

## Changes in Blood Biochemical Parameters in Rats Against the Background of Acute Combined Intoxication with Phenylhydrazine and Cobalt Nitrate and Drug Correction by “EPAM4”

Zhanat Kadyrbekovna Zhaznayeva<sup>1</sup>, Raikhan Rimbayevna Beisenova<sup>1</sup>,  
Marat Rashitovich Khanturin<sup>1</sup>, Gulnur Yerzhanovna Saspugayeva and  
Danagul Rimbayevna Beisenova

<sup>1</sup>L.N. Gumilyov Eurasian National University Republic of Kazakhstan, 010008, Astana, 2 Satpayevast.

<sup>2</sup>S.Seifullin Kazakh Agrotechnical University Republic of Kazakhstan, 010000, 62Pobedyave  
Karaganda State University of the name of academician E.A. Buketov, Republic of  
Kazakhstan, 100008, Karaganda, st.University 28

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The article presents the results of studies of the effect of acute combined intoxication with phenylhydrazine and cobalt nitrate in the blood chemistry of the experimental rats, as well as correction of the drug “EPAM4.” It is found that high doses of studied toxicant cause mitochondrial damage of the heart muscle, cause hypoglycemia, disruption of protein metabolism in the muscles, changes in the liver parenchyma. It is shown that during the correction by the drug “EPAM 4” there was a restoration of AST activity, creatinine level in the muscle of the experimental animals, restoration of disturbed formations of total bilirubin in the cells of thymol test, glucose production.

**Key words:** phenylhydrazine, heavy metals, cobalt, glucose, thymol test, creatinine, ALT - alanine aminotransferase, AST - aspartate aminotransferase, hypoglycemia.

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In the scientific literature there are many details that phenylhydrazine is included in the list of the main metgemoglobin producers. Hydrazine and its derivatives have liver toxicity<sup>1</sup>.

According to some researchers phenylhydrazine toxicity in rats resulted to the loss of body weight after chronic phenylhydrazine, after poisoning rats the volume of urine in the bladder decreased. During rats intoxication with phenylhydrazine the decrease in almost all major body fluids: blood plasma, lymph and urine output

was found. Against this background, in blood plasma, lymph and urine the content ratio of sodium ions, potassium and calcium is disturbed. Unsymmetrical dimethylhydrazine 1.1(UDMH) and its derivatives are a class of highly toxic compounds that cause acute and chronic intoxication, mainly affecting the liver, blood system and other organs of live animals. UDMH refers to a group of environmental toxicants. The process of biotransformation of hydrazine and its derivatives occurs mainly in the liver<sup>2</sup>.

Phenylhydrazine is called a hemolytic or blood poison that destroys red blood cells. Phenylhydrazine affects hemoglobin and turns it into carboxyhemoglobin or methemoglobin and leads to the development of hypochromic

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\* To whom all correspondence should be addressed.

sideroblasticgipersideremic anemia (lead)<sup>3</sup>.

According to G. A. Lazareva and other researchers intramuscular single administration of phenylhydrazine (80 mg / kg) and repeatedly (3 mg / kg) reduces the functional activity of mononuclear blood cells and immune reactivity. In the study of immune metabolic effects associated with single and multiple entry of hemolytic poison in the body, as well as the possibility of correcting the introduction of drugs that affect lipid metabolism - carnitine and biotin we revealed the following: carnitine and biotin have no significant effect on these immunological parameters at once phenylhydrazine administration and reduce the severity of changes in immunological functions at multiple injection of the hemolytic poison. A single injection of a large dose and multiple injections of small doses of phenylhydrazine reduce PMA of the mononuclear cells, inhibit the development of humoral immune response (GMOs) and delayed-type hypersensitivity (DTH), induce the appearance of immunosuppressive properties of the light red blood cells. According to the same research the appearance of immunosuppressive properties of the red blood cells after a single administration of large doses of phenylhydrazine due to the changes in the structure caused by the poison changes in the cell membranes structure. Immune suppressive properties of the light red blood cells after repeated administration of phenylhydrazine are induced by the tied with their membrane low-density lipoprotein and serum glycosaminoglycans<sup>4</sup>.

