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Original research

The effect of a new feed additive of humic nature on morphometric indicators of laboratory rats depending on the dose

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Abstract. The study of the effects of natural biologically active substances, manifestations and consequences of their application to different species of animals or under different conditions is being conducted. Peculiarities of the implementation of such effects when used separately or in combination, in particular, humic acids with mineral or organic acids, creates opportunities for optimal combination or solution to the complexities of the technological setting of feed additives. Changes in the morphometric parameters of internal organs in rats under the influence of the new feed additive of humic nature «Animal Forte» were detected. The established biochemical changes are confirmed at the histological level. In terms of the degree of expressiveness of the protective effect on the state of the liver parenchyma of rats, the dilution exceeds this dilution. No structural changes were detected in micropreparations of kidneys in animals of groups 2, 3, and 4, full blood and stasis in the capillaries of vascular glomeruli were observed. In the control and fifth experimental groups, the presence of damaged tubules, in which the brush border is rejected, was noted during the examination of kidney histopreparations. The morphometric parameters of the liver underwent the most pronounced changes in animals 1 and 2, intestines - 1 and 4, and kidneys and hearts - 2 and 5 of experimental groups, respectively. A smaller liver mass was found in the animals of the experimental groups, in particular, a decrease in the liver index in rats of the 1st and 2nd experimental groups by 21 % and 15 %, respectively. Kidneys in animals of experimental groups 2 and 5 had 14 % less weight, but the kidney index did not have significant changes in rats of all experimental groups compared to the control. Almost similar changes were related to heart weight, animals of experimental groups 2 and 3 also had probable changes by 13 %, and 5 groups by 14 %. Intestinal mass was significantly smaller in animals 1 (by 14 %), 3 (by 10 %) and 4 (by 11 %) of experimental groups, but the index was reduced only in animals of experimental group 1 by 16 %.

Keywords: «Animal Forte»; laboratory rats; morphometric parameters

Вплив нової кормової добавки гумінової природи на морфометричні показники лабораторних щурів залежно від дози

Анотація. Проводиться вивчення ефектів природних біологічно активних речовин, прояви та наслідки застосування різним видам тварин або за неоднакових умов. Особливості реалізації таких ефектів при окремому або комбінованому використанні, зокрема гумінових кислот із мінеральними або органічними кислотами, створює можливості оптимального поєднання або вирішення складнощів технологічного задавання кормових добавок. Виявляли зміни морфометричних показників внутрішніх органів у щурів за впливу нової кормової добавки гумінової природи «Animal Forte». Встановлені біохімічні зміни підтверджуються на гістологічному рівні. За ступенем вираженості протекторної дії на стан печінкової паренхіми щурів розведення перевищує таке розведення. Структурних змін щодо мікропрепаратів нирок у тварин 2, 3 і 4 груп не виявлено, спостерігаються повнокров'я та стази в капілярах судинних клубочків. У контрольній та п'ятій дослідній групах при дослідженні гістопрепаратів нирок відзначали наявність пошкоджених канальців, у яких відбувається відторгнення щіткової облямівки. Найбільш виразних змін морфометричні показники печінки зазнали у тварин 1 і 2, кишечника – 1 і 4, а нирок і серця - 2 і 5 дослідних груп відповідно. Виявлено меншу масу печінки у тварин дослідних груп, зокрема зниження індексу печінки у щурів 1 і 2 дослідних група на 21% та 15% відповідно. Меншу маси на 14% мали нирки у тварин 2 та 5 дослідних груп, проте індекс нирок не мав суттєвих змін у щурів усіх дослідних груп проти контролю. Майже аналогічні зміни стосувались маси серця, вірогідні зміни мали також тварини 2 і 3 дослідних груп на 13%, а 5 групи на 14%. Маса кишечника було вірогідно меншою у тварин 1 (на 14%), 3 (на 10%) і 4 (на 11%) дослідних груп, але індекс виявився зниженим лише у тварин 1 дослідної групи на 16%.

