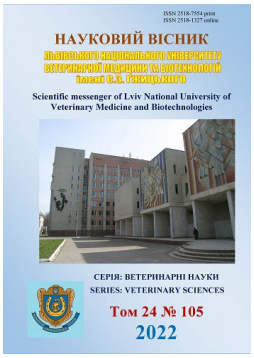

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Experimental assessment of the toxicity of a cardiac drug based on a phosphodiesterase-3 inhibitor and ethylmethylhydroxypyridine succinate

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The study aimed to establish the parameters of chronic toxicity of the newly developed drug based on phosphodiesterase-3 inhibitor and ethylmethylhydroxypyridine succinate in experiments on laboratory animals. The analysis was performed on white sexually mature young male Wistar rats weighing 170–185 g. Four groups of white rats were formed. The first experimental group was administered Bendamine based on a phosphodiesterase-3 inhibitor and ethylmethylhydroxypyridine succinate at a therapeutic dose. Rats of the second experimental group were injected with the experimental drug in a 5-fold dose. Rats of the third experimental group were administered the drug in a 10-fold dose. The fourth group served as control. The study of chronic toxicity of Bendamine in white rats indicates that long-term 30-day administration in therapeutic doses or 5-fold dose does not cause clinical signs of poisoning, as evidenced by the physiological limits of fluctuations in the studied morphological and biochemical parameters of blood rats. Prolonged administration of Bendamine to rats in a 10-fold dose is accompanied by a slight suppression of the body's physiological state, as indicated by a decrease in total erythrocytes and hemoglobin by 10.1 % against an increase in white blood cells by 59.8% ($P < 0.001$). In addition, there was a decrease in the functional state of the liver, as evidenced by a probable reduction in total protein by 8.0% and urea – by 13.5 %, as well as an increase in ALT, AST, and alkaline phosphatase by 31.6 %, 7.4 %, and 53.9% respectively. Probable changes in the coefficients of liver and spleen mass have been established. When administered intramuscularly to rats with the drug Bendamine for 30 days, the macroscopic and microscopic structure of the studied internal organs is preserved in all groups of animals. The second experimental group revealed reversible moderate histostructural changes in the liver and kidneys. In rats treated with ten times the therapeutic dose of the drug, histologically found hemodynamic disorders and alterations in dystrophic nature, mainly of protein origin, with focal localization in the parenchyma of the liver, kidneys, and myocardium, which in most cases are reversible and result from the compensatory response. Macroorganism on the introduction of a high dose of the study drug.

Key words: *rats, toxicology, pharmacology, ethylmethylhydroxypyridine succinate, a phosphodiesterase-3 inhibitor, Bendamine.*

Експериментальна оцінка токсичності кардіологічного препарату на основі інгібітора фосфодиестерази-3 та етилметилгідроксипіридину сукцинату

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Метою роботи було у дослідах на лабораторних тваринах встановити параметри хронічної токсичності новоствореного препарату на основі інгібітора фосфодиестерази-3 та етилметилгідроксипіридину сукцинату. Дослідження проводили на білих статево-зрілих молодих щурах-самцях лінії Вістар масою 170−185 г. Було сформовано чотири групи. Першій дослідній групі вводили препарат "Бендамін" на основі інгібітора фосфодиестерази-3 та етилметилгідроксипіридину сукцинату у терапевтичній дозі. Щурам другої дослідної групи вводили дослідний препарат у 5-ти кратній дозі. Щурам третьої дослідної групи вводили препарат у 10-ти кратній дозі. Четверта група служила контролем. Дослідження хронічної токсичності Бендаміну на білих щурах вказує на те, що тривале 30-добове його введення у терапевтичній дозі або у 5-кратній дозі, не викликає клінічних ознак отруєння, про що свідчать фізіологічні межі коливань досліджуваних морфологічних та біохімічних показників крові щурів. Тривале введення щурам дослідного препарату в 10-кратній дозі супроводжується незначним пригніченням фізіологічного стану організму, на що вказує зниження загальної кількості еритроцитів і вмісту гемоглобіну на 10,1 % на тлі підвищення кількості лейкоцитів на 59,8 % (P < 0,001). Крім цього встановлено зниження функціонального стану печінки, про що свідчить вірогідне зменшення вмісту загального протеїну на 8,0 % і сечовини – на 13,5 %, також підвищення активності АлАТ, АсАТ і лужної фосфатази на 31,6 %, 7,4 % і 53,9 % відповідно. Встановлено вірогідні зміни коефіцієнтів маси печінки та селезінки. За внутрішлункового застосування щурам препарату "Бендамін" упродовж 30 діб макроскопічна та мікроскопічна структура досліджуваних внутрішніх органів збережена у всіх групах тварин. У другій дослідній групі виявлені помірні гістоструктурні зміни в печінці і нирках, які мали зворотній характер. У щурів, яким застосовували 10-кратну терапевтичну дозу препарату, гістологічно встановлено порушення гемодинаміки та зміни дистрофічного характеру, переважно білкового походження, із вогнищевою локалізацією в паренхімі печінки, нирок та міокарду, що носять у більшості випадків зворотний характер і є наслідком компенсаторної реакції з боку макроорганізму на введення підвищеної дози досліджуваного препарату.

Ключові слова: *щури, токсикологія, фармакологія, етилметилгідроксипіридин сукцинат, інгібітор фосфодиестерази-3, Бендамін.*

Introduction

Cardiovascular diseases in dogs are widespread, but their causes are not always known. Cardiac pathology in animals may not be clinically evident for a long time, making it difficult to diagnose (Borgarelli & Buchanan, 2012; Varkholiak & Gutyj, 2018). In the case of detection of cardiovascular pathology in dogs, it is important to establish the degree of hemodynamic disorders and diseases of other organs that may be crucial for the course and prognosis (Borgarelli et al., 2007; Varkholiak et al., 2021). It is known that 10 % of all animals suffer from pathologies of the cardiovascular system of varying severity (Undhad et al., 2012). Animals of various breeds and age groups are admitted to the cardiologist. Cardiomyopathies are most often registered in large breeds of dogs (Trofimiak & Slivinska, 2021) (more often dilatation, less arrhythmogenic) and in small breeds – valvular pathology (endocardiosis of atrioventricular valves). Pericarditis and congenital disabilities are less common (Merveille et al., 2015; Ramírez et al., 2016; Yata et al., 2019; Morgan et al., 2020).

Disorders of the cardiovascular system lead to irreversible processes throughout the body, which in most cases end in death (Fox, 2012; Zhulikova, 2016; Tjostheim et al., 2019).

