



## Original Research Article

# Pathological Changes of Copper Ion Poisoning in Mice

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### ABSTRACT

In this study, the clinical manifestations of copper poisoning and the pathological changes of tissues and organs were observed by copper sulfate. The experimental mice were randomly divided into four groups: control group (0 mg / kg), low dose group (50 mg / kg), medium dose group (100 mg / kg), high dose group (200 mg / kg), 6 rats in each group, the test period was 2 weeks. The results showed that the mortality rate in the high dose group was higher than that in the middle dose group and the low dose group. Pathological observation of high-dose group showed hepatic steatosis and granular degeneration, hepatocyte showed honeycomb appearance, low dose group and middle dose group hepatocyte enlargement, cytoplasm filled with red stained fine particles; low dose group In the renal tubular degeneration, renal tubular epithelial cell swelling, so that the lumen becomes smaller or narrow, interstitial telangiectasia, congestion. In the middle dose group, there were obvious degeneration of the renal tubules, and there were many red dye protein in the lumen. Part of the renal tubular wall cell structure was destroyed. High dose group of renal tubular epithelial necrosis, disintegration, fall off in the lumen. The results show that copper poisoning mainly damage the liver, kidney and other tissues and organs, eventually leading to morbidity and even death.

**KEYWORDS:** Copper poisoning; pathological changes; mice

## 1. Introduction

Copper is common in animals, plants and microbes, and is one of the essential trace elements for all lower animals and vertebrates. Since the first report in 1925, the role of copper as a necessary trace element in animal and plant nutrition has been gradually recognized and continuously studied [1]. Studies have shown that the addition of an appropriate amount of copper in the feed can significantly improve the performance of the animal. This special biological role of copper has been widely used in modern animal husbandry and has achieved good economic benefits. In recent years, due to the excessive addition of copper in the feed caused by animal poisoning phenomenon often occur [4]. Many copper salts such as copper sulfate, copper acetate, copper carbonate, copper chloride, cuprous oxide, copper nitrate, copper acid, copper trichlorohydrin and the like as insects, soaking agents, fungicides, insect repellent, Molluscicides or wood preservatives [2] are widely used and often pose a significant potential risk to livestock farming, and copper poisoning occurs when animals eat copper. In addition, some plants containing pyrrole alkaloids of toxic plants (such as clover, thousands of miles of light, and days of buckwheat plants) can enhance the affinity of copper and liver, so that the accumulation of copper in the liver and cannot be normal discharge; others containing liver toxicity Alkaline plants can cause damage to animal liver cells, so that the metabolism of copper in the liver abnormalities, but also can make animals in the body too much copper poisoning [3].

Although the daily intake of copper in animals did not reach the toxic dose, but the animal dietary molybdenum content is very low or dietary copper / molybdenum ratio is too high (greater than 10), can also cause the accumulation of copper in animal liver, Accumulated overweight animals appear copper poisoning, there are reports of lambs in the feeding sunflower (molybdenum / aluminum ratio greater than 10), the emergence of chronic copper poisoning symptoms [18]. Copper poisoning occurs, often to the aquaculture industry to bring huge economic losses. Therefore, the timely grasp of animal copper poisoning after the clinical manifestations and its body tissues and organs of the pathological damage, to explore the pathogenesis of copper poisoning will be the clinical diagnosis and basic research to provide an important guiding significance [17].

Studies have shown that there is a large difference in the toxic effects of copper on copper and the sensitivity of copper [10]. Sheep were the most sensitive, ruminants followed, other animals [6]; poisoning dose reports are not the

same: lambs 25-50 mg / kg, sheep 20-110mg / kg [15], dairy cattle 200-880 mg Kg 200 mg / kg [8], laying hens 500 mg / kg, pigs 250-400 mg / kg [17]; rabbits 200 mg / kg [16]; horse 800 mg / kg, rat 1000 Mg / kg, broiler 300 mg / kg [11], duck 300mg / kg [9], goose 100 mg / kg [2]. Animal copper poisoning research focused on poisoning dose, clinical manifestations, pathological changes, blood biochemical indicators and pathogenesis, in the accumulation to a certain stage, will suddenly appear serious hemolysis, liver and kidney damage, and eventually died [14] The In the case of copper poisoning, 6-phosphate dehydrogenase and glutathione peroxidase activity were decreased, while alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase were increased [16].