Ключові слова: «Animal Forte»; лабораторні щури; морфометричні показники

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Introduction

Adjusting the metabolic processes in the body of animals and directing changes in exchange reactions in the desired direction to increase the productive qualities of animals is one of the main issues of the development of science at the current stage. A large number of biologically active substances of various nature are used, but the greatest preference is given to natural substances that are environmentally safe. One of these substances are organic compounds of humic nature.

A positive effect of humic feed additives on the antioxidant system of the liver and muscles was noted. The ration supplemented with biologically active additives of humic compounds contributes to the activation of the glutathione system of antioxidant protection of erythrocytes. However, the modulation of the antioxidant system in rodents and poultry liver under the influence of natural humic additives is different (Stepchenko et al., 2021).

Mixtures of glauconite, succinic acid, humic and fulvic acids, lactates of zinc, manganese, copper, cobalt and iron with feed are an effective means of preventing disorders of mineral metabolism in rabbits, have a positive effect on hematopoiesis, the state of the antioxidant system, protein metabolism and increase in body weight (Korniichuk et al., 2020, 2021).

Comparison of the effect of feed additives «Humilid», «Eko Impulse Animal» with vitamin E on the hematological parameters of the blood of rats indicates an almost synonymous effect of the investigated substances as antioxidants (Diachenko and Stepchenko, 2018). For Ibuprofen-induced stomach ulcers, Humilid promotes the healing and epithelization processes, stimulates mucus formation, hemoglobin concentration and blood oxygen capacity, normalizes the levels of platelets and leukocytes. This can be explained by the antioxidant, adaptogenic, membrane-stabilizing properties of humic substances (Lisna and Stepchenko, 2021, 2022).

In productive animals, humic acids help increase productivity. For example, in piglets, an increase in the mass of internal organs, namely the heart by 5.5 %, the lungs by 12.8 %, the liver by 8.4 %, and a decrease in the spleen by 7.5 % and kidneys by 5 %. In dairy goats, milk yield increased by 15.9 %, as well as milk protein content (Piskun, 2008; Horchanok et al., 2020).

During their life, animals often encounter adverse conditions, consume harmful substances, therefore there is a need to evaluate the negative effects of food additives or contaminants of food raw materials on the body of laboratory animals.

The systemic toxic effect of various types of plastic is caused by damage to the barrier function of the mucous membranes, which caused a massive penetration of autotoxins from the intestines into the internal environment of the body with the development of parenchymal hepatitis and myocardiodystrophy. An increase in the mass of the kidneys, liver and lungs and a decrease in the thymus and spleen are revealed. A decrease in the concentration of globulins and creatinine in the blood of the animals of the experimental groups is a sign of nephropathy or tubular nephrosis due to the degeneration of the epithelium of the urinary tubules. Addition of crushed polystyrene foam to the feed of rats causes a decrease in the weight of the heart by 15 %, intestines by 16 %, kidneys by 13%, but increases the weight of the liver by 17 % and the weight of the lungs by 24 %. This is caused both by the accumulation of microplastics in individual organs, and by the body's reaction to the appearance of such an environmental pollutant (Lieshchova et al., 2019; Bilan et al., 2022).

When fed a ration containing 30 % transgenic soy, an increase in the mass of the spleen and kidneys, but a decrease in the weight of the liver, was found in rats. The content of glycoproteins, medium-mass molecules and circulating immune complexes, alanine aminotransferase activity and the tendency to increase aspartate aminotransferase in the heart and liver increased in the blood compared to the control. There was an increase in detoxification processes in the liver tissues and inhibition of the development of internal organs in the animals of the experimental groups, as indicated by a higher level of free and acid-conjugated phenols in the liver and kidneys and a decrease in the weight of the kidneys, liver, spleen and heart compared to the control. No histological changes in the structure of the liver, spleen and kidneys were found. (Dolaychuk et al., 2012, 2013).

