

Change of Hepatobiliary System under the Influence of Hydrazine Derivatives

Saspugayeva G.Y.^{1*}, Beisenova R.R.¹, Zhaznaeva Zh. K. ², Meiramkulova K.S. ¹, Daribay A.O.¹, Tuleuova G.K.³

¹ L.N. Gumilyov Eurasian National University, Kazakhstan.

² S.Amanzholov East-Kazakhstan University, Kazakhstan.

³ Medical University of Karaganda, Kazakhstan

***Corresponding Author: Saspugayeva G.Y.**

Abstract

Hydrazine derivatives are used in different spheres, in aero-space activity, medical practice, laboratory-diagnosis practice, that is why environment is polluted with hydrazines in big areas. The question about influence of hydrazine derivatives, used as the rocket fuel, on the human organism and ways of its detoxication is very actual. In connection with appeared situations, we set the **goal**: to study the negative influence of hydrazine derivatives-hydrazine sulphur, nitrosodimethylamine (NDMA), phenylhydrazine, isonicotinic acid hydrazide (IAH) on several biochemical features of blood and correction of functional damages of organism with “Salsocollin” preparation.

Keywords: Isonicotinic acid hydrazide (IAH), N-nitrosodimethylamine (NDMA), ALAT-alanine aminotransferase, AsAT-aspartate aminotransaminase, Salsocollin

Introduction

Hydrazine derivatives are used in different branches of industry, as the rocket fuel, medicines, rust inhibitor materials, that are why studying of toxic influence of hydrazine derivatives is actual. In chronic intoxications with hydrazines there prevail the features of liver affection and, in a less degree, other systems (central nervous system, heart-vascular, excretory, hematopoietic) [1].

Results of complex expedition researches of Russia and Kazakhstan, carried out in places of fall of residual parts of space rockets showed the presence of 1, 1-nitrosodimethylamine and its oxidation products in soil, water and plants with concentration higher than maximum allowable [7].

Pathogenesis of affections with hydrazine derivatives is very complicated and is realized by means of damage of biochemical reactions of almost all known types of metabolism. However, among the plenty of biochemical processes, damage by hydrazine, it is possible to emphasize the number of

reactions, which have direct link with appearing and development of main pathogenetic effects of poisoning. First, enzymes, which catalyze the oxidation processes, processes of transamination, deamination and phosphorylation can be related to them. Blockage of ferments is mediated by interaction of hydrazines with co-factors subject to the type of formation of hydrazones, chelates, and regeneration of active functional groups [3, 6].

Beside the mechanisms, shown above, realization of toxic effect of hydrazine derivatives may be executed due to intermediate connection of radical nature. The main are superoxide radicals, which are formed in biotransformation of hydrazines in microsomes of liver. These radicals can influence different toxic effects, including the increasing of processes of lipid peroxidation.

Despite the natural abundance, the wide usage of hydrazines and knowledge of acute and chronic poisonings with hydrazine derivatives, their influence on morphological

structure of liver, is not known enough. The aim of research: examine the influence of several hydrazine derivatives (N-nitrosodimethylamine, sulphur hydrazine, isonicotinic acid hydrazide, 2, 4-phenylhydrazine) on morpho-function characteristic of human hepatobiliary system [2].

Based upon the experience of usage of plants in folk medicine and traditional medical systems, in last years Republic of Kazakhstan developed and offered to the practical healthcare the number of new herbal preparations, which have the liver-protection function. Liver-protective preparation "Salsocollin" was developed upon the base of the extract of hill-growing saltwort (*Salsola collina* Pall). In literature there are met the notices of effective usages of infusions and extracts of this plant as the vitamin-full, capillary-strengthening, reparative-regenerative, anti-bacterial, antipyretic, spasmolytic, antiviral, antitumour and cardiotropic means.

In folk medicine the extracts of hill-growing saltwort are used in the effective medicine for treatment of liver and gall-bladder [8]. Phytochemical examination of hill-growing saltwort raw materials showed the presence of great number of compounding: flavonoids, indispensable amino acids, carbohydrates, hardening agents, microelements.

Basing upon the structure and data on biological activity of flavonoids, it would be logically to suggest, the extract of hill-growing saltwort has cytoprotective features. Examination of antioxidant features of each component showed that effect of usage of extract is much higher, and, possibly, that is connected with synergism. So, complex of chemical and biological researches finally let us to develop the new medical preparation "Salsocollin", which has the expressed hepatoprotective features [4, 5].

Materials and Methods

The experiments were carried out with 510 white outbred rats with weight 250-300 gr. Two series of experience were made; in the first series there was examined the influence of acute doses of hydrazine derivatives-isonicotinic acid hydrazide (IAH), N-nitrosodimethylamine (NDMA), hydrazine sulphur and 2, 4-dinitrophenylhydrazine, and upon the base of correction with

"Salsocollin" preparation in the second series of experiments there were examined the chronic doses of hydrazine derivatives, upon the base of correction with "Salsocollin" preparation as well. Experimental animals in each series were divided into 9 groups. The first group (n=20) obtained the water, in volume 1 ml, the second group (n=10) obtained 40 mg/kg NDMA once in acute intoxication and 4 mg/kg NDMA in chronic one intragastrically during 3 months, the third group (n=10) was composed of animals, which obtained "Salsocollin" preparation in volume 50 mg/kg and 40 mg/kg of NDMA once in acute intoxication and 4 mg/kg of NDMA in chronic one during 3 months.

