The effects of lead on ATPase activity in liver of *Pelophylax nigromaculata*

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**Abstract.** Lead is a major heavy metal in sewage, which affects the growth, development, reproduction, endocrine and blood circulation of aquatic animals. The effects of lead on the activity of ATPase in liver of *Pelophylax nigromaculata* were studied. In vitro experiment, the grinding fluid of the liver of *P. nigromaculata* was mixed with the equal proportion of 0, 0.1, 10, 100, 1000 and 10000 μg/L lead. In vivo experiment, the liver was treated with 0, 10, 100 and 1000 g/L of lead for 30 d. The absorbance of the liver was measured by ATPase kit with enzyme labeling instrument and ATPase activity of the liver was calculated according to the formula. In vitro experiments, compared with the control group, the activity of ATP enzyme in lead treated group decreased significantly \(p<0.05\). With the increase of lead concentration, the activity value showed a fluctuating trend of first decreasing and then rising and then falling, and the activity value of 10 μg/L ATP in treatment group decreased most \(p<0.05\). With the increase of Pb concentration, the activity of ATP decreased first and then increased, and there was a significant difference among the treatment groups \(p<0.05\). The activity of 100 μg/L ATP decreased most in the treatment group. The results showed that lead treatment inhibited the activity of ATP enzyme in the liver of *P. nigromaculata*.

1. **Introduction**

A survey on the pollution of major rivers in China by Water Conservancy Departments has confirmed that more than 90% of rivers are seriously polluted. The main sources of pollution are urban domestic sewage and industrial wastewater discharged beyond the standard in industrial development. With the development of economy and society and the level upgrading of industrialization, industrial wastewater has become a more and more prominent social problem, which will lead to resource exhaustion, ecological destruction and other problems [1,2,3]. Heavy metals in sewage can be transformed into toxic compounds through the decomposition of microorganisms in water, and enriched in aquatic organisms through water flow, thus endangering the normal life activities of organisms. Biologically enriched heavy metals accumulate in organisms through food chain storage and their toxicological effects are strengthened with the extension of the food chain.

Lead is a major heavy metal in sewage. When lead ions are absorbed by organisms, lead-sulfur proteins are formed in vivo and selectively accumulated in the kidney and liver. When accumulated to
a certain extent, lead-sulfur proteins will cause varying degrees of damage to organisms [4,5]. For amphibians, their reproductive activities and larval development are carried out in water, which is the basis for their survival. When the pollution of heavy metals exceeds the self-purification capacity of water, the excessive heavy metals in water will directly affect their growth, development, reproduction, endocrine regulation and blood circulation, and affect the normal life activities of amphibians [6,7].

ATP is a coenzyme, also known as adenosine triphosphate, and it is an enzyme that catalyzes the hydrolysis of adenosine triphosphate to adenosine diphosphate and phosphate ions. ATP enzyme exists on the tissue membrane and organelle membrane, and is a protease on the bio membrane [8]. ATPase plays an important role in improving the metabolism of the body. It can participate in the metabolism of fat, protein, sugar, nucleic acid, nucleotide and so on. ATPase also plays an important role in material transport, energy conversion and information transmission. To some extent, the enzymatic reaction in this organism reflects the metabolic function of organism.

Pelophylax nigromaculata, a common frog in farmland is the research object. The effects of different concentrations of lead on ATPase of amphibians were studied in vitro and in vivo, which provided experimental basis for the protection of amphibians and ecological environment.

2. Materials and methods

2.1. Experimental animal
Healthy, mature and similar-sized male frogs were selected from non-pollution ponds.

2.2. Instruments and reagents
The instruments used include high-speed freezing centrifuge, constant-temperature water bath, enzyme labeling instrument, homogenizer, electronic balance, transfer liquid gun with 100 μl, 500 μl, 1000 μl, centrifuge test tube, enzyme plate, etc.

Pb(NO₃)₂, purity: 99.99%. The mother liquor of the two groups was prepared with distilled water 0.1 g/L and 100 g/L, and then the mother liquor of the two groups was used to prepare 0.1 μg/L, 1 μg/L, 10 μg/L, 100 μg/L, 1000 μg/L, 10000 μg/L lead solution, respectively.

2.3. Poisoned way

2.3.1. In vitro experiment. Six normal frogs were killed by double destruction and then they were anatomized. The livers were immediately extracted, the surface bloods were absorbed with filter paper and weighed on an electronic balance with a clean weighing paper. After weighed, the livers were put into the centrifuge test tube by forceps. The livers were cut into any size with scissors and then 0.5 g were taken. According to the weight ratio 1:9, nine times volume of normal salines were added to the centrifuge test tube. The liver tissue was broken into small particles to more uniform suspension with homogenizer. After centrifugation, 10 mL supernatant was poured into the test tube. 1 mL Pb²⁺ with concentration gradient was added to each test tube. Each concentration was repeated three times. The five lead concentrations were 0.1, 10, 100, 1000, 10000 μg/L, respectively.

2.3.2. In vivo experiment. Three lead concentration gradients (10, 100, 1000 μg/L) were set as experimental group and one control group, and a parallel group was set up. The solution was about 40 L. There were six individuals in each treatment group. The water temperature remained at room temperature, and the treatment time were 30 days. After the experiment, all the individuals were dissected for taking the liver and the enzyme activity was measured. The liver of each frog was gained 0.5 g to grind and centrifuge according to the method of in vitro experiment.