With the use of the herbicide «Roundup» in rats, there is a decrease in the weight of the heart, lungs and kidneys, but an increase in the liver and spleen. Feeding transgenic soy that had been treated with Roundup during growth had similar changes in heart, kidney and spleen weights, but increased lung and decreased liver weights. Which is probably connected with the arrival of the herbicide and its combination with biologically active substances of soybeans (Chorna and Dronik, 2018).

Glyphosate, sodium benzoate and saccharin have a noticeable immunosuppressive effect, which is confirmed by a decrease in the mass of the thymus. The change in the mass of animal organs under the influence of glyphosate is a consequence of irritation of the mucous membranes, increased desquamation of the intestinal epithelium with the development of catarrhal enteritis and severe pancreatitis. The main manifestations of the systemic effects of benzoate are the development of myocardial dystrophy, pulmonary edema, and hepatodystrophy. Saccharin causes the formation of degenerative changes in the liver parenchyma. Glyphosate causes granular dystrophy, and when combined with sodium benzoate and saccharin, signs of toxic hepatodystrophy were observed in rats (Lieshchova et al., 2018).

A decrease in the increase in live weight of rats with heavy metals intoxication was accompanied by hypo- and hypertrophy of internal organs, which are characterized by different intensity of metabolism. In animals exposed to cadmium, there was a tendency to increase the lungs by 2.8 %, the liver by 7.6 %, the brain by 18.7 %, the heart weight by 8.7 %, the kidneys by 2 %, 0 %, spleens - by 2.0 % compared to the control group. Rats injected with a 16.6 % aqueous solution of lead acetate at a dose of 200 mg/kg had an increase in heart weight by 2.6 %, lung weight by 4.9 %, kidney weight by 16.6 %, liver weight by 2.2 %, brain – 14.8 %, reduction of spleen mass by 4.4 %. Simultaneous administration of lead and cadmium salts to rats increased the heart by 7.3 %, kidneys by 17.6 %, liver by 9.4 %, brain by 21.1 %, lungs by 8.7 %, and spleen by 3.2 % (Lopotych et al., 2020). During preclinical or clinical research of drugs, general toxicity and possible morphological changes in laboratory animals due to the effects of such drugs and feed additives are revealed.

The established differences in the morphostructure of the internal organs of female F1 rats under long-term exposure to germanium citrate may be determined by the dose of its use. More pronounced morphological and histological changes were noted in the organs of female rats of the F1 generation under the influence of 200 mcg of germanium per 1 kg of body weight (Khrabko et al., 2017).

During the two-week drinking of citrates of nanoparticles of chromium and selenium, the body weight of rats increased by 14.8 % (from 251.3 g to 288.5 g), the liver by 54.7 % (from liver 8.08 g to 12.5 g), and the weight of the heart (1-1.1 g), lungs (1.8-1.9 g) and kidneys (1.8-2.3 g) did not change significantly (Khomyn et al., 2013).

During the study of the acute toxicity of the liquid extract of the herb of garden thyme, a macroscopic examination of the internal organs of rats was performed. According to the results of weighing the internal organs of animals and calculating their mass coefficients, it was found that the surface of the liver, kidneys and adrenal glands is smooth, the color, shape, and size of the organs are normal, the weight of the liver is 3.12-3.18, the heart is 0.32-

0.33, lungs 0.62-0.65, kidneys 0.64-0.68, spleens 0.39-0.43 g. The mucous membrane of the stomach has a pronounced relief and a typical anatomical structure. In the chest cavity, all organs are anatomically correct, typical in shape, size and color (Shanayda and Oleshchuk, 2017).

Humilid is non-toxic to protozoa at concentrations below 1 %, with a NOEC (no observed effect concentration) of 2 g/l and an EC 50 (half maximum effective concentration) of 104–124 g/l. Therefore, the results of the activity of Humilid against Paramecium caudatum allow it to be classified as a non-toxic substance, LC 50 is more than 5,000 mg/kg. In the conversion of the additive to the content of actual humic acids in the composition of the sample, LC 50 corresponds to 10.4–12.4 g/l (Stepchenko et al., 2019).