It should be noted that this pathology often takes a long time in a latent form, which does not manifest itself clinically and does not cause concern among owners. As a result, animals are referred to specialists during the period of decompensation of chronic heart failure. In addition, timely detection of pathology and the beginning of treatment can significantly extend the life of a sick animal (Hollmer et al., 2017).

The development, research, and implementation of new cardiac drugs for the prevention and treatment of animals with cardiovascular diseases, in the mechanism of which the development of oxidative stress, is on time and have significant prospects. It is advisable to use veterinary medicine for heart failure in animals with phosphodiesterase-3 inhibitors and antioxidants (Martyshuk, 2016; Lavryshyn et al., 2016; Martyshuk et al., 2016; Brett et al., 2019; Oldach et al., 2019; Martyshuk & Gutyj, 2019; Varkholiak et al., 2021; Martyshuk & Hutyi, 2021).

That is why the development of domestic cardio preparation for dogs for pathologies of the cardiovascular system and the study of its pharmaco-toxicological parameters is relevant.

The work aimed to establish the parameters of chronic toxicity of the newly developed drug based on phosphodiesterase-3 inhibitor and ethylmethylhydroxypyridine succinate in experiments on laboratory animals.

Materials and Methods

Experimental studies were conducted following the requirements of drug-biological experiments in selecting analogs, control, compliance with the same feeding and maintenance conditions during the investigation, and

accounting for results (Kotsiumbas et al., 2006; Todoriuk et al., 2018).

The study was performed on white sexually mature young male Wistar rats of 2–3 months of age, weighing 170–185 g, which were kept on the standard diet of the institute vivarium of the State Research Control Institute of Veterinary Drugs and Feed Additives. Four groups of white rats were formed. The first experimental group (R1) was administered the drug Bendamine in a therapeutic dose. The second experimental group (R2) of rats was administered the test drug in a 5-fold dose. The third experimental group (R3) of rats was administered Bendamine in a 10-fold dose. The fourth group served as control (K).

The drug in the above doses was administered at a particular time, daily for 30 days, orally, on an empty stomach, using a probe for laboratory animals.

Bendamine is an animal medicine that affects the cardiovascular system. The drug contains a phosphodiesterase-3 inhibitor and ethylmethylhydroxypyridine succinate. The latter has a pronounced antioxidant, antihypoxic, anxiolytic, membrane, and stress-protective activity.

At the experiment's beginning and end, the rats of the experimental and control groups were weighed. During the investigation, the clinical condition and behavior of the animals were monitored. Behavioral reactions of animals were studied according to the following criteria: horizontal motor activity, vertical motor activity, grooming, and vigorous activity.

On day 31 of the start of the administration, five white rats from each group were tested for liver detoxification function using a thiopental test. To do this, laboratory animals were administered 1 % sodium thiopental solution at a dose of 45 mg/kg. Then the average sleep time from when the animal took a lateral position was recorded (Kotsiumbas et al., 2006; Gutyj et al., 2016; 2017).

Simultaneously, the other five rats were tested for swimming by M. L. Rylova (Rylova, 1964). A glass aquarium was used for the experiment. The water column in the aquarium is 50 cm. The water temperature is 12–13 °C. Experimental animals were attached to the load (metal samples) – 5 % body weight. Before the experiment, the rat was weighed and attached to the tail of the load corresponding to its weight, then allowed to swim with experimental and control animals of approximately the same weight. Make sure that the animals are constantly swimming. An indicator of efficiency is when the animal can stay on the water. The animals swam the entire descent to the bottom.

The next day laboratory animals under light ether anesthesia were decapitated, performed hematological and biochemical studies according to generally accepted methods dissected, and determined the mass coefficients of organs compared with the control group (Malanin et al., 1988; Vlizlo et al., 2012).

All animal manipulations were performed following the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Official Journal of the European Union L276/33, 2010).

The analysis of research results was performed using the software package Statistica 6.0. Student's t-test assessed the probability of differences. The results were considered plausible at $P \leq 0.05$.

Results and Discussion

According to studies to determine the chronic toxicity of the newly developed cardiac drug, it was found that from giving white rats the drug in therapeutic doses and 5-fold and 10-fold therapeutic doses, no visible clinical signs of drug intoxication were observed. Throughout the experiment (30 days), appetite was maintained in experimental animals. Significant violations of appearance and general condition were also not detected. Probable changes in the behavior of experimental animals were not observed (Table 1), and the characteristic behavioral reactions reflected the normal functional state of the central nervous system. It is also worth noting the slight suppression of the body of rats of the third experimental group, which received ten times the dose of the drug.

Studies of emotional and behavioral responses of laboratory animals after administration of the drug “Bendamine” for 30 days in the therapeutic and five times higher than the therapeutic dose did not show a significant effect on the nervous system. Under these conditions, there was a tendency to suppress in rats given the drug “Bendamine” in a dose ten times higher than therapeutic, characterized by a decrease in horizontal (number of crossed squares) and vertical motor activity (number of lifts on the hind limbs), grooming (number washing). Under these conditions, the experimental (number of animal visits to the kidneys) and emotional (number of defecation and urination) activity did not differ from the animals of all experimental and control groups.

Table 1

Indicators of physiological condition and activity of white rats with 30-day administration of the drug “Bendamine” (M ± m, n = 6)

Group of animals	Indicators					
	Number of intersections	Number of racks	Number of visits to the burrows	Number of washes (grooming)	Number of defecations	Number of urinations
Control	13.5 ± 1.57	1.83 ± 0.60	4.17 ± 0.83	2.33 ± 0.42	2.33 ± 0.42	1.5 ± 0.43
First research	13.2 ± 1.57	2.0 ± 0.51	4.0 ± 0.93	2.5 ± 0.43	2.17 ± 0.31	1.16 ± 0.31
Second research	12.67 ± 1.33	1.5 ± 0.43	3.83 ± 0.70	2.16 ± 0.31	2.0 ± 0.45	1.33 ± 0.21
Third research	11.3 ± 1.08	1.0 ± 0.37	4.0 ± 0.68	1.66 ± 0.67	2.17 ± 0.40	1.67 ± 0.42

One of the integral indicators that reflects the level of metabolic processes in the body of animals in their intoxication is the change in body weight and individual internal organs. Owing to this, it is essential to study the toxicity of the drug "Bendamine" to determine individual internal organs' body weight and weight ratios. Figures 1 and 2 show body weight data and weight coefficients of individual internal organs of rats on the 31st day of the experiment to study the chronic toxicity of the newly developed drug. It was found that the introduction of the drug in a 10-fold dose caused a decrease in body weight of white rats by 5% compared with the control group (Fig. 1). Under these conditions, we noted that the rats of the first and second experimental groups tended to increase body weight slightly.

