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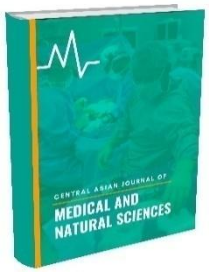

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Immunological Indicators in the Evaluation of the Effectiveness of Antioxidant Therapy for GEPAVID in Chronic Heavy Metal Poisoning in the Experiment

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Annotation: Compounds of heavy metals have a high biochemical activity, the ability to accumulate in the environment and living organisms. The important role of microelements as catalysts of many biological reactions and pathogenic effects of heavy metals on the human body has been revealed. The main sources of heavy metals entering the environment are emissions from industrial enterprises and vehicle emissions. High concentrations of heavy metals in the environment can lead to a decrease in the adaptive reactions of the body and the development of disease states [1].

Keywords: heavy metals, biochemical activity, immunological indicators, antioxidant therapy, GEPAVID.

Over the past decades, industrial production has increased, as well as the level of environmental pollution with various toxicants, including heavy metals [1]. Heavy metals enter the environment through industrial and agricultural activities, vehicles and boilers. Heavy metals make up a significant part of pollutant emissions, and their toxicity is a serious environmental problem [1,2]. At present, the problem of environmental pollution with heavy metals is relevant both throughout the world and in Uzbekistan. In many regions of the country, the content of toxic substances in soils exceeds the permissible levels, especially in areas with a developed mining and processing industry [1,8].

It is known that some metals, such as cobalt, copper, chromium, zinc, copper, iron, magnesium, nickel and selenium, are part of animal organisms and are necessary for various biochemical and physiological functions [2,4,6]. The lack of these elements can lead to the development of various diseases. However, excessive ingestion of heavy metals can cause acute poisoning, and chronic use of toxic doses can lead to chronic poisoning and even death [7,9].

Despite the biological role of heavy metals, all of them have a toxic effect on living organisms, since they can disrupt metabolic processes, cause mutagenic processes, and also affect reproduction, which can lead to carcinoma and, ultimately, death. Heavy metals have been reported to commonly exhibit chronic toxicity [4,6]. It is known that during intoxication various functional disorders occur, to which the immune system, endocrine and central nervous systems react, metabolism is disturbed [4,6].

Once in the body, salts of heavy metals decompose into ions, resulting in the formation of insoluble hydroxides and phosphates, which are absorbed in the gastrointestinal tract. Free ions can be quickly removed from the blood and accumulate in the skeleton [8,9].

In animals, heavy metals can be found in high concentrations and accumulate in the kidneys and liver. It is known that when toxicants enter the body, they can change their shape and form poorly soluble compounds. Compounds of the same metal can exhibit different toxic effects [4, 6]. There is experimental and epidemiological evidence that the combined effects of heavy metals can be significantly different from the effects caused by the action of only one metal. This is due to the fact that when interacting with other toxic substances in the environment, the resulting compounds cause higher toxicity.

It is known that heavy metals are usually deposited in tissues in the form of complex compounds with proteins and amino acids. However, their distribution in the internal organs is not the same. Sometimes this happens selectively. Summing up the facts mentioned above, it would be appropriate to study the depth of functional disorders in the internal organs. It is known that during intoxication various functional disorders occur, to which the immune system, endocrine and central nervous systems react, metabolism is disturbed [1,4,6].

To study organ damage and the effectiveness of the therapy, the method of quantitative registration of antigen-binding lymphocytes (ANTIGEN-BINDING LYMPHOCYTES), specifically sensitized to tissue antigens (TA) of various organs, was used, which is based on the use of the reaction of indirect rosette formation (PHRO) according to the method of GaribF.Yu. [3].

The essence of the method for determining ANTIGEN-BINDING LYMPHOCYTES to tissue antigens of various organs lies in the fact that when an organ is damaged of various genesis, intracellular processes are disturbed in its cells and dystrophy develops. An increase in the degree of dystrophy causes destruction and necrosis of cells. Molecules or fragments of structural and functional proteins with organ specificity enter the internal environment. Tissue proteins and molecules that are “foreign” to the internal environment acquire the status of tissue antigens (TA), and an immune reaction is launched aimed at their neutralization and elimination [2] .