The purpose of the work is to identify possible changes in the morphometric indicators of internal organs in rats under the influence of a new feed additive of humic nature «Animal Forte».

Material and methods

The experiment was conducted in the conditions of the university vivarium on white laboratory rats for 28 days. The animals were kept in standard cages on a balanced standard diet throughout the entire period of research and water intake was not restricted. To conduct the study, six groups of animals were formed according to the principle of analogues (one - control, five - experimental, 8 animals in each group). The experiment used two-month-old sexually mature male white rats. After a 10-day preparatory period, the animals of the experimental groups received daily, in addition to the main feed, the feed additive «Animal Forte» in the amount: the first group - 5, the second group - 10, the third group - 20, the fourth group - 30, the fifth group - 50 mg /kg mass. The supplement was administered per os using a special catheter with a dispenser. The calculated doses of the feed additive were dissolved in a 0.9 % sodium chloride solution so that the individual daily portion of each animal was 1 ml. Animals of the control group were similarly injected with 1 ml of 0.9 % sodium chloride solution.

All animals were removed from the experiment by dislocation of the cervical vertebrae after light anesthesia with diethyl ether. All manipulations with animals were carried out in accordance with the requirements of Directive 2010/63/EU on the use of animals in scientific experiments and the Law of Ukraine «On the Protection of Animals from Cruelty». Internal organs (heart, liver, intestines, kidneys) were taken from rats by anatomical preparation. Their absolute mass was determined by weighing on an AB224 analytical balance (Metrinco, China, 2021) with an accuracy of 0.0001, followed by determination of the organ mass index. An autopsy was performed, internal organs were examined and their mass coefficients (MC) were calculated. Mass measurements were carried out on laboratory scales after their verification, with an accuracy of 5 mg. The calculation of the MC of internal organs was carried out according to the formula:

MC = (m organ/M animal)*100 %.

The samples were fixed in a 10% solution of formalin in a phosphate buffer of pH 7.4 for 48 hours, then washed in running water, dehydrated in ethanol solutions of increasing strength, passed through a mixture of ethanol-chloroform, chloroform-paraffin, then embedded in paraffin. With the help of a sled microtome, sections 5-7 μm thick were prepared from paraffin blocks, made with the help of a sled microtome MC-2 with subsequent staining with hematoxylin and eosin according to classical methods, after which they were studied under a microscope by light-optical methods. Digital copies of the images were obtained using a Leica microscope and an Olympus C 740UZ camera (Horalskiy et al., 2019).

The obtained numerical data were processed using the standard package of Microsoft Excel statistical programs.

Result

Morphometric indicators of the internal organs of rats are shown in Table.

A smaller liver mass was found in the animals of the experimental groups, in particular, a decrease in the liver index in rats of the 1st and 2nd experimental groups by 21 % and 15 %, respectively. Kidneys in animals of experimental groups 2 and 5 had 14 % less weight, but the kidney index did not have significant changes in rats of all experimental groups compared to the control. Almost similar changes were related to heart weight, animals of experimental groups 2 and 3 also had probable changes by 13 %, and 5 groups by 14 %. Intestinal mass was significantly smaller in animals 1 (by 14 %), 3 (by 10 %), and 4 (by 11 %) of the experimental groups, but the index was reduced only in animals of experimental group 1 by 16 %.

Microscopic examination of the liver of rats of experimental groups 2, 3, and 4 revealed no deviations from the norm.