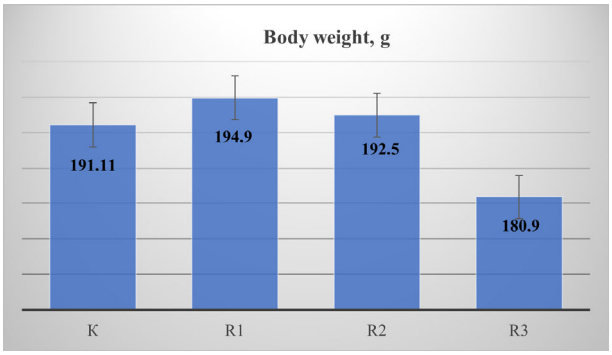

Fig. 1. Bodyweight of rats on the 31st day of the experiment to study the chronic toxicity of the drug "Bendamine" (M ± m, n = 6)

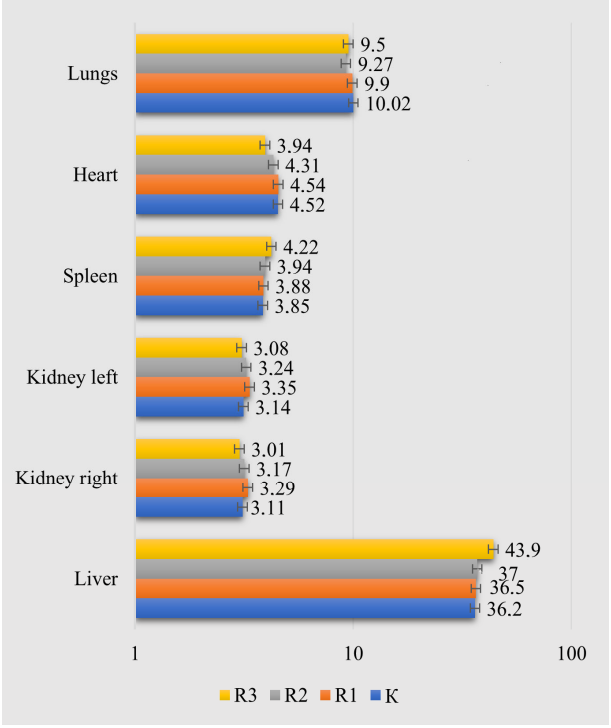

Fig. 2. Coefficients of mass of individual internal organs of rats on the 31st day of the experiment to study the chronic toxicity of the drug "Bendamine" (M ± m, n = 6)

In the study of relative weights of internal organs, it was found that the introduction of the drug in a 10-fold dose led to a possible change in the relative weight of the liver, spleen (43.9 ± 2.10; 4.22 ± 0.47) against the corresponding control values 36.2 ± 1.70; 3.85 ± 0.32).

The drug "Bendamine" in therapeutic and 5-fold doses contributed to a slight increase in the weight of the kidneys, while the introduction of the test drug at a much higher dose showed a decrease in the weight of the studied organs.

In the 10-fold dose of the drug relative to the therapeutic, a decrease in the heart rate of the third experimental group by 12.8% relative to the control group. The decrease in lung mass occurred after administration of the drug in 5- and 10-fold doses relative to the therapeutic, respectively, 9.27 ± 0.91 and 9.50 ± 0.75.

It is known that the duration of thiopental sleep in animals depends on the ability of microsomal liver enzymes to neutralize thiopental by glucuronidation. Usually, in clinically healthy rats, with a normal functional state of the liver, sleep lasts 25–30 minutes. The duration of sleep of animals was counted from the moment of their adoption of the lateral position to the first attempts to change it and expressed in minutes.

It was found that the rats of the control group had an average sleep time of 28.7 ± 1.66 minutes. In rats given the 10-fold therapeutic dose, the mean thiopental test increased by 23 %. The average swimming time of rats in this experimental group was also found to be 9.01 ± 1.32 min, while in the control group, the average swimming time was 12.58 ± 1.45 min (Fig. 3). In therapeutic and 5-fold doses, the drug did not affect the results of functional tests, as indicated by the average sleep time in rats of the second and third experimental groups, 29.4 ± 1.88 and 30.7 ± 0.65 min, and the average swimming time – 12.87 ± 1.57 and 11.69 ± 1.72 min.

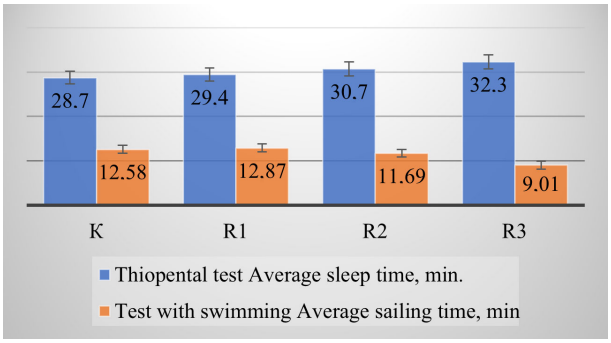

Fig. 3. The results of functional tests (M ± m, n = 5)

Therefore, the drug "Bendamine" after administration in therapeutic and 5-fold doses for 30 days does not significantly affect the functional state of the internal organs of experimental animals.

The study of hematological parameters in all animals administered the drug "Bendamine" showed a tendency to increase, compared with the control, the level of hemoglobin in the blood and the average hemoglobin content in erythrocytes, namely: in animals of group R1 by 10.3 and 4.7 %, groups R2 – by 5.1 and 4.1 % (Fig. 4).