#### **Phenylhydrazine causes selective damage of erythron<sup>5</sup>**

According to some researchers phenylhydrazine when administered in vivo induced the reduction of glutatino-S-transferase activity, more pronounced in the liver than in the kidney<sup>6</sup>.

In the literature, there is the evidence that phenylhydrazine content reduces macroenergy compounds (ATP and BFG) in red blood cells and functional metabolic activity of the peripheral blood neutrophils<sup>7</sup>.

Taking into account the above mentioned, the study of the effect of phenylhydrazine and its derivatives in the living organisms are of scientific and practical interest. According to the literature receipt in the living

organisms of this kind toxicant in chronic and lethal doses causes significant changes in the immune, hematopoietic and protective functions of the organism. It violates the biochemical processes in the circulatory, nervous and lymphatic systems.

For heavy metals, they accumulate and violate the function of vital organs and glands, such as the heart, brain, kidneys, bones, liver, etc. 8. In the terms antropoekosistem heavy metal pollution has a significant impact on the human body. The main sources of heavy metals in urban areas are industrial and energy enterprises, transport, housing and communal services<sup>9</sup> Worsening sanitary functions of urban soils poses a threat to the health and lives of the population, as a high content of heavy metals in the environment leads to various diseases<sup>10-15</sup>. Heavy metals are often used in personal care products, such as toothpaste<sup>16</sup> In studies of the spread of hypertensive disease among smokers there is a direct correlation between the accumulation of Zn in the tissues of the hair, scalp and urine<sup>17</sup>. If we talk about the carcinogenic effect of heavy metals, the incidence of breast cancer in Negiriek significantly increased during the last three decades in the parallel due to the rapid industrialization of the country's production and delivery of the lead (Pb) and other industrial metals.

The metal content in the hair and other biological media in the first place depends on the actual content of their dietary habits, but the accumulation of metals in the body of adolescents may be due to the inhalation as well as the chemical properties of the water intake in the area of residence. The processes of weathering of the soil and also lead to the accumulation of heavy metals in the hair. For cities where heavy metals are widespread, they mainly have the inhalation route of exposure<sup>18,19</sup>.

Thus, the soil, which is located at the intersection of transportation routes of migration of xenobiotics is the most sensitive indicator of geochemical conditions in the region<sup>18, 20</sup>. Hair composition primarily reacts to the changes in the concentration of some elements in the environment; metals in the hair are fixed well and reflect the stable indicators of the pollution<sup>15, 21, 22</sup>.

Cities with a diversified industry are characterized by the presence in the environment not specific pollutants and heavy metals

associations, capable to provide a combined effect on the body, in which the summation of effects and their potentiation may be observed<sup>23,24</sup>.

Dangerous levels of the pollution with heavy metals are in many industrial areas<sup>24-26</sup>. Compounds of heavy metals with a high toxicity towards living organisms are not destroyed in the soil, water, plants and animal organisms. They can store for a long time in the environment, migrate, accumulate in humans and animals, causing changes in the organs and tissues and cause irreparable damage to the health<sup>27,28</sup>.

Many heavy metals and their compounds in addition to the toxic effect, have carcinogenic and mutagenic effects and cause serious long-term consequences<sup>29</sup>. There are certain concentration ranges, in which trace elements, including metals, in particular cobalt, are needed for living organisms<sup>30,31</sup>. Serious interest in the biochemistry of the cobalt originated around 1934 y. in the connection with the severe disease of cattle and sheep in various parts of the world (Russia, Scotland, Australia, New Zealand, Canada). Animals lose weight, appetite, become lethargic, anemic and eventually died. The presence of anemia suggested the involvement in the iron deficiency. But it turned out that it is not in the iron, and in the presence in the iron compounds very small amounts of cobalt. The addition of cobalt into the feed completely eliminates all toxic symptoms<sup>31, 32</sup>. Physiological and pathophysiological effects of cobalt varied. There is information about its effect on the metabolism of carbohydrates and lipids<sup>33, 34</sup>, on thyroid function<sup>35,36</sup>, the state of the myocardium<sup>35, 37</sup>.

