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Original Research Article

Pathomorphological Changes in the Duodenum of Rats in Case of Subchronic Peroral Administration of Gadolinium Orthovanadate Nanoparticles Against the Background of Food Stress

Key words

rare earth metals;
gadolinium orthovanadate nanoparticles;
pathomorphological changes, duodenum;
white rats;
feed stress

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Abstract: In our research, we were interested in the actual presence of adaptive or negative reactions in the wall of the small intestine of white rats under the influence of gadolinium orthovanadate nanoparticles in the range of doses (≈0.03-0.3 mg/kg of body weight) under conditions of food stress (due to an excess of fiber and lack of protein in the diet) and their degree of manifestation, since this type of ration disproportion occurs quite often in Ukraine. Nanoparticles of gadolinium orthovanadate have a significant potential for use in animal husbandry and poultry farming, as in the range of doses of 0.03-0.15 mg/kg of body weight, they prevent negative effects on the intestinal mucosa, even in conditions of feed stress. It has been established that administration of gadolinium orthovanadate nanoparticles in doses of 0.03 and 0.15 mg/kg of body weight to white rats with drinking water for 56 and 28 days, respectively, leads to activation of the mechanical and immunological barrier of the mucous membrane, as indicated by an increase goblet cells, hyperplasia of enterocytes of some crypts, thickening of villi and infiltration by lymphocytes of the own plate, which reach the control level 14 days after stopping their administration. However, increasing the dose of gadolinium orthovanadate nanoparticles to 0.3 mg/kg of body weight in conditions of food stress leads to the depletion of the adaptive capabilities of the intestinal mucosa and excessive activation of the immunological barrier, which were manifested by dystrophic changes from the 14th day of administration, which deepened to the 56th day and do not level off after 14 days after stopping administration.

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Introduction

Rare earth metals (rare earth elements, REM) are a group of 17 elements that includes Lanthanum, Scandium, Yttrium, Gadolinium and lanthanides. All these elements are silvery-white metals, and they all have similar chemical properties (the most characteristic oxidation state is +3). The name "rare earth elements" was historically formed at the end of the 18th – beginning of the 19th century, when it was mistakenly believed that the mineral-containing elements of two

subfamilies: cerium (light – Sc, La, Ce, Pr, Nd, Pm, Sm, Eu) and yttrium (heavy – Y, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) are rarely found in the Earth's crust (1, 2, 3).

Currently, elements of the yttrium subgroup are used for the production of nanoparticles with good biocompatibility. These are, for example, nanoparticles based on Gd (such as gadolinium orthovanadate), which can be modified with Eu, Lu, Dy (to obtain fluorescent radiation, which is useful for the latest methods of magnetic resonance imaging), as well as a shell of silica to increase biocompatibility or various ligands for targeted delivery of nanoparticles (4, 5, 6).

Nanoparticles of gadolinium orthovanadate exhibit enzyme-like properties: inhibition of superoxide anion formation (similar to the action of superoxide dismutase) and acceleration of decomposition of hydrogen peroxide (similar to the action of catalase) were observed in aqueous solutions (7). The antioxidant properties of gadolinium orthovanadate nanoparticles were also observed during X-ray irradiation of aqueous solutions, despite the fact that the nanoparticles absorb the soft X-ray used in the experiment. In aqueous solutions, in the presence of nanoparticles, a decrease in the concentration of hydroxyl radicals was found as the main product of water radiolysis (8), and therefore, $GdVO_4:Eu^{3+}$ nanoparticles have radioprotective properties.

Significant progress in the study of this type of nanoparticles has been achieved in reproductive science. It was established that NP $GdVO_4:Eu^{3+}$ is a practically non-toxic compound (LD_{50} per os is more than 5000.0 mg/kg), and their effect, including on spermatogenesis, depends on the clinical condition of the animals, dose and duration of administration. Thus, in 6-month-old intact rats, when NP was used for 30 days in doses of 0.03, 0.3 and 3.0 mg/kg, only the proportion of pathological forms of spermatozoa increased statistically in the spermogram. After receiving 70 days of NP $GdVO_4:Eu^{3+}$ in minimum or maximum doses, no significant effect on spermatogenesis of intact rats was detected. The use of gadolinium orthovanadate NPs at a dose of 0.3 mg/kg led to a statistically significant decrease in the total concentration of sperm (by 47%), the concentration of morphologically normal gametes (by 50%), their motility (by 35%) and an increase in the percentage of pathological forms (by 50%) compared to the indicators of the control group. Whereas, in 10-month-old male rats with model hypofertility (in whose spermogram there is a lower total sperm concentration, a concentration of morphologically normal gametes and reduced fertility), administration of NP $GdVO_4:Eu^{3+}$ at a dose of 0.3 mg/kg for 70 days normalized all the studied spermogram indicators excluding cell motility, which decreased, and contributed to the normalization of fertility (9, 10, 11, 12, 13).

Due to a good bioavailability and antioxidant properties, one of the promising areas of application of REM nanoparticles is their use in agriculture, in particular, in poultry farming, since cerium dioxide nanoparticles have been proven to have a positive effect on the body of poultry through the intensification of egg production, their mass, and the fertilization of hatching eggs, and antioxidant action (14, 15, 16).

The assimilation and positive effect of minerals (including rare earth elements) depends on the quality of the diet: for example, fiber and some related substances have a strong ability to bind minerals or form complexes, so there is a suspicion that fiber impairs the assimilation of minerals (17). With an optimal amount of protein in the diet, protein components – amino acids and oligopeptides facilitate the absorption of trace elements, which confirms the greater availability of organic forms of trace elements (18, 19).

Disproportions of nutrients in feed can lead to so-called "feed stress" (by the way, this fact was described almost 65 years ago (20). Feed stress has a negative effect on the animal body (21), in particular, the first the digestive tract suffers (22). Along with this (23), a therapeutic dose of $GdVO_4:Eu^{3+}$ nanoparticles was established for induced mild intestinal inflammation – 0.02 mg/kg of body weight: administration of nanoparticles improved the morphology of small intestine, reduced the rate of infiltration of immune cells without affecting the intensity of apoptosis.

Studying the impact of stress on the poultry organism, three main stages were identified, which to some extent coincide with the stages of stress in the animal body according to H. Selye: the stage of stress detection (short-term stress regulation), the stage of development of resistance to stress and adaptation, the stage of exhaustion and the appearance of negative consequences (24, 25). In our research, we were interested in the actual presence of adaptive or negative reactions in the wall of the small intestine of white rats under the influence of $GdVO_4:Eu^{3+}$ nanoparticles in the range of doses (≈0.03-0.3 mg/kg of body weight) under conditions of food stress (due to an excess of fiber and lack of protein in the diet) and their degree of manifestation, since this type of ration disproportion occurs quite often in Ukraine (26, 27).

Therefore, the work aimed to determine the pathomorphological changes in the duodenum of rats in case ofsubchronic oral administration of gadolinium orthovanadate nanoparticles against the background of food stress.

Materials and methods

The experiments have been conducted in the laboratory for toxicological monitoring of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine"

(NSC "IECVM"), Kharkiv, Ukraine, and the research pathomorphology department of the State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) Kyiv, Ukraine.

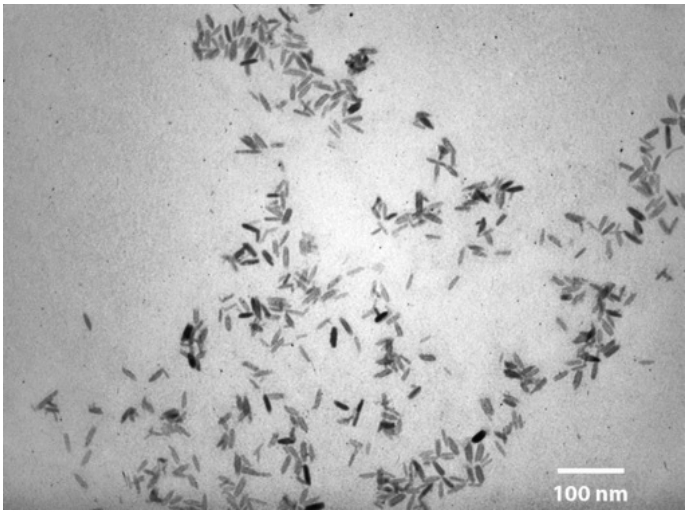

Figure 1: Photograph (transmission electron microscopy) of NP $GdVO_4:Eu^{3+}$ nanoparticles (30)

Experimental samples of gadolinium orthovanadate nanoparticles (NP $GdVO_4:Eu^{3+}$) (spindle-like geometry, size 8×25 nm) (Fig. 1) with an initial concentration of 1.0 g/dm^3 were used in the work.

Experimental samples of nanoparticles were synthesized and standardized by stability and size in the department of nanostructured materials named after Yu.V. Malyukin of the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine (28, 29).

Experiments on rats were conducted in the vivarium of the National Scientific Center "IECVM", 140 sexually mature male *Wistar* rats with an initial weight of 180.0-200.0 g were used as the object of research. Four groups of animals with 28 rats in each group were formed by the principle of analogs.

During the experiment, animals of the control group received drinking water without additives, rats of the first experimental group received a solution of gadolinium orthovanadate nanoparticles 0.2 mg/dm^3 (≈0.03 mg/kg of body weight); experimental group II – 1.0 mg/dm^3 (≈0.15 mg/kg body weight), and experimental group III – 2.0 mg/dm^3 (≈0.3 mg/kg body weight). Rats had received water or water with additives for 56 days, then the rats had been observed for another 14 days. Laboratory animals had free access to water and food.

A granulated grain mixture (with a disproportional content of nutrients) was used as a monofeed for rats to create food stress (Table 1). The content of nutrients in the diet was determined by the following normative documents.

Determination of crude protein content was carried out following the Kjeldahl method in accordance with DSTU ISO 5983:2003, crude fiber – in accordance with DSTU ISO 6865:2004, crude fat – in accordance with DSTU ISO 6492:2003, Calcium – in accordance with DSTU ISO 6490-1: 2004, Phosphorus – according to DSTU ISO 6491:2004. The content of vitamins – in accordance with DSTU 4687:2006, trace elements – in accordance with DSTU EN 14082:2019. The results of the experiment are summarized in Table 1.

