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RESEARCH ARTICLE

The hepatotoxic effects of copper-containing polymetallic dust and their correction with sorbents

Khamida Abdikadirova, Kymbat Amreyeva*, Xeniya Mkhitaryan, Nazgul Omarbekova, Bayan Yessilbayeva, Bayan Dyussenbekova, Kymbat Zhienbayeva, Bahyt Suleimenova

Non-Profit Joint-Stock Company «Karaganda Medical University»,
Gogolya 40, Karaganda 1000008, Kazakhstan.
*Corresponding Author E-mail: **kymbatamreeva@mail.ru**

ABSTRACT:

Industrial dust formed during the extraction and processing of polymetallic ores is a combination of various inorganic compounds. Of these, some inorganic compounds have a predominantly fibrogenic effect, while others have a local, resorptive and toxic-chemical effect. Therefore, the study of the features of morphological changes in the liver under toxic and fibrogenic effects of copper-containing polymetallic dust remains relevant. The morphofunctional state of liver tissue was studied in rats exposed to copper-containing polymetallic dust on the background of alimentary correction for 30 days. The experimental study was conducted on mongrel white male rats, which were divided into 3 groups and kept on a regular vivarium diet. Polymetallic dust of the Balkhash Mining and Metallurgical Combine with a 10% copper (Cu-10%) content ranging in size from 2 to 5 microns was injected once intratracheally in the form of a suspension of 50 mg of dust in 1.0 ml of saline solution according to a generally accepted method. Control animals were injected with 1 ml of saline solution. The rats received an alimentary correction at a dose of 150 g with food. The animals were slaughtered by instant decapitation. Morphometric analysis of the volume ratios of the structural components of the liver revealed an increase in the following indicators in the animals of group II compared to group I: Vv of necrosis – by 1 170 times ($p<0.001$), Vv of infiltrates – by 345 times ($p<0.001$), Vv of portal tracts – by 37.71% ($p<0.05$), Vv of dystrophically altered hepatocytes – by 24.13 times ($p<0.001$), Vv of fibrosis – 5.09 times ($p<0.01$). It should be noted that the Vv of two-core cells did not significantly differ from the level in group I. Based on the obtained data, the effect of polymetallic dust with a content of Cu-10% has a more expressed toxic effect on the structure and function of liver hepatocytes of experimental animals. The directed effect of alimentary correction on the system of microsomal oxidation of toxins in the liver and the mechanism of this product is based on stimulation of the formation of water-soluble compounds that are excreted with sorbents from the body.

KEYWORDS: Polymetallic dust, Copper, Liver, Antioxidants, Morphology, Histochemistry, Morphometry, Sorbents.

INTRODUCTION:

Heavy and non-ferrous metals are among the main pollutants or substances that have a very adverse effect on the body, the health of workers and the general population. These metals have a wide range of adverse biological effects (toxic, mutagenic, carcinogenic), which is why their presence in natural objects is strictly limited. Powerful industrial enterprises are able to create conditions for the formation of artificial biogeochemical provinces with all the ensuing consequences. The study of this issue is currently one of the most pressing problems[1-7].

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The dust factor is one of the main factors among the industrial hazards encountered in the mining industry. The dust has a complex chemical composition and contains impurities of various metals along with silicon dioxide. Copper is one of the representatives of non-ferrous metals. Copper is one of the most important trace elements for the animal and human body, since it is part of a number of mitochondrial cytochromes that provide hydrogen transport along the respiratory chain. Meanwhile, it is known that increased intake of copper into the body has an adverse effect [8-11].

The tissues of the liver and kidneys of rats have the ability to accumulate a huge amount of copper – 30-100 times higher than the level of its natural content in these organs. Copper primarily accumulates in soluble fractions of the liver, as well as in lysosomes. Then its amount increases in the nuclear fractions of the liver and kidneys. The appearance of peak concentrations coincided with the development of necrosis of liver cells and renal tubules. After discontinuation of administration, copper gradually disappeared from liver and kidney tissues. It should be noted that lysosomes play an important role in the neutralization and removal of copper from the cell. There is an assumption that copper damages lysosomal membranes and stimulates the release of enzymes from lysosomes due to a decrease in the number of mitochondria in the cell or due to their ferments inhibition [12-15].