The fact is that in some countries for several years (60-ies. XX century) to increase beer foaming cobalt was added (1.2 - 1.5 mg / L), and this resulted the serious illness or death among the fans of the drink. Cobalt may contribute to the

development of tumors [38, 39], it is even included in the list of carcinogenic agents IARC (Agency for Research on Cancer World Health Organization) [39], while its complex compounds have antitumor activity [40].

It is toxic [41, 42] (the first data on the toxicity of cobalt appeared in 1883 [43]), while at the same time and it can act as an antidote to the cyanide poisoning. [44]

There is information about the epileptogenic effect of cobalt [45].

The aim of the work was to determine the changes in blood biochemical parameters in rats allowing to assess functional disorders of the internal organs in phenylhydrazine acute intoxication and cobalt nitrate and their combined effect, also against the backdrop of hepatoprotective drug "EPAM4."

#### Methods of the study

The experiments were conducted on laboratory rats weighing 180-220 g. Acute intoxication was caused by single oral administration of phenylhydrazine (188 mg / kg), the combined administration of phenylhydrazine, and cobalt nitrate (188mg / kg, 434 mg / kg), also against the backdrop of ecotoxicants intoxication hepatoprotective drug "EPAM4" once orally (0.02 ml) was introduced. The scheme of the experiment is given in Table 1.

The results of studies of the rat blood biochemistry, statistically processed by parametric and parametric methods are presented in Table 2. Significant differences of the average values were assessed by the Student criterion.

## RESULTS AND DISCUSSION

Compared with the control group in the 2 group where laboratory rats were injected by phenylhydrazine parameter ALT increased by 612,3% ( $p < 0,001$ ), in group 3, where the animals

**Table 1.** The scheme of the experiment the of acute combined intoxication with phenylhydrazine and cobalt nitrate and drug correction by "EPAM4"

S. No	Number of rats	Name of the drug	Drug daily dose
1.	7	Water	1 ml
2	7	FH (phenylhydrazine)	188 mg/kg.
3	7	FH+ EPAM4 (cor.)	188 mg/kg + 0,02 ml
4	7	FH+ CiNO3 (cobalt nitrate)	188 mg/kg+434 mg/kg
5	7	FH+ CiNO3+ EPAM4	188 mg/kg+434 mg/kg

**Table 2.** Biochemical parameters of the rats' blood, on the background of the acute intoxication with phenylhydrazine and cobalt nitrate, with the drug "EPAM4" correction

Biochemical parameters	I gr Control group	II gr phenylhydra zine	III gr Phenylhydra zine + EPAM4	IV gr Phenylhydra zine + cobaltnitrate	V gr Phenylhydr azine + cobaltnitrate + EPAM4
ALT -alanineamino transferase	323,8±10 nmol/s*	2305,5±12,9***	2311,9±12,9***	254,4±13,6***	258,2±7,02***
AST - aspartateaminotransferase	323,8±10 nmol/s*	398,5±181,8*	513,6±66,9*	429,9±78,9*	415,6±67,5*
Glucose	6,4±0,3 mmol/l	4,2±0,49***	4,4±0,42***	11,61±0,46***	11,5±0,5***
Creatinine	54,8±0,25 mkmo/l	51±15,2*	21,7±6,8***	27±4,43***	38,67±8,1*
conjugatedbilirubin	3,35±0,16 mkmo/l	0,27±0,52***	0,16±0,52***	0,42±0,51***	0,29±0,52***
Bilirubin	13,8±0,68 mkmo/l	1,27±3,0***	2,56±3,0***	2,55±3,0***	8,38±5,2*
Tymoltest	1±0,06 un/mut	0,344±0,12***	0,392±0,15***	0,123±0,14***	0,21±0,14***

Note: \* - p<0,05; \*\* - p<0,01; \*\*\* - p<0,001

were injected byphenylhydrazine and re-seeded by the corrector, this parametr increases to 614.5% (p<0,001), in group 4, where the animals have acute combined injection with phenylhydrazine and cobalt nitrate ALT decreased by 21,4% (p<0,001), 5 group where animals had acute combined injection with phenylhydrazine and nitrate cobalt and re-injection with "EPAM4" index decreased by 20, 2% (p<0,001), (Figure 1).

AST compared with the control group is increased in the group 2 at 10,5% (p<0,05), in group 3 - 45,6% (p<0,05), in group 4 - 21.8% (p<0,05), in group 5 - 17,7% (p<0,05), (Figure 2).