In the presence of TA in the internal environment of the organ, antigen-binding lymphocytes (ABL) differentiate and circulate in the blood, capable of specifically binding to TA only of this organ. The level of ANTIGEN-BINDING LYMPHOCYTES to TA reflects the intensity of the processes of destruction and necrosis of structures in the organ: an increase in ANTIGEN-BINDING LYMPHOCYTES in dynamics indicates an increase, and a decrease in ANTIGEN-BINDING LYMPHOCYTES indicates a fading of the intensity of these processes, which makes it possible to assess the degree of organ damage, as well as the effectiveness of the therapy. The value of the method for determining ANTIGEN-BINDING LYMPHOCYTES to TA is its high sensitivity and specificity: the level of ANTIGEN-BINDING LYMPHOCYTES reaches a diagnostic level in the early stages and long before the onset of clinical signs of organ damage, which makes it possible to predict the risk of organ failure early. Establishment of the ANTIGEN-BINDING LYMPHOCYTES reaction with TA of several organs makes it possible to identify the multiple organ damage of the body during the development of pathology [5].

This chapter presents the results of studying the level of antigen-binding lymphocytes (ANTIGEN-BINDING LYMPHOCYTES) to tissue antigens (TAG) of the liver, kidneys and brain of rats in an experiment with exposure to heavy metal salts, as an indicator of the depth of pathological processes and a prognostic criterion for the effectiveness of the therapy.

The experiment was carried out on 30 white male rats weighing 160-200 g, kept in the usual microclimatic conditions of a stationary vivarium, in accordance with current standards, and received a standard diet.

Conducted 2 series of studies, in each of which the animals were divided into groups according to the objectives of the upcoming studies.

In the first series of 30 animals divided into 2 groups - intact (10 animals) and with simulated toxic hepatitis (20 animals) - the nature of the toxic effect of the studied combination of metals was studied.

In the second series (10 rats), after modeling the pathological process, a 2-week course of antioxidant therapy with the GEPAVIT dietary supplement was carried out.

In the 1st series of the experiment on 20 rats, subchronic poisoning was simulated by intraperitoneal injection (Elbekyan K.S., 2006) of a metal-containing mixture consisting of compounds of copper, manganese, molybdenum and chromium. The mixture was administered every other day for 3 weeks. The ratio of doses of metals in the mixture approximately corresponded to the ratio in soils in the area of the Almalyk mining and metallurgical plant [10] and amounted to Cu :Mn : Mo : Cr = 100:19:16:2.

At the end of each stage of the experiment, the rats were killed by the method of instantaneous decapitation. So, after a 3-week inoculation, 10 animals with simulated intoxication with salts of heavy metals and 10 animals from the control group were slaughtered. Animal blood samples were taken for the study.

When salts of heavy metals enter the body through the respiratory tract or orally, they enter the lungs and through the walls of the intestine through the blood are carried to various organs. Penetrating into the human body through the mucous membranes, skin, inhalation of poisoned air, with food, heavy metals are very easily absorbed from the digestive tract, enter the bloodstream, are transported to all vital organs and deposited in them. And most often this is a rather lengthy process. The main target organs (liver and kidneys) are the first to suffer from chemical poisoning. Since these substances are excreted from the body through them.

The largest amount of salts of heavy metals is concentrated in the liver, from where they are eliminated extremely slowly. They disrupt the course of numerous biochemical reactions in various organs. The most dangerous poisonings lead primarily to severe liver damage. Sharply disturbed detoxification functions of the liver and excretion of harmful metabolic products from the body lead to a general severe condition of all body systems. More often, liver damage is combined with the occurrence of severe clinical phenomena from the kidneys. General toxic effects on organs such as the brain , kidneys and liver can seriously affect the metabolic, physiological activity of organs.Because of their ability to reabsorb and store divalent metals, the kidneys are also a target organ for heavy metal toxicity. The degree of damage to the kidneys by heavy metals depends on the nature, dose, method and duration of exposure. Studies [11] have shown that both acute and chronic intoxication cause nephropathies with varying levels of severity, ranging from tubular dysfunction to severe renal failure, sometimes leading to death.