Before the administration of nanoparticles, the rats were kept on the above diet for 14 days. An indicator of the presence of food stress was considered to be the failure of all groups of rats to gain conditioned mass during the experiment.

During the experiment, the clinical condition of animals of all groups was observed: attention was paid to behavior, reaction to external stimuli, presence of appetite, skin condition, color of mucous membranes, frequency of breathing

Table 1: Qualitative composition of the diet of rats (granulated grain mixture)

Indicator	Value	Norm *	± to the norm
Carbohydrates, g/100 g	64,57	59,30	+ 5,27
Energy value, MJ	14,07	14,00	+ 0,07
Mass fraction of fat, %	3,12	4,40	– 1,28
Mass fraction of crude protein, %	12,50	19,60	– 7,1
Mass fraction of crude fiber, %	11,90	4,60	+ 7,3
Vitamin B_2, mg/kg	14,00	30,00	– 16,0
Vitamin A, IU/kg	4400,00	10000,0	– 5600,0
Vitamin E, mg/kg	137,50	100,00	+ 37,5
Selenium, mg/kg	0,46	0,10	+ 0,36
Copper, mg/kg	5,39	16,00	– 10,61
Zinc, mg/kg	42,26	60,00	– 17,74

Note * According to (Diet Meat Free Rat and Mouse Diet (SF00-100)) (31)

Table 2: Algorithm of tissue processing in the machine for histological processing of tissues of the carousel-type STP – 120

The name of the reagent	Concentration, %	Processing time, min.	Temperature, °C
Formalin	10, neutral	60	20-25
Tap water	-	60	20-25
Ethanol	70	90	20-25
Ethanol	80	90	20-25
Ethanol	90	90	20-25
Ethanol	96	60	20-25
Ethanol	96	60	20-25
Ethanol	96	60	20-25
Xylene	-	90	20-25
Xylene	-	90	20-25
Histological paraffin	-	120	62
Histological paraffin	-	120	62

Table 3: Algorithm of deparaffinization and staining of sections in a linear tissue staining machine

Reagent name	Concentration, %	Processing time, min.
Xylene	-	5
Xylene	-	5
Ethanol	96	5
Ethanol	96	5
Tap water	-	5
Hematoxylin	-	10
Tap water	-	5
Alcoholic eosin	0,3	1
Tap water	-	5
Ethanol	70	5
Ethanol	96	5
Ethanol	96	5
Carbol-xylene	3:1	5
Xylene	-	5

and defecation, changes in color and consistency of feces, etc. (32). 14, 28, 42 and 56 days after the start of the administration of nanoparticle solutions and 14 days after its termination, using CO_2 anesthesia 7 rats from each group were decapitated and samples of the small intestine (segment of the duodenum) were taken for histomorphological studies.

Experiments were conducted on the basis of specialized laboratories of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (protocol № 321). The research program was reviewed and approved by the Bioethics Commission of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" in the current order. Animal experiments do not contradict the current legislation of EU (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, 22 September 2010).

Histomorphological studies were carried out following generally accepted methods in the research pathomorphological department of the SSRILDVSE.

Fixation and cutting of pieces of patmaterial

Pieces were cut from different parts of the organ. In the presence of visible pathomorphological changes in the organs (tissues), pieces were cut on the border of the area with visible pathomorphological changes and without visible changes.

To preserve the tissue and cellular structure, pieces of organs were fixed. For this, a 10% aqueous solution of neutral formalin was used, the volume of which should be (20-40) times greater than the volume of the sampled material. Laboratory ware was tightly closed and left in a fume cupboard at room temperature. After a day, the fixing liquid was changed. After (5-7) days, pieces (2-3) mm thick were cut through the entire thickness of the organ (tissue) and placed in plastic cassettes. The latter were labeled and placed in a fixing liquid (10% aqueous solution of neutral formalin) for another day.

The rest of the organs (tissues) were packed, labeled (indicating the number of the work protocol, the date of receipt of the sample) and stored in a 10% formalin solution until the results of the research were obtained.

The algorithm for histological processing of the selected samples is given in Table 2.

Formation of paraffin blocks

Paraffin blocks were formed using a paraffin pouring station, using special molds and cassettes. The forms were transferred to the filling platform of the dosing unit, the piece was placed in the desired position with tweezers, covered with a cassette and filled with paraffin. The form was transferred to a cooling platform until the paraffin solidifies completely. The formed paraffin block was removed.

Microtoming

Before microtoming, slides were prepared. They were degreased in a mixture of equal volumes of ethanol and ether, followed by flaming in the flame of spirit lamp.

Table 4: Algorithm for Van-Gieson staining of sections

Reagent name	Concentration, %	Processing time, min.
Xylene	-	5
Xylene	-	5
Ethanol	96	5
Ethanol	96	5
Tap water	-	5
Weigert hematoxylin	-	3-5
Tap water	-	5
Tap water	-	5
Picrofuchsin	10:1	2-3
Tap water	-	10-15 сек.
Ethanol	96	2-3
Ethanol	96	2-3
Carbol-xylene	3:1	5
Xylene	-	5

The glass was marked, indicating the number of the work protocol, the date of receipt of the samples and the index of the piece.

Sections with a thickness of (5-7) μm were made using a rotary microtome, a section transfer system and a water bath.

The obtained sections were smoothed out on the surface of water (temperature +45 °C), then they were transferred to a prepared glass slide and left to dry overnight.

Hematoxylin and eosin staining of preparations

Directly before staining, paraffin was removed from the sections pasted on the glass. Deparaffinization was performed with a paraffin solvent (xylene, an organic solvent). To remove solvent residues, the sections were transferred to alcohol, after which they were ready for staining (the procedure was carried out in the HMS 70 staining apparatus, which is installed in the fume hood). The program is designed for 1 hour 10 min (Table 3).

Differential staining was also used in the work (Table 4).

Administration of histopreparations

The stained section was placed in the final medium, covered with a cover glass and left to dry overnight.

Light microscopy of drugs

Stained preparations were examined under a light microscope at low (ob. ×5, 10, 20) and high (ob. ×40) magnification using an Axioskop 40 microscope ("Carl Zeiss", Germany) and software for making photos.

Statistical analysis

The results were processed by variation statistics using the analysis of variance software package (ANOVA) StatPlus 5 (6.7.0.3) (AnalystSoft Inc., USA). The reliability of the obtained results was evaluated by Tukey's test (HSD mean difference) at a reliability level of 95.0% ($p < 0.05$).

Results

Clinical observations of the rats of both the control and experimental groups I and II showed that the general condition of the animal bodies during the 56-day administration of gadolinium orthovanadate nanoparticles was satisfactory: the rats were mobile and responded adequately to external stimuli.

No violations of appetite, breathing, urination, defecation and appearance (fur was shiny, smooth, clean) were observed in rats. While after the administration of gadolinium orthovanadate nanoparticles at a dose of 2.0 mg/dm^3, starting from the 28th day of administration, a decrease in the body weight of animals was observed, on the 42nd and 56th days along with this a violation of defecation was noted – the dilution of feces in 71.4 and 23.8% of animals, respectively, the rats were not active enough, the fur was dull and disheveled, and on the 14th day after the cessation of the administration of nanoparticles, the weight of the rats did not differ from the control, the appearance also came to the level of the control group. It should be noted that no animal deaths were recorded during the entire observation period in all experimental groups.

Fig. 2 shows the dynamics of the post-slaughter weight of rats during the experiment. Thus, in case of the administration of gadolinium orthovanadate nanoparticles at a dose of 0.2 mg/dm^3 of drinking water during the entire experiment, no reliable deviations of the post- slaughter weight of rats from the control group were observed. In case of administration of nanoparticles at a dose of 1.0 mg/dm^3 of drinking water from the 14th to the 56th day, no changes were noted in the post- slaughter weight of rats, while after stopping the administration of gadolinium orthovanadate nanoparticles, the post-slaughter weight of rats exceeded the control by 7.4% ($p<0.05$).

Dynamics of the post-slaughter weight of rats in the experimental group III that received gadolinium orthovanadate nanoparticles at a dose of 2.0 mg/dm^3 of drinking water was as follows: on the 14th day, no changes were noted in the post-slaughter weight of rats from the control, on the