A well-defined lobular and lamellar structure of the liver was established, where a central vein is located in the center of each lobe, from which liver plates (beams) formed by rows of hepatocytes depart radially. Hepatocytes have a round-polygonal shape with

Table - Morphometric indicators of internal organs of the experimental groups (M ± m, n = 8)

Indicator	Groups					
	Control	Experimental				
		1	2	3	4	5
Liver	9,91±0,45	8,4±0,37*	6,9±0,32*	7,7±0,28*	8,8±0,25*	7,9±0,54*
Liver index	3,66±0,16	2,9±0,13*	3,1±0,18*	3,3±0,2	3,5±0,1	3,2±0,22
Kidneys	1,74±0,09	1,7±0,09	1,5±0,05*	1,5±0,1	1,7±0,09	1,5±0,05*
Kidney index	0,64±0,04	0,6±0,03	0,6±0,03	0,7±0,05	0,7±0,03	0,6±0,03
Heart	0,92±0,03	1,0±0,05	0,8±0,03*	0,8±0,04*	0,9±0,03	0,7±0,03*
Heart index	0,34±0,02	0,3±0,02	0,4±0,02	0,3±0,02	0,4±0,01	0,3±0,01
Intestine	21,52±0,81	18,6±0,62*	18,8±1,25	19,4±0,52*	19,1±0,74*	19,5±0,61
Intestine index	7,94±0,25	6,7±0,29*	8,3±0,61	8,2±0,34	7,6±0,18	8,0±0,21

* statistically significant differences compared to control group animals $p<0.05$

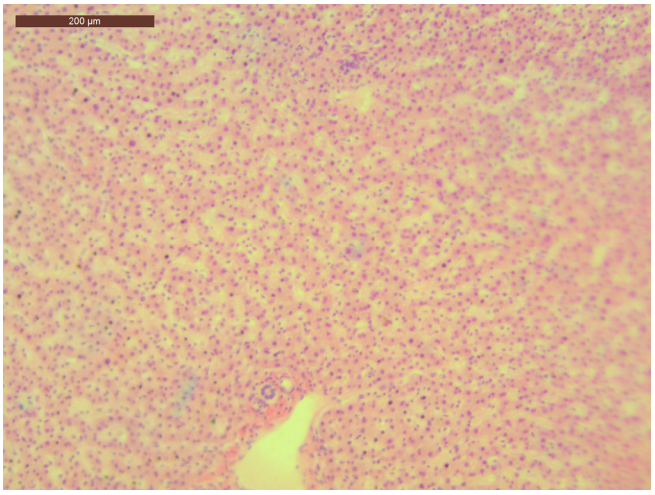

Fig. 1. Microstructure of rat liver (hematoxylin-eosin). Histostructure of the liver lobe (hepatocytes without abnormalities in animals of groups 2, 3, 4).

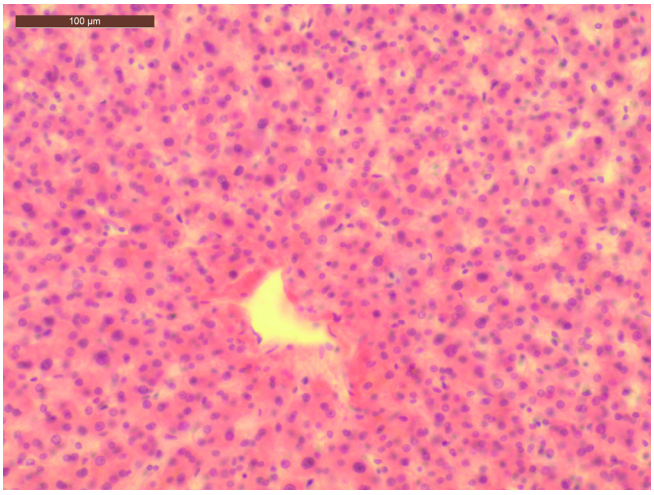

Fig. 2. Microstructure of rat liver (hematoxylin-eosin). Change in the structure of the lobule (samples from animals of groups 1 and 5).

large round nuclei. The nuclei are normochromic, located centrally and contain one, sometimes two nucleoli. The number of cells with a diploid nucleus is moderate.