In chronic poisoning, heavy metals accumulate in various tissues and then have a toxic effect on the body. These substances in small doses have a non-specific effect, which is realized through the accumulation of asymptomatic changes in tissues and organs, and also manifests itself in the frequency and complications of somatic pathology.

Considering the above and bearing in mind that antigen-binding lymphocytes appear in the body at an early stage, when there are no visible clinical manifestations yet, we determined the level of ANTIGEN-BINDING LYMPHOCYTES, specifically sensitized to tissue antigens of the liver, kidneys and brain, as the most vulnerable organs in chronic poisoning. salts of heavy metals. The level of damage to the liver, kidneys, brain was determined by the detection of antigen-binding lymphocytes (ANTIGEN-BINDING LYMPHOCYTES), specifically sensitized to tissue antigens (TA) of the organs under study in the laboratory of clinical immunology (head of the laboratory - MD AkhmedovaKh.Yu.) of the Republican Specialized Scientific -Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases.

Analysis of the level of antigen-binding lymphocytes specifically sensitized to tissue antigens of the liver, kidneys, brain in the experiment after subchronic poisoning of a metal-containing mixture consisting of compounds of copper, manganese, molybdenum and chromium for 3 weeks relative to control values showed a significant increase in antigen-binding lymphocytes to liver tissue antigens (26.60 ±2.04 and 4.60 ±0.31%, respectively, P >0.05), to renal tissue antigens (23.60 ±3.19 and 4.50 ±0.31%, respectively, P >0, 05), to tissue antigens of the brain (22.20 ±1.95 and 3.60 ±0.56%, respectively, P > 0.05), indicating a pronounced inflammatory process and deep destructive changes in the studied organs (Fig. 1).

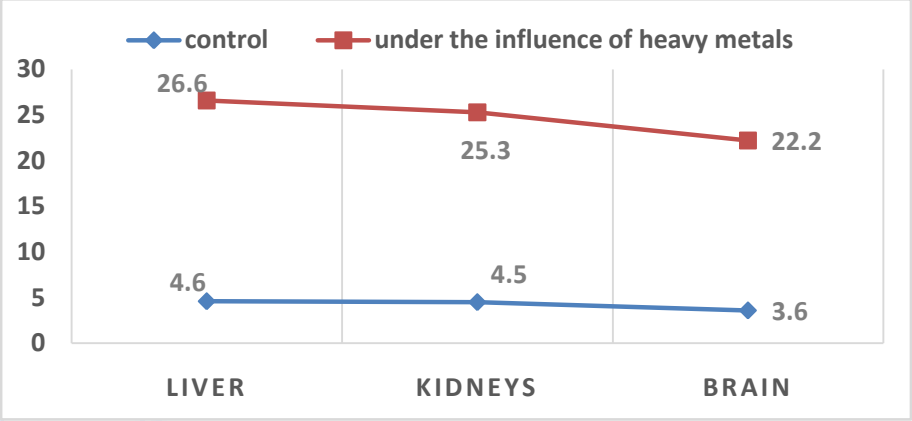


Fig.1 Dynamics of antigen-binding lymphocytes totissue antigens in the liver, kidneys and brain of rats under the influence of salts of heavy metals

We analyzed the parameters of the general blood test, which showed that in the group of animals with an experimental model of chronic intoxication with salts of heavy metals, a pronounced inflammatory reaction was noted. An increase in the inflammatory reaction could be judged by moderate leukocytosis and a regenerative shift of the leukocyte blood formula to the left. With an increase in the total number of leukocytes by 28%, a neutrophilic shift was noted with a predominance of young forms: the number of stab neutrophils increased by 2 times, with a parallel decrease in segmented neutrophils by 2.6 times. The number of lymphocytes increased by 1.9 times. ESR increased 2.4 times. (Table)

The hemoglobin level decreased to an average of 102.00 ±4.38, while the indicators in the control group were 137.86 ±0.83 g / l , there was a decrease in the number of erythrocytes to an average of 3.40 ±0.18 (x10 ^{12} / l), while in intact rats 4.79 ±0.03 (x 10 ^{12} /l). The reason for this picture of red blood may be a toxic effect on erythropoiesis, which develops with intravascular hemolysis of erythrocytes.