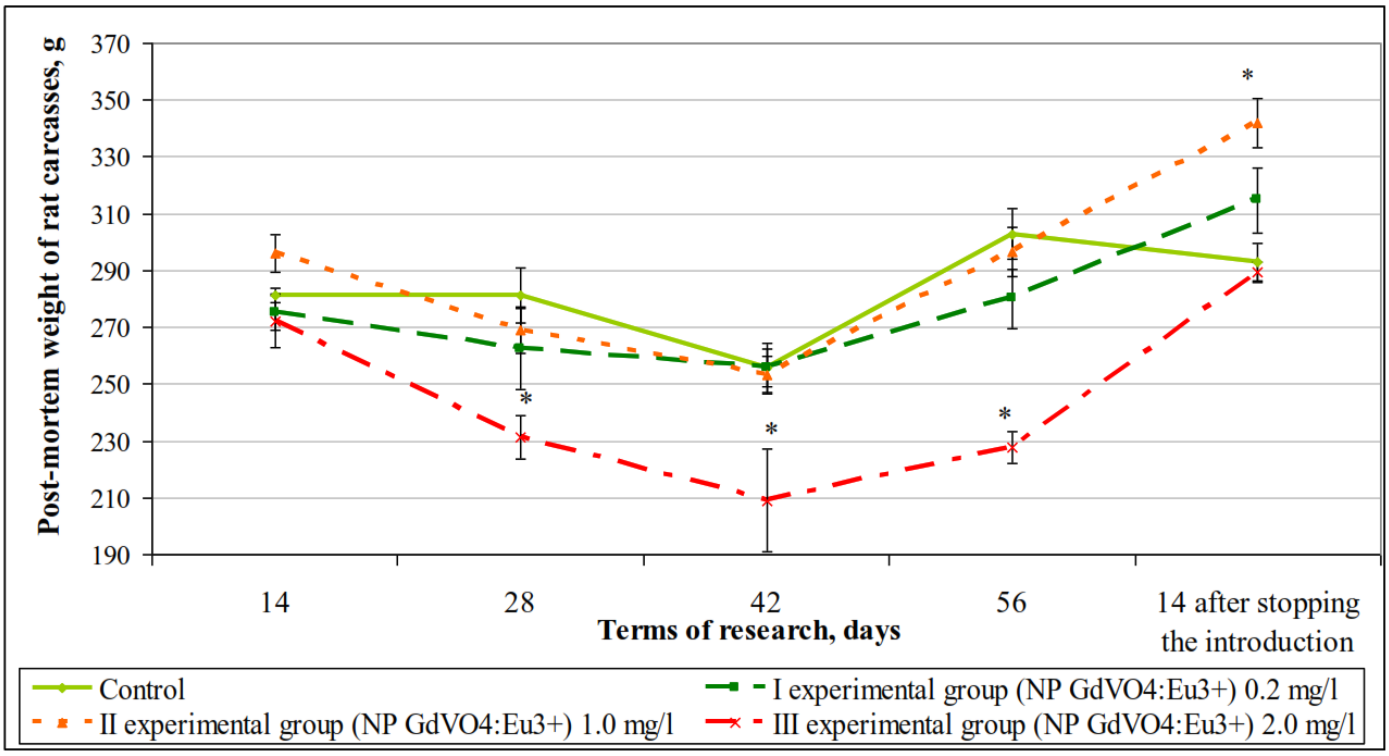

Figure 2: Dynamics of the post-slaughter weight of rats under conditions of administration of different doses of gadolinium orthovanadate nanoparticles with water (M±m, n=7, *– p<0.05 – relative to the control)

28th and 42nd days its decrease (p<0.05) was noted – by 17.8 and 18.3%, while on the 56th day the decrease was 24.8% (p<0.05), and after stopping the administration of gadolinium orthovanadate nanoparticles, the post-mortem weight of rats did not differ reliably from the control.

No organic changes were recorded during the post-mortem examination in the experimental groups I and II at all periods of the experiment. The appearance of the bodies of laboratory animals before dissection: the color of the coat was white, shiny; changes in visible mucous membranes, discharge from the oral (nasal) cavity and anus were not noted.

At the autopsy (relative to the control group), there were no changes in the mucous membranes of the oral cavity, trachea, pharynx, and esophagus; food remains were observed in the stomach; hyperemia of the subcutaneous tissue was not noted; the heart was not enlarged in volume, cone-shaped, the consistency of the myocardium was elastic; the liver was brown, elastic; consistency, not increased in volume; spleen and pancreas – unchanged; kidneys of brown color, not increased in volume; the vessels of the mesentery of the small intestine were not filled with blood, signs of inflammation in the stomach, small and large intestines were not detected.

The exception was the large intestine dilatation in rats of the experimental group II on the 56th day of the experiment. Whereas in the rats of the experimental group III (received gadolinium orthovanadate nanoparticles at a dose of 2.0 mg/dm^3 of drinking water), starting from the 42nd day of the experiment, signs of inflammation were observed in the small intestine, and on the 56th day of the experiment, in addition to this, the liver was light color, slightly increased in volume, flabby consistency, intestinal distention was also noted.

It should be noted that these organic changes disappeared 14 days after stopping the administration of the nanoparticle solution (Fig. 3).

The morphological characteristics of the intestinal samples of the control and experimental groups are given below. Thus, on the 14th day of the experiment, histological studies of the fragments of the duodenum of the control group of rats it was found that the demarcation of the layers was well expressed, the villi were intact, and the epithelium covered the surface evenly. Nuclei of enterocytes were moderately basophilic, rounded, equal in size, located at the basal pole of the cells. Goblet cells were contoured, vacuoles were transparent, rounded. Acidophilic cells were well defined. The crypt lumen was free. Lymphocytes, plasma cells and fibroblasts of the lamina propria were evenly distributed and had a contoured, basophilic nucleus. Lacteal lumen was moderate. The muscle plate was intact, the cytoplasm of the cells was oxyphilic, the nuclei were contoured, basophilic. The vessels of the submucosal base were moderately filled with blood. Reticular fibers were oxyphilic, evenly stained. The number of fibrocytes and lymphocytes in the submucosal base was moderate. The muscle layers were intact, structured, the cell nuclei ere well

contoured, basophilic, the cytoplasm was oxyphilic, and the layer between the fibers was defined. The color of the preparation was even. Slight fuchsinophilia was observed in the structures of the submucosal base and muscle layers. The nuclei of epithelial cells, lymphocytes, and fibroblasts were stained brown-black, the intercellular substance was light brown. Connective tissue structures were painted in red (Fig. 4).

In the experimental group I on the 14th day of administration of gadolinium orthovanadate nanoparticles at a dose of 0.2 mg/l of drinking water, it was established that the demarcation of the layers was good, the villi were intact, and evenly covered with a layer of epithelium. Some villi were thickened due to to an increase in lymphocytes and plasma cells of the main plate.

The goblet cells of the crypts were enlarged. Enterocytes of individual crypts had signs of hyperplasia: the nuclei were hyperchromic, closely adjacent to each other, and their number was significantly increased. The lacteals were narrowed. The nuclei of enterocytes were basophilic, contoured, the cytoplasm was intensely neutrophilic. Acidophilic cells differentiated. The lamina propria contained the nuclei of lymphocytes and plasma cells. Lymphocytic infiltration of the lamina propria was observed in individual villi. The submucous base was structured, oxyphilic. The lumen of individual Brunner glands was enlarged, the cytoplasm of mucocytes was weakly oxyphilic and contained a significant number of vacuoles. Cell nuclei were basophilic. The muscle layers were well demarcated (Fig. 4).

In the experimental group II on the 14th day of administration of gadolinium orthovanadate nanoparticles at a dose of 1.0 mg/l of drinking water, it was established that the layers were well demarcated. At the tops of individual villi, peeling of the epithelium was sometimes observed. Goblet cells of villi and crypts were slightly enlarged, vacuoles contained basophilic granules. Acidophilic cells were determined. The number of plasma cells, lymphocytes and fibroblasts of the lamina propria was moderate. The muscle plate was intact. Elastic fibers of the submucous base were structured, integral, oxyphilic. The circular muscle layer was slightly thickened, the staining was uneven, the oxyphilicity of the cytoplasm of myocytes was less pronounced (Fig. 4).

In the experimental group III on the 14th day of administration of gadolinium orthovanadate nanoparticles at a dose of 2.0 mg/l of drinking water, it was established that the separation of layers was good. The villi had signs of necrosis – the internal structure was lost, the stained tissue is neutrophilic. In the case of preservation of the own plate, the latter was infiltrated by lymphocytes. The crypts were enlarged, the nuclei of enterocytes were basophilic, tightly adjacent to each other. Goblet cells were also enlarged. Acidophilic cells differentiated poorly. The reticular fibers of the submucosal base were weakly basophilic and

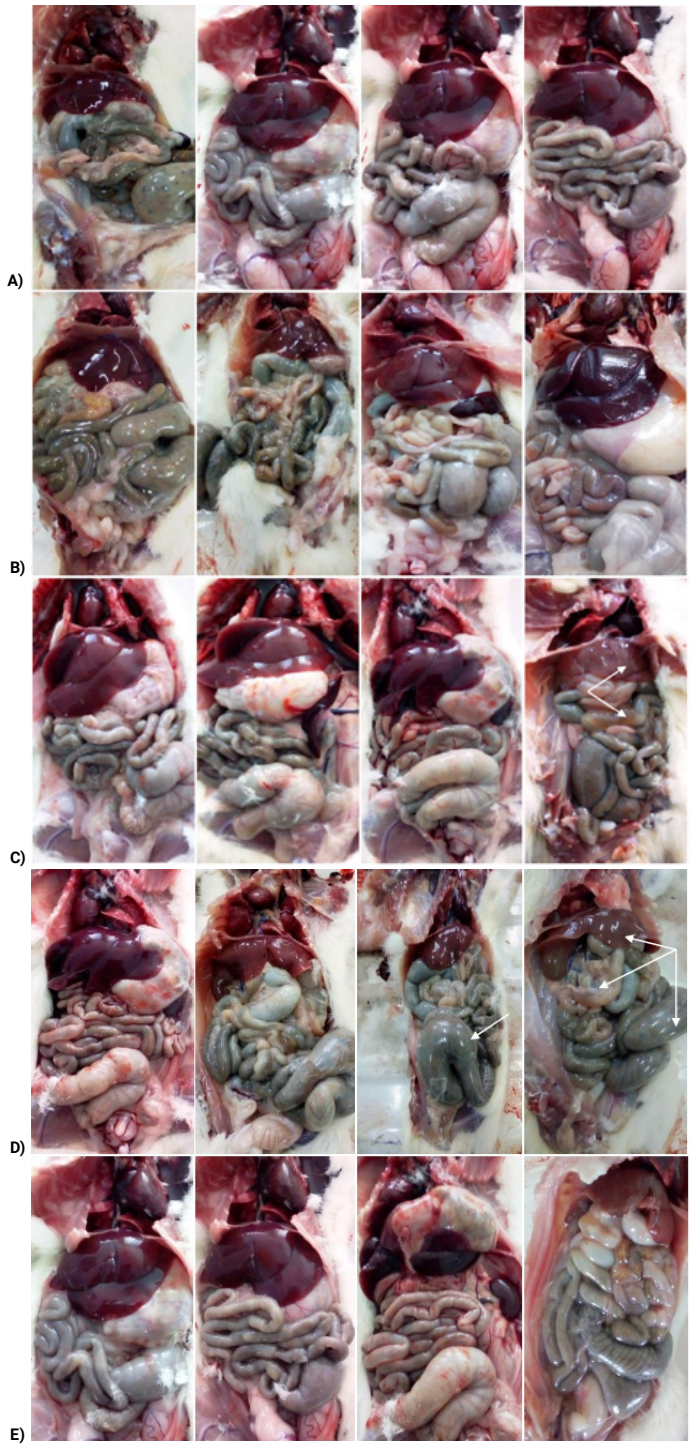

Figure 3: Patho-anatomical picture of internal organs of rats: A) on the 14th day of theexperiment; B) on the 28th day of the experiment; C) on the 42nd day of the experiment; D) onthe 56th day of the experiment; E) 14 days after stopping the administration of the nanoparticle solution