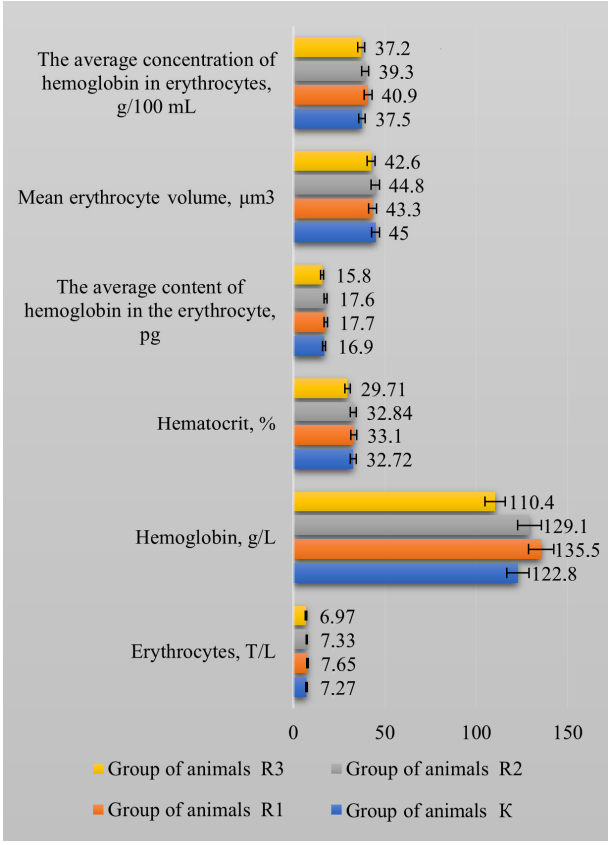

Fig. 4. Morphological parameters of the blood of white rats on the 31st day of the experiment to study the chronic toxicity of the drug "Bendamine" (M ± m, n = 6)

After administering the drug to rats at ten times the therapeutic dose, the level of hemoglobin in the blood of rats of the experimental group R3 decreased to 110.4 ± 3.2 g/L, and the average hemoglobin content in erythrocytes decreased by 6.5 % relative to the control group of rats. The number of erythrocytes in the blood of experimental groups R1 and R2 increased by 5.2 and 1 %, while the experimental group R3 decreased by 4.1 % compared to the control group. The mean erythrocyte volume was lowest in the R1 and R3 rats. In experimental group R2, the mean erythrocyte volume was close to that of the control group of rats.Administration of the drug "Bendamine" to animals of the first experimental group for 30 days led to an increase in hematocrit to 33.10 ± 1.50 %. The lowest hematocrit was in the third experimental group, 29.71 ± 2.53 %.

After administering the test drug to rats of the experimental groups, an increase in the number of leukocytes in the blood of rats R1, R2, and R3 were observed by 4.5, 26.9, and 59.7 %, respectively. The number of platelets in the blood increased only in the first experimental group. The number of lymphocytes in the blood of rats of the experimental groups increased relative to the administered dose. Thus, in the blood of the first experimental group, the number of lymphocytes increased by 1.7 %, in the blood of the second – by 4.1 %, and in the third – by 10 % relative to the control group (Table 2).

In the leukogram of the blood of rats, there was a decrease in the relative number of monocytes to 1.90 ± 0.49 % in the blood of experimental group R3, to 2.12 ± 0.25 % in the blood of rats of group R2 and up to 2.50 ± 0.50 % in the blood of rats of group R1.

The leukogram also showed a slight decrease in the number of rod and segmental neutrophils in the blood of rats of experimental groups R1 and R2. The lowest data were in rats of experimental group R3, which were fed the drug "Bendamine" at a dose ten times higher than the therapeutic, whereas compared to the control group, it decreased by 1.4 and 2.9 %.

The percentage of eosinophils and basophils was lowest in the third experimental group, where it was 1.40 ± 0.25 and 1.91 ± 0.24 %, respectively. In comparison, it was significantly higher in the control group of rats and ranged from 2.50 ± 0.50 and 1.97 ± 0.26 %.

Table 2

Leukogram in white rats on the 31st day of the experiment to study the chronic toxicity of the drug "Bendamine" (M ± m, n = 6)

Indexes	Control group	Research groups		
		First	Second	Third
Leukocytes, G/L	7.28 ± 1.13	7.61 ± 1.65	9.24 ± 2.10	11.63 ± 1.10**
Platelets, G/L	499.5 ± 67.4	553.0 ± 75.0	489.8 ± 97.1	497.0 ± 62.4
Lymphocytes, %	61.46 ± 1.93	62.50 ± 2.22	64.00 ± 3.05	67.60 ± 1.33*
Monocytes, %	2.60 ± 0.41	2.50 ± 0.50	2.12 ± 0,25	1.90 ± 0.49
Eosinophils, %	2.50 ± 0.50	2.00 ± 0.57	1.67 ± 0.66	1.40 ± 0.25*
Basophils, %	1.97 ± 0.26	1.83 ± 0.16	1.93 ± 0.22	1.91 ± 0.24
Neutrophils (segmental), %	25.30 ± 2.67	25.51 ± 1.64	25.00 ± 2.00	22.40 ± 1.17
Neutrophils (rod-shaped), %	6.17 ± 1.74	5.66 ± 0.90	5.28 ± 1.35	4.79 ± 1.50

In addition, a slight increase in the nonspecific part of the immune system of white rats, namely: phagocytic activity of neutrophils and phagocytic index (Fig. 5).

It was found that the highest phagocytic activity of neutrophils was in the blood of the third experimental group, where it increased by 2.43 % compared to the control group. Similar changes were found after the study of the phagocytic index, which was higher in the blood of experimental groups R1 and R3 by 2.1 and 21 %. In comparison, in the blood of rats of the experimental group, R2, this figure was lower by 7.6 % relative to the control group.

The results of biochemical studies of serum of rats on the 30th day of administration of the drug "Bendamine"

in different doses are given in the table. 3. It was found that at the therapeutic dose of the drug, there were no deviations in blood parameters that characterize the functional state of the liver.

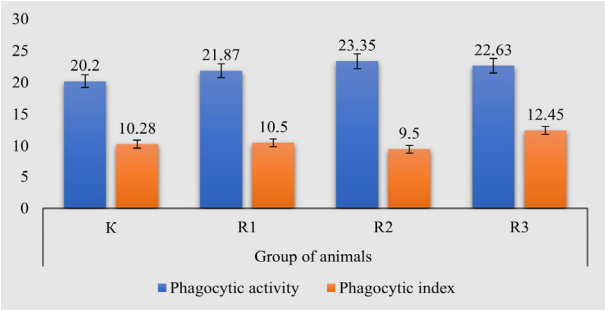

Fig. 5. Indicators of nonspecific immunity in white rats on the 31st day of the experiment to study the chronic toxicity of the drug "Bendamine" (M ± m, n = 6)

With the introduction of the experimental drug in 5-fold and 10-fold doses, rats of experimental groups R2 and R3 showed increased alanine aminotransferase activity by 12.7 and 31.6 % relative to the control group of rats. Similar changes were found in the determination of serum aspartate aminotransferase activity, which increased by 3.9 % in experimental group R2 and 7.4 % in experimental group R3 in rats of the control group. The serum of experimental rats found an increase in alkaline phosphatase activity by 11 % (R1), 25 % (R2), and 54 % (R3), respectively.

