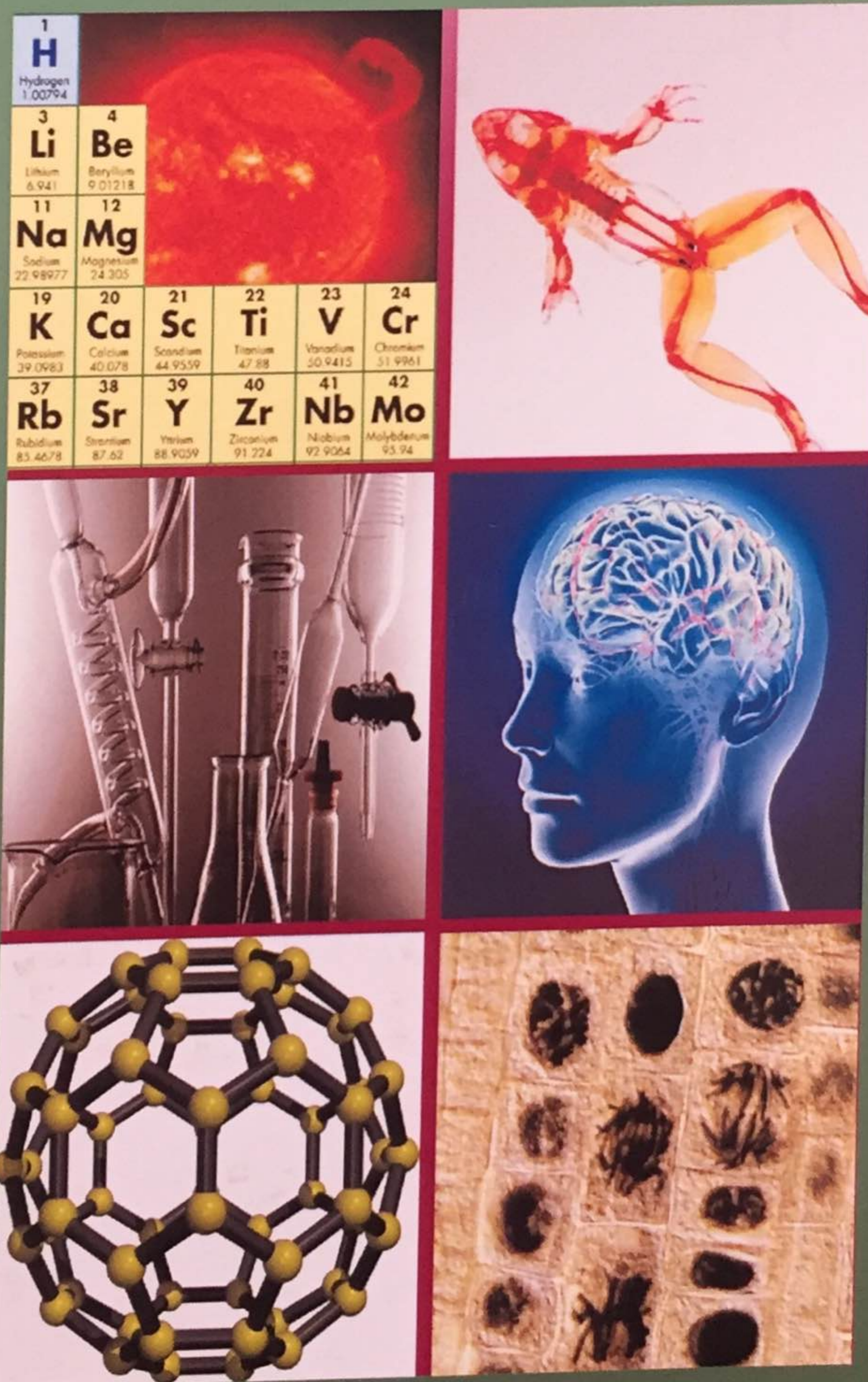


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### Contents

<i>D. Orazbayeva, B. Kenessov, J.A. Koziel</i> Quantification of naphthalene in soil using solid-phase microextraction, gas-chromatography with mass-spectrometric detection and standard addition method.....	4
<i>A.N. Imangaliyeva, G.A. Seilkhanova, A.Zh. Ondasheva, M.S. Telkhozhayeva</i> Adsorption studies of Cu (II), Pb (II) and Cr (VI) by chitosan/unitiol composite.....	12
<i>D.E. Aisina, A.T. Ivashchenko, R.E. Niyazova, S.A. Atambayeva, E.N. Imyanitov</i> Characteristics of interaction of miRNA with mRNA of breast cancer candidate genes.....	18
<i>A.M. Alexandrova, O.V. Karpova, R.M. Nargilova, R.V. Kryldakov, A.S. Nizkorodova, A.V. Zhigaylov, E.M. Yekaterinskaya, S.V. Kushnarenko, R.Zh. Akbergenov, B.K. Iskakov</i> Distribution of potato ( <i>Solanum tuberosum</i> ) viruses in Kazakhstan.....	33
<i>G.A. Shalakhmetova, S. Nayekova, M. Sagi, Z. Alikulov</i> The effect of presowing saturation with molybdenum and presence of nitrate on the allantoin content in sprouted wheat grain.....	41
<i>M.A. Altay, M.S. Kulbaeva, S.T. Tuleukhanov, Yu.A. Kim, L.K. Baktybaeva, T.T. Kulbaev, B.K. Kairat, A.I. Zhumadilla</i> Study of the influence of tonsillitis on other organs based on biophysical properties of skin biologically active points.....	49
<i>L.K. Baktybayeva, M.K. Tauassarova, B.K. Darrell, V.K. Yu., A.G. Zazybin</i> Immunostimulating properties of the azaheterocyclic compounds BIV-3, BIV-4, BIV-7.....	57
<i>Mursaleen, Syed Zahir Shah, Liaqat Ali, Nadeem Ahmad, Izaz Kuram, S. Barinova</i> Bioindication of water quality by algal communities in the Mardan River, Pakistan.....	65
<i>G.D. Daulet, G.K. Atanbaeva, S.T. Tuleukhanov, A. Ydyrys, A. Baishanbo, S.N. Abdireshov</i> Injection of sorbent and subsequent analysis of blood cells.....	82
<i>K.S. Koshkimbayev, E.M. Isakaev, M.V. Kulemin, M.Zh. Halykova, G.A. Utebayeva</i> Spring fauna of blackflies ( <i>Diptera, Simuliidae</i> ) of Syrdaria river middle reaches.....	89
<i>M.S. Kurmanbayeva, N.M. Mukhitdinov, A.D. Serbayeva, G. Sramko, M.K. Makhambet</i> Anatomical features of the rare species of <i>Erysimum croceum</i> M.Pop. from Trans-Ili Alatau.....	97
<i>S.T. Tuleukhanov, O.I. Salatova, Z.Zh. Zhanabayev, A.N. Oralbek, Iu.A. Kim</i> Entropic index of diurnal dynamics of systolic and minute volumes of human blood before and after exercise stress.....	106
<i>A.A. Sazanova, M.S. Kulbaeva, S.T. Tuleukhanov, Yu.A. Kim, A.I. Zhussupova, G.K. Atanbaeva, N.T. Ablaykhanova, Zh.O. Oralkhanova</i> Study of the influence of mobile phones on the functional condition of students' cardiovascular system during the examination period by Holter methods.....	112
<i>G.I. Yemazarova, S.K. Turasheva, A.A. Sartayeva, S.B. Orazova, A.A. Bazargaliyeva, E.M. Imanova, G.K. Omarova, S. Yancheva</i> Absorption of chromium by mono- and mixed cultures of microalgae.....	119
<i>B.A. Ussipbek, N.T. Ablaykhanova, M.K. Murzakhmetova, V. Isachenko, J.B. Ryskulova</i> Study of qualitative and quantitative indicators of the spermatogenesis for determination of the effectiveness of cryopreservation.....	127
<i>A.B. Ibraimov, M.M. Sergazina, M.B. Alimzhanova, M.B. Abilev</i> Analysis of tobacco products by chromatography methods: review.....	133
<i>A.M. Tuiebayeva, M.M. Sergazina, M.B. Alimzhanova, Zh.S. Mukataeva</i> Determination of the chemical composition of tea by modern physico-chemical methods: a review.....	142
<i>A.N. Azhkeyeva, R.K. Rakhmetullayeva, G.Zh. Yeligbayeva, Ye.M. Shaikhutdinov, U. Nakan, M. Abutalip, K. Park</i> Synthesis of novel thermosensitive copolymers based on ethylacrylate.....	153
<i>A.N. Azhkeyeva, R.K. Rakhmetullayeva, G.Zh. Yeligbayeva, Ye.M. Shaikhutdinov, M. Abutalip</i> Complexation of new linear copolymers based on ethyl acrylate.....	163
<i>Zh.Zh. Akzhigitova, A.M. Baiseitova, Yang Ye, J. Jenis</i> Investigation of chemical constituents of <i>Artemisia absinthium</i> .....	169
<i>A.A. Kapar, M.B. Alimzhanova, M.M. Sergazina, A.P. Abdykarimova</i> Determination of hazardous components in dairy products by chromatographic methods: a review.....	178
<i>A.K. Nurlybekova, Yang Ye, J. Jenis</i> Investigation of liposoluble constituents from the root of <i>Ligularia narynensis</i> .....	189
<i>G. Tuleushov, Martin Attfield</i> Synthesis of Metal Organic Framework materials by performing linker exchanges using solvothermal procedure.....	198