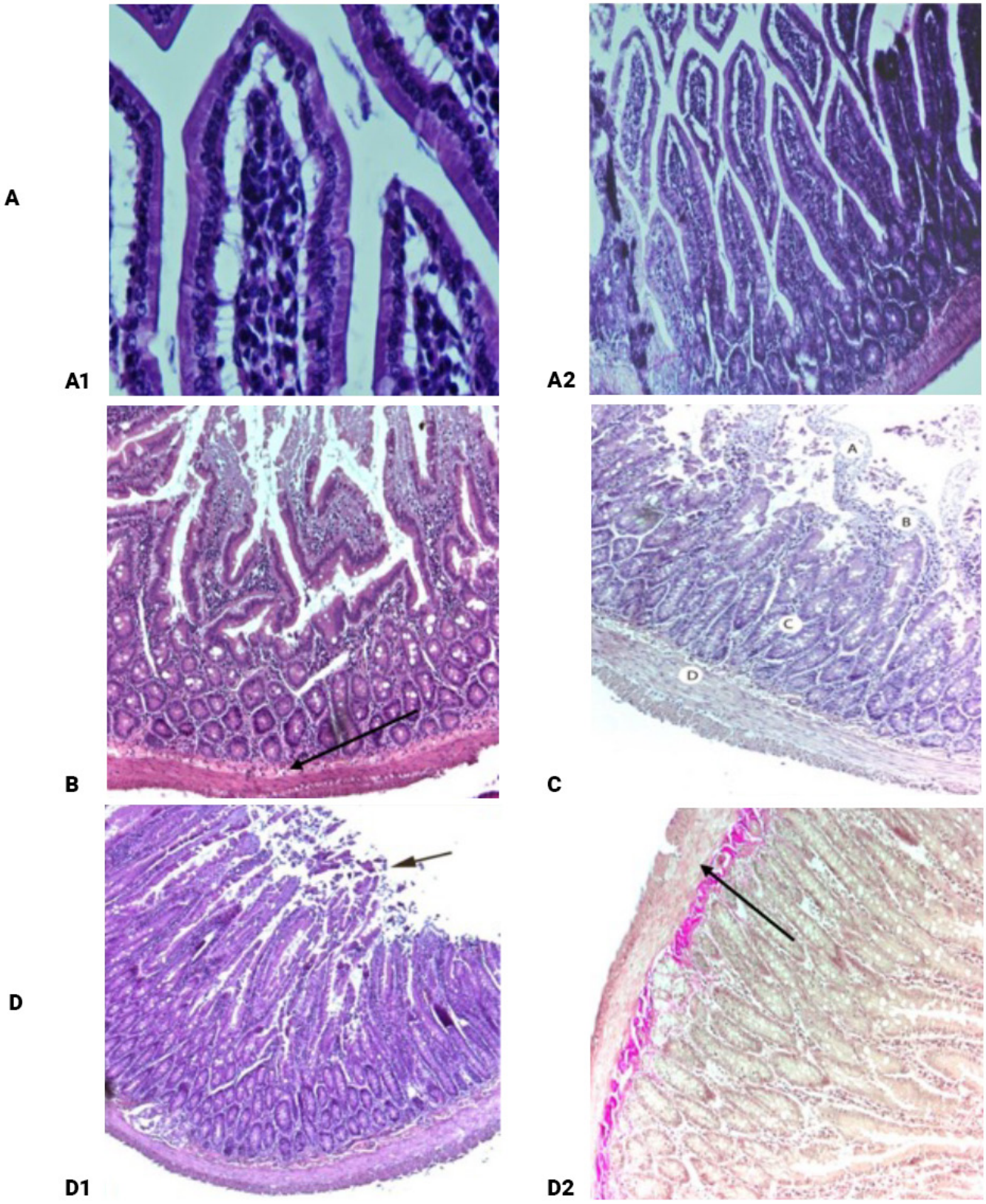

Figure 4: The structure of the duodenum of rats on the 14th day of the experiment: A) control group: A1) Villus of the proximal part, longitudinal section. Hematoxylin and eosin, ×400; A2) Proximal part. Hematoxylin and eosin, ×100; B) I experimental group (NP $GdVO_4$:Eu^{3+} 0,2 mg/l drink water). Lymphocytic infiltration of the lamina propria Hematoxylin and eosin, ×100; D) II experimental group (NP $GdVO_4$:Eu^{3+} 1,0 mg/l drink water). D1) Desquamation of the epithelium of the villi, ×50; D2) Thickening of the circular muscle layer. Van Gieson, ×100; C) III experimental group (NP $GdVO_4$:Eu^{3+} 2,0 mg/l drink water). Dystrophy and necrosis of the villous epithelium (A). Lymphoid infiltration of the lamina propria (B), crypt hyperplasia (C). Edema of the muscle layer (D). Hematoxylin and eosin, ×100

fragmented. The circular muscle layer was thickened, swollen, weakly oxyphilic (Fig. 4).

On the 28th day of the experiment, histological studies of the duodenum fragments of the control group of rats showed that the layers were well demarcated. Villi were whole, evenly covered with a layer of epithelium. The nuclei of enterocytes were basophilic, contoured, the cytoplasm was neutrophilic. Goblet cells were well defined, vacuoles were transparent.

Acidophilic cells differentiated. The nuclei of lymphocytes and plasma cells of the lamina propria were contoured and basophilic. The vessels of the submucosal base were moderately filled with blood. The connective tissue of the submucosal base was oxyphilic and structured. The muscle layers were well demarcated (Fig. 5).

In the experimental group I on the 28th day of administration of gadolinium orthovanadate nanoparticles at a dose of 0.2 mg/l of drinking water, it was established that the layers were demarcated. Sometimes, the tips of the villi were destructured, the epithelial layer was fragmented or peeled off. In some places, nuclei and cell boundaries werenot defined. Slight destructuring of the own plate of the tip of the villi. The basal part of the villi and the crypts were preserved. The main plate was somewhat filled with lymphocytes and plasma cells. The nuclei of enterocytes were basophilic, contoured, the cytoplasm was neutrophilic. Goblet cells were well defined, some of them were enlarged. Acidophilic cells differentiated. The submucous base was structured. Fibers were oxyphilic, cell nuclei were basophilic, contoured. The muscle layers were painted evenly (Fig. 5).

In the experimental group II on the 28th day of administration of gadolinium orthovanadate nanoparticles at a dose of 1.0 mg/l of drinking water, it was established that the demarcation of layers was good. Villi were whole, evenly covered with a layer of epithelium. The nuclei of enterocytes were basophilic, contoured, the cytoplasm was intensely neutrophilic. Goblet cells contained transparent vacuoles. Acidophilic cells differentiated. The lamina propria contained the nuclei of lymphocytes and plasma cells. Lymphocytic infiltration of the lamina propria was observed in some villi. The submucosal base was structured, oxyphilic (Fig. 5).

In the experimental group III on the 28th day of administration of gadolinium orthovanadate nanoparticles at a dose of 2.0 mg/l of drinking water, it was established that the layers were demarcated. The villi had signs of apical destruction: the epithelial layer was fragmented or absent, the enterocytes of the apices were pyknomorphic, the lamina propria was homogenized, neutrophilic and infiltrated by lymphocytes. In some cases, the villi were completely destroyed. Acidophilic cells were determined. Goblet cells were slightly enlarged.

Crypts were enlarged and elongated. The epithelium of the crypts had signs of hyperplasia – the nuclei were hyperchromic, densely located. The vessels of the submucosal base were dilated, filled with blood cells. Reticular fibers were acidophilic, structured. The muscle layers were well demarcated. There was a lot of mucus in the intestinal lumen (Fig. 5).

On the 42nd day of the experiment, histological studies of the fragments of the duodenum of the control group of rats established that the demarcation of the layers was well defined, the villi were intact, and the epithelium covered the surface evenly. Nuclei of enterocytes were moderately basophilic, rounded, equal in size, located at the basal pole of the cells. goblet cells were contoured, vacuoles were transparent, rounded. Acidophilic cells were well defined.

The crypt lumen was free. Lymphocytes, plasma cells and fibroblasts of the lamina propria were evenly distributed and had a contoured, basophilic core. Lacteal lumen was moderate. The muscle plate was intact, the cytoplasm of the cells was oxyphilic, the nuclei were contoured, basophilic. The vessels of the submucosal base were moderately filled with blood. Reticular fibers were oxyphilic, evenly stained. The number of fibrocytes and lymphocytes in the submucosal base was moderate. The muscle layers were intact, structured, the cell nuclei were well contoured, basophilic, the cytoplasm was oxyphilic, and the layer between the fibers was defined. Enterocytes of the crypts were hyperchromic, the nuclei were densely located. Acidophilic cells differentiated. The vessels of the submucosal base were dilated, the connective tissue was oxyphilic and structured. The muscle layers were well demarcated and structured. The nuclei of epithelial cells, lymphocytes, and fibroblasts were stained brown-black, the intercellular substance was light brown (Fig. 6).

In the experimental group I, on the 42nd day of administration of gadolinium orthovanadate nanoparticles at a dose of 0.2 mg/l of drinking water, it was established that the demarcation of the layers of the wall of the small intestine was good. Villi were thickened. Sometimes, the epithelial layer was unevenly distributed. The lamina propria was focally infiltrated with lymphocytes. Enterocytes of the crypts were hyperchromic, nuclei were densely arranged, acidophilic cells were differentiated. Some goblet cells were hypertrophied. In the submucosal layer, there was defibrillation of the structures, the fibers were oxyphilic. The muscle layers were well demarcated, the cytoplasm of the cells was oxyphilic (Fig. 6).