The fourth group (n=10) was injected with 143 mg/kg IAH once in acute intoxication and 14,3 mg/kg IAH during 3 months in chronic one intragastrically, the fifth group (n=10) was composed on animals, which were injected the "Salsocollin" preparation in volume 50 mg/kg and 143 mg/kg of IAH once in acute intoxication and 14,3 mg/kg of IAH during 3 months in chronic one.

The sixth group (n=10) was composed of animals, which were injected 188 mg/kg of phenylhydrazine intragastrically once in acute intoxication and 18, 8 mg/kg of phenylhydrazine during 3 months in chronic one, the seventh group (n=10) was composed of animals, which were injected "Salsocollin" preparation intragastrically in volume 50 mg/kg and 188 mg/kg of phenylhydrazine once in acute intoxication and 18, 8 mg/kg during 3 months in chronic one. The eighth group (n=10) of animals was injected with 100 mg/kg of hydrazine sulphur once in acute intoxication and 10,0 mg/kg of hydrazine sulphur during 3 months in chronic one, the ninth group (n=10) was composed of animals, which were injected with "Salsocollin" preparation intragastrically in volume 50 mg/kg and 100 mg/kg of hydrazine sulphur once in acute intoxication and 10,0 mg/kg of hydrazine sulphur during 3 months in chronic one, the third, the fifth, the seventh and the ninth groups in chronic intoxication, in the last month of primer, "Salsocollin" preparation was injected in volume 50 mg/kg every day.

Results and Discussion

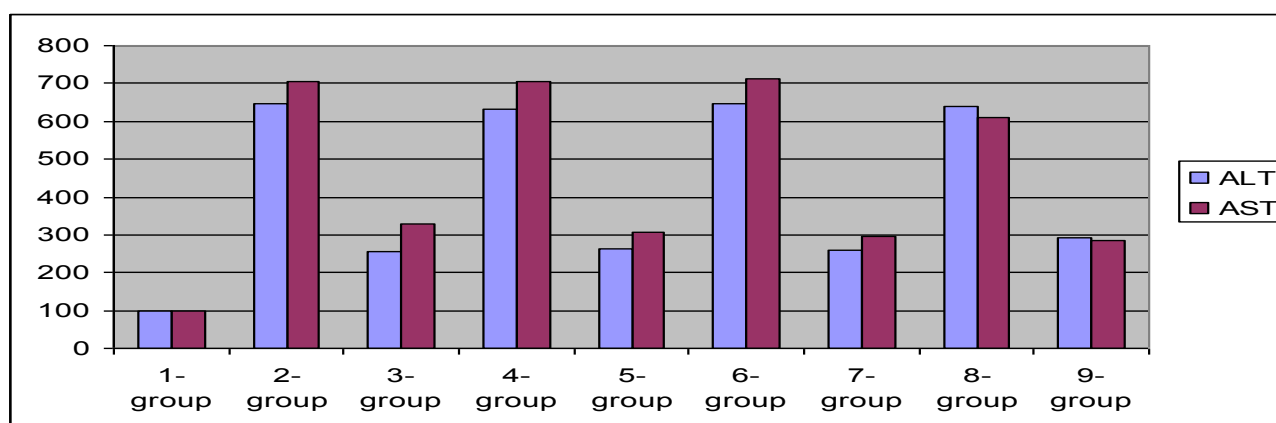
According to the result of experiments, the thymol probe, which shows the pathology of

liver, was moderately increased in all the groups, poisoned with hydrazines. In acute intoxication NDMA was increased on 20%, IAH-on 39% ($P<0,01$), phenylhydrazine-on 55% ($P<0,05$), hydrazine sulphur on 120% ($P<0,001$). In injection of "Salsocollin" with the background of acute intoxication was higher in the third group on 16%, in fifth group-on 36% ($P<0,01$), in the seventh-on 54%, in the ninth-on 105% ($P<0,001$).