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## Injection of sorbent and subsequent analysis of blood cells

**Abstract:** This article indicates the adaptive reaction of the blood and lymph system that is affected by the extreme factor which is toxic substance. Change of the viscosity and overall protein composition of the blood and lymph, the erythrocytes and leukocytes of the rats which were receiving toxic substance for a long period of time and small changes in concentration of ions were observed. SUMS-1 (1g/kg) was used as a detoxicant in the experiment. The impact of toxic substances has decreased and animal state has improved after intake of SUMS-1. Regarding the composition and flow of the lymph, physico-chemical and biochemical parameters of blood cells were positive. When using the organic compounds lymph flow declined and composition reduced, whereas application of enterosorbent detoxifiers resulted conversely. After introduction into abdominal cavity SUMS-1 displayed high sorption properties. Sorbents are characterized by their ability to absorb toxic substances. In recent years, sorbents are widely used in various diseases, so it has great importance in the treatment and prevention. It is noticed that the physical and chemical properties of the animals blood receiving the sorbent improved. As can be seen from these data, the excretion of toxic substances from the body during sorbent reception was significant higher. The data obtained during the experiment showed that the decrease in erythrocytes was observed simultaneously with the reduction of hematocrit, which showed that the plasma portion of the blood increased and the appearance of hydrobialysis. It should be noted that the addition of enterosorbent to rats leads to a restoration of the volume of plasma and blood cells. The results provide strong evidence that SUMS-1 supplementation is beneficial in protecting the kidneys from CCl<sub>4</sub> toxicity. This kind work of research in this area should be continued.

**Key words:** composition of lymph, blood flow, SUMS-1, sorbent, biochemical parameters of blood, hematological parameters of blood, tetrachloromethane.

### Introduction

According to the forecasts of the World Health Organization experts (WHO, Geneva, 2003), the XXI century faces the global spread of diseases of cardiovascular system, liver and kidneys [1]. Due to the impact of anthropogenic factors, the appearance of malfunction, disabilities are constantly increasing, now they are on the first rank of social importance and will not lose urgency continue in future.

Heavy metal toxicity has proven to be a major threat and there are several health risks associated with it. The toxic effects of these metals, even though they do not have any biological role, remain present in some or the other form harmful for the human body and its proper functioning. They sometimes act as a pseudo element of the body while at

certain times they may even interfere with metabolic processes [2]. Few metals, such as aluminum, can be removed through elimination activities, while some metals get accumulated in the body and food chain, exhibiting a chronic nature. Various public health measures have been undertaken to control, prevent and treat metal toxicity occurring at various levels, such as occupational exposure, accidents and environmental factors. Metal toxicity depends upon the absorbed dose, the route of exposure and duration of exposure, for example acute or chronic. This can lead to various disorders and can also result in excessive damage due to oxidative stress induced by free radical formation [3].

Metals are substances with high electrical conductivity, malleability, and luster, which voluntarily lose their electrons to form cations. Metals are found

naturally in the earth's crust and their compositions vary among different localities, resulting in spatial variations of surrounding concentrations. Heavy metals are generally referred to as those metals which possess a specific density of more than 5 g/cm<sup>3</sup> and adversely affect the environment and living organisms. These metals are quintessential to maintain various biochemical and physiological functions in living organisms when in very low concentrations, however they become noxious when they exceed certain threshold concentrations. Although it is acknowledged that heavy metals have many adverse health effects and last for a long period of time, heavy metal exposure continues and is increasing in many parts of the world [4].