In the experimental group II on the 42nd day of administration of gadolinium orthovanadate nanoparticles at a dose of 1.0 mg/l of drinking water, it was established that the demarcation of the layers of the wall of the small intestine was good. The tips of some villi were with signs of necrosis of the epithelium and lamina propria. Enterocyte cells were weakly stained, some nuclei were missing, and the

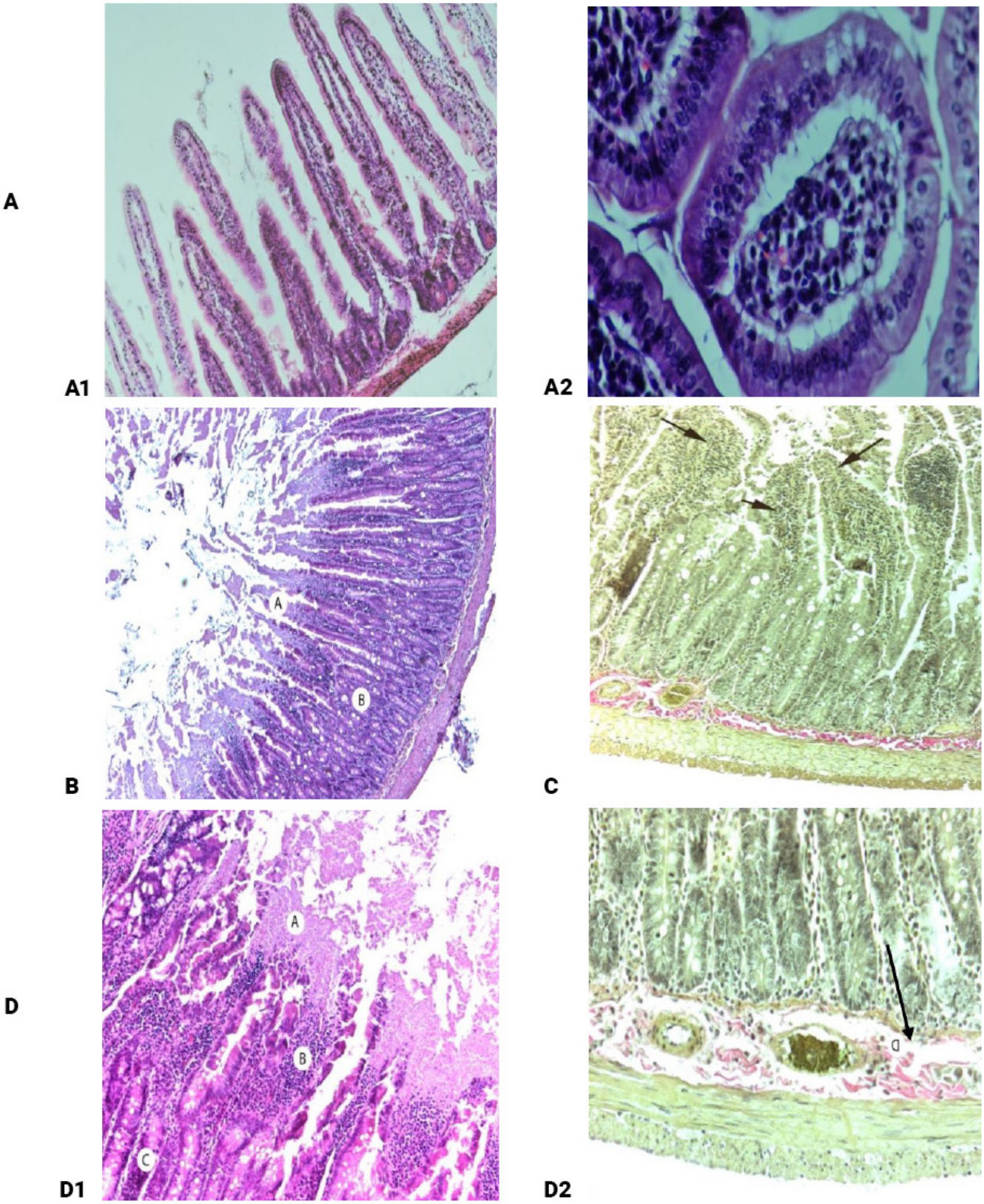

Figure 5: The structure of the duodenum of rats on the 28th day of the experiment.: A) control group: A1) Rat duodenum. Hematoxylin and eosin, ×100; A2) Villus of the proximal part, transverse section. Hematoxylin and eosin, ×400; B) I experimental group (NP $GdVO_4$:Eu^{3+} 0,2 mg/l of drinking water). Necrosis of the tips of the villi (A). Hypertrophy of goblet cells (B). Hematoxylin and eosin, ×50; C) II experimental group (NP $GdVO_4$:Eu^{3+} 1,0 mg/l of drinking water). Lymphocytic infiltration of individual villi. Van Gieson, ×100; D) III experimental group (NP $GdVO_4$:Eu^{3+} 2,0 mg/l drinking water): D1) Dystrophy and necrosis of the villous epithelium (A). Lymphoid infiltration of the main plate (B). Hyperplasia of the crypt epithelium (C). Hematoxylin and eosin, ×100; D2) Edema of the submucosal base. Van Gieson, ×200.

cytoplasm had a fragmented structure. The lamina propria was infiltrated with lymphocytes. In most villi, the structure was preserved, enterocytes had basophilic contoured nuclei. Goblet cells were well defined and had transparent vacuoles. Acidophilic cells differentiated. The muscular plate was intact, the submucous base was oxyphilic and structured. The muscle layers were well demarcated (Fig. 6).

In the experimental group III on the 42nd day of administration of gadolinium orthovanadate nanoparticles at a dose of 2.0 mg/l of drinking water, it was established that the demarcation of the layers of the wall of the small intestine was good. Infiltration of the lamina propria by lymphocytes was observed. At the tips of the villi, the epithelium was exfoliated in many cases, the tips of the villi were thickened due to lymphoid infiltration. Fragments of villi tissues were observed in the intestinal lumen. In some cases, only the shells of the basal membrane and vessel walls remained from the villi, while atrophy of the mucous layer as a whole was observed. Goblet cells were well contoured, their vacuoles were transparent. Enterocytes of crypts were hyperchromic, acidophilic cells differentiated in individual crypts. The connective tissue of the submucosal base was oxyphilic, with signs of defibrillation. The muscle layers were structured, oxyphilic, and evenly stained (Fig. 6).

On the 56th day of the experiment, histological studies of duodenum fragments of rats in the control group established that the demarcation of the layers was well defined, the villi were intact, and the epithelium covered the surface evenly. Nuclei of enterocytes were moderately basophilic, rounded, equal in size, located at the basal pole of the cells. Goblet cells were contoured, vacuoles were transparent, rounded. Acidophilic cells were well defined. The crypt lumen was free. Lymphocytes, plasma cells and fibroblasts of the lamina propria were evenly distributed and had a contoured, basophilic nucleus. Lacteal lumen was moderate. The muscle plate was intact, the cytoplasm of the cells was oxyphilic, the nuclei were contoured, basophilic. The vessels of the submucosal base were moderately filled with blood. Reticular fibers were oxyphilic, evenly stained. The number of fibrocytes and lymphocytes in the submucosal base was moderate. The muscle layers were intact, structured, the cell nuclei were well contoured, basophilic, the cytoplasm was oxyphilic, and the layer between the fibers was defined. The color of the drug was uniform. Slight fuchsinophilia was observed in the structures of the submucosal base and muscle layers. The nuclei of epithelial cells, lymphocytes, and fibroblasts were stained brown-black, the intercellular substance was light brown. Connective tissue structures were stained in red (Fig. 7).

In the experimental group I on the 56th day of administration of gadolinium orthovanadate nanoparticles at a dose of 0.2 mg/l of drinking water, it was established that the demarcation of layers was good. Desquamation of the epithelial layer, loss of its structure, was sometimes observed on the tips of the villi. The central part of the villi was preserved. The epithelial layer was structured, the nuclei of endotheliocytes were basophilic, the cytoplasm was neutrophilic, weakly basophilic. In the lamina propria, nuclei of lymphocytes, plasma cells, and blood vessels were observed. Goblet cells were contoured, alveoli were transparent. In some crypts, goblet cells were enlarged in size (Fig. 7).

In the experimental group II on the 56th day of administration of gadolinium orthovanadate nanoparticles at a dose of 1.0 mg/l of drinking water, it was established that the demarcation of the layers of the wall of the small intestine was good. The tips of the villi had signs of epithelium desquamation and necrosis of the lamina propria. The tissue had neutrophilic staining and was homogenized. The middle part of the villi was better preserved: a layer of enterocytes was defined. Cell nuclei were basophilic, contoured. The cytoplasm was neutrophilic or weakly basophilic. Nuclei of lymphocyte, plasmocytes, and vascular structures were observed in the lamina propria. Goblet cells were well defined, their vacuoles were transparent.

Enterocytes of the crypts were intensely basophilic, acidophilic cells were weakly differentiated.

The muscular plate was intact, the tissue of the submucous base had a fibrous structure and oxyphilic coloration. On some preparations, the layers between the fibers were enlarged. The circular muscle layer was slightly thickened (Fig. 7).

In the experimental group III on the 56th day of administration of gadolinium orthovanadate nanoparticles at a dose of 2.0 mg/l of drinking water, it was established that the demarcation of the layers of the wall of the small intestine was good. There was thickening of the tips of the villi, homogenization of the structures of the lamina propria, and desquamation of the epithelium. Villous enterocytes had a contoured basophilic nucleus and neutrophilic cytoplasm. The number of goblet cells of villi was reduced. Lymphocytic infiltration was observed at the base of the villi, in some areas. Enterocytes of individual crypts were hyperchromic, their nuclei were quite densely arranged. The goblet cells of the crypts were slightly enlarged. Acidophilic cells differentiated. The muscle plate was intact. The submucous base was structured, oxyphilic. The vessels of the submucosal base had collapsed. The muscle layers were well demarcated and structured (Fig. 7).