At a higher magnification of the microscope, liver macrophages are clearly visible - Kupffer star cells, located between hepatocytes and sinusoids. The zones of the triads are narrow. The condition of the epithelium of blood vessels in triads and other vessels is within normal limits. Intralobular sinusoidal hemocapillaries are moderately expanded and contain formed elements of blood. Hepatocytes of the central and peripheral parts of the liver lobe do not differ in their structure. Under the influence of humic acids, the morphological structure of the liver parechyma, the glycogen-forming function and the processes of lipogenesis are preserved.

In the first and fifth research groups, during the examination of histopreparations of the liver, in some places, the overflow of blood in most of the central veins of the lobules, discomplexation of the liver plates (beams) on the periphery of the lobules, where the majority of hepatocytes are swollen, polymorphic, their cytoplasm is unevenly colored, granular. In the central part, the radial structure of the liver plates is preserved. The border between cells is preserved, the cytoplasm of hepatocytes is moderately stained, the nuclei are round. An increase in the number of cells with a diploid nucleus is observed. The expanse of Dice is expanded, the sinusoidal hemocapillaries are expanded and infiltrated. Connective tissue strands of the liver skeleton, penetrating the parenchyma, surround the expanded portal tracts. Many lymphocytes and fibroblasts are detected perivascularly.

The histostructure of the kidneys of intact rats corresponded to the norm. The capsule, cortical and brain layers are clearly visible. In the cortical layer, numerous glomeruli of nephrons are clearly visible, having a spherical shape with a slightly uneven surface. The glomeruli are enclosed in capsules, the lumen of which has a crescent shape or surrounds the glomerulus in the form of a ring. Capsule cavities are free of contents. The space between the glomeruli is represented by homogeneously colored tissue with numerous round sections of convoluted tubules and vessels of the cortical substance.

The epithelium of the tubules tightly adheres to the surface of the basement membrane and is represented by a continuous single-cell layer of endothelial cells. The nuclei of these cells are rounded and located in the center of the homogeneous cytoplasm. The tubule lumen is gaping, free of contents. The brain substance is represented by homogeneously colored parenchyma with parallel tubular

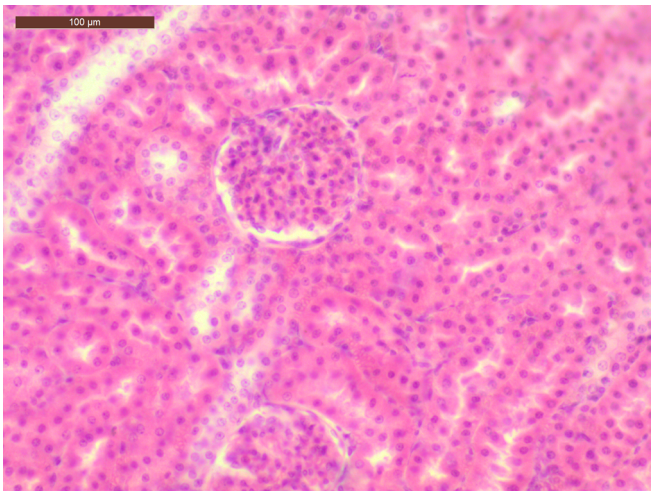

Fig. 3. Microstructure of rat kidneys (hematoxylin-eosin).

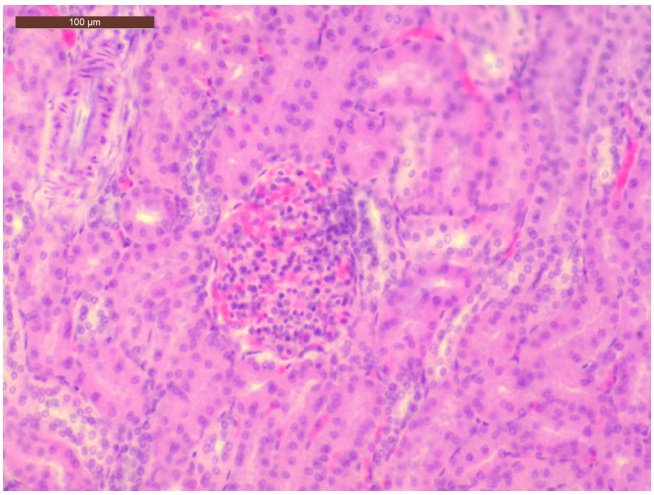

Fig. 4. Microstructure of rat kidneys (hematoxylin-eosin) of group 2 animals.