Carbon tetrachloride (CCl<sub>4</sub>) is a potent nephrotoxin, as it causes acute as well as chronic toxicity in kidneys. CCl<sub>4</sub> is a toxic chemical, widely used in the dry cleaning industry, in filling fire extinguishers, in the fumigation of grains, and as an insecticide [5]. Recent studies have shown that CCl<sub>4</sub> is associated with advanced production of free radicals leading to dysfunction of several organs [6]. Chronic CCl<sub>4</sub> treatment is a common practice to induce hepatic fibrosis, renal, pulmonary and testicular injuries, and cardiac tissue damage in rats as an experimental model.

Tissue damage by CCl<sub>4</sub> depends on the amount of dosage and duration of exposure of the experimental animals to this toxicant. Its action is based on membrane lipid peroxidation and induction of trichloromethyl radical ( $\bullet\text{CCl}_3$ ), resulting in severe cell damage [7-9]. It is evidenced that metabolic activation of CCl<sub>4</sub> by cytochrome P450 resulted in the production of trichloromethyl radical ( $\bullet\text{CCl}_3$ ) and peroxy trichloromethyl radical ( $\bullet\text{OCCl}_3$ ) that, in turn, initiate subsequent lipid peroxidation, responsible for injuries in various organs such as liver and kidney [10]. Therefore, it can be stated that CCl<sub>4</sub> is the well-characterized tool for the study of oxidative stress trials as it consistently generates free radicals with the implication of pathological environment.

These free radicals damage the integrity of liver cell membranes by releasing the cytosolic enzymes such as alanine transaminase, aspartate transaminase, alkaline phosphatase, and lactate dehydrogenase into the blood stream and elevating thiobarbituric acid reactive substances (TBARS) level with subsequent necrosis and inflammatory cell infiltration; affect physical parameters of kidney such as urinary and serum profile; increase lysosomal enzymes activities of testis and kidney; and decrease the activity of a diagnostic marker enzyme creatinine phosphokinase

(an enzyme responsible for ATP regeneration) in cardiac tissue [11].

Oral intake of magnesium also has beneficial effects on lipid metabolism and efficiency of insulin in maintaining glucose homeostasis in human subjects [12; 13]. Mg deficiency is known to decrease the level of GSH in erythrocytes and even inhibit its biosynthesis, and in agreement with these findings, magnesium supplementation was shown to induce a significant increase in GSH in kidney of mice treated with cadmium [14]. Magnesium intake is capable of decreasing the blood concentration of vanadate in rats and the cadmium level in blood, kidney, spleen, and bone marrow in rabbits. In addition, both oral and intraperitoneal supplementation of magnesium acetate were effective against cadmium toxicity.

Benzene is among the most widely used chemicals in the world. It is mainly used to make materials such as plastics, rubbers, dyes, detergents and pesticides. It can also be found in automobile and industrial fumes [15]. Its toxic effects on blood cells are well documented and it is known to cause different kinds of leukemia, multiple myeloma and non-Hodgkin lymphoma. However, the exact mechanisms involved in its toxicity are not yet understood [16].

Researchers from Universiti Kebangsaan Malaysia built on previous research that shows that benzene is metabolized in the liver, then its metabolites are further metabolized in the bone marrow to produce 1,4-benzoquinone (1,4-BQ), which is known for its toxic effects on blood cells. The team studied the effects of 1,4-BQ on haematopoietic stem cells (HSCs are stem cells found in the bone marrow that can give rise to any kind of blood cell) and haematopoietic progenitor cells (the bone marrow can differentiate into only one specific type of blood cell) in mic [17].

Adsorption at various interfaces has concerned scientists since the beginning of this century. This phenomenon underlies a number of extremely important processes of utilitarian significance. The technological, environmental and biological importance of adsorption can never be in doubt. Its practical applications in industry and environmental protection are of paramount importance. Crucial progress in theoretical description of the adsorption has been achieved, mainly through the development of new theoretical approaches formulated on a molecular level, by means of computer simulation methods and owing to new techniques which examine surface layers or interfacial regions [18; 19].

An effective method for removing dissolved organic substances that cause tastes, odours, or colours is adsorption by activated carbon. Adsorption is the

capacity of a solid particle to attract molecules to its surface. Powdered carbon mixed with water can adsorb and hold many different organic impurities. When the carbon is saturated with impurities, it is cleaned or reactivated by heating to a high temperature in a special furnace. The adsorbed molecules are usually referred to collectively as the adsorbate. An example of an excellent adsorbent is the charcoal used in gas masks to remove poisons [20-22].

During the study, the lymphatic system revealed the results of studies of toxic substances. During long-term use of organic poisons, one can notice changes in lymphatic flow, arterial pressure, total protein composition, lymphatic and viscous blood pressure, and changes in potassium ion concentrations in rats. In experiment we used sorbent SUMS-1 (1 gr/kg) as detoxicant. In general it has positive effect on lymphatic and lymphatic components, on blood cells, on the physico-chemical and biochemical outcome.

The regeneration of blood cells after the action of sorbents significantly influenced the positive results in the lymphocyte counts in the blood cells. The use of enterosorbents rapidly decreased the negative effects of organic poison to lymphodynamics and lymphatic structure. After the sorbents, the biochemical and physico-chemical properties of the plasmids and lymphs improved. This determines the highest concentration of sorbent in SUMS-1 [23].

### Materials and methods

55 laboratory rats with weight of 220-250 g served the objects of the study. They were divided into 4 groups. The first group was a control group ( $n=10$ ), the second group ( $n=15$ ) and the third group ( $n=15$ ) were experimental group. Experiments were conducted after 10 and 30 days afterwards injecting intoxication, this group's rats received 0.3 mL of  $CCl_4$  three times a week. The fourth group ( $n=15$ ) obtained the SUMS - 1 sorbent (1/kg) after the injection of  $CCl_4$ .

Research work was carried out in the laboratory of physiology and lymphatic systems of the Institute of Human and Animal Physiology, MES RK and in the Laboratory of Human and Animal Physiology, Department of Biophysics and Biomedicine, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University. Morphology of the blood cells: erythrocyte, platelet, leukocyte indices were defined by using Sysmex KX-21 - hematological analyzer (HORIBA, Japan). Amount of blood oxygen and Ph index were defined with the help OSMETECH

OPTI™ CCA-hematological analyzer (OPTI, USA). All animals were identified to have electrolytes from blood plasma, lymph and urine by ion analyzer. Experimental data was processed using the Microsoft Excel for Windows 2007 application, spreadsheet Excel 7.0.