When penetrated, copper begins to bind to specific proteins in the blood, such as ceruloplasmin, transcuperein, and other proteins and amino acids. After absorption by the liver, copper can accumulate in this organ. Apparently, the binding of copper in the liver requires the participation of glutathione. Metallothioneins are depots of copper for the formation of a number of enzymes and, above all, cytochromes. However, with excess copper cleaves from metallothioneins and can react with hydroxyl radicals. In this case, substances with cytotoxicity are formed [16, 17].

The importance of copper intake into the endoplasmic reticulum was noted. At the same time, the microtubule swelling and the formation of Malory bodies were observed in it. At the same time, it was revealed that the copper-bound protein entered into topographic associations with hyaline structures of bodies. It was found in experiments on rats that the level of metallothionein increases with dietary overload, and it is found in the liver and duodenum. It was also found in experiments on white mice that the intake of copper inevitably leads to the formation of Mallory bodies, which determines the effect of copper on nucleic acids [18-21].

The analysis of domestic and foreign literature on this problem has shown that the materials devoted to the comprehensive study of the direct effect of copper-containing polymetallic dust on the structure and function of the liver are few and fragmentary. In this regard, experimental studies of these problems seem relevant.

THE AIM OF THE RESEARCH:

To evaluate the effectiveness of the hepatoprotective properties of alimentary correction when exposed to polymetallic dust with a Cu-10% content at an early stage of the experiment.

THE OBJECTIVES OF THE RESEARCH:

1. To study morphofunctional changes in the liver of rats under toxic and fibrogenic effects of polymetallic dust with Cu-10% content against the background of alimentary correction for 30 days in comparison with control group.
2. To study the morphometric, histochemical and ultrastructural features of the qualitative and quantitative composition of the cellular reactions of the liver of rats when exposed to polymetallic dust with Cu-10% content against the background of alimentary correction for 30 days compared with the control group.

MATERIALS AND METHODS:

Objects and methods of research:

The experiment was performed in the laboratory of the Scientific-research center of the Non-Commercial Joint-Stock Company «Karaganda Medical University» and in the toxicological laboratory of the National Center for Labour Hygiene and Occupational Diseases of the Ministry of Health of the Republic of Kazakhstan. The study is a fragment of the scientific research work MT 0103KR00361 «Distribution and elimination of dust particles in the body, intracellular adaptation to the effects of radioactive dust containing silicon dioxide, study of the mechanisms of creation of substances that increase the dust resistance of the body» of the basic plan of the National Center for Labour Hygiene and Occupational Diseases of the Ministry of Health of the Republic of Kazakhstan.

Chemical and semi-quantitative spectral analysis of dust from the copper smelting shop of the Balkhash Mining and Metallurgical Combine was carried out in the laboratories of the Production Geological Association «Tsentrkazgeologiya».

Spectral analysis DFS-8 (evaporation method) was used to study the chemical composition of dust. According to the results of chemical analysis, the content of free silicon dioxide in dust was 2.5%; AL_2O_3 – 11%; Fe_2O – 20%; MgO – 0.03%; Na_2O – 0.05%; K_2O – 4%; TiO_2 – 1.2%; P_2O_5 – 0.1%; MnO – 0.1%.

The main constituent elements of polymetallic dust belong to hazard class 1-2 and are capable of causing significant destructive changes at all levels of liver tissue, having a toxic effect [22, 23].

Experimental animals:
The experimental study was conducted on mongrel white male rats with an initial weight of 120-170 g. The animals were divided into 3 groups. The animals of group 1 were used for control observation. The animals of group 2 were subjected to intratracheal dusting with copper-containing polymetallic dust. And the animals of group 3 took a specialized product.

Experimental protocols:
The experimental male rats were kept in vivarium conditions throughout the experiment (temperature 25±22 ºC, relative humidity from 40 to 45±10%, light/dark cycles 12:12). The animals were acclimatized within 1 week before the study and had free access to standard food and vivarium water without restrictions. Animal maintenance and experiments were conducted in accordance with the international rules «Guide for the Care and Use of Laboratory Animals» (Strasbourg, 1986). All experimental procedures were conducted in accordance with the principles of care and use of laboratory animals in research and were approved by the Institutional Committee on Animal Ethics (ACP/IAEC/2018/01). The protocols were approved by the local ethics committee of Non-Commercial Joint Stock Company «Karaganda Medical University» (Certificate of ethical approval of the publication No.1 dated 02.02.2024).