Protein-synthesizing and detoxifying functions of the liver in animals of the experimental groups did not change significantly, and the indicators that characterize them tended to increase slightly. Thus, the total protein level in the serum of the first experimental group increased by 4 %, and the concentration of urea increased by 1 %. Only in the third experimental group were inhibition of protein-synthesizing and detoxifying function of the liver, which indicates a decrease in serum total protein to 61.32 ± 4.02 g/L and urea concentration to 4.89 ± 0.47 mmol/L.

Table 3

Biochemical parameters of the blood of white rats on the 31st day of the experiment to study the chronic toxicity of the drug "Bendamine" (M ± m, n = 6)

Indicators	Control group	Research groups		
		First	Second	Third
ALT, unt/L	53.4 ± 2.45	54.2 ± 3.16	60.2 ± 4.10	70.3 ± 3.34**
AST, unt/L	173.4 ± 3.85	178.2 ± 5.01	180.1 ± 3.50	186.2 ± 4.24*
LF, mkkat/L	1.80 ± 0.30	2.00 ± 0.42	2.25 ± 0.25*	2.77 ± 0.42*
Creatinine, μmol/L	74.07 ± 2.66	67.4 ± 2,11*	70.16 ± 2.85	86.6 ± 3.14*
Urea, mmol/L	5.65 ± 0.20	5.70 ± 0.32	5.76 ± 0.26	4.89 ± 0.47
Total protein, g/L	66.65 ± 2.70	69.36 ± 3.55	66.91 ± 4.10	61.32 ± 4.02
Albumins, g/L	26.30 ± 1,82	31.00 ± 3.64	25.04 ± 3.36	18.95 ± 3.11*
Globulins, g/L	40.35 ± 3.61	38.36 ± 4.85	41.87 ± 5.20	42.37 ± 5.31

After studying the protein fractions in the serum of rats of experimental groups, it was found that the level of albumin in rats of the first experimental group increased by 17.9 %, while in the third experimental group decreased by 17.9 %. The level of globulins in the blood of rats of the first experimental group decreased by 5 %. In contrast, in rats of the second and third experimental groups, globulins increased by 4 and 5 % relative to the control group.

Probable ($P < 0.001$) increase in serum of rats of the third experimental group of creatinine level of 86.6 ± 3.14 μmol/L and a decrease in urea concentration to 4.89 ± 0.47 mmol/L by 20.5 % indicates the presence of systemic disorders of not only the liver but also the kidneys.

Therefore, summarizing the results of clinical, morphological, and biochemical parameters, it can be argued that the introduction of the experimental drug "Bendamine" in therapeutic and 5-fold doses for 30 days does not cause visible clinical signs of intoxication, and the studied hematological and biochemical parameters are not obtained animals of the control group.

After a pathological autopsy in the carcasses of rats of control and experimental groups, no external damage was found; the fur is uniform, smooth, shiny, well kept in the hair follicles, and natural openings are closed without a discharge.

An internal examination revealed that the location of the thoracic and abdominal cavities was anatomically correct. Peritoneum smooth, shiny, moist, without layers. Content in the thoracic and abdominal cavities - little, transparent, watery consistency. Lungs pale pink, loose, divided into lobes, pulmonary and costal pleura smooth, shiny without layers. The heart is conical; the pericardium is transparent without layers, and the myocardium is elastic and uniformly colored red. Stomach, small intestines are moderately filled with food masses, large intestines - with formed fecal masses, mucous membrane pale pink, shiny, moist, without layers. Liver dark red, smooth capsule, sharp edges, with a characteristic structure in section, elastic consistency, in rats receiving ten times the therapeutic dose of Bendamin, noted the presence of single foci of light brown color with a smoothed pattern of parenchyma on the incision.

Bean-shaped kidneys, dark red, the edges of the incision converge, the boundary between the cortical and cerebral areas is preserved, and the capsule is easily removed; in some rats receiving ten times the therapeutic dose of Bendamine, observed a slight increase in organ with the uneven color of the subcapsular surface. Spleen dark cherry color, sharp edges, the cut structure is preserved, the scrape is small or moderate in various individuals. The bladder is

moderately filled with urine; the wall is not thickened, and the mucous membrane is pale pink, without layers.

Histological examination of the liver structure of all studied groups of rats was preserved and represented by a lobular structure. The radial placement of liver beams around the central vein, triad zone without structural features, and organ vessels devastated or moderately filled (Fig. 6). Hepatocytes are polygonal; the cytoplasm is homogeneous, well-colored, with round nuclei, clearly contoured (Fig. 7).

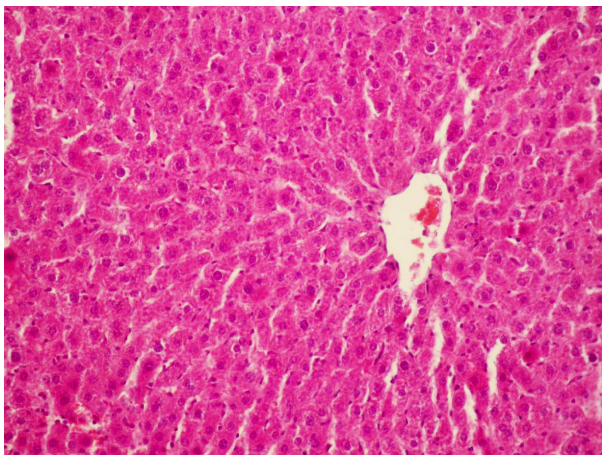

Fig. 6. Histological structure of the rat liver of the control group. Hepatocytes are radially arranged. Hematoxylin and eosin. ep. 10, lens 20

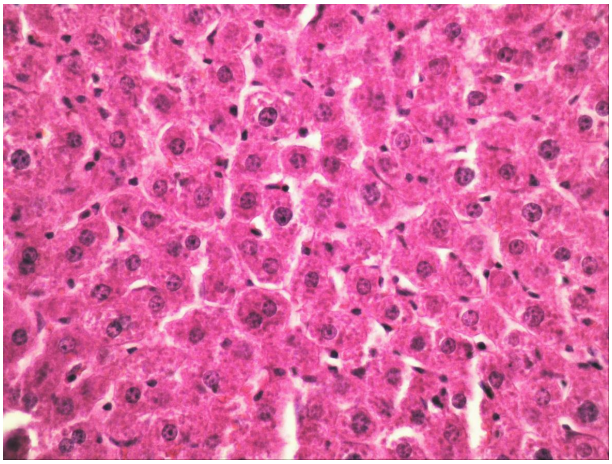

Fig. 7. Rat liver of the control group. Hepatocytes are polygonal; the cytoplasm is homogeneous, and the nucleus is preserved. Hematoxylin and eosin. ep. 10, lens 40

In rats of the second experimental group, given a 5-fold dose of the drug, we observed moderate dilation of Intra lobule capillaries and venous lumen, granular dystrophy of hepatocytes in the centrilobular part of the lobe.