Physical-chemical indicators of blood cells were determined by using the method of Sukharev, its viscosity is decided by the VK-4 viscosimeter and used well-known haemotoxic method. Total protein, urea, and creatinine concentrations in the lymph and plasma were measured by using Bio-Lachema-Test kits. Plasma activities of ALT and AST and bilirubin content were measured and thymol test was carried out by the Routine methods. The homeostasis disorders caused by  $CCl_4$  were corrected by adsorbent (1 g/kg), shown on Figure 1.

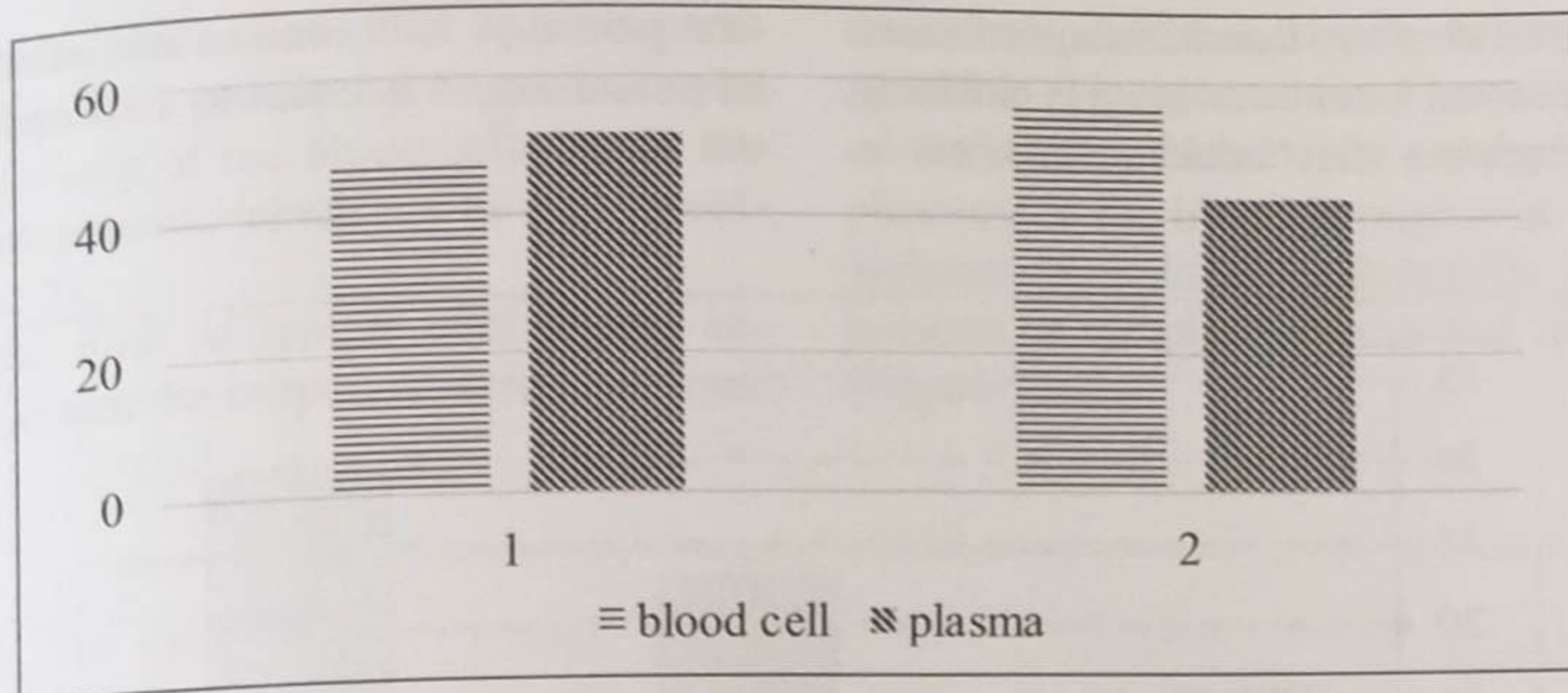
### Results and discussion

Also known by several other names, hematocrit is the most important determinant of the whole blood viscosity. It is the volume percentage of red blood cells in blood and normally counts  $47\pm 5\%$  for men and  $42\pm 5\%$  for women. It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count. Hematocrit levels can indicate possible disease. An abnormally low hematocrit may suggest anemia, a decrease in the total amount of red blood cells, while an abnormally high hematocrit is called polycythemia. Both are potentially life-threatening.

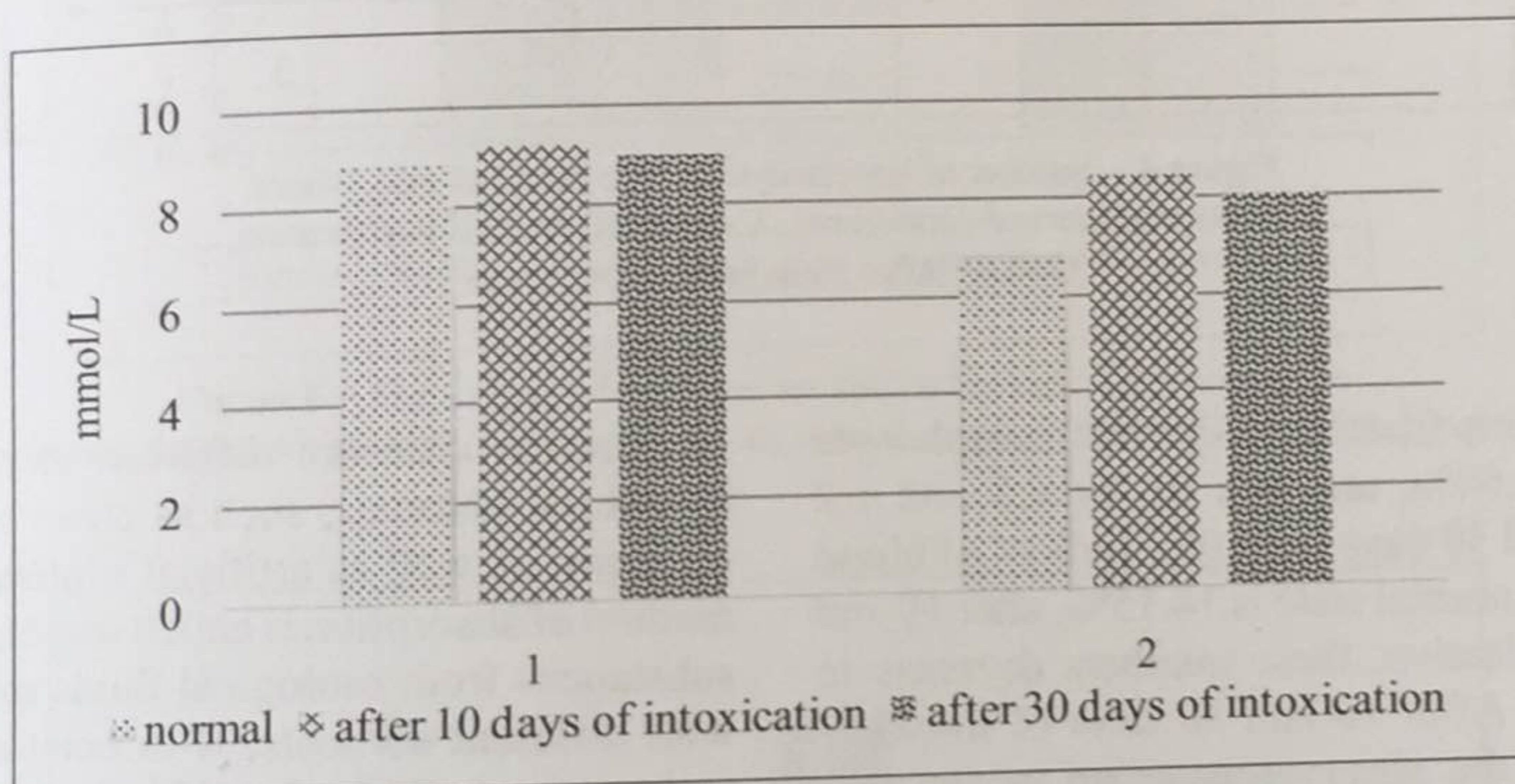
Blood viscosity and vascular resistance affect total peripheral resistance to blood flow, which is abnormally high in the established phase of primary hypertension. In accordance with hematocrit indices of plasma portion of the blood was decreased. When various changes appear, blood cells perform several functions in accordance with features (Figure 1).