Technical conditions have been prepared for the preparation of a specialized product «Adapt-TM», which have an effect in a certain direction (TS 3510 RK 39550455–RGKP-01-2004 «Specialized food products based on wheat bran, alfalfa and lipid-protein extracts of animal brains «ADAPT»). The composition of the specialized product includes the following components; milk – 10%, bran – 30%, *Camelina sativa* – 15%, brain – 15%, alfalfa – 15%, livicin – 5%, licorice – 6.5%, starch – 2%, sugar – 1.5%, rutin – 0.3 g.

Morphological examination of the preparations:
For histological and morphometric examination, liver tissue was fixed in a 10% solution of neutral formalin, and then poured into paraffin according to a generally accepted technique. Microtomic sections 5-7 microns thick were prepared from paraffin blocks and were stained (with hematoxylin-eosin and picrofuxin according to Van Gieson) by review methods. A benzidine sample was also placed on the paraffin sections to detect deposited copper in hepatocytes [24].

Morphometric examination:
Morphometric examination of liver tissue was performed with calculations in volume fractions (Vv) to study the qualitative and quantitative composition of cellular reactions. The calculation was carried out in each studied case in at least 100 fields of view, which is sufficient for the representativeness of indicators at the second level of probability [25]. The calculation and differentiation of cellular elements was carried out according to the method of G. G. Avtandilov (1973, 1980, 1990) [26-28] with a microscope magnification of 15x20. The calculation of dust inclusions in the hepatocytes of the organ was also carried out at the rate of 100 cells per each field of vision.

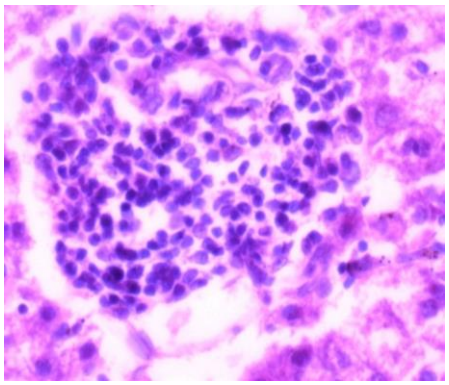
Histochemical examination:
For histochemical examination of liver tissue, the pieces were frozen in a cryostat and sections 5-10 microns thick were made, then they were stained to determine the activity of enzymes. To quantify the activity of acid phosphatase, lactate dehydrogenase, succinate dehydrogenase, glucose-6-phosphate dehydrogenase and glycogen in liver cells, a cytophotometry method using a photometric prefix MFEL-I (probe 0.1) was used. The wavelength of the light was 580 nm and was expressed in conventional units. The standard for comparison was the average content of enzymes in the cytoplasm of intact rat hepatocytes. In each preparation, 50 cells were taken for morphometric analysis of enzymes. The results of the study were processed statistically [29].

Ultrastructural examination of hepatocytes:
For electron microscopic examination, pieces of liver tissue were placed in 2.5% glutaraldehyde solution on phosphate buffer with pH of 7.2-7.4. Additionally, the material was fixed in buffered 1% osmium tetrachloride solution for two hours and poured into a mixture of epon and araldite. Ultrathin sections of 40-60 nm were obtained on ultramicrotomes LKB-III (Sweden), Reichert (Austria), contrasted with 2.5% uranyl acetate solution and Reynold lead citrate. The study was carried out on electron microscopes JEMA 100B, JEMA 100XX (Japan) and EVM-100L at an accelerating voltage of 75-80 kv. The shooting was carried out on FT-40 film and such photographic plates for nuclear research as MK or MR.

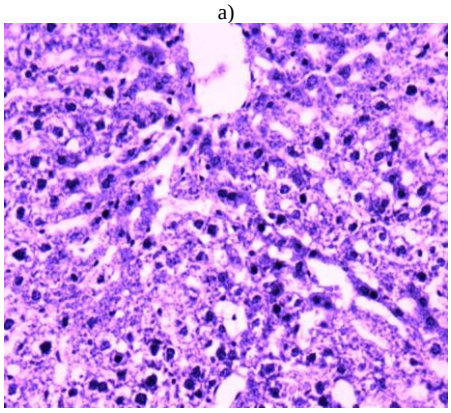
Statistical analysis:
The results of the study were subjected to statistical processing on a personal computer using the software package «Statistics» V.10.0. The reliability of the differences between the tools was evaluated according to the Student's criterion. The differences were considered statistically significant at $p<0.05$.