Rats of the third experimental group, which received a 10-fold therapeutic dose of the drug Bendamine, more often found a discomplexation of the lamellar structure of the liver lobes. Hepatocytes were placed out of order, sinusoidal capillaries dilated. The presence of hepatocytes with heterogeneous cytoplasmic color and granularity was observed over the entire area of the lobes. Enlightened hepatocytes with enlightened cytoplasm were more common. Among them were cells with lysed and pyknotic nuclei and blurred cell contours. Isolated clusters of round-cell elements were observed in the triad region. The identified changes indicate the development of protein dystrophy and necrobiotic processes (Fig. 8–11).

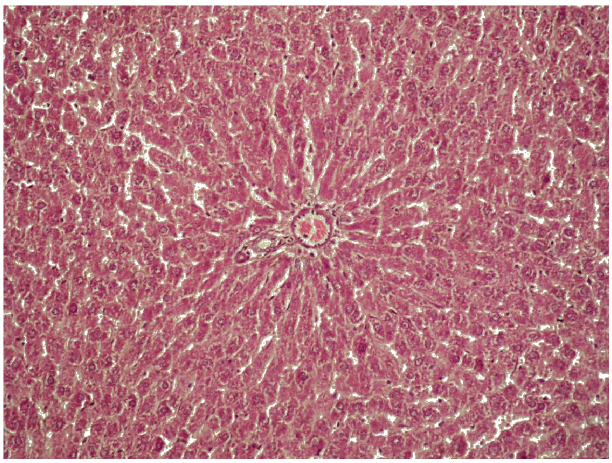

Fig. 8. Histological structure of rat liver of the second experimental group. Triad. Moderate varicose veins. Hematoxylin and eosin. ep. 10, lens 10

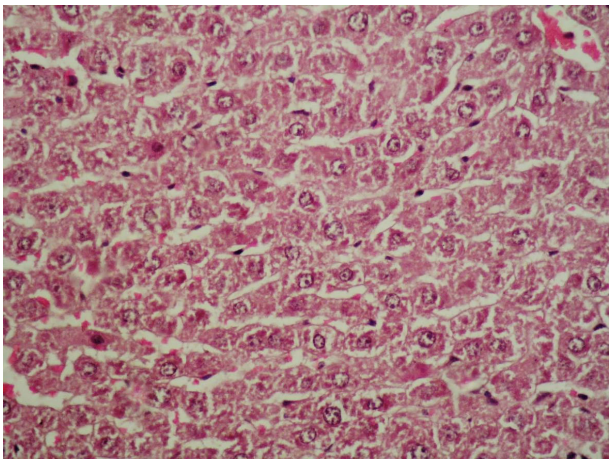

Fig. 9. Rat liver of the third experimental group. Discomplexation of the lamellar structure of the lobe. Sinusoidal capillaries are dilated. Hematoxylin and eosin. ep. 10, lens 20

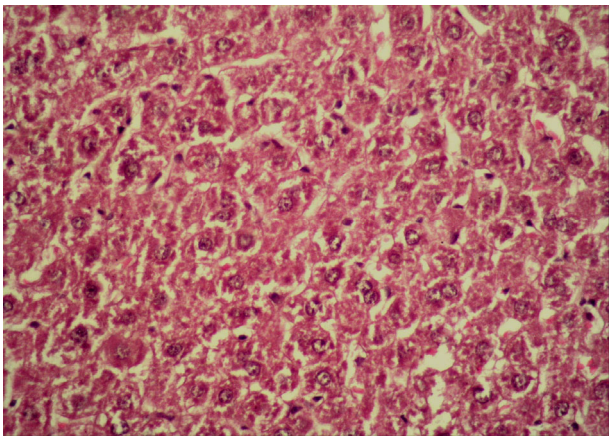

Fig. 10. Rat liver of the third experimental group. The cytoplasm of hepatocytes is granular, unevenly stained, devastated; some nuclei are hypertrophied. Hematoxylin and eosin. ep. 10, lens 20

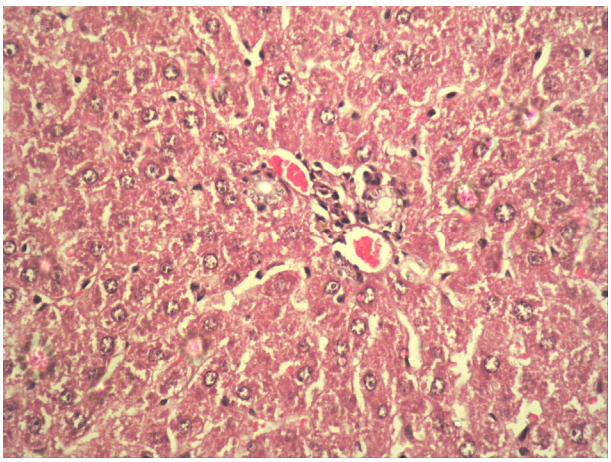

Fig. 11. Rat liver of the third experimental group. Minor cellular infiltration in the hepatic triad. Hematoxylin and eosin. ep. 10, lens 20

The histological structure of the kidneys of rats of all study groups is preserved. No changes were detected in rats of the first and second experimental groups (Fig. 12–14).