Presence of moderate leukocytosis (9.70 ±0.43 with indicators in the control group 4.27 ±0.04 (x 10 ^{9} /l) , an increase in the number of lymphocytes (68.39 ±2.23 and in the control group 37.54 ±1.10%) ,

an increase in ESR (20.29 ±1.94 and in the control group 10.19 ±0.92 mm/s) indicates the progression of the inflammatory reaction (Fig. 2).

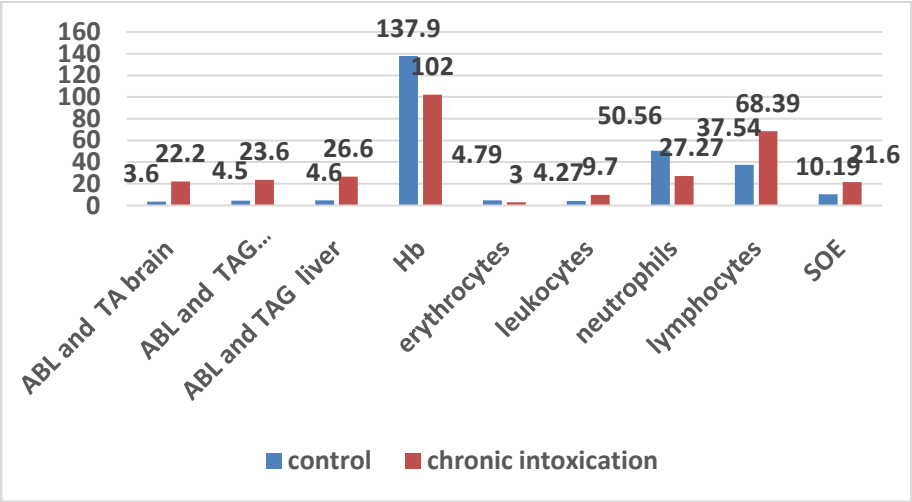
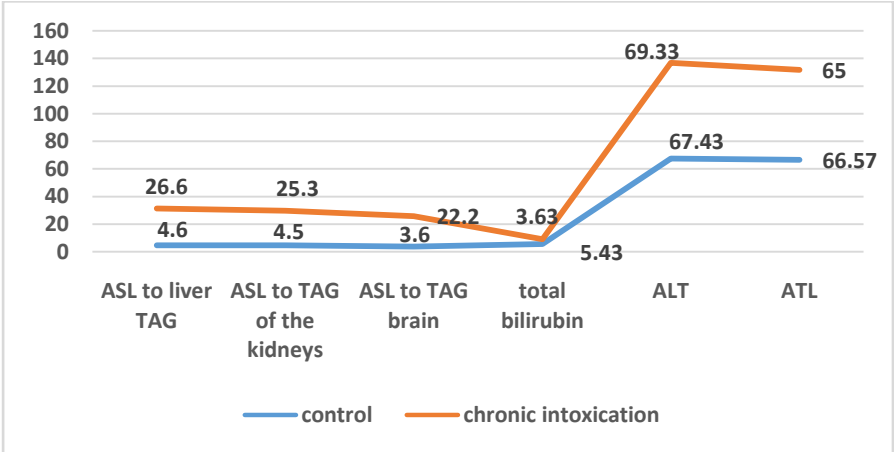


Fig . 2 Dynamics of antigen-binding lymphocytes to tissue antigens of the liver, kidneys and brain of rats and some indicators of blood hemostasis under the influence of salts of heavy metals.