According to the results, hematocrit indices that the amount of blood cells in poisoned rats has been decreased obviously and amounted to an average of 11-15%.

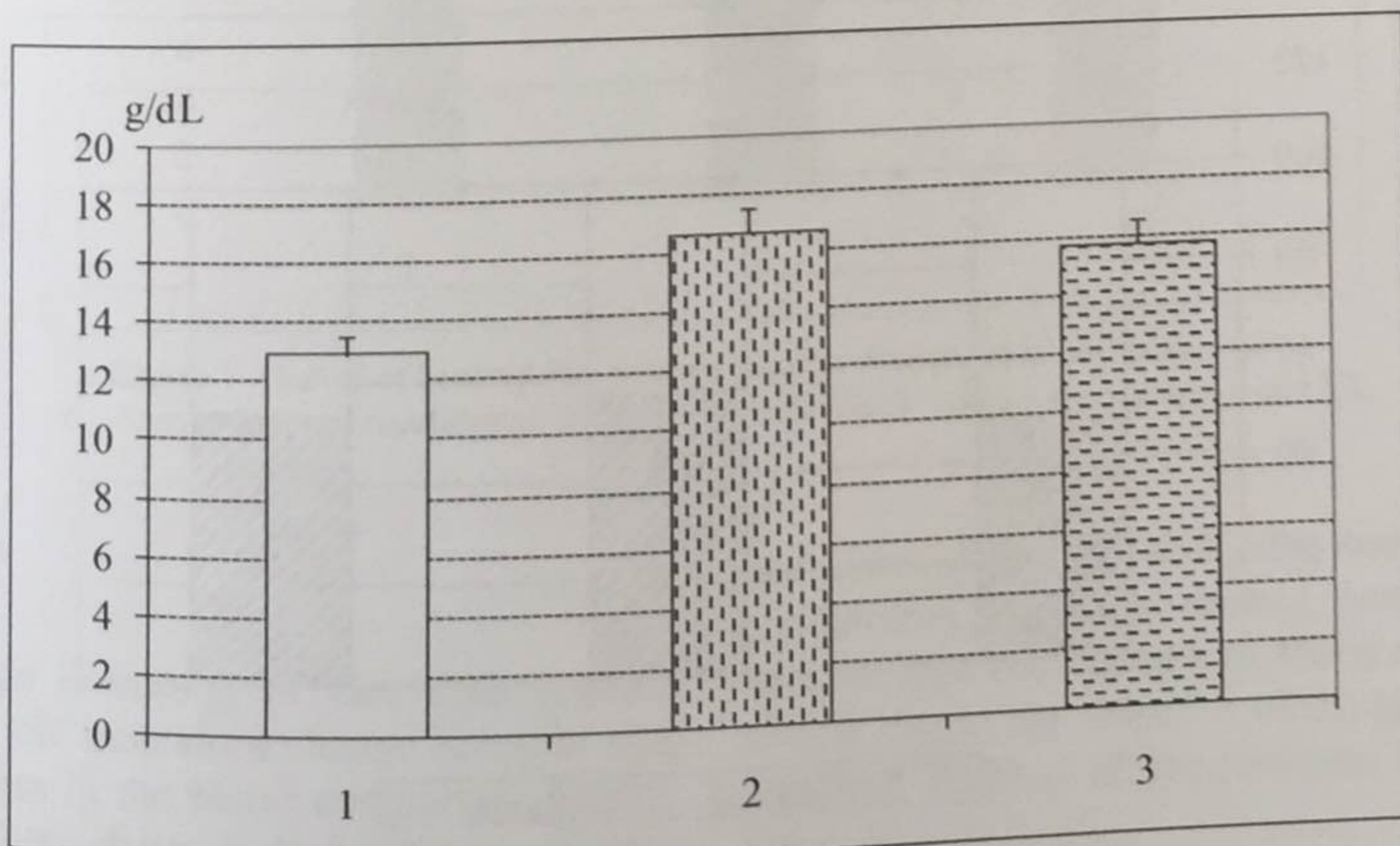
As can be seen from the Figure 2, 10 days since poisoning the number of the red blood cells decreased by 8.9%, and after 30 days, it rose to 17.14% (in the observation group  $8.87 \pm 0.1$  mmol/L). Normally, the number of leukocytes  $6.79 \pm 0.2$  mmol/L, after 10 days poisoning  $4.62 \pm 0.1$  mmol/L, and after 30 days, the number of leukocytes rose considerably to 80.47% (Figure 2).