Animal copper poisoning habits are divided into acute poisoning and chronic poisoning. Acute copper poisoning is mainly manifested in the clinical symptoms of gastroenteritis, manifested as nausea, salivation, diarrhea, severe abdominal pain [13]. Chronic copper poisoning mainly hemolytic symptoms as the main clinical symptoms, according to domestic reports, Wang Youming and Wang Changqing and other pig poisoning research done, the clinical manifestations of the following symptoms of poisoning, loss of appetite, vomiting, abdominal pain, diarrhea, muscle relaxation, Row of black thin manure, stomach and duodenal mucosa see bleeding, ulcers, necrotic lesions, liver enlargement was soil yellow [5]. Xu Shanliang and Yang Yunlin made goat copper poisoning research, the specific symptoms are as follows: copper poisoning pathological changes in the changes of gastrointestinal mucosal erosion, ulcers, bleeding is characterized [12]. Party Xiaopeng and Qi Changming made sheep research, the specific symptoms are as follows: epicardial hemorrhage, gallbladder enlargement of the liver, kidney surface bleeding [15]. Shu Gangliang, Zhai Guodong and Zhu Xiuqin made the chicken research, the results are as follows: acute copper poisoning the last cough and death, poisoning clinical symptoms: diarrhea, shrink the head to the appetite waste, mental depression, leg muscle paralysis, breathing difficulties Check the liver enlargement, was massive yellow soil mucosal congestion, which is more severe duodenum, gallbladder swelling [11].

In this study, we investigated the effects of copper ions on the heart, liver, spleen, lung and kidney of mice by studying the copper poisoning in mice. The pathologic damage of copper poisoning to organ tissues was studied and the pathogenesis was discussed. Research to provide scientific basis.

## 2. Materials and methods

### 2.1. Test material

#### Test animals:

Kunming mice (24, weight 18g-22g), purchased in Hunan Jishou University Medical College Experimental Animal Center. Feeding room temperature 23 C ~ 27 C, 12 hours a day light.

#### Experimental reagents and instruments:

(Shanghai Jiangdu Reagent Co., Ltd.), xylene (Shanghai Experimental Reagent Co., Ltd.), Hematoxylin (Shanghai Specimen Model Factory), Yihong Dyeing Pigment (Shanghai Reagent Factory), Sliced Paraffin (Shanghai Specimen Model Factory), Neutral Gum (Shanghai specimen model factory), formalin, lithium carbonate saturated liquid and so on.

Motic Olympos Microscopic System, YD202 Paraffin Slicer.

### 2.2. Experimental methods

#### Animal grouping

After one week of incubation, the mice were randomly divided into four groups: the control group, the low dose group (50mg / kg), the middle dose group (100mg / kg) and the high dose group (200mg / kg). Copper sulfate solution by oral administration of mice, the control group with the same amount of saline instead of copper sulfate. Every day at noon for gavage, not limited to drinking water, free feeding, a total of continuous infusion of 15 d. During the test, the temperature was 22 C~ 26 C, illumination 12 h.

#### Clinical observation

Clinical observation during the trial period, record clinical symptoms. On the 10th and 11th day of the trial, 3 mice were killed in the high dose group and 1 mouse died on the 14th day in the middle dose group. Death of the mice immediately take the day of the organ, the remaining mice at the end of the trial using artificial necrotic death to kill, and coring, liver, spleen, lung, kidney observation, and then into the Fulma Fixed in liquid fixation.

## **Histopathological observation**

(1) Drawn: take the lesion and normal tissue at the junction of the physical organs (heart, liver, spleen, lung and kidney).

(2) Fixation: the sick material set 10% of the formalin fixation solution fixed 48 h, fixed after 6h hours, the tissue block repair. The repair of the tissue block in a small white box, according to set a good procedure for dehydration, transparent, dip wax, the specific procedures for the washing process → 80% alcohol 1h → 90% alcohol 1.5h → 95% Alcohol 2h → 95% alcohol 2h → 100% alcohol 1h → 100% alcohol 1.5h → 100% alcohol 1.5h → xylene 0.5h → xylene 1h → paraffin 2h → paraffin 2h.

(3) Embedding: remove the lid of the small bag, remove the tissue from the bag with a small tweezers, remove the attention will still be cut face down, flat on the paraffin has been drops of stainless steel It is very important to embed the box in the center of the box, otherwise it will cut the surface of the cut, put the tissue block, the bottom of the small white box embedded in the top of the embedded stainless steel, and then drop the paraffin. And then will have been wrapped in a good stainless steel package box cooling to solidification. When the tissue block has been fully integrated with the paraffin solidification, the stainless steel box is gently removed from the bottom of the tissue block to be cut, and the paraffin on the side of the white bag with tissue blocks is gently scratched.