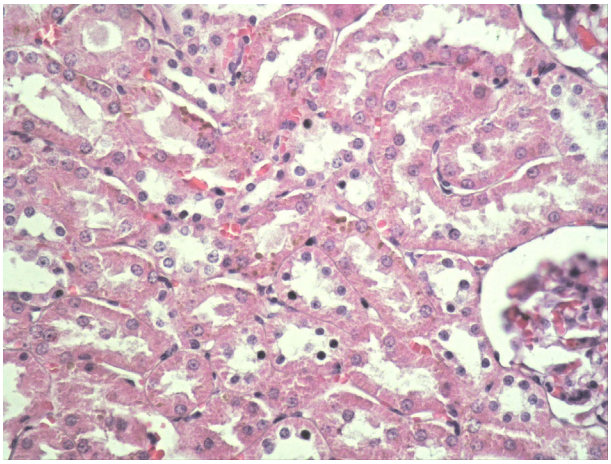

Fig. 12. Tortuous renal tubules of the rat control group. Hematoxylin and eosin. ep. 10, lens 20

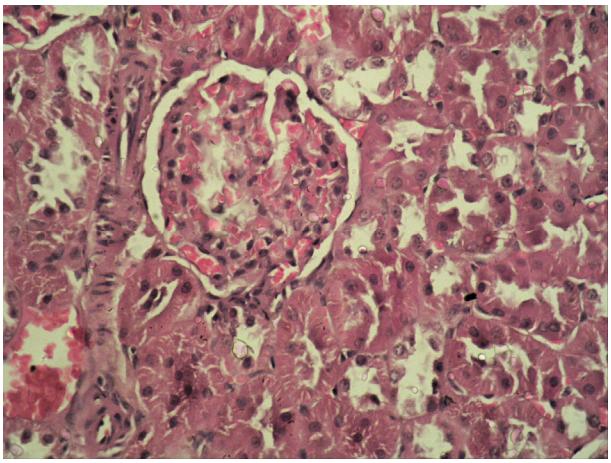

Fig. 13. Renal glomerulus and tortuous canal rats of the first experimental group. Hematoxylin and eosin. ep. 10, lens 40

In the kidneys of rats treated with a 10-fold therapeutic dose of the drug "Bendamine" for 30 days, focal granular protein dystrophy of the epithelium of the tortuous renal tubules with narrowing of their lumen was detected. The nuclei of some nephroepitheliocytes are enlarged, some with signs of karyopyknosis and karyorrhexis. The capillary network of individual glomeruli is compacted, the lumen between the Shumlyansky-Bowman capsule is enlarged, and the contents are absent (Fig. 12–16).

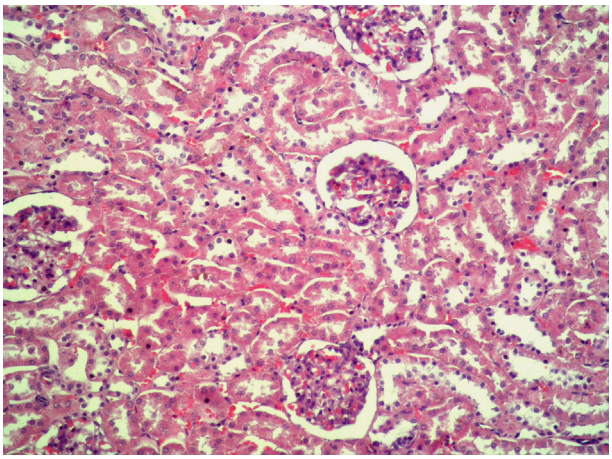

Fig. 14. Histological structure of the rat kidney of the second experimental group. Hematoxylin and eosin. ep. 10, lens 10

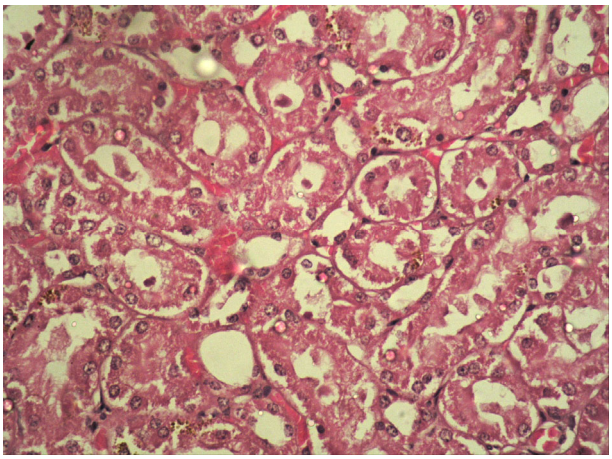

Fig. 15. Rat kidney of the third experimental group. Granular dystrophy of the epithelium of the tortuous renal tubules. Hematoxylin and eosin. ep. 10, lens 20

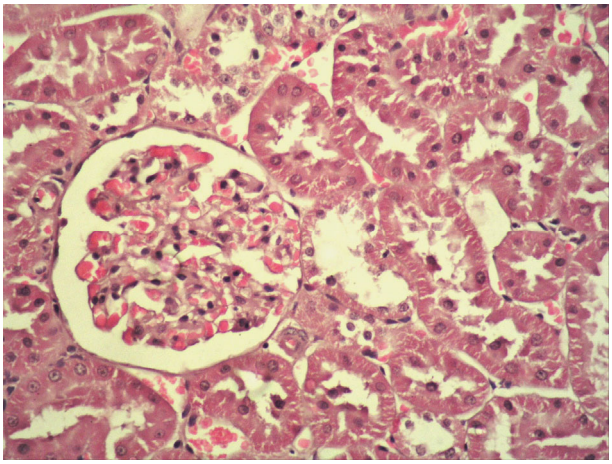

Fig. 16. Rat kidney of the third experimental group. Sealing of the capillary network of the renal glomerulus. ep. 10, lens 40

The myocardium of rats of the control and the first and second experimental groups is represented by bundles of muscle fibers with preserved transverse striation; the nuclei of cardiomyocytes are clear and located in the center of the cell (Fig. 18). A moderate accumulation of transudate was observed in the intermuscular lumen (Fig. 19). In the myocardium of rats of the third experimental group, foci of fibrosis, thickening, fragmentation of muscle fibers, and loss of transverse and longitudinal striation were observed.

Sarcoplasm in some places contained acidophilic granularity; the nuclei are mostly preserved, some swollen with low chromatin content, intermuscular lumen moderately dilated, impregnated with weakly oxyphilic transudate, indicating the development of moderate edema and dystrophic processes in the body (Fig. 17–21).