14 days after stopping the administration of nanoparticle preparations, histological studies of the fragments of rat duodenum in the control group revealed that the demarcation of the layers was good, the villi were intact, and the epithelium covered the surface evenly. The nuclei of enterocytes were moderately basophilic, rounded, of the same size, located at the basal pole of the cells. Goblet cells were contoured, vacuoles were transparent, rounded. Acidophilic cells were well defined. The crypt lumen was free. Lymphocytes, plasma cells and fibroblasts of the lamina

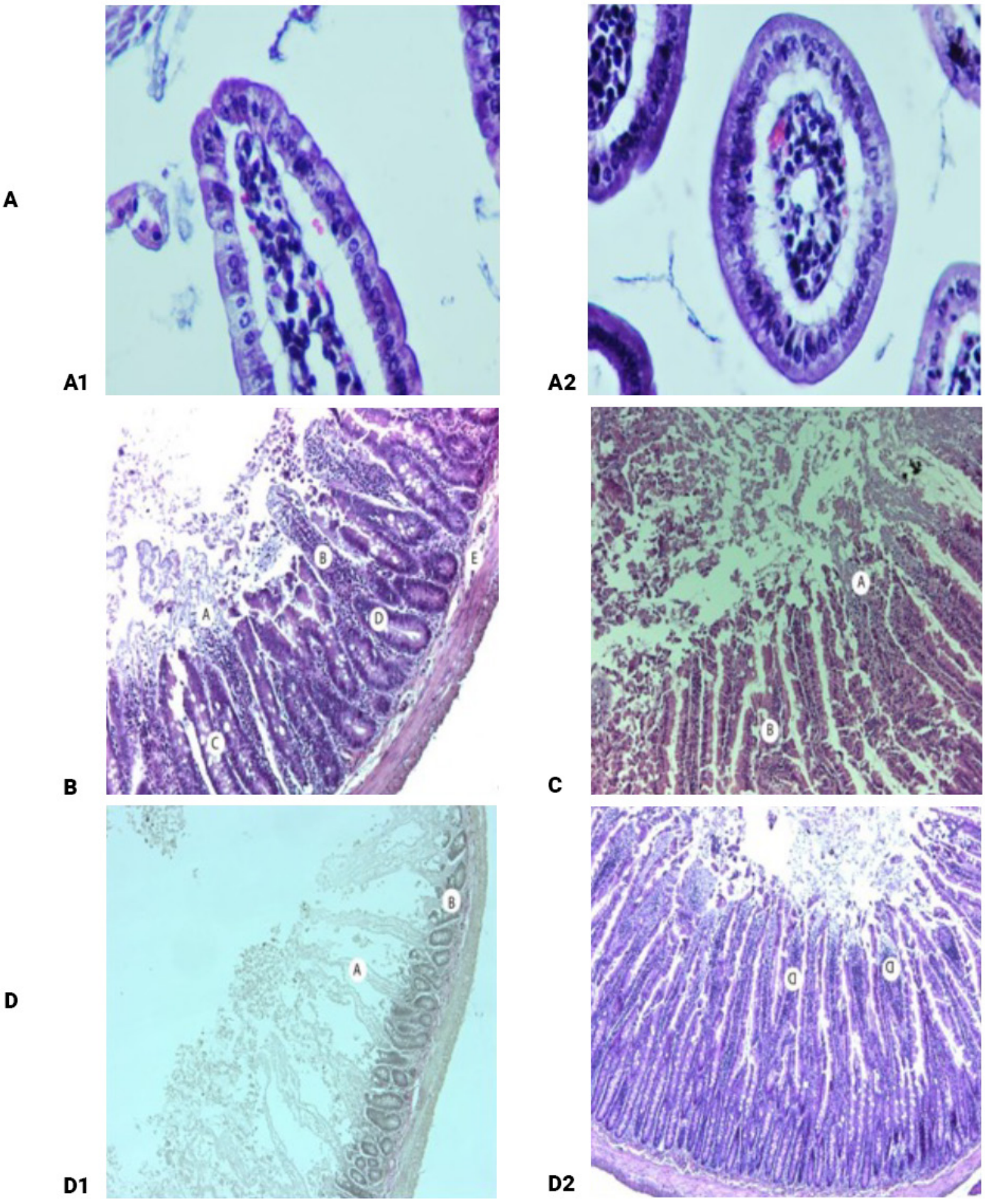

Figure 6: The structure of the duodenum of rats on the 42nd day of the experiment: A) control group: A1) The tip of the villi, longitudinal section. Hematoxylin and eosin, ×400; A2) The tip of the villi, cross section. Hematoxylin and eosin, ×400; B) I experimental group (NP $GdVO_4$:Eu^{3+} 0,2 mg/l of drinking water). Destruction of the epithelium and own plate of villi (A). Lymphocytic infiltration of the lamina propria (B), hypertrophy of goblet cells (C) and hyperplasia of crypt enterocytes (D). Edema of the submucosal base (E). Hematoxylin and eosin, ×100; C) II experimental group (NP $GdVO_4$:Eu^{3+} 1,0 mg/l of drinking water). Necrosis and desquamation of the epithelium of the tips of some villi (A). Lymphoid infiltration of the lamina propria of some villi (B). Hematoxylin and eosin. ×100. D) III experimental group (NP $GdVO_4$:Eu^{3+} 2,0 mg/l of drinking water): D1) Necrosis of the villi (A) and mucosal atrophy (B). Van Gieson, ×50; D2) Lymphoid infiltration of the lamina propria (D). Hematoxylin and eosin, ×50.

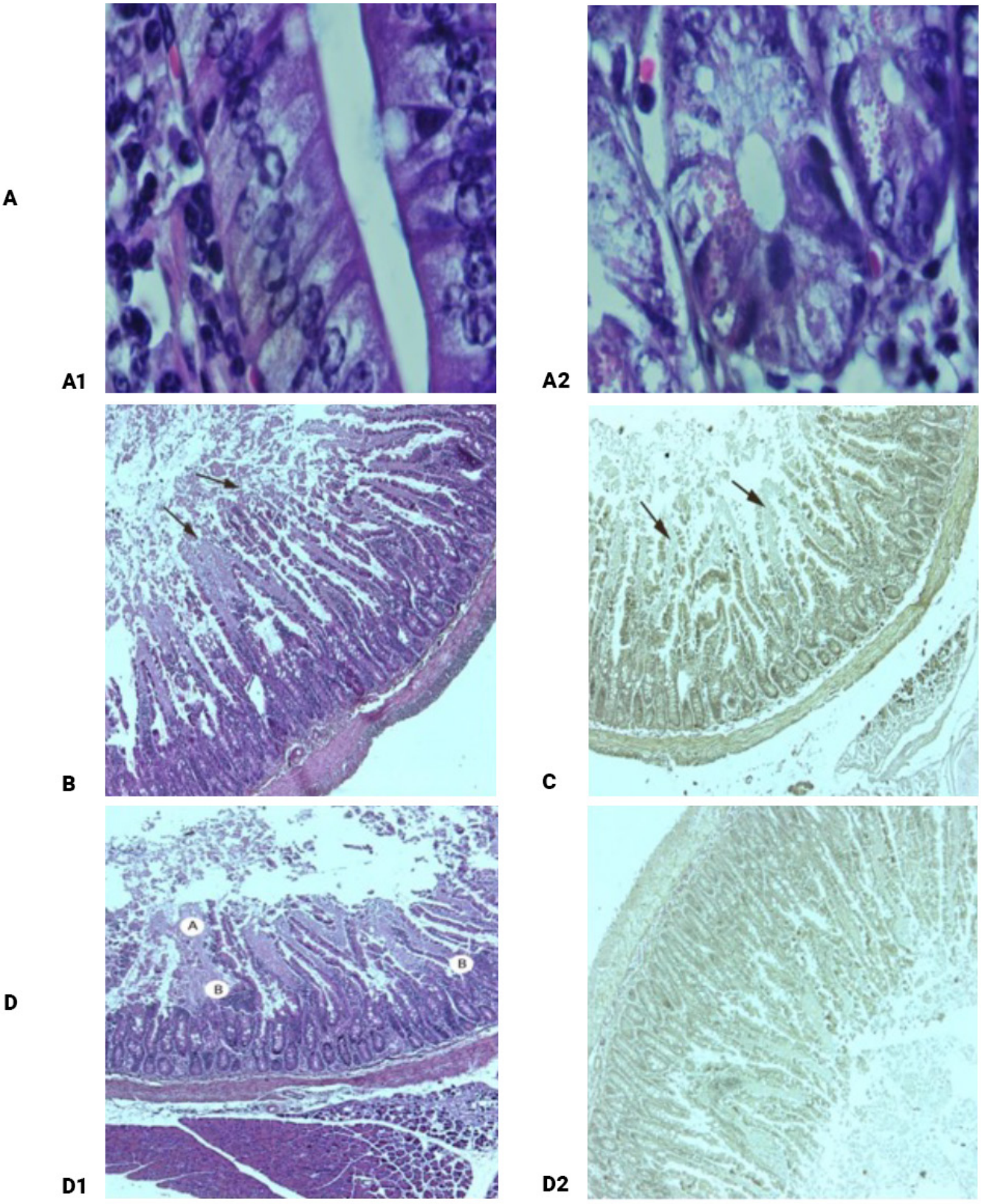

Figure 7: The structure of the duodenum of rats on the 56th day of the experiment: A) controlgroup: A1) Acidophilic cells of the crypt. Hematoxylin and eosin, ×1000; A2) Enterocytes of villi. Hematoxylin and eosin, ×1000; B) I experimental group (NP $GdVO_4$:Eu^{3+} 0,2 mg/l of drinking water). Desquamation of the epithelium of the villi. Hematoxylin and eosin, ×50; C) II experimental group (NP $GdVO_4$:Eu^{3+} 1,0 mg/l of drinking water). Desquamation of the epithelium and necrosis of the lamina propria of the tips of the villi. Van Gieson, ×50; D) III experimental group (NP $GdVO_4$:Eu^{3+} 2,0 mg/l of drinking water): D1) Desquamation of the epithelium, necrosis of the tips of the villi (A) and lymphocytic infiltration of the lamina propria (B).