(4) Slices: the tissue block retained in the white small box is fixed on the paraffin slicer, the first repair, when the repair to the complete tissue, the slice thickness adjusted to 5-7 $\mu$ m, and then sliced, sliced Pay attention to force evenly, continuous slicing.

(5) Film, chip and drying: the tissue tissue will be cut with a brush gently provoke, while gently with small tweezers will cut the beginning of the tissue tissue clip, into the water bath pot exhibition Sheet, the temperature is 45-50 °C. The temperature is too high can be sliced melting, too low, the slice should not stretch. When the slices are placed in the magazine, use the small tweezers to gently flatten the area where they are shrinking. At this point, the prepared slides are stretched vertically under the surface of the slide. / 3 will be thin slices slowly ups and downs, slices can be automatically attached to the slide, the slide on the slicer, and then on the shelf. Finally, the slicer will be transferred to the oven drying (oven temperature of 76 °C), the general duration of 40min.

(6) Dewaxing, dyeing and sealing: put the glass slide into the xylene I soak 10 min → xylene II soak for 10min (with full dewaxing) → 100% alcohol 5min → 100% Alcohol 5 min → 95% Alcohol 5 min → 85% Alcohol 5 min → 80% Alcohol 5 min → Hematoxylin Dyeing 5 min → Washing (Place the slices in a pre-prepared water for 5 min) → 1% Alcohol Differentiation (this step is very critical, its main purpose is to take off the cytoplasm of the coloring, too long can make the color of the nucleus off.) Into the quickly after the removal, the time generally in 4-6 seconds? Tap water slowly washed 15-30 min → Yihong dye dyeing 1-3 minutes → 95% alcohol 3s → 100% alcohol 3s → 100% alcohol 3s → xylene I 3s → xylene II 3s. At this point, the HE staining process is finished, the slides on the dyeing frame are taken out, the xylene liquid is dipped with a clean gauze, and the neutral gum is dropped on the above, and the coverslips are sealed and labeled And then into the slice box, observe the pathological changes of each organ.

## **3. Results and Analysis**

### **3.1. Clinical symptoms and pathological changes**

High-dose group of clinical manifestations of appetite waste, the spirit of depression, shrinking, body tremor, lying down, row of dark brown feces, spleen volume reduction, weight loss, color fades, liver was brown, crisp fragile, gallbladder Swelling is full of blue and green bile, death cases can also see the kidney mild swelling. The middle dose group showed loss of appetite, mental depression, spleen volume reduction, weight loss, color fades, liver was brown. There was no significant change in the low-dose group.

### **3.2. Histopathological changes**

Liver: low dose group and middle dose group hepatocyte enlargement, cytoplasm filled with red stained fine particles, central vein, hepatic sinus and interstitial small blood vessels filled with red blood cells. High-dose group of liver showed significant degeneration and steatosis, hepatocyte enlargement, cytoplasm filled with a large number of red stained fine particles or round vacuoles, cells showed honeycomb appearance (Figure 1). Part of the cases can be seen focal necrosis, the arrangement of liver cell cable disorder, hepatocyte cytoplasm red staining, the nucleus appears to shrink and fragmentation (Figure 2). Central venous red blood cells are not clear, I saw red dye mesh material filled with blood vessels.

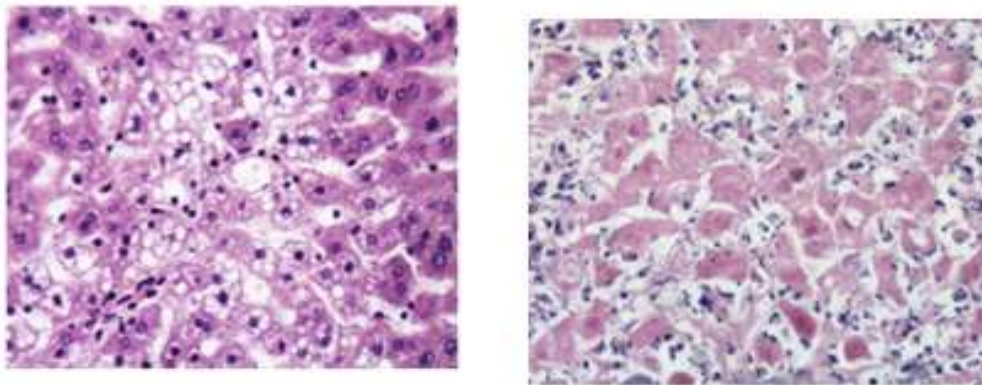


Figure 1 Hepatocytes were honeycomb

Heart: In the middle dose group and high dose group visible myocardial fiber swelling, there are many red stained fine particles (Figure 3). Myocardial fibrous capillaries are filled with red blood cells.