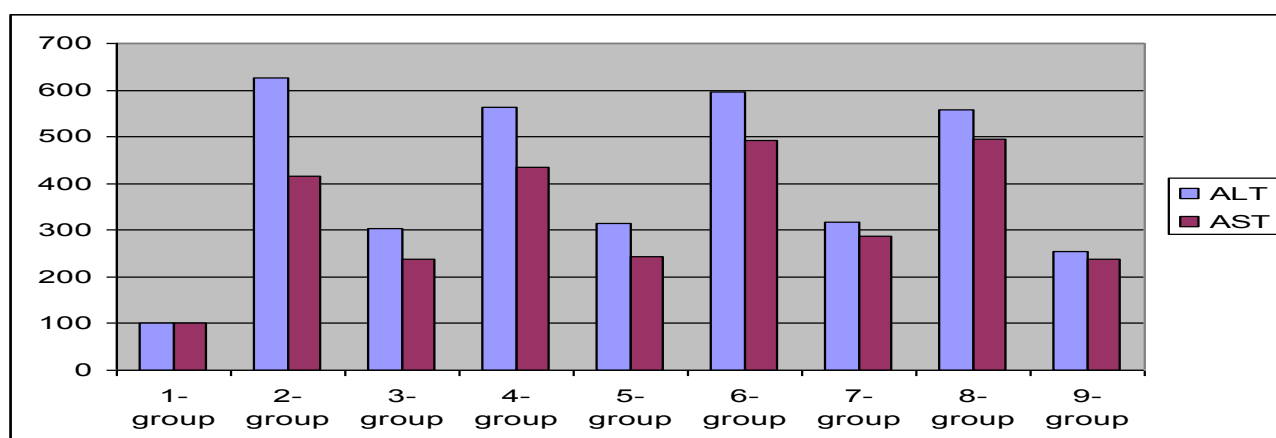
In chronic intoxication with NDMA the thymol probe was positive, in the second group was higher on 55, 4% ($P<0,01$), in the fourth group insignificantly lower, in the sixth group higher on 82, 6% ($P<0,01$) and in the eighth group on 41, 7% higher than control information. In injection of "Salsocollin" preparation with the background of chronic intoxication, the thymol probe was slightly hastened to control data. Positive thymol probe in intoxication

with hydrazines shows the significant changes in liver pulp. Activity of aminotransferase in experimental groups was truly higher than control data. Activity of AlAT in group, which obtained the acute doses of hydrazines is 6 times ($P<0,001$) higher, than in control group, activity of AsAT is increased as well in all the groups of animals, poisoned with acute doses of hydrazines 6-7 times ($P<0,001$).

Activity of AlAT in group, which got the chronic doses of hydrazines 5-6 times ($P<0,001$) higher, than in control group, activity of AsAT is increased as well in all the groups of animals, poisoned with chronic doses of hydrazines 4-5 in times ($P<0,001$). In injection of "Salsocollin" activity of AlAT was lower than level in poisoned groups, higher than level of control group twice, but lower than poisoned groups in several times (Picture 1, 2).



Picture 1-Activity of aminotransferase in acute intoxication with hydrazine derivatives



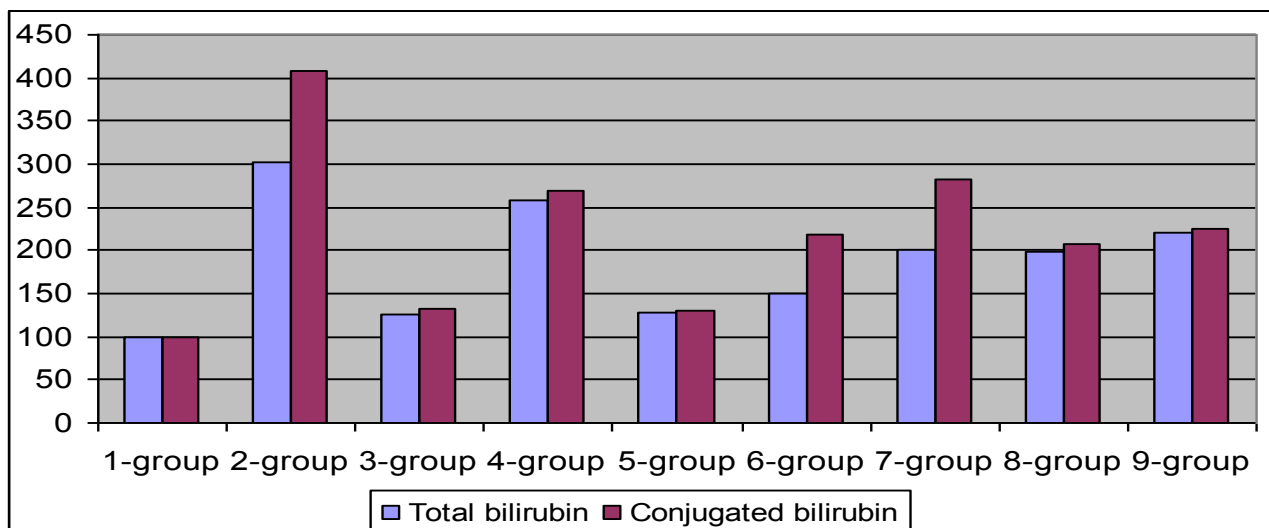
Picture 2: activity of aminotransferase in chronic intoxication with hydrazine derivatives

