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Histomorphological changes in various rat tissues following chronic exposure to copper-zinc-pyrite ore

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Abstract

Aim. To assess the histomorphological state of lung, gastrointestinal and kidney tissues following exposure to copper-zinc-pyrite ore in the long-term model experiment.

Methods. The study was performed on 60 outbred albino male rats, aged 3–4 months, weighting 200±30 g. The toxic effect of heavy metal salts of copper-zinc-pyrite ore on the animal's body was analyzed by the model of dosed oral injection of water with ore in a dose 600 mg/kg body weight for 120 days. Pieces of the stomach, small and colon, liver, lungs and kidneys were taken from control and experimental rats for histomorphological study on the 30th, 60th, 90th and 120th day of the experiment.

Results. The structural disorders of the stomach lining were observed on the 30th day of the experiment: desquamated and dilapidated epithelial cells appeared in the preparations. On the 120th day, along with signs of epithelial desquamation and diffuse lymphocytic infiltration, the preparations contained large lymphoid follicles that occupy the full thickness of the gastric mucosa. The epithelial layer of the small intestine mucosa was disrupted on the 60th day. At day 120 diffuse infiltration and necrotic changes in the lining of the small intestine were recorded. Lymphomacrophagia infiltrations were observed during portal triad and inside the liver wedges of experimental animals on the 30th day of the experiment. By the end of the experiment, toxic hepatocyte dystrophy developed. On the 60th day, signs of bronchopneumonia appeared in the lung tissue. After 3 months, tubulopathy and tubulointerstitial nephritis were observed in the experimental animals.

Conclusion. Prolonged administration of ore has led to pronounced inflammation and degenerative changes in the gastrointestinal tract, liver, kidney and lung tissues, accompanied by lymphocytic tissue reaction.

Keywords: copper-zinc-pyrite ore, rat, intoxication, heavy metals.

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Background. The basis for studying the impact of heavy metals composed of copper-zinc pyritic ores on humans is due to the presence of one of the largest mining enterprises, the Uchaly mining and processing plant, in the territory of the Republic of Bashkortostan. The main factor responsible for the harmful effect of the industrial environment on the workers of this enterprise is the copper-zinc pyrite fine dust, which consists of sulfur, lead, cadmium, mercury, arsenic, antimony, and chromium as accompanying components. Cadmium and lead are the most metals dangerous for health of all the listed chemical elements [1, 2].

In lead intoxication, the synthesis of erythropoietin in the kidneys decreases, proliferation and differentiation of bone marrow stem cells are impaired, bone marrow erythropoiesis is inhibited, hemoglobin synthesis is impaired, and the osmotic resistance of erythrocytes is decreased [3, 4].

Cadmium, which accumulates in the cells of parenchymal organs, binds the carboxyl, amino, and sulfphydryl groups, leading to the disruption of intercellular interactions, necrosis, and apoptosis [5, 6].

The geochemical specificity of mining is known to have a negative impact on the health of the people working and living on the territory of the ore deposits [7]. Regarding copper-zinc pyritic ores, most studies are conducted to assess the occupational morbidity in mining enterprises, while not enough attention is paid to the experimental histomorphological analysis of the changes in tissues under the influence of non-ferrous metal ores,

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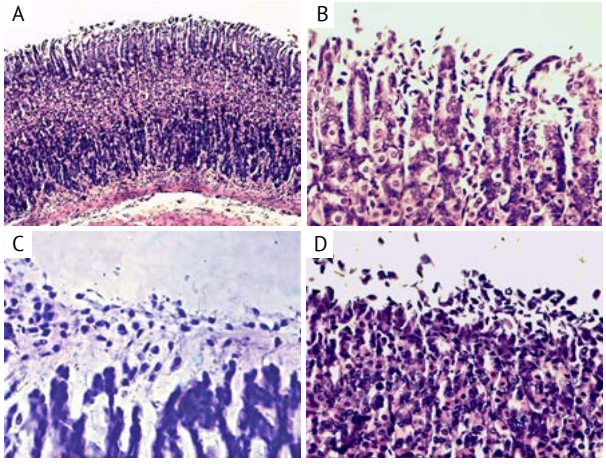

Fig. 1. Mucous membrane of the stomach: A—control; B—day 30, the initial stage of epithelial desquamation; C—day 60, desquamated epithelial cells on the surface of the mucous membrane; D—day 120, desquamation of the epithelium and infiltration of the mucous membrane with lymphoid cells. Staining with hematoxylin and eosin. Photomicrography. Ocular lens 10×, camera lens 40×.