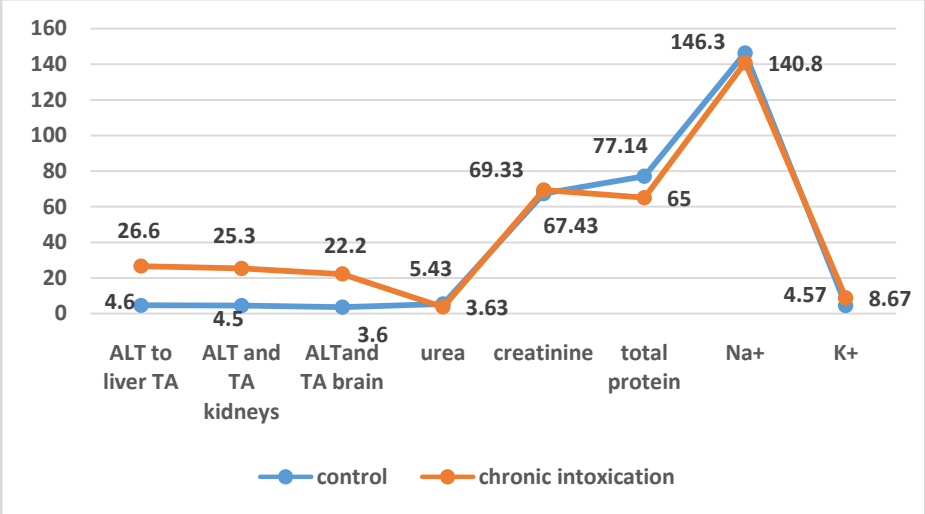
In a comparative analysis of a group of animals exposed to salts of heavy metals, bilirubinemia was noted: an increase in the total fraction was 67.13 ±2.66, while the indicators in the control group were 10.57 ±1.62 μmol/l , mainly due to an increase in the direct fraction (23.76 ±1 .06 and in the intact group 2.70 ±0.21 μmol/l) , which indicates the presence of toxic hepatitis. ALT activity in the blood serum increased slightly compared to the indices of animals of the intact group (84.67 ±28.90 and 61.14 ±9.86 units/l, respectively) , with a significant increase in AST activity (752.83 ±308.26 and 46.57 ±5 .34 U/L, respectively) . This resulted in an increase in the de Ritis coefficient (the ratio of the activity of serum AST and ALT. The value of the coefficient in the norm is 1.33 ± 0.42 or 0.91-1.75) . So, in the group of intact animals, the de Ritis coefficient was 1.33 ±0.42 , while in the group with subchronic exposure to a mixture of heavy metals, the coefficient was significantly higher than the permissible norms (6.31±0.98) (Fig. 3).

The dynamics of the activity of the main enzymes of cytolysis revealed by us indicates a violation of the integrity of the membrane structures of hepatocytes and the depletion of the functional capabilities of the liver.



Rice. 3 Dynamics of antigen-binding lymphocytes to tissue antigens of the liver, kidneys and brain and some indicators of bilirubin and liver enzymes in rats under the influence of salts of heavy metals

As you know, the liver is the main organ where the metabolic transformations of amino acids, ammonia, urea and other nitrogenous substances take place. In our studies, in connection with the violation of urea formation due to the toxic effect of a combination of metals, a significant level of urea in the blood (3.63 ± 0.37 and 5.43 ± 0.23, respectively) was revealed, relative to the indicators in the control , which indicated violation of protein metabolism with damage to the synthetic function of the liver, since the synthesis of urea is one of the most important stages of protein metabolism, and testified to the progression of liver failure. There was an increase in creatinine (154.33 ± 9.31 and 67.43 ± 6.37, respectively) , which indicated the associated renal failure.