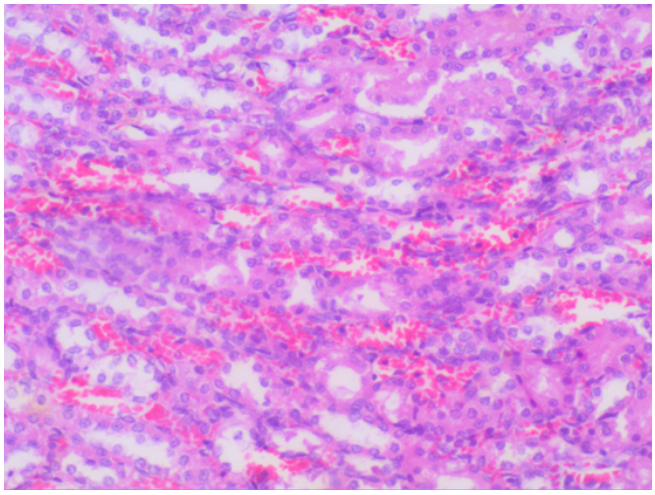

Fig. 5. Microstructure of rat kidneys (hematoxylin-eosin) of group 1 animals.

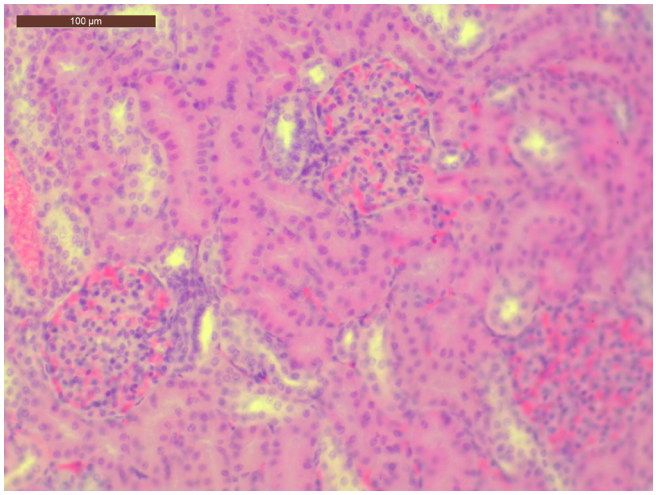

Fig. 6. Microstructure of rat kidneys (hematoxylin-eosin) of group 5 animals.

apparatus. Tubular lumens gape, free of contents. The epithelium of the collecting tubules is represented by a single layer of cells closely adhering to the basement membrane, the nuclei of the cells are uniformly basophilic-stained and round in shape.

No structural changes were detected in micropreparations of kidneys in animals of groups 2, 3, and 4, full blood and stasis in the capillaries of vascular glomeruli were observed.

In the first and fifth experimental groups, during the examination of histopreparations of the kidneys, the presence of damaged tubules was noted, in which the rejection of the brush border occurs.

Discussion

The obtained effects from the use of natural biologically active substances of different groups allow comparing both biochemical and morphological indicators with the aim of generalizing them.

Excess fat in the diet of rats disrupts the work of the excretory system, which is manifested in a decrease in the kidney mass index, a high creatinine content, and a decrease in the level of urea in the blood. The addition of succinic acid has a positive effect on the functional state of the liver and kidneys, especially when taken for a long time. Another consequence is a violation of the function of the organs of the circulatory system and immune protection, which is accompanied by a decrease in the relative mass of the thymus and spleen, a low content of hemoglobin and the number of erythrocytes, but does not have a significant effect on the content of other cellular elements of the blood (Lieshchova et al., 2020).