Currently, ALT and AST are the most reliable markers of the damage to the liver parenchyma. The activity of these enzymes in serum is usually increased by the liver pathology. Experimental results show the activity of ALT-alanine in 2 and 3 groups, this suggests that acute intoxication with phenylhydrazine activates this enzyme. Acute intoxication with phenylhydrazine causes disturbances of the internal structure of hepatocytes and increases the permeability of the cell membranes, in connection with which parameter ALT in these groups was very high. In the 4 group ALT - alanine aminotransferase was lower than the benchmarks. It is possible that the combined effect of phenylhydrazine with cobalt nitrate reduces the permeability of the membranes of hepatocytes and release of ALT into the blood decreases. However, under the correction by the drug "EPAM4" in the 5 group dynamics of this index is closer to normal.

Parameters of AST-activity in all groups are higher than those in the control group, where the intact animals were given water. However, it should be noted that 5 group where in addition to acute intoxication combined with phenylhydrazine and cobalt nitrate drug "EPAM4" was performed in comparison with a group of 4 AST-aspartate parametr was lower. In the 5 group compared with the 4 group parametr AST is 17.7% and 21.8% in the fourth. This suggests that the drug "EPAM4" delaysmitochondrial cells damage. Since it is known that in some patients with acute liver failure caused by Wilson's disease, AST activity in serum may be higher than the activity of ALT, this reflects the mitochondrial damage.

The index of glucose compared to the control group is less in the second group 65,2% (p

<0,001), in the third group 31,3% (p <0,001), increased in the fourth group to 81,9% (p <0,001), in the fifth group 79,1% (p <0,001), (Figure 3).

The experimental results showed that in Groups 2 and 3 there are signs of hypoglycemia. Norma of the blood glucose is  $6,4 \pm 0,3$ , while the

results of the experiments in these groups observed glucose content below normal. Blood glucose levels below 3.3 mmol / L., is a sign of hypoglycemia. Possibly, in the experiments animals had a stress from acute exposure to toxicants that leads to hypoglycemia. In acute intoxication with