**Figure 1** – Changes in hematocrit indices in normal rats and in rats poisoned with  $CCl_4$ , where: ordinate axis – the percentage of hematocrit; X-axis: 1 – normal group, 2 – after intoxication with  $CCl_4$



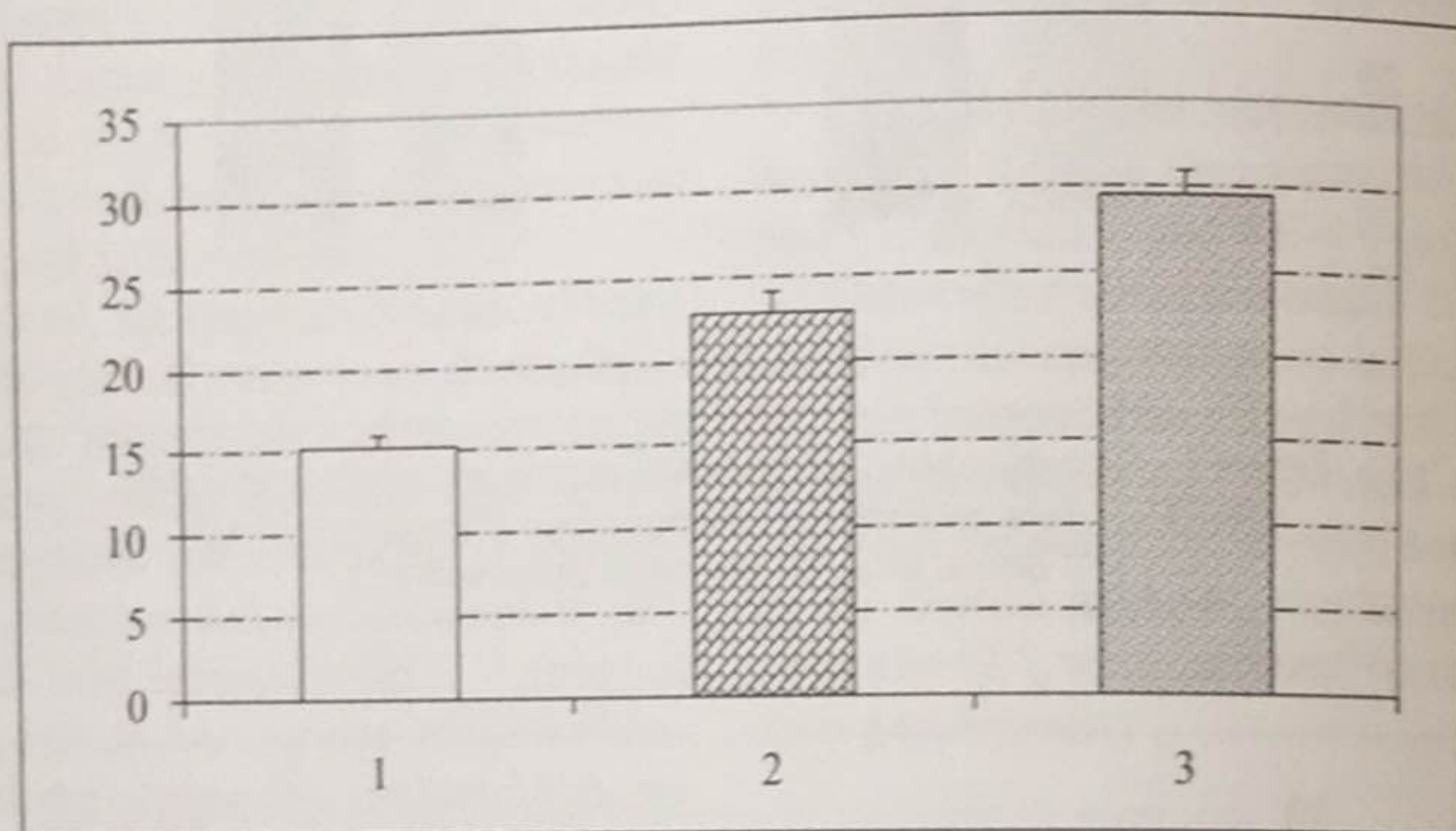
**Figure 2** – Changes of erythrocytes and leukocytes after intoxication, where: ordinate axis – amount of blood cells, mmol/L; X-axis: 1 – red blood cells; 2 – white blood cells



**Figure 3** – The amount of blood hemoglobin of normal and poisoned rats, where: 1 – control (normal conditions), 2 – after 10 days of intoxication, and 3 – after 30 days of intoxication

Figure 3 shows that after 10 and 30 days of intoxication, hemoglobin and hematocrit level is climbing. The level of hemoglobin of rats during the observa-

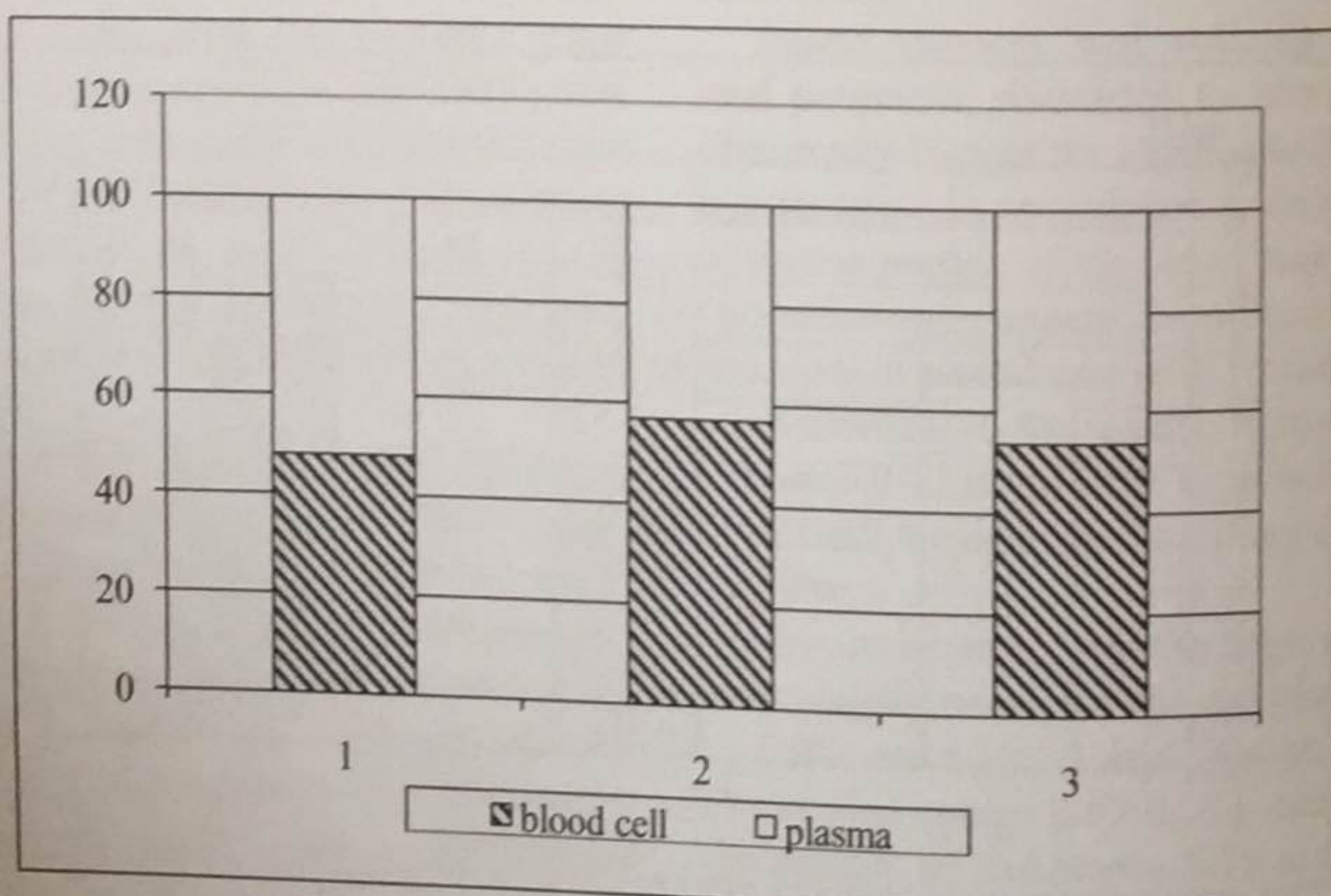
tion period is 12.9, and in rats after 10 and 30 days of poisoning, it is equal to 16.7 and 15.8 g/dL (Figure 3).