Rice. 4 Dynamics of ANTIGEN-BINDING LYMPHOCYTES to tissue antigens of the liver, kidneys and brain and some indicators of the biochemical analysis of the blood of rats under the influence of salts of heavy metals

Comparative analysis of the obtained results of the dynamics of ANTIGEN-BINDING LYMPHOCYTES to tissue antigens of the liver, kidneys and brain with the data of the general analysis and biochemical parameters of the blood of intact rats and rats with chronic heavy metal intoxication showed certain correlations. Thus, an increase in leukocytes in the experimental group with chronic intoxication with heavy metal salts relative to the indicators in the control group (9.70 ±0.43 and 4.27 ±0.04%, respectively, P <0.05), a decrease in the number of erythrocytes (3.40 ±0.18 and 4.79 ±0.03%, respectively, P < 0.05), hemoglobin (102.0 ±4.38 and 137.86 ±0.83%, respectively, P < 0.05), marked decrease in neutrophils (27.27 ±1.44 and 50.56 ±1.10%, respectively, P <0.05), along with a pronounced increase in lymphocytes (68.39 ±2.23 and 37.54 ±1.10%, respectively, P <0.05) , as indicators of the presence of an inflammatory process in the body, an increase in total bilirubin (67.13 ±2.66 and 10.57 ±1.62, respectively, μmol/l ,P <0.05), liver enzymes AST (84.67 ±28.90 and 61.14 ±9.86 U / l, respectively) , with a significant increase in AST activity (752.83 ±308.26 and 46.57 ±5.34 units/l, respectively, P <0.05) , urea (3.63 ±0.37 and 5.43 ±0.23, respectively) , indicating an existing pathological process in the liver and cretinin dynamics (154.33 ±9 .31 and 67.43 ± 6.37, respectively, P < 0.05), indicating kidney damage, correlate with high levels of ANTIGEN-BINDING LYMPHOCYTES to brain TA (22.20 ± 1.95 and 3.60 ± 0.56, respectively, P <0.05) , liver (26.60 ±2.04 and 4.60 ±0.31, respectively, P <0.05) and kidneys (25.30 ±2.82 and 4.50 ± 0.31, respectively,P <0.05) in dynamics relative to the indicators of intact animals (Fig. 4).

Based on the results obtained, we also studied the immunological evaluation of the effectiveness of the antioxidant therapy of the GEPAVIT dietary supplement in chronic heavy metal poisoning in an experiment obtained at the Tashkent Research Institute of Vaccines and Serums by Professor Kamilov

H.M., which consists of various proportions of turmeric, milk thistle, corn silk, anise. The study was carried out on 10 rats with simulated chronic poisoning with salts of heavy metals, which were injected with the drug using a probe once a day at a dose of 1 ml/100 g of body weight for 3 weeks. At the end of the experiment, the rats were killed by the method of instantaneous decapitation. Animal blood samples were taken for the study.

In the dynamics of the experiment after 2 weeks of antioxidant therapy with dietary supplement "GEPAVIT"a comparative analysis of the average values of ANTIGEN-BINDING LYMPHOCYTES to TA of the liver, kidneys and brain in this group of rats amounted to - 10.0 ±0.99%; 5.30 ±0.64% andP ±<±_ ±_ ±_ _ _ 0.05). However, if the indicators of ANTIGEN-BINDING LYMPHOCYTES to TA of the kidneys (5.30 ±0.64%) and the brain (5.10 ±0.65%), after the course of treatment practically did not differ from the corresponding indicators of intact rats (4.50 ±0.31% and 3.6 ±0.56%, respectively, P >0.05), the level of ANTIGEN-BINDING LYMPHOCYTES to liver TAg, tending to decrease (10.0 ±0.99%), 2.7 times lower than in rats with chronic poisoning (26 .60 ±2.04%, P <0.05), retains a significant difference with the indices of intact rats (4.60 ±0.31%, P <0.05) (Table 1).