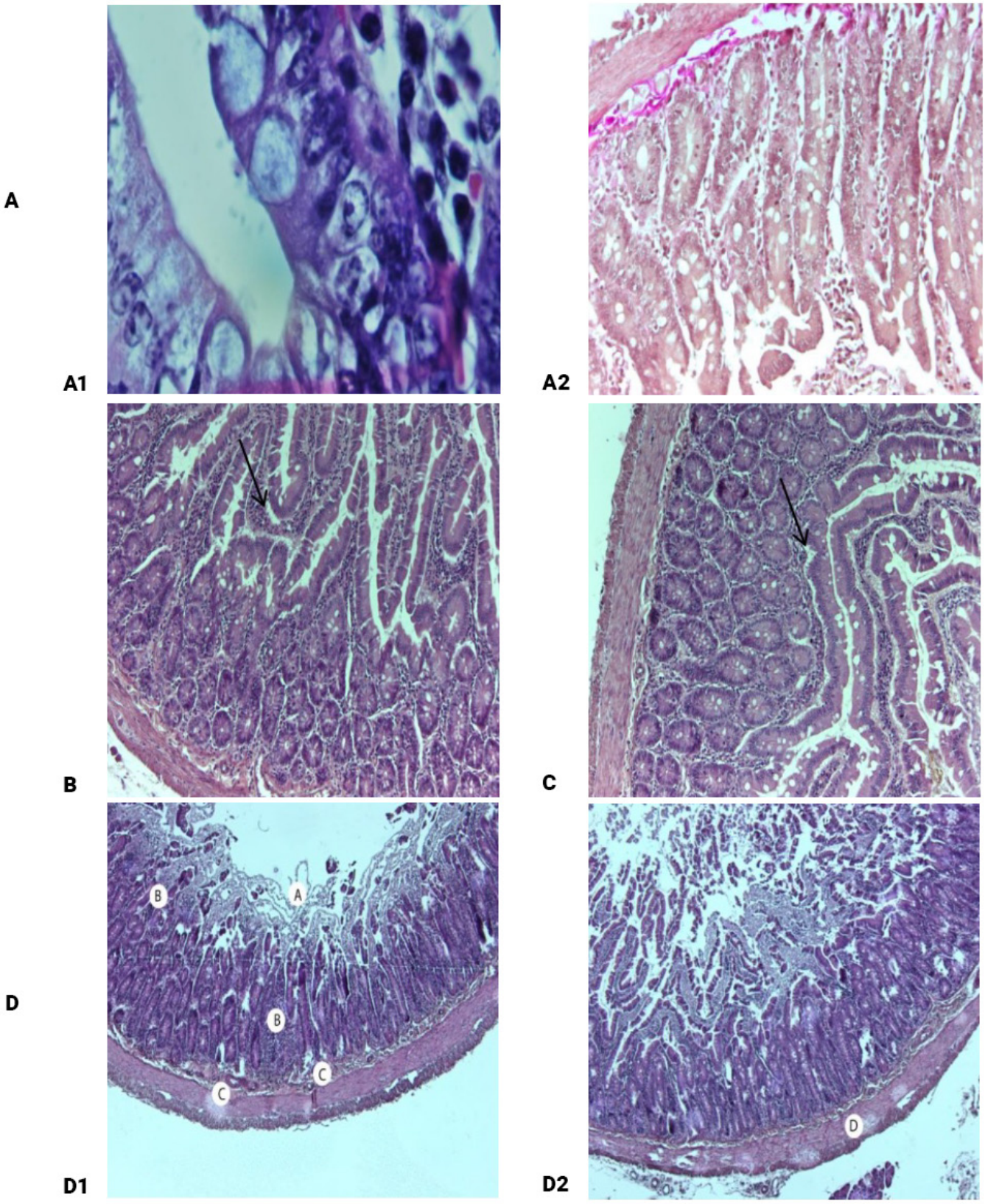

Figure 8: The structure of the duodenum of rats 14 days after stopping the administration of nanoparticle preparations: A) control group: A1) Goblet cells. Hematoxylin and eosin, ×1000; A2) Rat duodenum. Van Gieson, ×200; B) I experimental group (NP $GdVO_4$:Eu^{3+} 0,2 mg/l of drinking water). Slight infiltration of the lamina propria. Hematoxylin and eosin, ×100; C) II experimental group (NP $GdVO_4$:Eu^{3+} 1,0 mg/l of drinking water). Rat duodenum. Lymphocytic infiltration of the lamina propria. Hematoxylin and eosin, ×100; D) III experimental group (NP $GdVO_4$:Eu^{3+} 2,0 mg/l of drinking water): D1) Necrosis of mucosal villi (A) and lymphoid infiltration of the lamina propria (B). Edema of the submucosal base (C). Hematoxylin and eosin, ×50; D2) Edema and infiltration of the circular muscle layer (D). Hematoxylin and eosin, ×50

propria were evenly distributed and had a contoured, basophilic nucleus. Lacteal lumen was moderate. The muscle plate was intact, the cytoplasm of the cells was oxyphilic, the nuclei were contoured, basophilic. The vessels of the submucosal base had collapsed, there were blood cells in the lumen of some of them. Reticular fibers were oxyphilic, evenly stained. The number of fibrocytes and lymphocytes in the submucosal base was moderate. The muscle layers were intact, structured, the cell nuclei were well contoured, basophilic, the cytoplasm was oxyphilic, and the interlayer between the fibers was defined (Fig. 8).

In the experimental group I, 14 days after stopping the administration of gadolinium orthovanadate nanoparticles at a dose of 0.2 mg/l of drinking water, it was established that the layers of the wall of the small intestine were well demarcated. Villi were whole, evenly covered with a layer of epithelium. The nuclei of enterocytes were basophilic, contoured, the cytoplasm was neutrophilic. Goblet cells were well defined, vacuoles were transparent. Acidophilic cells differentiated. The nuclei of lymphocytes and plasma cells of the lamina propria were contoured and basophilic. Slight infiltration of the lamina propria was noted. The vessels of the submucosal base were moderately filled with blood. The connective tissue of the submucosal base was oxyphilic and structured. The muscle layers were well demarcated (Fig. 8).

In the experimental group II, 14 days after stopping the administration of gadolinium orthovanadate nanoparticles at a dose of 1.0 mg/l of drinking water, it was established that the layers of the wall of the small intestine were well demarcated. Villi werewhole, evenly covered with a layer of epithelium. Individual villi were thickened due to an increase in lymphocytes and plasma cells of the main plate. The goblet cells of the crypts were enlarged. Enterocytes of individual crypts had signs of hyperplasia: the nuclei were hyperchromic, closely adjacent to each other, and their number was significantly increased. The lacteals were narrowed. The nuclei of enterocytes were basophilic, contoured, the cytoplasm was intensely neutrophilic. Acidophilic cells differentiated. The lamina propria contained the nuclei of lymphocytes and plasma cells. Lymphocytic infiltration of the lamina propria was observed in some villi. The submucous base was structured, oxyphilic. The lumen of individual Brunner glands was enlarged, the cytoplasm of mucocytes was weakly oxyphilic and contained a significant number of vacuoles. Cell nuclei were basophilic. The muscle layers were well demarcated (Fig. 8).

In the experimental group III, 14 days after stopping of administration of gadolinium orthovanadate nanoparticles at a dose of 2.0 mg/l of drinking water, it was established that the demarcation of the layers of the wall of the small intestine was good. Necrosis of the lamina propria was observed in almost all villi of the mucous membrane, while separate fragments of the epithelial cover and basement membrane were preserved. The crypts were elongated, the cells were hyperchromic, the lumen of the glands was insignificant. Goblet cells were contoured. Areas of lymphoid infiltration were observed between the crypts. The muscle plate was fragmented. The submucous base was weakly oxyphilic or neutrophilic. The vessels of the submucosal base had collapsed. The circular muscle layer was thickened, oxyphilicity was reduced, the color was uneven (Fig. 8).

Discussion

At the beginning of the discussion, we would like to note that this research is the first regarding this type of nanoparticles, the terms and conditions of their administration (young animals and the presence of a stress factor). First of all, we would like to focus on the control group of animals, because a logical question arises: why did food stress not cause significant pathological changes in the duodenum of rats compared to the use of nanoparticles?

Let's recall the structure of the intestinal mucosa of animals, which includes several protective barriers. This is quite well reflected in the scheme proposed by Chinese researchers (33). They distinguish four barriers: the first is microbiological (the so-called "good" microflora), the second is chemical (includes neutral and acidic mucin), the third is a mechanical barrier (intestinal epithelial cells are tightly connected by proteins), and the fourth is immunological (includes macrophages and cytokines) (Fig. 9).

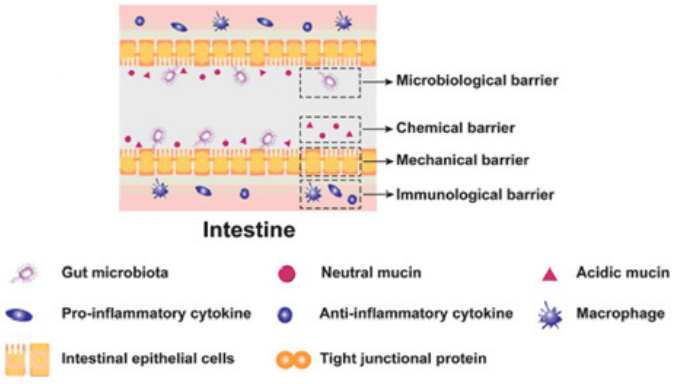

Figure 9: Schematic structure of the mucous membrane of the small intestine of animals (33)

In our opinion and based on the available literature data (34, 35, 36), the absence of pathological changes in the control group is associated with the presence of adaptive reactions of the intestinal wall to excessive fiber, which caused subchronic mechanical irritation, namely: by increasing the synthesis and secretion of mucin, which neutralized inflammatory processes. This mechanism can be roughly depicted as follows (Fig. 10): due to mechanical action, the fiber peels off the microbiological chemical barrier and interacts with the epithelium of the mucous membrane, stimulating the formation of mucin, which in turn has anti-inflammatory properties (37) and increasing its quantity prevents further

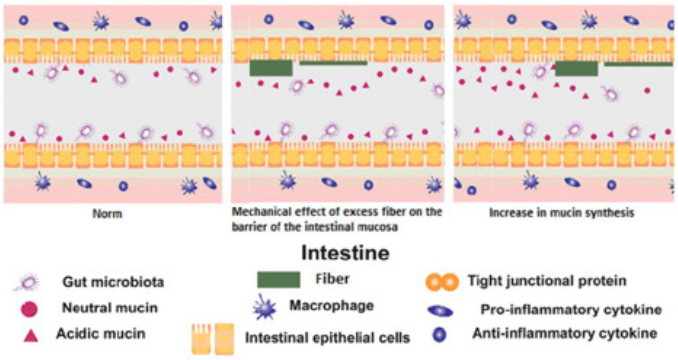

Figure 10: The mechanism of action of excess fiber on the intestinal mucosa

destruction of the mechanical barrier. Besides, some authors have observed no or only minor apparent effects on small and large intestinal morphology in response to high fiber feeding in pigs and rats (38, 39, 40, 41).