**Figure 4** – Number of lymphocytes in the blood of rats, where: 1 – control (normal conditions), 2 – after 10 days of intoxication, and 3 – after 30 days of intoxication

As can be seen from the Figure 4, lymphocyte indices reach 59.08%, and rise up by 1.3 and 1.2 times after 10 and 30 days ( $p < 0.05$ ). Indices of blood monocytes in the normal state is 14.15%, after 10 and 30 days of intoxication, these numbers decrease to 35.8 and 47.9%. After 10 and 30 days of intoxication, platelets of the observation group increase by 2.3 times in compare to control.

The sorbents are different in nature, presented by natural betonies, such as clean soil consisting of minerals, as well as artificial synthetic sorbents. The method of absorption is called sorption therapy. Toxic substances from biological fluids might be removed with different sorbents, with enterosorption playing an important role in the reduction of the pathological condition of the body.

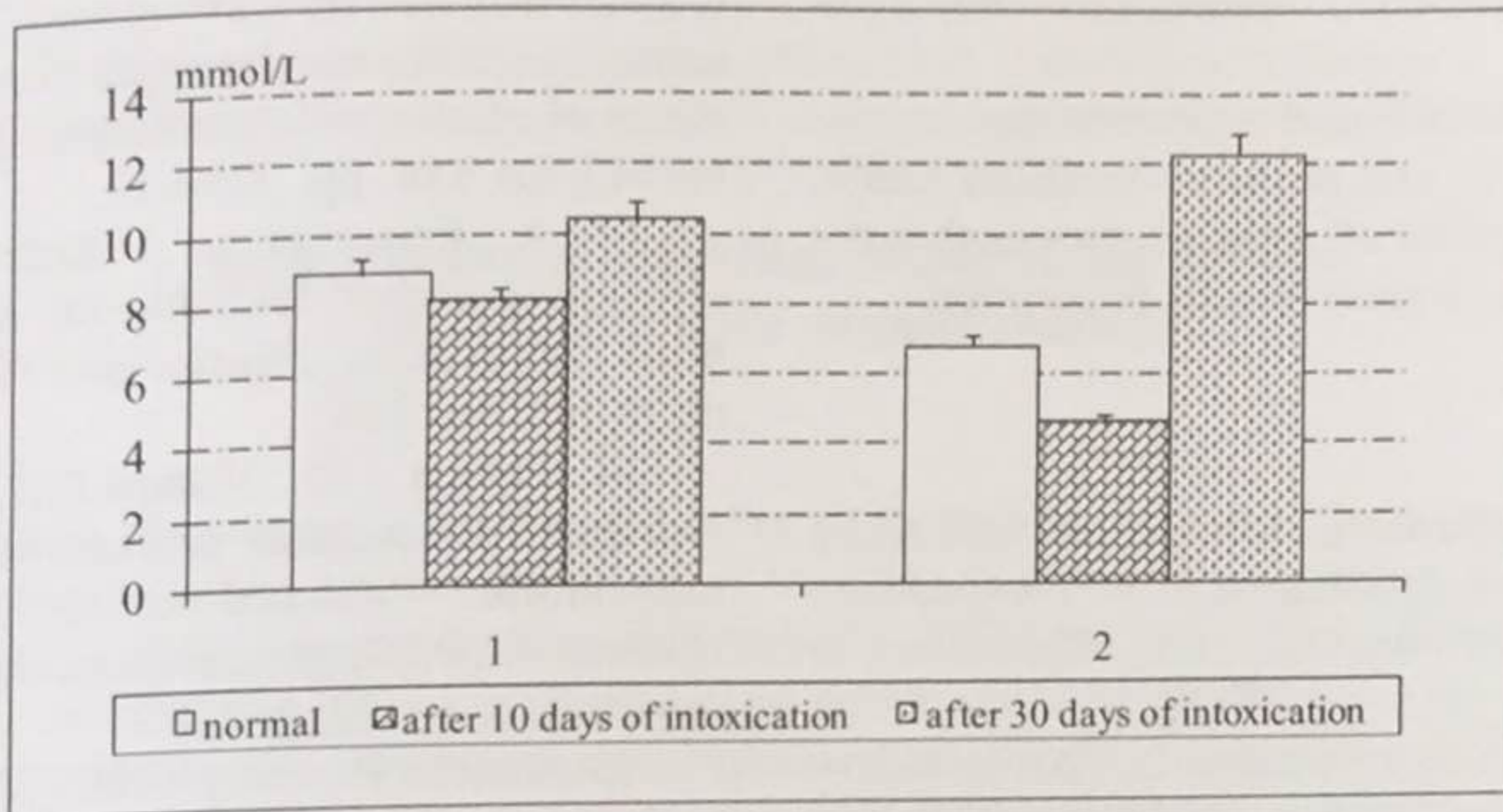


**Figure 5** – Hematocrit indices of intoxication with dichloromethane and the perception of enterosorbent, where: ordinate axis: the percentage of hematocrit; X-axis: 1 – control (normal conditions), 2 – after intoxication, 3 – taking the sorbent with CCl<sub>4</sub>