Addition of crushed dry shoots of S. officinalis to the diet of rats led to a sharp increase in their body weight (up to 130.8 % of the initial over 30 days of the experiment), an increase in daily body weight gain to 253.1 %, and S. sclarea – its decrease to 27.8 % of the daily increase in the control group. In the group receiving S. officinalis, the specific weight of the brain (77.7 % of the control), spleen (80.4 %) and thymus (59.1 %) decreased. In the S. sclarea group, the relative mass of the thymus decreased (43.4 %), and the relative mass of the colon increased (159.7 % compared to the control group). The consumption of sage led to a significant decrease in the relative weight of the organs of the immune system (thymus gland and spleen), but no changes in blood composition (total number of leukocytes and leukoformula) were found (Lieshchova et al., 2021).

Morphological changes in the liver, myocardium, and kidneys of rats after a mechanical experimental craniocerebral injury on the third day showed impaired blood circulation of the ischemic type with subsequent dystrophic changes, which led to necrotic changes on the fifth day (Kholodkova and Prus, 2018).

Biologically active additives of humic nature have a pronounced positive effect on the condition and functional activity of the liver, kidneys, and heart.

For example, the activity of catalase, aspartate aminotransferase, and the amount of cytochrome C increased in the liver of gerbils due to increased antioxidant protection of cells by strengthening adaptation processes. Adding ascorbic acid to the Humilid solution enhances its antioxidant properties (Dyomshina et al., 2017).

Humilid (individually and in combination with ascorbic acid) and Eco-Impulse have hepatomodulating properties. The formation of the appropriate response of the liver cells of gerbils to the ingestion of the active substances of the Eco-Impulse preparation was accompanied by an increase in the amount of TBC-active products and cytochrome C (Serova et al., 2016).

Cold-pressed amaranth oil simultaneously with Humilid has a positive effect on the filtration and excretory function of the kidneys, does not cause changes in the activity of blood plasma enzymes, and enhances the energy supply of metabolic processes (Harashchuk et al., 2021).

Fulvic acids of leonardite (produced in Hungary) increased the content of iron in the liver and kidneys, copper in the liver, and zinc in the kidneys, unlike humic acids. Both groups of biologically active substances did not significantly affect the concentration of manganese in the liver, kidneys and femurs, stimulated the absorption of zinc and copper (Szabó et al., 2017; Hullar et al., 2018).

«Animal Forte» feed supplement accelerates metabolic processes, activates a hematopoietic function, especially erythropoiesis, and increases hemoglobin content and the number of erythrocytes. Thanks to this, tissue respiration, redox processes, liver work, and functional properties of kidneys improve, thanks to which the content of urea and creatinine in the blood of rats decreases (Stepchenko et al., 2020].

The use of corvitin and humilid reduces free radical oxidation in the heart muscle. The activity of glutathione peroxidase and glutathione reductase decreases in animals with adrenaline-induced myocardial ischemia, but remains elevated compared to intact animals (Paronik et al., 2015).

Conclusions

The established biochemical changes are confirmed at the histological level. In terms of the degree of expressiveness of the protective effect on the state of the liver parenchyma of rats, the dilution exceeds this dilution. No structural changes were detected in micropreparations of kidneys in animals of groups 2, 3, and 4, full blood and stasis in the capillaries of vascular glomeruli were observed. In the first and fifth experimental groups, during the examination of histopreparations of the kidneys, the presence of damaged tubules was noted, in which the rejection of the brush border occurs. The morphometric parameters of the liver underwent the most pronounced changes in animals 1 and 2, intestines - 1 and 4, and kidneys and hearts - 2 and 5 of experimental groups, respectively.

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