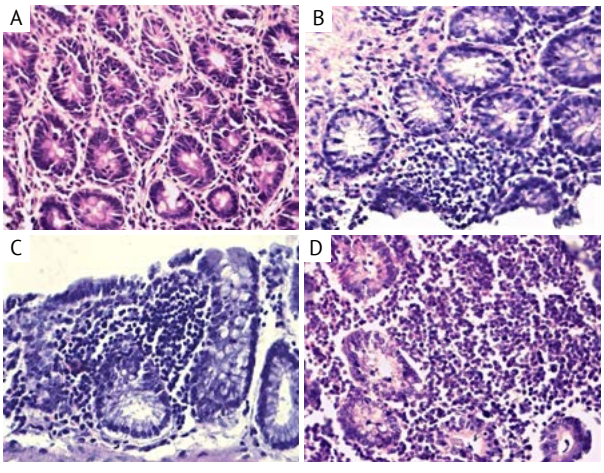

Fig. 2. Small intestine: A—control; B—day 60, accumulation of lymphocytes between the crypts; C—day 90, lymphoid infiltration of the mucous membrane; D—day 120, formation of lymphoid follicles and signs of necrosis. Staining with hematoxylin and eosin. Photomicrography. Ocular lens 10×, camera lens 40×.

as contemporary literature presents only the results of metabolism and the structural reorganization of bone tissue and liver [1,8,9].

Our **study aimed** to analyze the chronic effects of copper-zinc pyritic ore on the histomorphological state of lung tissues, organs of the gastrointestinal tract, and kidneys in a long-term model experiment.

Material and methods. An experimental study was performed using 60 white nonlinear male rats aged 3–4 months and having an initial body weight of 200 ± 30 g. The work was conducted in accordance with the principles of the Basel Declaration. According to the international rules for the ethical treatment of animals, the rats were kept in the university vivarium in standard cages (n = 6), with free access to water and food, and at an air temperature in the vivarium of 24 ± 2°C, in accordance with the rules of SP 2.2.1.3218 and Directive 2010/63/EU on the protection of animals used for scientific purposes. The study was approved by the local ethical committee of the Bashkir State University and the Ministry of Health of the Russian Federation, protocol No. 5 dated 09/13/2017.

To evaluate the toxic effects of the heavy metal salts of copper-zinc pyritic ore on the animal body, a model of dosed oral administration of crushed ore was used [8, 10]. A sample of the ore under study was provided by the Uchaly mining and processing plant (Uchaly, Russia). The studied ore sample comprised 0.06% of lead and 0.0088% of cadmium.

To create an experimental model of chronic intoxication, an aqueous suspension of copper-zinc pyritic ore powder at a dose of 600 mg/kg of animal body weight was injected *per os* daily to an experimental group of animals (n = 40) for 4 months, one hour before standard feeding. The administered dose was calculated based on the maximum allowable concentration of heavy metals in grain and bread [11]. During the experiment, the dose of ore was adjusted each time after every weighing of the animals, which was performed every 14–15 days. The control group consisted of 20 rats that were not exposed to the ore.

For histomorphological studies on days 30, 60, 90, and 120 of the experiment, samples of the stomach, small and large intestine, liver, lungs, and kidneys measuring 0.5 × 0.5 cm were collected from the control and experimental rats. Tissues were fixed in 10% buffered formalin solution, and after appropriate histological tracing, 7 μm thick sections were prepared, followed by staining with hematoxylin and eosin (ECOlab, Russia) [10, 12]. Visual histomorphological analysis of micropreparations was performed on an AXIO Lab.A1 microscope (ZEISS, Germany) with the lens magnification at 40×. A total of 100 micropreparations were examined.

Results. In the histomorphological studies of the rat organs subjected to chronic intoxication with copper-zinc pyritic ore, we observed signs of inflammatory and degenerative processes, the dynamics of which different organs had their own characteristics.