RESULTS:

A comprehensive histological examination of the experimental material in the group with alimentary correction showed less expressed alterative changes on the 30th day of the experiment than in the comparison group. Against the background of the continued fullness of the central veins and sinusoids, necrosis of hepatocytes in the center of the lobules was represented by small foci that were infiltrated by mononuclears. The portal tract was focally infiltrated by mononuclears and neutrophilic leukocytes. The hepatocytes in the lobule showed signs of hydropic dystrophy. The cells were enlarged and swollen. The cytoplasm was with the expressed vacuoles, discharged (Figure 1a). At the same time, an increased number of regenerating cells was registered in the liver tissue. The benzidine test for copper remained positive, but as shown by quantitative analysis, its severity decreased markedly (Figure 1b).



a)



b)

Figure 1: Exposure to polymetallic dust with a copper concentration of 10% during 30 days of the experiment against the background of alimentary correction

a) focal infiltration of the portal tracts by mononuclears with single neutrophilic leukocytes, with small focal hydropic changes of hepatocytes in the lobule. Stained with hematoxylin and eosin.

b) benzidine test for deposited copper. Reduction of copper in liver hepatocytes. Magnification: 16 x approx. 7.

Figure 2 contains a comparative characteristic of the amount of dust particles during intratracheal exposure and in the dynamics of the experiment against the background of alimentary correction. Thus, it can be seen that 7 dust particles are detected in hepatocytes when exposed to copper-containing polymetallic dust. Against the background of alimentary correction, the number of dust particles decreases by almost 2.33 times than in the dusty groups and their specific gravityis 36.4%, respectively.

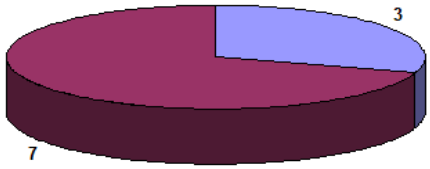


Figure 2: Quantitative ratio of dust particles in liver tissue under the influence of copper-containing polymetallic dust and after alimentary correction

Morphometric parameters of liver tissue are presented in Table 2. A decrease in Vv of necrosis by 31.11% ($p<0.05$), Vv of infiltrates by 39.13% ($p<0.05$), and Vv of dystrophically altered hepatocytes by 57.3% ($p<0.001$) was recorded in animals of group 3 compared with animals of group 2. However, the Vv of the binuclear cells in group 3 animals was 2.05 times higher ($p<0.001$) relative to group 2. Relative to the control group, there was a continued increase in the level of morphometric indicators: Vv of necrosis by 806 times ($p<0.01$), Vv of infiltrates by 210 times ($p<0.01$), Vv of portal tracts by 48.13% ($P<0.05$), Vv of dystrophically altered hepatocytes by 10.3 times ($p<0.01$).

Table 1: Morphometric parameters of animal liver tissue under the influence dust on the background of alimentary correction on the 30th day of the experiment

Parameters	Group 1	Group 2	Group 3
	n=6	n=6	n=6
Vv of necrosis	0.010± 0.0001	11,700± 0.370***	8,060± 1.180***#
Vv of infiltrates	0,010± 0.008	3,450± 0.240***	2,100± 0.330***#
Vv of portal tracts	4,800± 0.230	6,610± 0.570*	7,110± 0.770*
Vv of dystrophically altered hepatocytes	1,090± 0.010	26,300± 0.710***	11,230± 2.280**###
Vv of dual-core cells	0.010± 0.001	0.190± 0.120	0.390± 0.210
Vv of fibrosis	0.110± 0.005	0.560± 0.080***	0.370± 0.160

Note:

1. The reliability of the differences between control group and experimental groups: *p<0.05; **p<0.01; ***p<0.001
2. The reliability of the differences between groups 2, 3; #p<0.05; ##p<0.01;###p<0.001

During this period of the experiment, histochemical examination of liver tissue against the background of alimentary correction showed a significant increase in glycogen in liver cells and the activity of Glucose-6-phosphate dehydrogenase (Fig. 3 a, b).