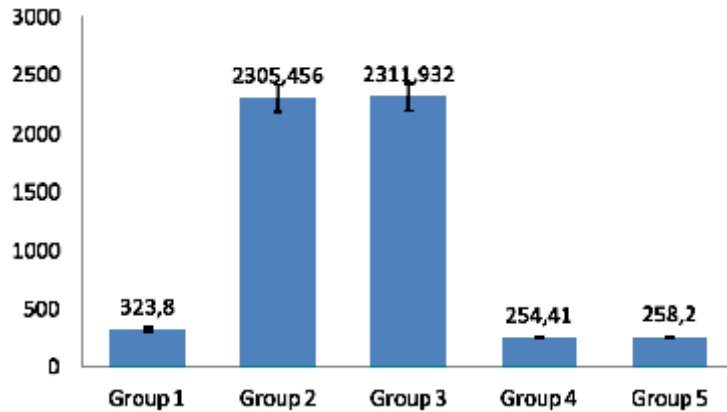


Fig. 1. ALT-alanine aminotransferase parameters in the rats blood

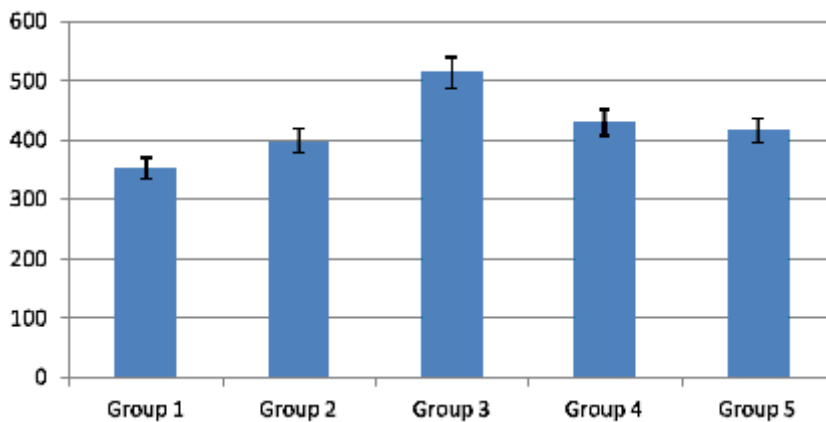


Fig. 2. AST - aspartate aminotransferase in the rats blood

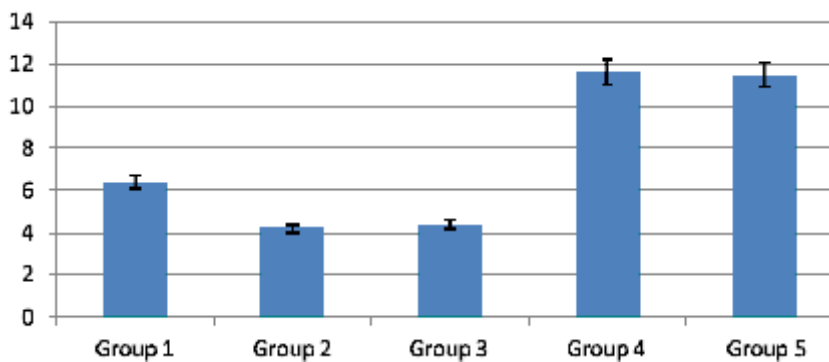


Fig. 3. Parameters of the glucose in the rats blood.

phenylhydrazine in Group 2 glucose index was lower monitoring data, however, the correction of the drug “EPAM4” show improvement results, in group 3 where the correction with the drug “EPAM4” was index of glucose is higher than in group 2. Combined intoxication with phenylhydrazine and cobalt nitrate in group 4 hyperglycemia was observed, but with the correction by the drug “EPAM4” improvements in the group 5 are noticeable, namely the approach to the control data. This condition is the result of possible violations of pancreatic function and the production of the hormone insulin, which plays a transport function in the transport of glucose.

Creatinine compared with the control group, there is a decrease in group 2 to 6,9% ( $p < 0,001$ ), in group 3 to 60,6% ( $p < 0,001$ ), in group 4 to 49,3% ( $p < 0,001$ ), in group 5 to 29,7% ( $p < 0,001$ ), (Figure 4).

Creatinine is the end degradation product of creatine, which plays an important role in energy metabolism in muscle and other tissues. Elevated levels of serum creatinine indicate a decrease in the filtration function of the kidney. Reduced levels of creatinine in the blood are rare. Increased creatinine levels depend mainly on the filtration function of the kidneys, and in hypocreatinemia their condition is not important. The emergence of state of hypocreatinemia says about violation of metabolic processes, accompanied by deep disorders of protein metabolism in the body or in the muscle tissue. In group 4 compared to group 2 creatinine level is lower, it means the violation of metabolic processes in combined intoxication with phenylhydrazine and cobalt nitrate. In Group 5 where was the correction with the drug “EPAM4” against the background of acute intoxication with phenylhydrazine and cobalt nitrate compared to