Table 1. Dynamics of ANTIGEN-BINDING LYMPHOCYTES indicators to TA in the liver, kidneys and brain of rats in an experiment with simulated exposure to salts of heavy metals

Groups	ANTIGEN-BINDING LYMPHOCYTES indicators (M±m) (%)		
	liver	kidneys	Brain
Control (n =10)	4.60 ±0.31	4.50 ±0.31	3.60 ±0.56
Before treatment (n =10)	26.60 ±2.04*	23.60 ±3.19*	22.20 ±1.95*
After treatment (n =10)	10.00 ±0.99 *•	5.30 ±0.64 *	5.10 ±0.65 *

Note: *- P <0.05 significance of differences in indicators in relation to the control group; • - P <0.05 significance of differences in indicators in relation to group 1

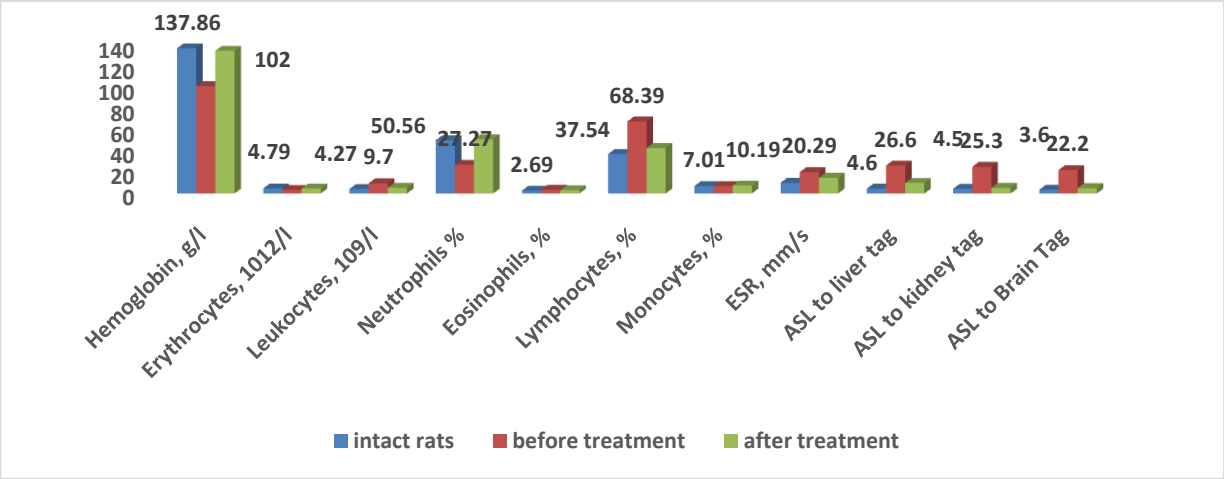
Thus, the decrease in the dynamics of ANTIGEN-BINDING LYMPHOCYTES to TA of the liver, kidneys and brain in the dynamics of the experiment indicates the high efficiency of the GEPAVIT dietary supplement herbal tea in restoring functional activity and reducing inflammation in the relevant organs. At the same time, the remaining high ANTIGEN-BINDING LYMPHOCYTES value to liver TA indicates that a pathological process is still ongoing in this organ, which requires longer treatment (Table).

We have carried out a comparative analysis of hemostasis parameters in rats with simulated chronic poisoning after a course of treatment with dietary supplement "GEPAVIT" . It was found that the animals had no signs of anemia (Hb-135.57±0.72 g/l), and the level of red blood cells remained within the physiological norm (4.73±0.04x10 ^{12} /l). A slight leukocytosis persists (5.39±0.25x10 ^{9} /l). There was a clear trend towards a decrease in lymphocytosis (43.14±2.38%) and normalization of ESR (14.96±1.43 mm/s). (Table 3.1.1). The remaining slight leukocytosis and lymphocytosis indicates an ongoing pathology in the body, which is also indicated by high ANTIGEN-BINDING LYMPHOCYTES values for liver TAg (Table 2, Fig. 5).