The introduction of nanoparticles slightly changes the mechanism of adaptation of the intestinal wall, depending on the dose and time of introduction: the fiber peels off the microbiological chemical barrier, which allows the nanoparticles to come into direct contact with the epithelium of the mucous membrane, resulting in the activation of both villus cells and the immunological barrier. In the future, due to the increase in mucin secretion, part of the nanoparticles probably binds to its components, part interacts with bacteria, and part enters the general bloodstream, which causes a general effect on the body (Fig. 11).

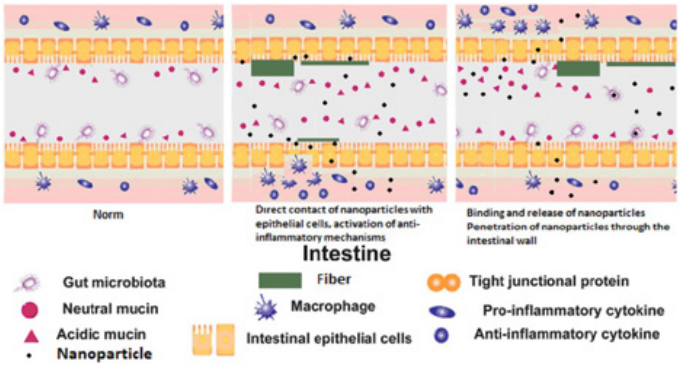

Figure 11: The mechanism of action of excess fiber together with nanoparticles on the intestinal mucosa

With a 5-fold increase in the dose of gadolinium orthovanadate nanoparticles to 1.0 mg/l of drinking water (≈0.15 mg/kg of body weight), the processes of activation of the mechanical barrier continue up to the 42nd day of administration, which is manifested by an increase in goblet cells of villi and crypts, and the processes of activation of the immunological barrier – during the entire period of administration by the presence of lymphocytic infiltration of the lamina propria. From the 42nd to the 56th day of administration, there is a certain exhaustion of adaptation processes in the cells of the epithelium of the intestinal mucosa of rats, which is indicated by the presence of signs of necrosis of the epithelium of some villi tips, fragmentation of the cytoplasm, desquamation of the epithelium and necrosis of the lamina propria.

It should be noted that these changes were also reversible, since the 14th day after the cessation of the administration of nanoparticles, the restoration of the integrity of the villi was observed, but some villi still remained thickened due to the increase in lymphocytes and plasma cells of the main plate. Also, the goblet cells of the crypts remained enlarged, and the enterocytes of individual crypts had signs of hyperplasia. So, we can also assert the adaptogenic effect of NP $GdVO_4$:Eu^{3+} at a dose of ≈0.15 mg/kg of body weight (1.0 mg/l of drinking water), but it is limited in time –28 days.

When NP $GdVO_4$:Eu^{3+} was administered to rats at a dose of ≈0.3 mg/kg of body weight (2.0 mg/l of drinking water), the processes of exhaustion of the adaptive capabilities of the intestinal mucosa and excessive activation of the immunological barrier were observed, which were manifested by dystrophic changes starting from the 14th day of administration: necrosis and loss of the internal structure of villi, increase of crypts, thickening and swelling of the circular muscle layer, excessive lymphocytic infiltration of the lamina propria. As a result of nanoparticles administration for 28 days, dystrophic processes progressed: the villi had signs of tip destruction (in some cases they were completely destroyed), homogenization of the lamina propria, increase of goblet cells and elongation of crypts, hyperplasia of the epithelium, which caused excessive mucus formation and general clinical manifestation in the form of liquefaction of feces on the 42nd and 56th days of administration, respectively. Accordingly, at the given period of the experiment, significant signs of destruction of the intestinal mucosa of rats were also noted: fragments of villi tissue were noted in the lumen of the intestine, in some cases only the shells of the basement membrane and vessel walls remained from the villi, atrophy of the mucous layer as a whole was observed. Significant infiltration of mucosal elements by lymphocytes was also noted, that is, existing inflammation. It should be noted that 14 days after the stopping of administration, only partial signs of restoration of the mucous membrane structure were observed: some fragments of the epithelial cover and basement membrane were preserved, there were areas of lymphoid infiltration between the crypts, and in general, lamina propria necrosis occurred in almost all villi of the mucous membrane. Based on the above, it is possible to assert the toxic effect of NP $GdVO_4$:Eu^{3+} at a dose of ≈0.3 mg/kg of body weight (2.0 mg/l of drinking water) under the conditions of feed stress.

In addition to the dose and external factors (increase or decrease in direct access to cells), the toxicity of nanoparticles depends on their properties. So, for example, gold nanoparticles smaller than 6 nm effectively penetrated the cell nucleus, while large NPs (10-16 nm) penetrated only through the cell membrane and were located only in the

cytoplasm. This suggests that NPs smaller than 10 nm may exhibit more toxic effects than those larger than 10 nm (42). The dependence of toxicity on their size was also established using the example of gold nanoparticles: 15 nm NPs were 60 times less toxic than 1.4 nm NPs for fibroblasts, epithelial cells, and macrophages (43). NPs smaller than 5 nm usually cross cell barriers nonspecifically, for example, by translocation, while larger particles enter cells by phagocytosis; a NP size of around 25 nm is thought to be optimal for pinocytosis (44). That is, the investigated gadolinium orthovanadate nanoparticles are quite accessible to cells (size 8×25 nm), and theoretically can have a negative effect on cells.

Another likely factor in the toxicity of the investigated nanoparticles in a larger dose is their shape (spindle-shaped). For example, a comparison of the effects of hydroxyapatite NPs of different shapes (acicular, lamellar, rod-like, and spherical) on cultured BEAS-2B cells showed that rod-shaped and acicular NPs cause more cell death than spherical and rod-shaped NPs (45). The cytotoxic potential of rod-like (65 ± 15 nm) ZnO nanoparticles was greater than that of spherical (60 ± 20 nm) ZnO nanoparticles when applied to human peripheral blood mononuclear cell culture and was limited to proliferative lymphocytes. At the same time, rod-shaped ZnO NPs produced more more active forms of oxygen compared to spherical ones and caused significant DNA damage in the above cell culture (46). However, the most destructive effects in the body are caused by spindle-shaped nanoparticles. Also, when affecting the body, the dose-effect relationship is clearly visible (47, 48).

Conclusions

Based on the conducted subchronic toxicological experiment on white rats under conditions of feed stress, taking into account the results of clinical and pathohistological studies, a safe and effective range of doses for further use in farm animals can be considered a concentration of gadolinium orthovanadate nanoparticles of 0.2-1.0 mg/dm^3 of drinking water (≈ 0.03-0.15 mg/kg of body weight), and the administration period – 42-28 days, respectively.

Nanoparticles of gadolinium orthovanadate under certain conditions (food stress, young animals) have a rather small range of therapeutic effect, since when the dose was increased to 2.0 mg/dm^3 of drinking water (≈0.3 mg/kg of body weight), destructive processes occurred in the intestines of rats .

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Patomorfološke spremembe v dvanajstniku podgan ob subkroničnem peroralnem dajanju nanodelcev gadolinijevega ortovanadata ob prehranskem stresu

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Izvleček: V naši raziskavi nas je zanimala dejanska prisotnost prilagoditvenih ali negativnih reakcij v steni tankega črevesa belih podgan pod vplivom nanodelcev gadolinijevega ortovanadata v razponu odmerkov (≈ 0,03–0,3 mg/kg telesne teže) v pogojih prehranskega stresa (zaradi presežka vlaknin in pomanjkanja beljakovin v prehrani) in njihova stopnja izražanja, saj se tovrstna nesorazmernost obrokov v Ukrajini pogosto pojavlja. Nanodelci gadolinijevega ortovanadata imajo pomemben potencial za uporabo v živinoreji in perutninarstvu, saj v območju odmerkov 0,03–0,15 mg/kg telesne teže preprečujejo negativne učinke na črevesno sluznico tudi pri stresu zaradi krme. Ugotovljeno je bilo, da dajanje nanodelcev gadolinijevega ortovanadata v odmerkih 0,03 in 0,15 mg/kg telesne teže belim podganam s pitno vodo 56 oziroma 28 dni povzroči aktivacijo mehanske in imunološke pregrade sluznice, kar se kaže v povečanju števila čašastih celic, hiperplaziji enterocitov nekaterih kript, zadebelitvi resic in infiltraciji limfocitov, ki 14 dni po prenehanju dajanja dosežejo kontrolno raven. Vendar pa povečanje odmerka nanodelcev gadolinijevega ortovanadata na 0,3 mg/kg telesne teže pri prehranskem stresu povzroči izčrpavanje prilagoditvenih sposobnosti črevesne sluznice in pretirano aktivacijo imunološke pregrade, kar se je od 14. dneva dajanja pokazalo z distrofičnimi spremembami, ki so se poglobile do 56. dne in se po 14 dneh po prenehanju dajanja niso izravnale.

Ključne besede: redke zemeljske kovine; nanodelci gadolinijevega ortovanadata; patomorfološke spremembe, dvanajstnik; bele podgane; krmni stres