De Ritis coefficient, which shows the proportion of aminotransferase, in groups of acute intoxication is higher than 1, that can show the significant damages of cardiac muscle together with liver damages. Increasing of activity of denoted ferments in chronic intoxication and De Ritis coefficient

lower than 1 may show the significant unfavorable variants in liver pulp cells. "Salsocollin" preparation corrects the liver transformations, which is certified with the information about activity of transferase of blood plasma. Contents of total and conjugated bilirubin in blood are changed in

blood plasma of experimental animals in acute intoxication. In the second group the level of total bilirubin was increased on 260,5% ($P<0,001$), and conjugated-two times ($P<0,001$), in the fourth group-on 61,1 % and 42,15% ($P<0,01$), in the sixth group-on 84,7%, and on 85,1% ($P<0,05$), in the eighth group

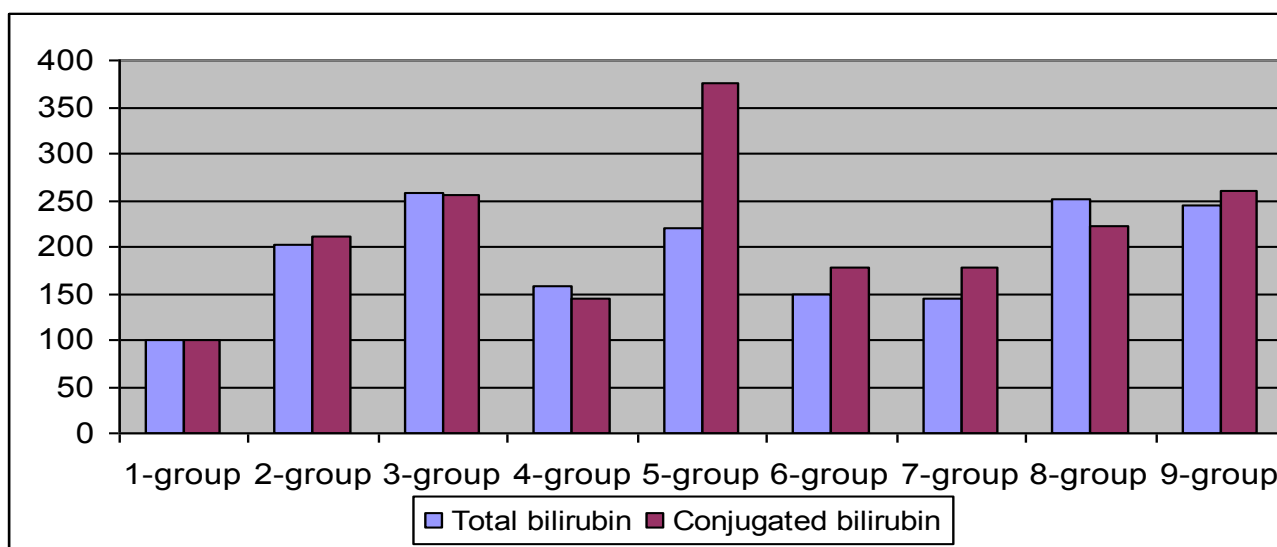
these indications were higher on 156,5% and on 125,1% ($P<0,01$) correspondingly than in control group. Upon the base of correction with “Salsocollin” preparation, in the third, the fifth groups were approximated to the control data, and in the seventh and the ninth groups they were kept on the level of data of poisoned groups.



Picture 3: Content of bilirubin in acute intoxication with hydrazine derivatives

In chronic intoxication in the second group, level of total and conjugated bilirubin was twice higher ($P<0,001$), in the fourth group 1, 5 and 1, 6 times ($P<0, 01$) higher, in the sixth

group total bilirubin was higher on 57%, and conjugated-twice ($P<0, 01$) higher, in the eighth group both total and conjugated bilirubin is twice higher than control data.



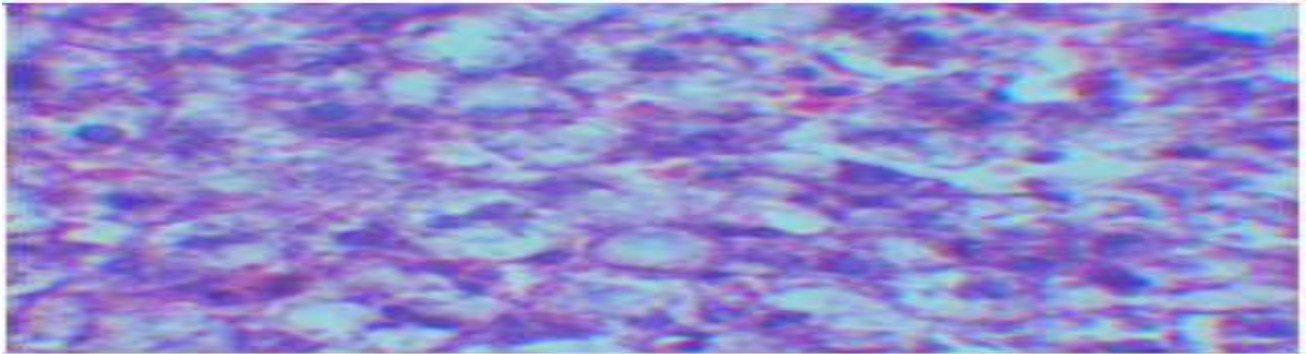
Picture 4: Content of bilirubin in chronic intoxication with hydrazine derivatives 2-series