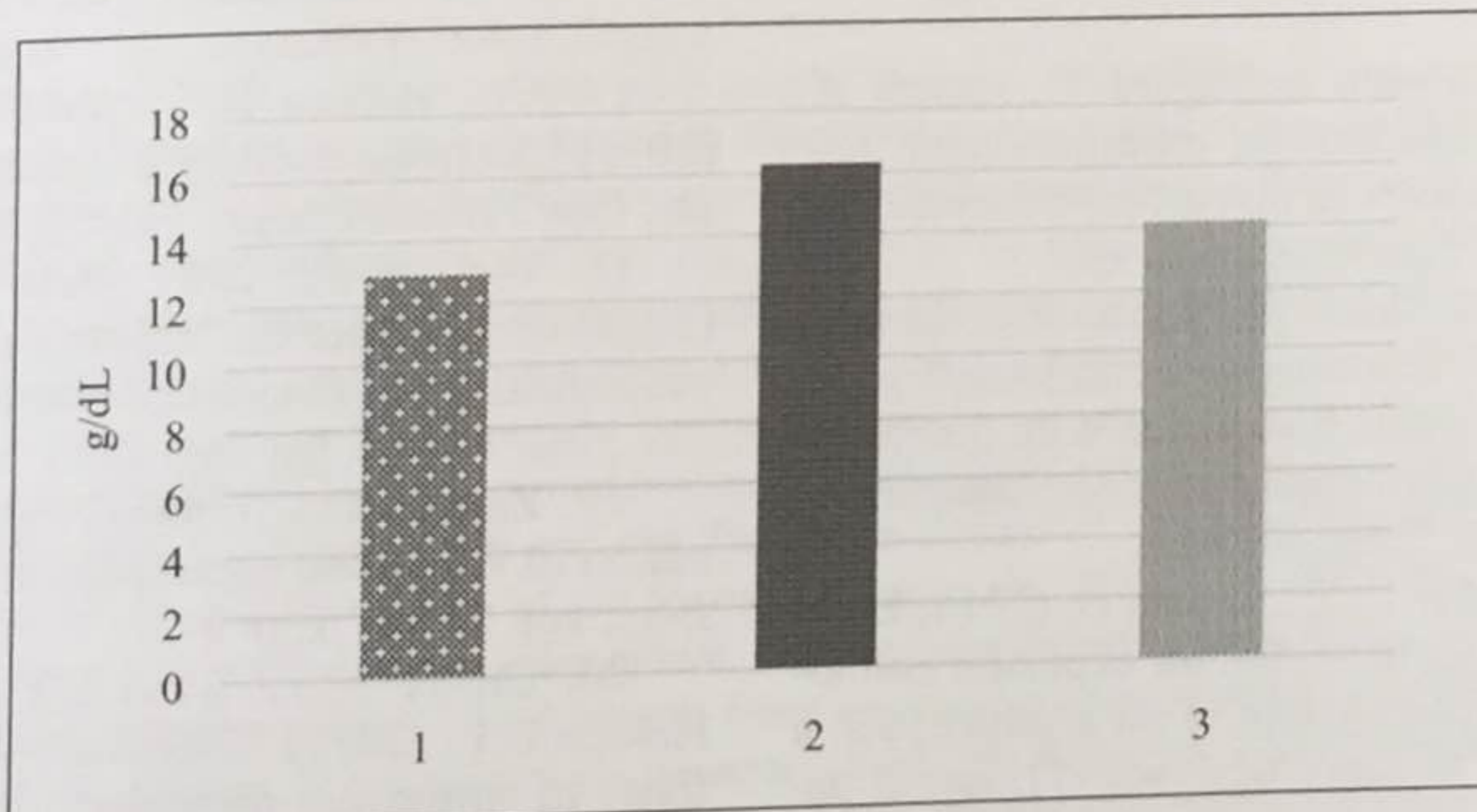
The experimental data shows that by hematocrit indices, levels of plasma part in the blood increased and decreasing volume of red blood cells shows the appearance of polyplasmia which can be seen simultaneously (Figure 5).

Improving the flow of lymph after taking enterosorbent accelerates the output of organic poisons

from microcirculation zone. In experiment, sorbent SUMS-1 (1g/kg) was used as detoxicant. Influence of the sorbent blood cells restored, indices of lymphocytes in the blood is improved. Usage of enterosorbents immediately reduced the impact of organic poisons on lymph dynamics and lymph in the blood (Figure 7).



**Figure 6** – The indices of erythrocytes and leukocytes in the perception of sorbent after intoxication with dichloromethane, where: 1 – red blood cells, 2 – white blood cells



**Figure 7** – Level of hemoglobin in animals after enterosorbent influence, where: 1 – control (normal conditions), 2 – after intoxication, 3 – taking the sorbent with CCl<sub>4</sub>

## Conclusion

1. During the poisoning of rats with organic toxicants, blood pH indicators change towards acidosis. The changes in the blood compared with the period of experiment shows that after 10 days erythrocytes number decreased by 8%, and after 30 days it increased by 17%, as well as the first 10 days the leukocytes number decreased by 31%, and after 30 days increased to 20%. From these result, we can see

the more influence of CCl<sub>4</sub>, the detrimental effect on the internal state of the animal, lymph dynamics and biochemical composition of blood and lymph. In addition, due to the influence of the sorbent blood cells restored, indexes of lymphocytes in the blood improved.

2. Usage of enterosorbents significantly reduced the adverse impact of organic poisons on lymph dynamics and composition of lymph. The regeneration of blood in rats after the injection of sorbents prove



that the sorbent SUMS-1 has a good quality of sorption. Sorbent SUMS-1 showed that it is possible to restore changes in the organism after intoxication. This shows that the work needs further researches. In experiment, applied sorbent SUMS-1 (1g/kg) as detoxicant which led to an improvement in the state of change which was the result of organic poisoning, in general terms – it became known that it has a positive effect on lymph flow, the cell lymph, blood cell elements, physical-chemical and biochemical parameters.

The regeneration of blood plasma in rats and renewal of biochemical and physical-chemical lymph indices after the applied of sorbents proves that the sorbent SUMS-1 has a good quality of sorption.

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