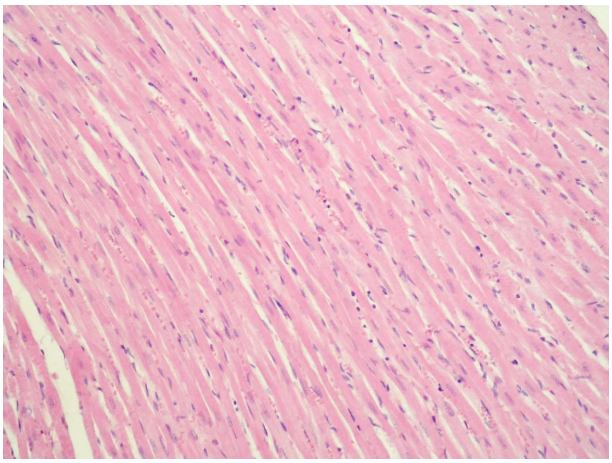

Fig. 17. Histological structure of the rat myocardium of the control group. Hematoxylin and eosin. ep. 10, lens 10

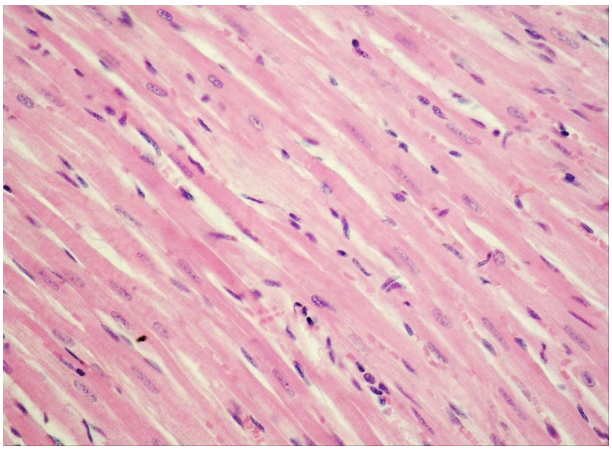

Fig. 18. Rat myocardium of the control group. The sarcoplasm of cardiomyocytes is homogeneous, the nuclei are clearly delineated. Hematoxylin and eosin. ep. 10, lens 20

Therefore, when administered intramuscularly to rats with the drug "Bendamine" for 30 days, the macroscopic and microscopic structure of the studied internal organs is preserved in all groups of animals. The second experimental group revealed reversible moderate histostructural changes in the liver and kidneys. In rats treated with ten times the therapeutic dose of Bendamine, histologically revealed hemodynamic abnormalities and alterations in dystrophic nature, mainly of protein origin, with focal localization in the parenchyma of the liver, kidneys, and myocardium, which in most cases are reversible and result from compensatory response macroorganism on the introduction of a high dose of the study drug.

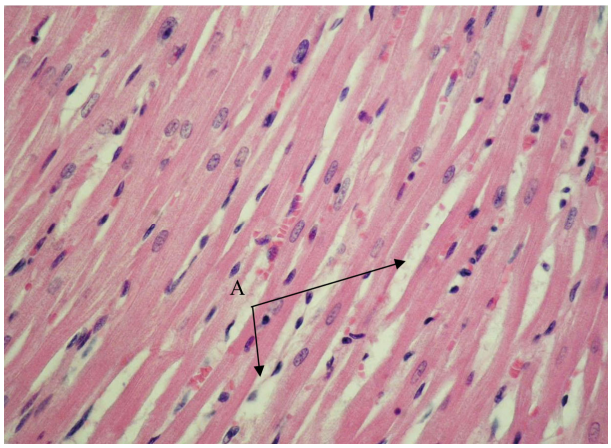

Fig. 19. Histological structure of the rat myocardium of the first experimental group. A – moderate intermuscular edema; Hematoxylin and eosin. ep. 10, lens 20

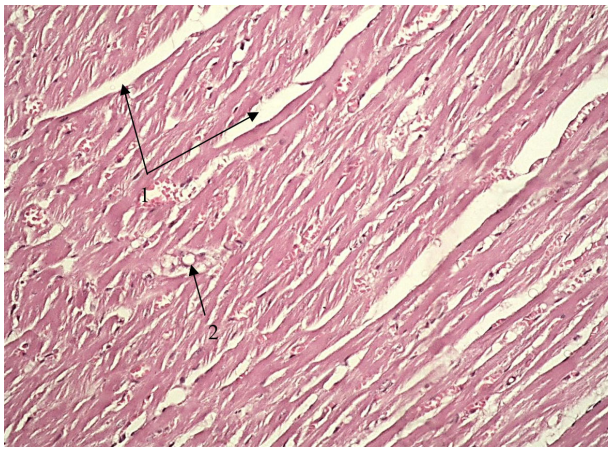

Fig. 20. Rat myocardium of the third experimental group. Loosening of connective tissue fibers, thickening of muscle fibers. Perivascular edema. Hematoxylin and eosin. ep. 10, lens 10

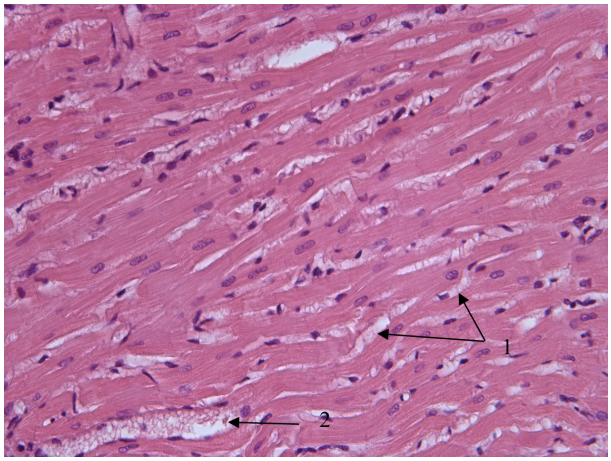

Fig. 21. Rat myocardium of the third experimental group. Permeation of intermuscular fibers with transudate. Hyperemia. Hematoxylin and eosin. ep. 10, lens. 40

Conclusions

1A study of the chronic toxicity of the drug "Bendamine" in white rats indicates that prolonged 30-day administration in therapeutic doses or 5-fold dose does not cause clinical signs of poisoning, as evidenced by physiological limits of fluctuations in morphological and biochemical parameters of blood rats.

2. Prolonged administration to rats of the drug "Bendamine" in a 10-fold dose is accompanied by a slight suppression of the physiological state of the body, as indicated by a decrease in total erythrocytes and hemoglobin by 10.1 % against an increase in white blood cells by 59.8 % ($P < 0.001$). In addition, there was a decrease in the functional state of the liver, as evidenced by a probable decrease in total protein by 8.0 % and urea – by 13.5 %, as well as an increase in ALT, AST, and alkaline phosphatase by 31.6 %, 7.4 %, and 53.9 % respectively. Probable changes in the coefficients of liver and spleen mass have been established.

3. Under the conditions of administration of 10 times the therapeutic dose of Bendamine to rats, there is a violation of hemodynamics of various organs on the background of dystrophic changes, protein origin, with focal localization in the parenchyma of the liver, kidneys, and myocardium. The established changes are a consequence of the compensatory reaction of the macroorganism to the introduction of an increased dose of the study drug and are mostly reversible.

Conflict of interest

The authors declare that there is no conflict of interest.

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