In the gastric mucosa, under chronic exposure to copper-zinc pyritic ore, structural disorders developed as early as day 30, since desquamated and dilapidated epithelial cells appeared in the prepa-

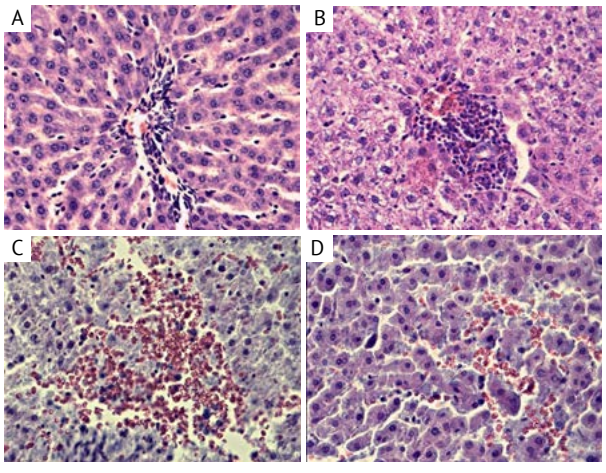

Fig. 3. Liver: A—control; B—day 60, hydropic degeneration of the liver; C—day 90, plethora of sinusoidal capillaries and fatty degeneration of hepatocytes; D—day 120, focal hyperemia of intralobular sinusoidal capillaries, and signs of autolysis and necrosis of the hepatocytes. Staining with hematoxylin and eosin. Photomicrography. Ocular lens 10×, camera lens 40×.

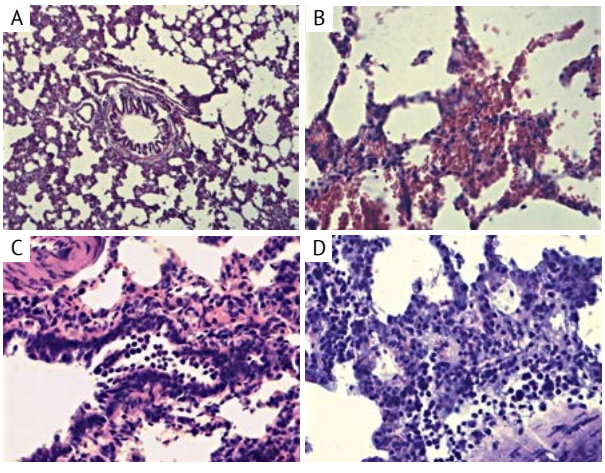

Fig. 4. Lungs: A—control; B—day 30, focal leukocyte infiltration of interstitial tissue; C—day 60, desquamation of bronchial epithelium, infiltration of their walls with lymphocytes; D—day 120, pronounced infiltration of interstitial tissue and blood vessels with lymphocytes and macrophages. Staining with hematoxylin and eosin. Photomicrography. Ocular lens 10×, camera lens 40×.

rations, the number of which increased by day 60 (Fig. 1). At the same time, lymphoid infiltration was revealed in the proper layer as well as submucous tissue of the gastric mucosa. By day 120, along with signs of epithelalial desquamation and diffuse lymphocytic infiltration, the preparations contained large lymphoid follicles occupying the entire thickness of the gastric mucosa.

In the small intestine of animals, the first signs of morphological changes were found later on day 60 of copper-zinc pyritic ore exposure (Fig. 2). They manifested in the destruction of the epithelial layer of the mucous membrane and were accompanied by a noticeable infiltration of the intestinal wall with lymphocytes. In the colon wall, the number of individual lymphocytes and solitary lymphoid follicles also increased. By day 120, in the experimental animals, diffuse lymphocytic infiltration and focal necrosis were registered in the mucous membranes of the small and large intestines.

Inflammatory and degenerative processes caused by the administration into the body of heavy metal compounds in the composition of copper-zinc pyritic ore manifested themselves most clearly in the liver of the experimental animals (Fig. 3). Signs of structural reorganization of the organ appeared as early as on day 30 of intoxication, when along the hepatic triad and inside the liver lobules, foci of accumulation of lymphoid cells were formed. By day 60, hydropic degeneration of hepatocytes appeared in the preparations, and the liver tissue was saturated with lymph macrophage infiltrates. By day 90 of the chronic exposure to copper-zinc ore, fatty degeneration developed in the liver of experimental rats. By day 120, signs of necrosis were revealed in the liver, and in the center of the lobules, there were foci of autolytic cell disintegration and fatty protein detritus of different area, while along the periphery of the lobules, there were hepatocytes in a state of fatty degeneration.

Despite the fact that the experimental animals received copper-zinc ore orally in our experiment, inflammatory changes occurred not only in the organs of the gastrointestinal tract, but also in the lung tissue (Fig. 4). On day 30 of the experiment, an accumulation of lymphoid cells and stagnation of blood in the capillaries surrounding the alveoli were recorded in the lungs, and the interstitial connective tissue was infiltrated by neutrophils and macrophages. By day 60 of chronic exposure to copper-zinc pyritic ore, signs of bronchopneumonia appeared in the lung tissue in the presence of inflammatory phenomena. On day 90, edema and cellular infiltration of the walls of the bronchioles and the alveolar space developed in a significant part of the lung tissue. All of the listed signs of an inflammatory process in the lungs were also noted on day 120 of the experiment.