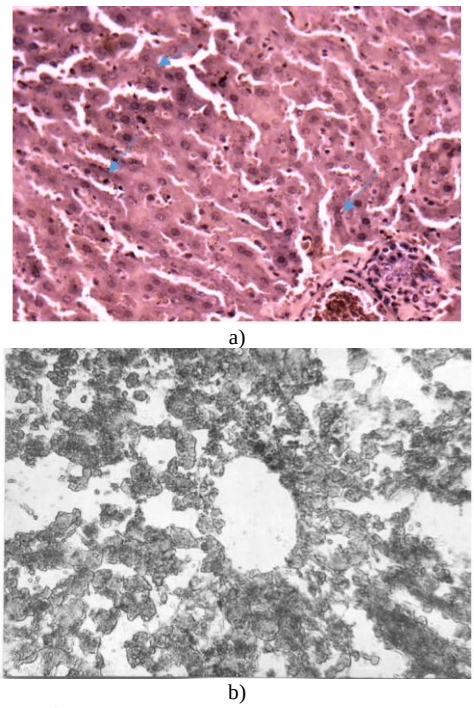


Figure 3: After alimentary correction against the background under the influence of copper-containing polymetallic dust during 30 days of the experiment:
a) increase in the glycogen content in cells. Staining: reaction with schiff-iodic acid.
b) expressed increase in the activity of glucose-6-phosphate dehydrogenase in hepatocytes. Staining: histochemical reaction. Magnification: 16 x approx. 7.

The parameters of enzyme activity are presented in Table 2. It was noted that in animals of group 3, compared with animals of group 2, the glycogen level increased by 50.53% ($p<0.01$), the enzyme level did not significantly change.

Table 2: Parameters of enzyme activity under the influence of copper-containing polymetallic dust on the background of alimentary correction on the 30th day of the experiment

Parameters	Group 1	Group 2	Group 3
	n=6	n=6	n=6
Acid phosphatase	0.422± 0.022	0.192± 0.0102	0.214± 0.012
Succinate dehydrogenase	0.436± 0.024	0.232± 0.0149	0.248± 0.011
Lactate dehydrogenase	0.474± 0.022	0.204± 0.0139	0.221± 0.013
Glucose-6-phosphate dehydrogenase	0.318± 0.033	0.202± 0.0114	0.219± 0.014
Glycogen	0.802± 0.041	0.190± 0.0165	0.286± 0.022**

Note:
*the significance of the differences between groups 2 and 3 (the difference in comparison with the control is statistically significant according to the Student's t-test ($p<0.05$)

The ultrastructure of the liver at this stage of the experiment was characterized by the following picture. The nuclei of hepatocytes were large, with great amounts of condensed chromatin. The nuclear shells had wavy contours. Hyperplasia was determined, as a result of which numerous tubules of the smooth endoplasmic reticulum (SER) were located in the hyaloplasm. The SER tubules were narrow or slightly expanded with a large number of fixed ribosomes, were located in the hyaloplasm. In some hepatocytes, signs of dystrophy persisted, expressed in the expansion, degranulation and partial destruction of the SER tubules.

The Golgi complex was active and represented by a well-developed membrane and vesicular particles. As a result of degranulation of fixed ribosomes, numerous vesicles of the agranular reticulum were visible. Large lipid inclusions were found in the field of view. Mitochondria were hyperplasized, polymorphic in shape and size. At the same time, most mitochondria were characterized by a low-density matrix, well-preserved crystals, which indicated a high level of phosphorylation. Regenerative shifts also developed – mitochondrial hyperplasia and hypertrophy, activation and hypertrophy of the Golgi apparatus, and ат expansion of the perinuclear space of the nucleus (Fig. 4).

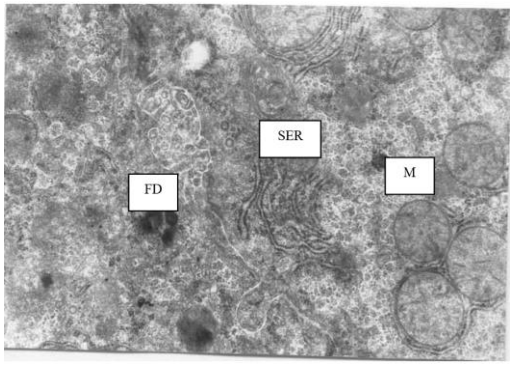


Figure 4: Mitochondrial hypertrophy. A large number of GER. Lipid inclusions. Magnification: 16 x 400

DISCUSSION:

Thus, in comparison with the experimental groups, changes in liver tissue were characterized mainly by dystrophic changes in the parenchyma and moderately pronounced inflammatory manifestations in the stroma. Ultrastructural analysis showed a combination of moderate destructive and expressed intracellular regenerative processes in the cell. Destructive changes in organelles disappeared, and active forms of mitochondria appeared. It is clearly seen that there was an increase in the processes aimed at restoring mitochondria, granular endoplasmic reticulum and Golgi complex, which characterizes the strengthening of protein-synthesizing and detoxification functions of the liver.