Upon the base of correction with “Salsocollin” preparation there was no significant improvements in metabolism of bilirubin. These improvements in metabolism of bilirubin, i.e. bilirubinemia show the damage of liver pulps, bilirubin enters the blood through the damaged liver cells. It is widely known that in colestasis replete bile vessels are damaged and let bilirubin flow into blood, and significant changes of biochemical blood

features show the development of toxic hepatitis, when strong changes in protein-formation, bile-formation functions of liver take place. “Salsocollin” influences this change favorably only in case of acute intoxication with NDMA and IAH. In microscopic examination of liver of experimental animals, which got different chemical compounds of hydrazine for 3 months, stereotyped pathomorphological

changes were found, which varied only in manifestation rate of heaviness of pathological process and depended on type of used chemical substance. In whole they were characterized with hemolymphatocirculating damages, phlogistic infiltration of both hepatoportal ducts and inside of acinus with mononuclear cells, dystrophic and destructive changes in functional liver pulp cells, which

occurred with damage of hystoarchitectonics of organ and development of fibroplastic processes of different manifestation rate. So, microscopic and morphometric examination of histological preparations showed, that in case of usage of phenylhydrazine in experiment, hemomicrocirculating damages came to the foreground, especially those of system of blood drainage from the organ pulp.



Picture 5: Adipose and hydropic degeneration of hepatic cells of liver Colouring: with hematoxylin and Eosin. Increased in 10 times, volume 40

Focuses of colliquative necrosis. Focuses of necrosis of hepatic cells were of mosaic nature. Alternative changes of hepatic cells were correspondingly followed with development of both portal and periportal, and in acinus of perinecrotic phlogistic

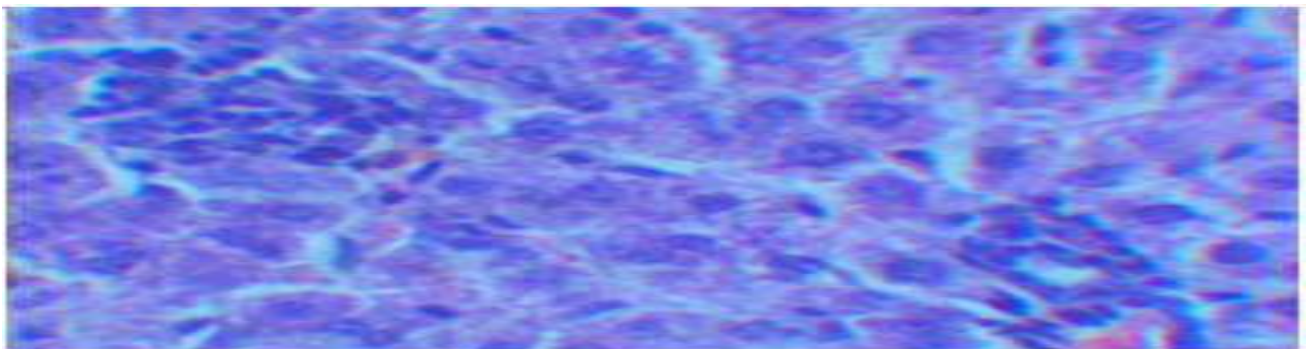
infiltrates, which mainly were of lymphocytic nature with adding of segmented cells and plasmatic cells. Portal ducts were widened, lymphocytic infiltrate penetrated into surrounding liver pulp, where piecemeal necroses were visible.



Picture 6: Piecemeal necroses of periportal zone. Swelling of stroma and phlogistic-cellular infiltration Colouring: with hematoxylin and Eosin. Increased in 10 times, volume 40

Together with morphological features of damage of organ, there were noted the enlargements of granulation tissue in the