Analysis of the kidney histomorphological structure revealed the inflammatory process in the tissue surrounding the tubules of the nephron as early as on day 30 of intoxication with the components of copper-zinc pyritic ore (Fig. 5). By day 60 of the experiment, the rats developed tubulointerstitial nephritis with focal infiltration of the interstitial tissue. In the subsequent days, the severity of the destructive processes in the kidneys of the experimental animals increased. By day 120, his-

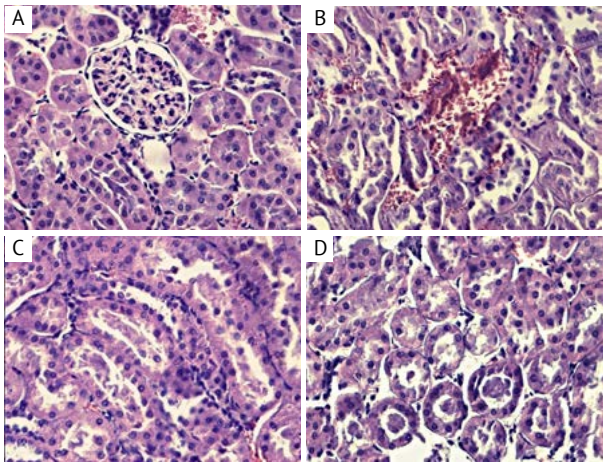

Fig. 5. Kidneys: A—control; B—day 30, venous hyperemia in the area of the renal tubules; C—day 60, colloid-like contents in the tubules of the kidney nephron; D—day 120, cylinders in the renal tubules. Staining with hematoxylin and eosin. Photomicrography. Ocular lens 10×, camera lens 40×.

tological sections revealed changes in the microvasculature (venous hyperemia followed by an increase in the permeability of the capillary walls, increased blood plasma exudation, migration of leukocytes into the perivascular zone), as well as multiple focal leukocyte infiltration of the interstitial tissue. Thus, chronic intoxication with copper-zinc pyritic ore led to the development of pronounced tubulointerstitial nephritis and tubulopathy with signs of chronic pyelonephritis.

Discussion. Our data suggests a pronounced toxic effect of the components of copper-zinc pyritic ore on the mammalian organism. In addition to copper, zinc, and sulfur, the ore contains lead, selenium, tellurium, cadmium, nickel, cobalt, arsenic, antimony, thallium, and barium. Heavy metals and their compounds enter the body through air, water, and food, accumulate in it, and have toxic effects on various organs and tissues.

It has been shown that chronic administration of ore components in experimental animals disrupts the functions of the endocrine system; particularly, the levels of testosterone, iodine-containing thyroid hormones, and parathyroid hormone decrease, and the synthesis of glucocorticoids increases [8]. At the cellular level, under the influence of zinc, lead, and cadmium, lipid peroxidation is activated, membrane permeability changes, cell adhesion capacity decreases, and the proliferation and differentiation of hematopoietic cells, liver cells, and bone tissue are inhibited [1, 9, 10, 13-15].

The histomorphological changes described in our study are the result of the nonspecific damaging action of the components of the copper-zinc pyritic ore, which apparently causes signs of a systemic inflammatory response in various tissues.

Particular attention should be given to the phenomenon of parenchymal organs and mucous membranes infiltration by lymphoid cells during chronic intoxication of animals with copper-zinc pyritic ore. It is known that lymphocytes do not only protect the body from foreign antigens, but also regulate morphogenesis, supporting the mechanisms of tissue renewal under physiological conditions and triggering, if necessary, a reserve program of reparative regeneration [16, 17].

CONCLUSIONS

1. Long-term oral administration of heavy metals to rats as part of copper-zinc pyritic ore led to pronounced inflammatory and degenerative changes in the lung tissue, organs of the gastrointestinal tract, and kidneys.

2. Intoxication with copper-zinc pyritic ore causes a pronounced lymphocytic tissue reaction.

Authors' contributions. Z.K.R. conducted the experiment, analyzed the data obtained, wrote and prepared the article; K.A.F. was the study head; K.F.A. and F.M.Ya. performed the morphological examination, description of the histological preparations, and preparation of photomicrographs.

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Conflict of interest. The authors declare no conflict of interest related to the article presented.

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