When tissues are exposed to large amounts of lead for long periods, it may cause rapid and irreversible damage. Extensive studies have been conducted on the different effects of lead in different tissues of the body (Perottoni et al., 2005). Oyagbemi et al. (2015) conducted a study entitled "Oxidative damage in kidney tissue of rats with lead acetate". They showed that different tissues such as the liver, kidney, brain, bone, bone marrow, fetus, muscles, ovary, and especially testicular tissue can be affected by lead. The kidney has been reported as one of the first targets of lead contamination (Oyagbemi et al., 2015). Alwaleedi (2016) examined hematological and biochemical changes in experimental poisoning of albino, male, and female rats. The results revealed that even low doses of lead acetate have harmful effects on animals owing to blood and biochemical changes (Alwaleedi, 2016).

Koohi et al. investigated the possible changes in the onset of pain sensation and the intensity of the response to it in mice after long-term exposure to lead acetate. They showed that the animals exposed to lead exposure showed a delay in response to pain caused by heat at a low dose (52 %) and a high dose (59%). The intensity of thermal pain decreased by 63% at low doses and 82% at high doses of lead acetate ( $P < 0.05$ ). The delay in response to chemical pain showed a decrease of up to 68% and the intensity of response to pain decreased by up to 80%, none of which was statistically significant. The blood cortisol level was almost unchanged in the groups. It suggests a delay in the response to painful stimuli seen after long-term exposure to lead acetate. It may be considered a disorder that weakens the warning role of pain (Koohi et al., 2016).

Johari et al. (2014) induced experimental poisoning by lead and investigated the effect of garlic extract in all newborn rats. The results showed that the reduction in serum lead load was significant in groups B1 and B2 compared to group C ( $P < 0.05$ ). However, it was not significant in groups A1 and A2 ( $P > 0.05$ ). In kidney tissue, the decrease in the lead was significant in all groups except group A1. Also, in the liver and bone tissues, it was significant in all groups compared to group C ( $P < 0.05$ ). This study suggests that the administration of at least 250 mg of fresh garlic per kilogram of body weight daily can reduce the amount of lead in serum, liver, and bone tissue. However, the same dose of garlic tablets is not effective on serum and kidney. Administrating at least 500 mg of pills per kilogram of body weight daily is also effective on kidney tissue. It is due to the antioxidant property of garlic and its protective effect on the toxic effect of lead on the kidney tissue (Johri et al., 2014). In the present study, lead poisoning is defined as an independent variable, and changes in biochemical interpretations including (liver: LAD enzyme, ASD, kidney: urea and creatine, pancreas: glucose) are defined as a

dependent variable. The present study investigates the biochemical changes in tissues examined in previous studies in the form of experimental lead poisoning induction in the rabbit animal model.

#### Materials and Methods

In the present study, 14 New Zealand white male rabbits were divided into experimental (N=7) and control (N=7) groups. They were kept in metal cages with the same water and food and the laboratory conditions in the animal house of the veterinary school of Azad University, Sanandaj branch, to adapt to the environment with an average weight of 2700 gr to 3500 gr. The experimental group was injected with 6.5 mg/kg of lead acetate and 3 ml of distilled water intraperitoneally every other day for 7 weeks to induce experimental poisoning (Gholamhosseini et al. 2008). The control group was injected with physiological serum every other day for 7 weeks intraperitoneally. At the end of the seventh week, to relax the

muscles of the rabbits and ease the injection, and to avoid the anxiety of the rabbits, ... was injected. Then, blood samples were collected directly from the hearts of the rabbits using a normal syringe. The collected blood was poured into 2 test tubes. The first tube was immediately centrifuged in the laboratory without using an anticoagulant to separate the serum. The second tube, which contained the anticoagulant (EDTA) was sent to the veterinary diagnostic laboratory to measure hematology factors. ALT, AST, glucose, creatine, and urea enzymes were determined by a photometric method using a kit..... made in Iran with an auto-analyzer made in Iran.

#### Results

The rabbits of the two groups were examined in terms of changes in vital and clinical reflexes during the study (7 weeks). The results are shown in Table 1.