Table 2. Dynamics of indicators of hemostasis in rats in an experiment with simulated exposure to salts of heavy metals

Indicator	Control (intact rats)	Chronic intoxication with salts of heavy metals	
		before treatment	after treatment
Hemoglobin, g/l	137.86±0.83	102.00±4.38*	135.57±0.72•
Erythrocytes, $10^{12}/_{l}$	4.79±0.03	3.40± 0.18 *	4.73±0.04•
Leukocytes, $10^{9}/_{l}$	4.27±0.04	9.70± 0.43 *	5.39±0.25 * •
Neutrophils %	50.56±1.10	27.27±1.44*	51.51±0.43•
Eosinophils, %	2.69±0.16	3.60± 0.24 *	3.1 1±0.28•
Lymphocytes, %	37.54±1.10	68.39±2.23	43.14±2.38*•
Monocytes, %	7.01±0.32	7.06 ± 0.63	7.53±0.22
ESR, mm/s	10.19±0.92	20.29±1.94	14.96±1.43*•

Note: *- P <0.05 significance of differences in indicators in relation to the control group; • - P <0.05 significance of differences in indicators in relation to group 1



Rice. 5 The level of indicators of hemostasis and ANTIGEN-BINDING LYMPHOCYTES to TAg of the liver, kidneys and brain in the dynamics of the experiment

The study of some biochemical parameters in the dynamics of the experiment showed that the level of urea in the blood of the animals of the study group was within the physiological norm (5.39±0.15 μmol/l) , which confirms the emerging improvement in the state of protein metabolism (Table 3.).

Table 3: Dynamics of indicators of pigment metabolism and activity of cytolysis enzymes in case of metal poisoning and after a course of therapy for hepatitis

Indicator	Control (intact rats)	Chronic intoxication with salts of heavy metals	
		before treatment	after treatment
Bilirubin total, μmol/l	10.69±1.64	67.1±2.66 *	31.43±2.01*•
AST activity, U/l	46.57±5.34	762.0±305.48*	59.0±1.31*•
ALT activity, U/l	61.14±9.86	95.17±26.04 *	70.0±7.78•
De Ritis coefficient	0.84±0.08	6.31±0.98*	0.77±0.06•
Total protein, g/l	77.14±1.03	65.0±11.43*	76.71±1.04•
Creatinine, μmol/l	67.43±6.37	154.33±9.31*	63.57±4.85•
Urea, μmol/l	5.43±0.23	3.55±0.31*	5.39±0.15•

Note: *- P <0.05 significance of differences in indicators in relation to the control group; • - P <0.05 significance of differences in indicators in relation to group 1

As a result of the treatment, we did not observe a statistically significant change in the level of bilirubin (31.43±2.01 μmol/l) , however, there was a clear trend towards its decrease, which indicated the emerging positive changes in the state of the liver parenchyma. The activity of cytolysis enzymes (AlT70.0 ± 7.78 U/l) in blood plasma decreased and did not differ from that in healthy animals. The activity of the studied transaminases (AST - 51.00 ± 4.08 U/l) in the liver tissue also decreased, but these changes were not statistically significant. Nevertheless, the results obtained allow us to speak about the emerging positive dynamics and a decrease in the intensity of the pathological process in the liver (table).

Conclusions:

1. A significant increase in antigen-binding lymphocytes specifically sensitized to tissue antigens of the liver, kidneys, and brain in the experiment after subchronic poisoning with heavy metal salts relative to control values indicates a pronounced inflammatory process and profound destructive changes in the organs under study;
2. The results of the studies and statistical analysis of the depth of the inflammatory process in the organs of rats with simulated chronic intoxication with salts of heavy metals relative to intact animals, which is reflected in the indicators of antigen-binding lymphocytes, specifically sensitized to tissue antigens of the liver, kidneys and brain, show a direct, strong significant correlation with indicators of intoxication and inflammatory reaction of the general and some data of the biochemical analysis of the blood of this group of rats;
3. The decrease in the dynamics of ANTIGEN-BINDING LYMPHOCYTES to TA of the liver, kidneys and brain in the dynamics of the experiment indicates the high efficiency of the GEPAVIT dietary supplement herbal tea in restoring functional activity and reducing inflammation in the relevant organs. At the same time, the remaining high ANTIGEN-BINDING LYMPHOCYTES value to liver TA indicates that a pathological process is still ongoing in this organ, which requires a longer treatment.

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