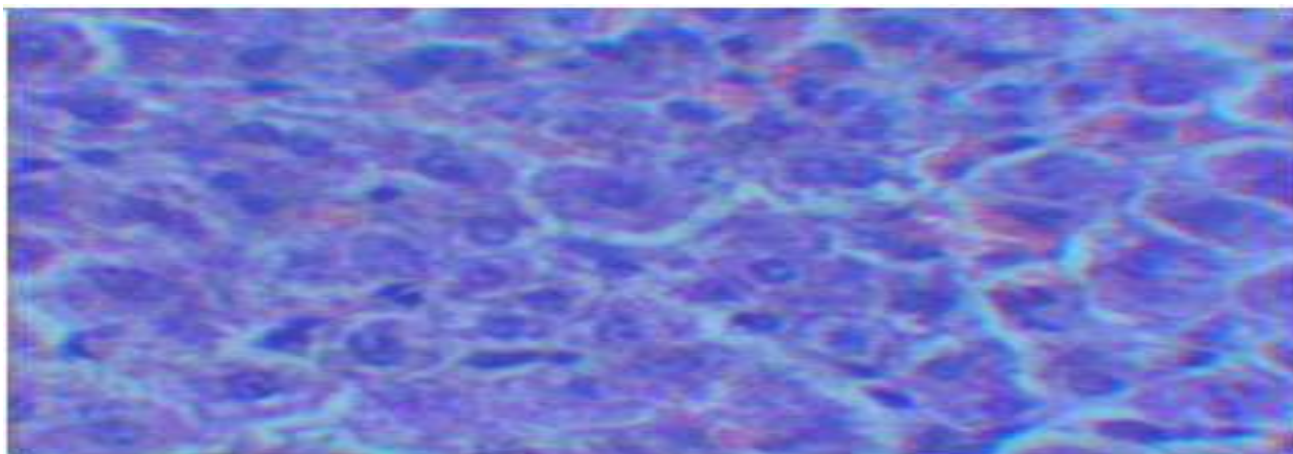
place of necrotized hepatic cells with apparent fibroplastic reaction.



Picture 7: Small-focused coagulation necroses of hepatocytes of periportal zone. Lymphatic-macrophagic infiltration. Colouring: with hematoxylin and Eosin. Increased in 10 times, volume 40

Sometimes single necroses of liver cells were met in other parts of acinus. Though Vv part of dystrophically changed hepatocytes did not distinguish significantly from the group,

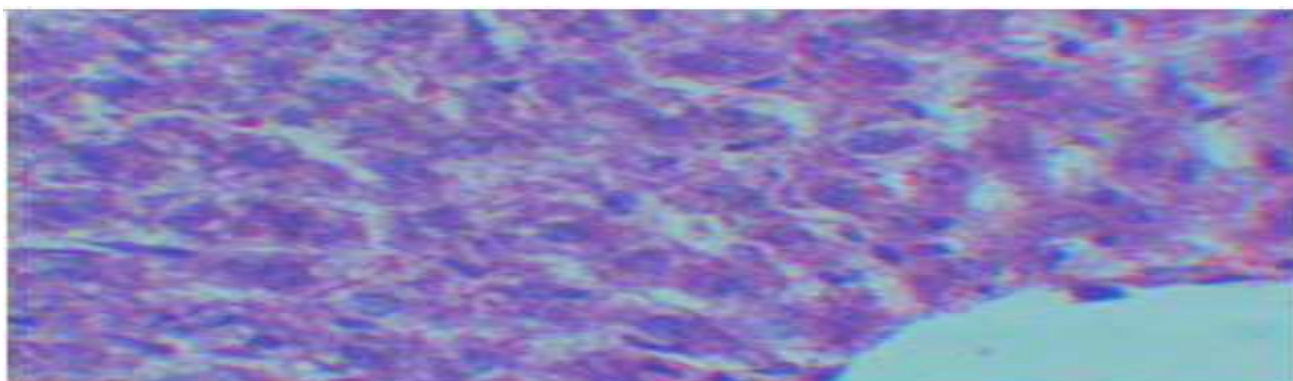
which was getting phenylhydrazine. Granular and hydropic degeneration of liver cells prevailed.



Picture 8: Moderately expressed plethora of sinusoids, granular and hydropic degeneration of liver cells. Colouring: with hematoxylin and Eosin. Increased in 10 times, volume 40

Liver macrophages were moderately proliferated. As morphometric analysis shown, the tendency of decreasing of quantitative-qualitative indicators of destructive changes in organ pulp was kept

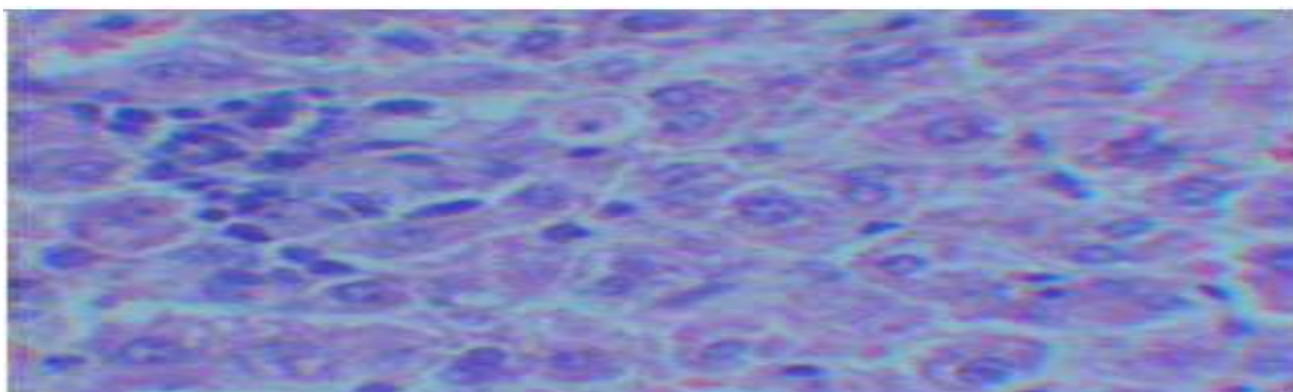
and increased. Granular dystrophy prevailed in hepatocytes; it was displayed with accumulation of protein granules in cytoplasm of liver cells. Single cells with hydropic degeneration were noted.