Spleen: In the middle dose group and high dose group, the spleen area of the spleen was smaller, the number of lymphocytes in the central lymphocytes and the marginal area decreased, the reticular cell proliferation and the red sinus sinus dilation were congested (Fig. 4). In the high dose group, some cases of spleen white lymphocytes in the spleen were necrotic.

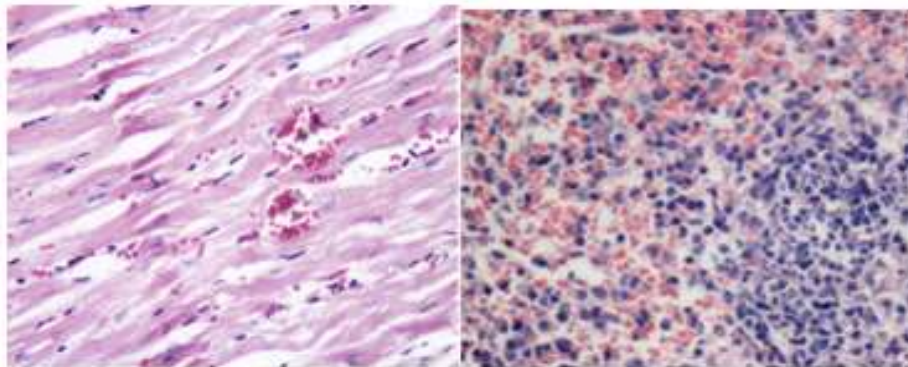


Figure 2 Muscle fiber swelling and fine particles

Lung: In the middle dose and high dose group, small blood vessels and capillaries were dilated and filled with red blood cells (Fig. 5). High-dose group of alveolar cavity in a small amount of red dye slurry, partial alveolar expansion of alveolar emphysema (Figure 6).

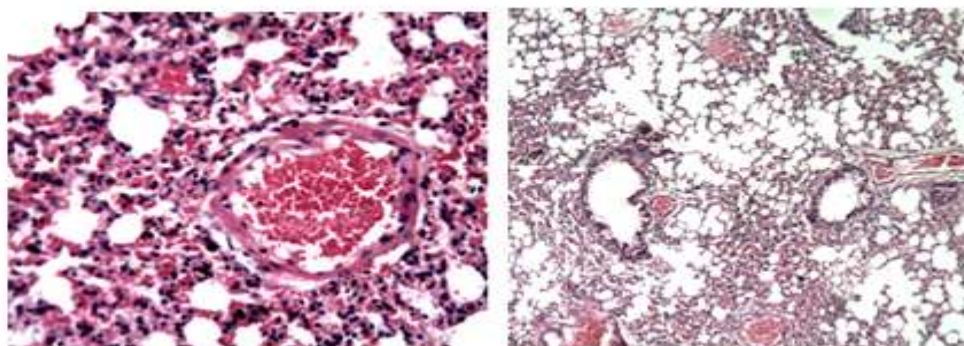


Figure 3 Small blood vessels and capillaries were significantly expanded, filled with red blood cells

Kidney: Low-dose group of renal tubular degeneration, renal tubular epithelial cell swelling, so that the lumen smaller or narrow (Figure 7), interstitial telangiectasia, congestion. In the middle dose group, there were obvious



degeneration of the renal tubules, and there were many red-stained protein substances in the lumen, and the tube type appeared (Fig. 8). Part of the renal tubular wall cell structure was destroyed. High dose group of renal tubular epithelial necrosis, disintegration, fall off in the lumen.

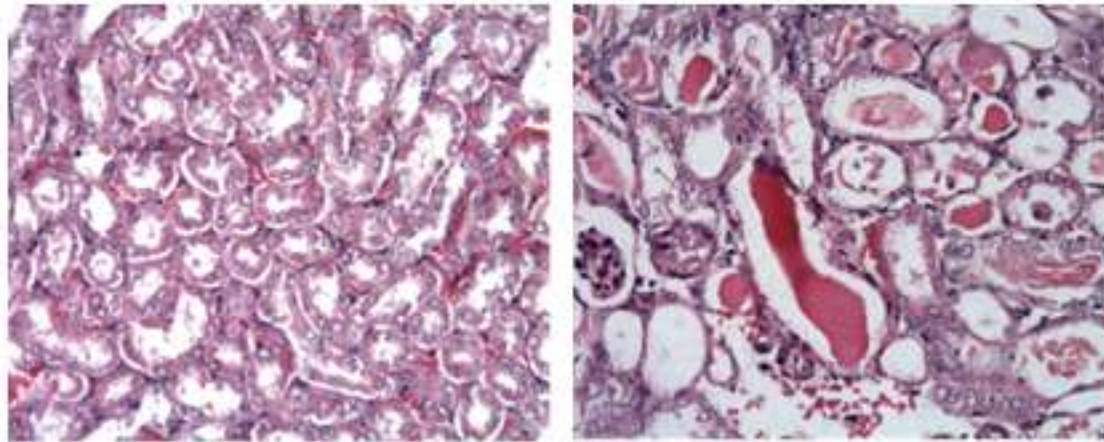


Figure 4 Renal tubular degeneration, epithelial cell swelling, so that the lumen smaller or narrow