2.4. Enzymatic reaction
The liver tissue protein of the sample was pretreated. The concentration of tissue protein was measured by Coomassie brilliant blue reagent, and the curve was drawn by computer. The standard curve of tissue
protein was obtained by passing the curve across the origin. The optimum concentration of diluted concentration is 10-20 times.

The treated samples and corresponding blank test tubes were divided into experimental group and control group, and they were placed on the same frozen EP box to prepare the dosage. According to the order of sodium-potassium ATP reagent kit, the drugs were added to the centrifugal test tube of the experimental group and the control group with the pipette gun. After mixing, the supernatant was centrifuged to determine phosphorus.

The supernatants of the experimental group and the control group were added with chromogenic reagents, mixed, sat quietly at room temperature for 2 minutes, and then the corresponding reagents in the kit were added.

Prepare a standard tube and a blank tube, and add phosphorus standard solution and color reagent to the standard tube, at the same time add double steaming water and color reagent to the blank tube, mix all the tubes, sat quietly at room temperature for 2 minutes, then add the corresponding reagent in the kit.

Using a range of 500 μL pipette gun, 300 L of each solution in the centrifuge tube was respectively injected into the 96-hole labelling plate. After connecting the enzyme labeling instrument with the computer, the enzyme labeling board was put into the enzyme labeling instrument. The operation procedure was set as incubation for 5 minutes and vibration for 3 minutes. Then the absorbance values of each hole in the enzyme labeling board were measured. The ATP activity values of each pore were calculated, compared and analyzed.

3. Results

3.1. In vitro experiment

Compared with the control group, the activity of ATP enzyme in lead treated group decreased significantly \( p<0.05 \). With the increase of lead concentration, the activity value showed a trend of first decline, then rise and then decrease. There was no significant difference in ATPase activity between 0.1 μg/L and 100 μg/L, 1000 μg/L and 10 000 μg/L treatment groups \( p>0.05 \), but there was significant difference among other treatment groups \( p<0.05 \) (Fig.1). Compared with the control group, ATP activity decreased by 21.14%, 45.63%, 18.22%, 40.28% and 36.34% respectively in 0.1, 10, 100, 1000 and 10 000 μg/L treatment groups, among which ATP activity in 10 μg/L decreased most.

![Figure 1. The effects of lead on ATPase activity in liver of P. nigromaculata in vitro experiment](image-url)
3.2. In vivo experiment
Compared with the control group, the activity of ATP enzyme in lead treated group decreased significantly ($p<0.05$). With the increase of lead concentration, the activity value showed a fluctuating trend of decreasing first and then rising, and there was a significant difference among the treatment groups ($p<0.05$) (Fig. 1).

Compared with the control group, the ATP activity of 10, 100, 1000 μg/L treatment group decreased by 27.04%, 84.23% and 57.27% respectively, and the ATP activity in 100 μg/L treatment group decreased the most.

![Figure 2. The effects of lead on ATPase activity in liver of *P. nigromaculata* in vivo experiment](image)

The accumulation of lead in organisms often causes serious damage to tissues and cells. In this study, we found that the liver of *P. nigromaculata* was damaged in function and morphology by the lead in vitro and in vivo experiment. The results showed that the organs, tissues and enzyme activities of *P. nigromaculata* were affected to some extent, when exogenous chemicals entered the body. At the same time, it was observed that the surface of the liver which was not infected with lead or with low concentration of lead showed bright red color, while that of the liver which was infected with high concentration of lead became dark. This phenomenon means that when a high concentration of lead solution is added, lead will partially deposit on the surface of the liver with the blood flow, or cause blood lead concentration to a certain extent increased with the blood circulation.

The water environments with high concentration or long-term pollution of lead can cause the heavy metal enters the liver through osmosis, causing certain histopathological damage to the liver, which can result in the destruction of the structure of tissue cells, and eventually lead to the decline of ATPase activity. With the increase of lead concentration, ATP enzyme activity showed a certain volatility. It may be that a certain concentration of heavy metals stimulates the organism to produce a protective mechanism to enhance the activity of enzymes to achieve self-protection, but beyond this concentration, the protective mechanism will be lost and the enzyme activity will decrease again.
4. Conclusion
The mechanism of the decrease of ATPase activity caused by heavy metal lead may be: 1) the accumulation of lead ions in the liver destroys the ion balance on the cell membrane; 2) the ion imbalance destroys the function of biomembrane, weakens the defense ability of organisms against free radicals to a certain extent, and affects the dissociation of some groups of ATP enzyme active center; 3) The nucleophilic catalysis of the enzyme activity center are also affected, resulting in the decrease of ATPase activity. However, how the external environment stimulates the production of protective mechanisms and how the heavy metal lead directly acts on the liver leads to the damage of cells remains to be further studied.

ATPase drives energy reactions, participates in metabolic energy reactions, exports toxins, metabolic wastes and other substances that may hinder cell processes [8]. Our experimental results also reflect that the higher the lead concentration, the more obvious effect on the physiological activity of amphibians. This may be due to the change of ATP content in ATP pool when physiological state is under stress, which destroys the energy supply and ion balance, followed by the change of ATPase activity, and ultimately affects the living conditions. However, the safety range of lead to amphibians is still to be further studied.

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