Alimentary correction for 30 days had a protective effect on liver hepatocytes. Destructive organelle changes have disappeared. Active forms of mitochondria appeared with a high level of oxidative phosphorylation, which ensures the performance of biosynthetic processes in liver cells. Intracellular regenerative processes aimed at restoring mitochondria, granular and agranular reticulum and Golgi complex were intensified. As a result, the protein and detoxification function of hepatocytes was enhanced. The results obtained confirm the data of our previous works[14, 15], as well as the data of other researchers [30-42].

The effect of the product used in alimentary correction is based on the restoration of membrane structures as a result of the use of lysine and a phospholipid component. A certain importance for the restoration of these structures may be associated with the high level of carotene and polyunsaturated fatty acids in the composition of *Camelina sativa*, which, after conversion to esterified forms of retinol, can also contribute to increasing the stability of membrane structures and their restoration. This product leads to normalization of metabolism in liver tissue under the action of polymetallic dust. The use of rutin in the product content was also aimed at stabilizing membrane structures. The use of processed bran as a product with a high content of components with the ability to sorption, which contributes to the removal of copper and quartz from the liver, could have a certain significance [30, 31,36,41].

It was noted that with the insufficiency of the protein component in food, there is a significant decrease in the content of cytochrome P-450 in rat liver microsomes as a result of a violation of the relationship of the components of the monooxygenase system [43], in connection with which impaired protein assimilation and impaired protein metabolism during intoxication are often the cause of a decrease in the function of the monooxygenase system.

To maintain the high efficiency of the liver and other organs and tissues, the animals received products containing high-grade easily digestible protein (milk). To improve the quality of the protein component of the diet, the specialized product was enriched with livicin (a product of biological synthesis with a predominant content of lysine (85%) and other essential aminoacids). Lysine enhances the utilization of fatty acids by mitochondria. The process of enterosorption in the intestine plays an extremely important role when exposed to xenobiotics. For this purpose, products with a high content of cellulose, hemicellulose and lignin (bran, alfalfa) 28-30 were used. These products are known to stimulate bile production. Licorice was also used for these purposes. Preparations based on *Glycyrrhiza uralensis* were also hepatoprotectors for toxic liver damage with carbon tetrachloride, reducing the activity of lipid peroxidation in liver tissue homogenate and blood serum. They showed antioxidant properties in acute lung surfactant damage by total irradiation, exerting adaptagenic, anti-stress, anti-inflammatory and lipid-lowering effects, regulating cellular metabolism [33- 36,44].

It was found that during morphometric examination, the components of a specialized product significantly reduce the development of the volume fraction of dystrophic and necrobiotic changes in hepatocytes, and a decrease in the volume fraction of infiltration into liver tissue.

CONCLUSIONS:

1. On the 30th day of the experiment, against the background of alimentary correction, a decrease in the number of dust particles in the rats' hepatocytes by 2.33 times was registered in comparison with the dusty group.
2. Morphometric examination of liver tissue showed that the volume fraction of binuclear cells increased by 2.05 times ($p<0.001$) against the background of alimentary correction in relation to the dusty group. There was also an increase in the volume fraction of glycogen by 50.53% ($p<0.01$). Ultrastructural examination found a combination of moderate destructive and pronounced intracellular regenerative processes in hepatocytes.

So, in the conditions of the experiment, the preventive and antihepatotoxic effect of the specialized product was recorded, which allows us to state the membrane-stabilizing and antioxidant effect of the components of the specialized product.

AUTHOR CONTRIBUTIONS:

Conceptualization, Kh.A. and K.A.; methodology, Kh.A.-B.S; software, N,O., X.M; formal analysis, N.O., X.M; investigation, K.Zh.; resources B.S, B.Ye.; writing—original draft preparation, B.Ye and B.D.; writing—review and editing, B.D.; visualization, K.A.; supervision, K.Zh. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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