Picture 9: Small-focused hydropic degeneration of liver hepatocytes. Colouring: with hematoxylin and Eosin. Increased in 10 times, volume 40

In different parts of liver acines there are met small focuses of pulp necrosis with destruction of argyrophil stroma and accumulation of macrophages and

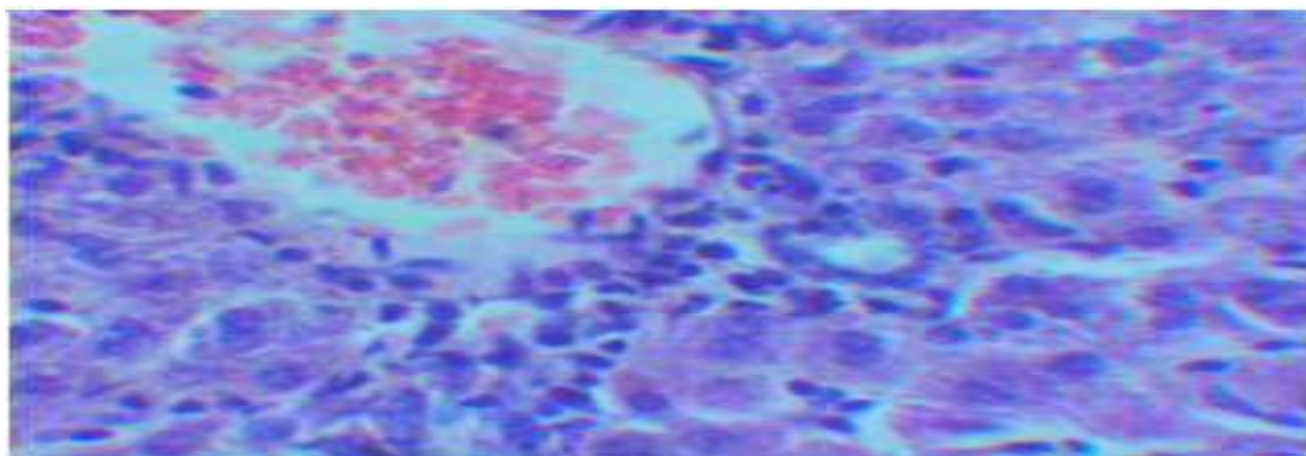
lymphocytes of segmentated cells in this place. Proliferation and hypertrophy of Kupffer's cells is expressed.



Picture 10: Small-focused necrosis of hepatocytes of intermediate part of liver acinus with lymph-macrophagic infiltration. Colouring: with hematoxylin and Eosin. Increased in 10 times, volume 40

PAS-reaction to glycogen reflected the decreasing of its concentration in central parts, which created its irregular distribution in acinus.

In peripheral parts of acinus its concentration was high and decreased in intermediate and central parts.



Picture 11: Hypostasis, lymphocytary infiltration of stroma of portal ducts. Vessels plethora. Colouring: with hematoxylin and Eosin. Increased in 10 times, volume 40

Intoxication was displayed in different manifestation rate, mainly in granular degeneration. Hydropic degeneration was discovered in separated cells of liver tissue. All the denoted types of degeneration were localized mainly in periportal and intermediate zones. Necroses of hepatocytes were isolated, mainly of colliquation nature, and were met in intermediate zone. Proliferate activity of Kupffer's cells was of focused nature and with different manifestation rate. Glycogenization of hepatocytes seemed to be relatively regular and was slightly lowered in central parts of acinus.

Hystoarchitectonics of liver did not suffer from significant changes. Vv of fibrosis amounted 3, 3 ± 0 , 7. To the end of experiment with injection of treatment dose of "Salsocollin" our complex research of histological preparations, as it is seen in table, shown above, showed the real decreasing of volume indications, which reflect the destructive changes in organ pulp, and increasing of indications, which reflect the reparative processes in liver tissue. So, the amount of binuclear hepatocytes increased significantly. The great number of hypertrophied liver cells appeared which reflected the intracellular reparative processes.

In microscopic examination of liver tissue in comparison with non-treated group, pathomorphological changes were expressed fewer. Necroses of pulp in group, which got

phenylhydrazine, were of small focus or focal nature, volume ratio of which was significantly less than comparison group, and in groups, which got NDMA and isoniaside was not found. Portal ducts looked widened, slightly or moderately infiltrated.