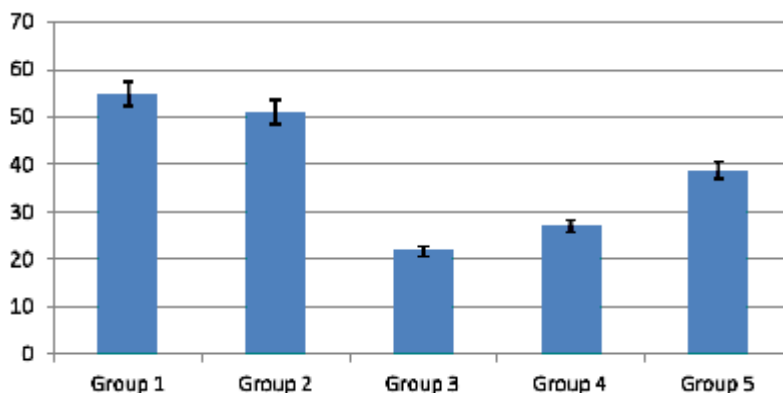


Fig. 4. Parameters of creatinine in the rats blood

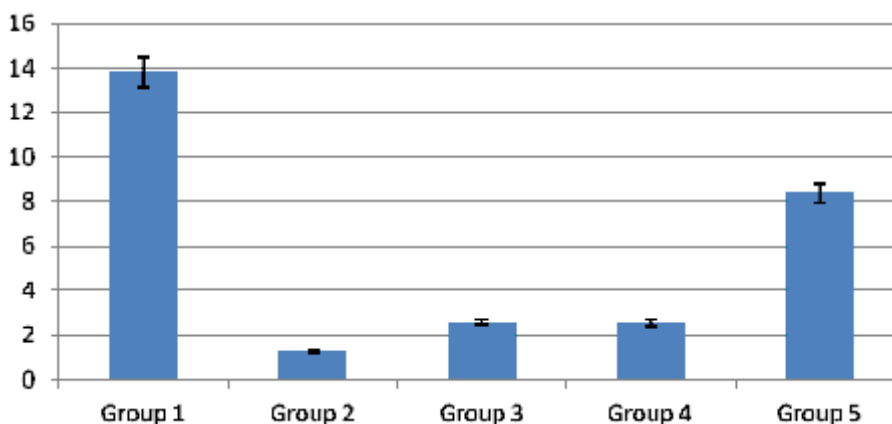


Fig. 5. Indicators of total bilirubin in the rats' blood

the group 4 creatinine was close to the reference data. It shows a positive trend of normalization of metabolic processes result the correction with “EPAM4.”

There is a decrease in all 4 groups in the parameters of Total bilirubin and conjugated bilirubin compared with the control group in which animals were given only water (Figure 5).

Bilirubin is the major bile pigment. Changes in the total bilirubin are because there are signs of disorders of bilirubin in the cells of the monocyte-macrophage system marrow and subsequent elimination from the circulation by hepatocytes.

The level of the total bilirubin in all groups dropped sharply. Low levels of the total bilirubin in the blood of experimental animals to that of the control group may indicate

destabilization of the chromogenic liver function. The index of the total bilirubin in group 2 was much lower than in the control group at the 90,8% ( $p < 0,001$ ), but the correction with the drug “EPAM4” in 3 group it is lower than control data for 81,4% ( $p < 0,001$ ), so it was more close to the reference data. The index of the total bilirubin in group 4 was lower than in the control group to 81,5% ( $p < 0,001$ ), but the correction with the drug “EPAM4” in 5 group the rate of total bilirubin was lower than in the control group to 39,3% ( $p < 0,05$ ) and closer to the reference data. In the groups where was the correction with the drug “EPAM4” the rate of total bilirubin in the blood significantly increased, and was close to the benchmarks (see Figure 5).

Indicator of the conjugated bilirubin in all groups is lower than the control data. However, in group 4 which had combined acute intoxication