## 4. Discussion

In this study, three groups of different concentrations of copper on the mice were attacked, respectively, low dose, medium dose and high dose, the time is half a month. The mice in the high-dose group died during the trial. The clinical symptoms were manifested as loss of appetite, mental depression, shrinking, body tremor, lying down, dark brown feces, reduced spleen volume, weight loss, the liver was brown, crisp and fragile, gallbladder swelling is full of blue and green bile, death cases can also see the kidney mild swelling. Moderate dose of appetite for the waste, the spirit of depression, spleen volume reduction, weight loss, color fades, liver was brown, low dose eye no significant change. The results obtained in this experiment for animal copper poison system research provides an animal model, but also for clinical or pathogenic diagnosis and treatment of copper poisoning as a reference from the onset of the mice, the course of the disease, the comprehensive analysis of the death of the author that copper poisoning Low dose and medium dose caused by chronic copper poisoning. Copper poisoning caused by high doses of acute copper poisoning of mice, indicating that the toxic pathology of copper due to animal species and copper poisoning dose varies.

Histopathological studies have shown that the target organ of copper poisoning in mice is liver, kidney, digestive tract and immune organs, with copper salt stimulation and corrosion, copper in the body tissue distribution and accumulation characteristics and copper emissions are closely related. As the copper salt has a strong stimulating and corrosive effect, copper salt into the digestive tract will cause severe reactions and cause serious pathological damage, manifested as muscle thickening of the stratum corneum, cracks and even hairy, intestinal mucosal epithelial necrosis, intestinal Villous rupture, which is the direct effect of copper salt on the gastrointestinal mucosa, leading to impaired gastrointestinal function, copper absorption into the body after the main accumulation in the liver, with the increase in the amount of copper increased significantly. Once the accumulation of copper over the liver of its use, in the release of blood before the liver cells will cause direct damage, causing hepatocyte enlargement, granular degeneration and steatosis, hepatocyte enlargement, cytoplasm filled with a large number of red of the fine particles or round vacuoles, the cells appear honeycomb appearance. As a result of the large amount of copper released into the blood caused by blood increased blood copper, liver and copper content has not decreased, and showed a strong time - dose effect, so liver copper can be used as a sensitive indicator of copper status. Liver copper accumulation to a certain extent, a large number of release into the blood caused by elevated blood copper, while. Renal copper concentration also increased, resulting in renal tubular epithelial cell damage, leading to impaired renal function.

## 5. Conclusion

This study combined with the current research progress of copper poisoning, from the etiology, clinical symptoms and poisoning after the body of the structure of the organs of the multi-angle detailed description of the artificial replication of copper poisoning pathology, artificial replication of copper poisoning Prevention and diagnosis provides an extremely important technical basis.

Occurs when the copper poisoning, resulting in a wide range of physical organs damage and bleeding, especially the liver, intestinal and kidney pathological damage is more obvious, the liver: low dose group and middle dose group

of hepatocytes, Red stained fine particles, central vein, hepatic sinus and interstitial small blood vessels filled with red blood cells. High-dose group of liver obvious degeneration and steatosis, hepatocyte enlargement, cytoplasm filled with a large number of red stained fine particles or round vacuoles, cells showed honeycomb appearance, some cases can be seen focal necrosis, internal liver cells Cable arrangement disorder, hepatocyte cytoplasm red staining, the nucleus appears to shrink and fragmentation, the central vein of red blood cells is not clear, I saw red dye mesh material filled with blood vessels. Kidney: kidney: low dose group of renal tubular degeneration, renal tubular epithelial cell swelling, so that the lumen becomes smaller or narrow, interstitial capillaries expansion, congestion. In the middle dose group, there were obvious degeneration of the renal tubules, and there were many red dye protein in the lumen. Part of the renal tubular wall cell structure was destroyed. High dose group of renal tubular epithelial necrosis, disintegration, fall off in the lumen. Therefore, in the copper ion as a feed additive, to determine the content of copper.

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