The cells of lymphocytary row were mainly defined in infiltrate. Infiltrates, as a rule, did not excess the limits of portal stroma, which was focused-sclerotic in this group. Volume indications of phlogistic cell infiltration were significantly decreased in both portal ducts and inside of acines. Volume ratio of dystrophically changed hepatocytes decreased significantly and was of protein-granulated nature. No features of adipose or hydropic degeneration were found. In carrying out of PAS-reaction to glycogen, its regular distribution in cytoplasm was found. Particles of glycogen of middle size were found more often.

Conclusion

In acute and chronic intoxication with hydrazines the increasing of activity of aminotransferases was noted, which certifies the unfavorable changes in liver pulp cells. Positive thymol probe in hydrazines intoxication certifies the significant changes in liver pulp. In injection of "Salsocollin" preparation against the background of chronic intoxication, the thymol probe was slightly approximated to control data, activity of AlAT and AsAT was approximated to control data, which certifies the favorable influence of "Salsocollin" on liver.

Bilirubinemia, which is noted in all the groups, poisoned with hydrazine, shows the damage of liver pulp, bilirubin penetrates into blood through the damaged liver cells. It is widely known, that overfilled bile vessels are damaged and let bilirubin penetrate into blood, and significant changes of biochemical features show the development of toxic hepatitis, when the severe changes in protein-formation and bile-formation functions of liver take place.

“Salsocollin” influences it favorably only in case of acute intoxication with NDMA and IAH. So, in long-term injection of different chemical compositions of hydrazine there were noted pathomorphological changes, which started with hemomicrocircular damages, expressed with veins plethora, damage of penetrability of vascular walls, extravasation into surrounding tissues with further development of destructive changes in functional pulp. Width and manifestation rate of morphological changes directly depended on type of used chemical composition of hydrazine.

The most expressed destructive changes of liver, followed with intensive infiltration of portal ducts and inside the acinus with mononuclear cells, and with development of post-necrotic fibrosis were found in

intoxication with phenylhydrazine and hydrazine sulphur, and, in lesser degree, NDMA and isoniaside. In injection of “Salsocollin” preparation, manifestation rate of morphological changes of liver was significantly lower than in intoxication with hydrazines, which certifies the favorable influence of this preparation on liver. Upon the results of experiments in acute and chronic intoxication with hydrazines, there was noted the increasing of activity of aminotrasferases, positive thymol probe, bilirubinemia, which certifies the unfavorable changes in cells of liver pulp. Pathomorphological changes, which started from hemomicrocircular damages, expressed with veins plethora, damage of penetrability of vascular walls, extravasation into surrounding tissues with further development of destructive changes in functional pulp.

The most expressed destructive changes of liver were found in intoxication with phenylhydrazine and NDMA and in lesser degree with hydrazine sulphur and isoniaside. In injection of “Salsocollin” preparation, manifestation rate of morphological changes of liver was significantly lower than in intoxication with hydrazines, which certifies the favorable influence of this preparation on liver.

References

1. Avakyan AK (1990) New molecular criteria for assessing the toxic effects of hydrazine derivatives. Reactive oxygen species as key agents in the mechanism of toxicity/ Pharmacology and Toxicology, 53: 70-73. Russia
2. Beisenova RR, Ibrayeva AO, Khanturin MR (2008) Changes in the behavior of laboratory animals under the influence of hydrazine derivatives and correction. Bulletin of Eurasian National University named after L.N. Gumilyev. Series of natural and technical sciences, 6 (67): 46-53.
3. Bogdanov NA (1990) Pathology, clinic and therapy of lesions of liquid rocket fuel. VMOLA 36-38. Saint-Petersburg, Russia
4. Beisenova RR, Khanturin MR (2009) Pathomorphological changes in the kidney under intoxication of hydrazine derivatives. Bulletin of Kazakh National University named after Al-Pharaby. Series of ecological sciences, 2 (25)59-67.
5. Portyannaya NI, Osipenko BG, Moskadina GA, Novokhatski NK (1998) To the biochemical mechanism of toxic action hydrazines. 1st Congress of Toxicologists of Russia. Abstracts, 306. Moscow, Russia
6. Saspugayeva GY, Beisenova RR, Khanturin MR (2011) Dynamics of plasma proteins under the influence of hydrazine and vanadium oxide derivatives //Journal of Environmental Science and Engineering. USA, 1155-1161
7. Tazitdinova R, Beisenova R, Saspugayeva G, Aubakirova B, Nurgalieva Z, Zhandibay A, Fakhrudinova I, Kurmanbayeva A (2018) Changes in biochemical parameters of rat blood under the combined effect of chronic intoxication with such heavy metals as copper, zinc, arsenic. Advances in animal and veterinary sciences, 6 (11): 492-498.
8. Zhaznayeva Zh K, Beisenova RR, Khanturin MR, Saspugayeva GY,

Beisenova DR (2015) Changes in Blood Biochemical Parameters in Rats against the Background of Acute combined Intoxication with Phenylhydrazine and

Cobalt Nitrate and Drug Correction by “EPAM4”. Biosciences biotechnology research Asia, 12: 731-740.