Table 1: The results of clinical symptoms in the studied and control samples

Study sample		Protective response in the eye		Pupil response to light radiation in the eye	Movement disorders	Examination of mucous
		Same side	Both sides			
Cage 1	Sample 1	No	No	Negative	Positive	Pale
	Sample 2	No	No	Negative	Positive	Pale
	Sample 3	No	No	Negative	Positive	Pale
Cage 2	Sample 4	No	No	Negative	Positive	Pale
	Sample 5	No	No	Negative	Positive	Pale
	Sample 6	No	No	Negative	Positive	Pale
	Sample 7	No	No	Negative	Positive	Pale
Control group	Sample 1	Yes	Yes	Positive	Negative	Normal
	Sample 2	Yes	Yes	Positive	Negative	Normal
	Sample 3	Yes	Yes	Positive	Negative	Normal
	Sample 4	Yes	Yes	Positive	Negative	Normal
	Sample 5	Yes	Yes	Positive	Negative	Normal
	Sample 6	Yes	Yes	Positive	Negative	Normal
	Sample 7	Yes	Yes	Positive	Negative	Normal

Based on the results obtained (Table 1), in the experimental group, there is no protective response in the eye, and the response of the pupil to the light radiation in the eye is

negative, the rabbits have movement disorders, and are pale. However, in the control group, they did not have any of the symptoms.

Table 2: Mean and standard deviation of biochemical symptoms by sample

Sample	Experimental group	Control group	Sig
Symptoms	3.125±37.166	2.145±48.151	0.024
Biochemistry	6.794±18.166	5.546±86.177	0.335
Aspartate aminotransferase	31.086±112.166	28.025±117.135	0.891
Alanine aminotransferase	6.177±96.166	5.168±81.145	0.072
urea	0.081±1.766	0.060±1.947	0.191

According to the results obtained from the above table, there is a significant difference between the experimental and the control groups regarding aspartate aminotransferase at a significance level of 0.05. There is no significant difference between them regarding the rest of the biochemical symptoms.

### Discussion

Lead has a high affinity for bonding with sulfhydryl, carboxyl, and amine groups, so it disrupts the function of many vital enzymes in the body (Peterson & Talcott, 2004). Alwaleedi (2016) examined the hematological and biochemical changes in the experimental poisoning of albino, male, and female mice. The results revealed that even low doses of lead acetate have harmful impacts on animals owing to blood and biochemical changes. These findings are consistent with the results of blood and biochemical changes in the present study. The kidney is one of the significant tissues in lead poisoning. It is stated that kidney-specific proteins that bind to lead and can facilitate the transfer of lead into the nucleus and binding to DNA, may act as tissue-specific receptors for lead. Thus, the accumulation of these proteins in renal tubules causes degenerative-necrotic changes and cell death (Fowler and Duval, 1991). Kidney damage and Fanconi syndrome (in which the proximal tubules are functionally damaged) are other toxic lead injuries (Clark et al., 2004, (Kantiz et al., 1999). Lead quickly enters the blood and is deposited in organs including the kidneys which have a higher blood flow and higher metabolism (Garibotto et al., 2009). Recent studies have shown that long-term exposure to lead causes many histopathological damages, such as reduced density of microvilli and loss of integrity of the epithelium of the lumen of the proximal tubules (Ceruti et al., 2002).

Acute kidney inflammation affects ureters, blood vessels, interstitial tissues, and cells, especially podocytes and the vascular loop of glomeruli (Cerulli et al., 2006). Also, it has been observed that long-term exposure to lead acetate causes changes in the small structures of kidney tissues such as increased density of mitochondria, expansion of the endoplasmic reticulum, and thickening of the base membrane

of the urinary filtration barrier (Deveci et al., 2011). Karimfar et al. (2016) also conducted a study entitled “The long-term exposure to lead acetate in rabbit kidney tissue”. They showed that long-term exposure to lead acetate causes histopathological effects, including dilatation, congestion, nuclear heterochromatic effects, increased diameter of the renal tubules, and thickening of the urinary barrier in the kidney tissue of rabbits. Quantitative and qualitative results of long-term exposure to lead acetate have shown many histopathological side effects, especially in the urinary barrier (Karimfar et al., 2016, Azarnia et al. 2001). Some enzymes are particularly vulnerable and sensitive to lead. They include gamma ALA dehydratase and ferrochelatase. The conflict with ferrochelatase prevents the formation of protoporphyrin and results in the accumulation of non-metallated porphyrins, including protoporphyrin, uroporphyrin, and coproporphyrin. Uroporphyrin and coproporphyrin are excreted through feces and urine. The conflict with the activity of ALA dehydratase might be responsible for lead-related brain damage due to the conflict with heme metabolism and the change in the function of other half-life proteins (Smith, 2001).

### Conclusion

According to the obtained biochemical results, there is a significant difference between the experimental and control groups regarding aspartate aminotransferase. There is no significant difference between these groups regarding the rest of the biochemical symptoms.

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