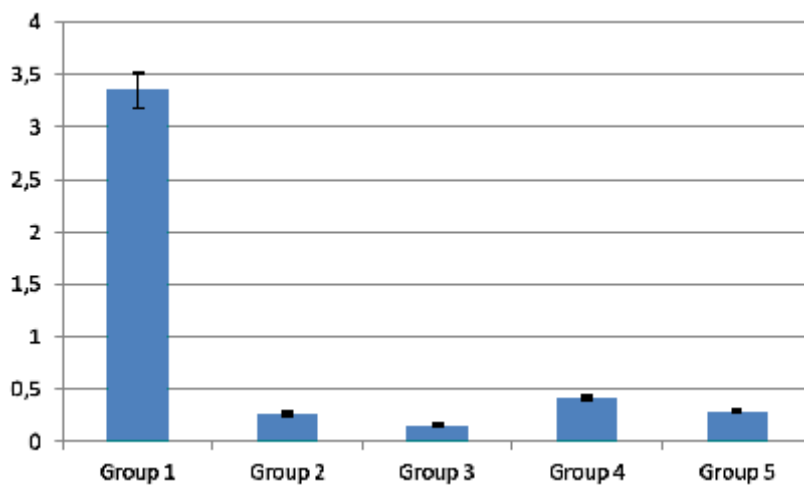


Fig. 6. Indicators of conjugated bilirubin in the rats' blood

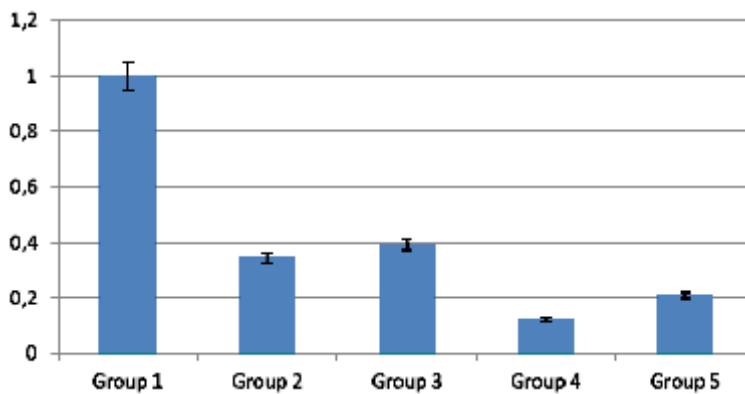


Fig. 7. Indicators of the thymol test in the rats' blood



with phenylhydrazine and cobalt nitrate conjugated bilirubin index was higher than in the other groups (Figure 6).

The indicator of the thymol test in group 2 is lower than the control at 65,43% ( $p < 0,001$ ), in the 3 group at 61,13% ( $p < 0,001$ ), in the 4 group at 87,71% ( $p < 0,05$ ), 5 group at 79,18% ( $p < 0,05$ ), (Figure 7).

Thymol test is designed to detect changes in the composition of serum proteins in various diseases. Positive thymol test indicates changes in the liver parenchyma. Most patients with hemophilia in the blood group 0 (I) deviation from the norm in the thymol test can be in the negative direction. Obstructive jaundice due to the violation of the outflow of the bile thymol test can also be negative. The experimental results showed that the level of thymol test in all experimental groups is below normal. In group 2 indicator of the thymol test is higher than in group 4 and closer to the norm, so the combined acute intoxication with phenylhydrazine and cobalt nitrate can cause a decrease of thymol test in the negative direction below the norm. When the correction with the drug "EPAM4" in group 3 compared with group 2 the thymol test has a trend to the growth and approaches to the norm, the same trend is observed in combined acute intoxication in 5 group with the respect to group 4. When correcting with the drug "EPAM4" in two groups, namely group 3 compared to group 5 the trend towards the improvement i.e. parameters' growth is closer to the norm expressed more.

## CONCLUSION

Studies of changes of blood biochemical parameters in experimental animals as a result of the combined effects of phenylhydrazine and cobalt nitrate in the background of the acute intoxication allow to make the following conclusions.

Index ALT-alanine was below the control and after correction with the drug "EPAM4" was higher. It is possible that the combined effect of phenylhydrazine with cobalt nitrate reduces hepatocyte membrane permeability and the release of ALT in the blood decreases. It is worth noting that the heart muscle from all enzymes AST aspartate aminotransferase is the most specific.

Activity AST index is higher than control that may reflect mitochondrial damage and can cause acute liver failure. The drug "EPAM4" corrects the activity of AST, as the results show a decrease in the level of AST in the blood.

The index of blood glucose is higher than control so it shows a possible violation of the pancreas. However, the drug "EPAM4" corrects and reduces the rate of glucose in the blood.

Creatinine is higher than control and it is considered to be a violation of the filtering function of the kidneys, but in our experiment it is lower benchmark data. When combined effects of phenylhydrazine and cobalt nitrate in the background of acute intoxication creatinine was lower than normal, perhaps this is due to a violation of metabolic processes, accompanied by deep disorders of protein metabolism in the body or in the muscle tissue. The drug "EPAM4" corrects these disorders and increases the content of creatinine in the blood. The index of total bilirubin was below normal this can indicate destabilization of chromogenic liver function. The drug "EPAM4" corrects the result and total bilirubin rises. Thymol test is lower than control, it may be the result of obstructive jaundice due to the violation of the outflow of bile. Thymol test can also be negative. Correction with the drug "EPAM4" indicator of the thymol test increases and it is close to normal.

Thus, on the basis of the obtained data, we can conclude that the study of blood biochemical parameters is an additional objective test determines the extent of the functional state of living organisms in the combined acute intoxication with phenylhydrazine and cobalt nitrate. The drug "EPAM4" is an effective drug correcting functional changes in the body.

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