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## The study of acute and chronic toxicity of the sodium-, calcium-, iron-polygalacturonate pharmacological substance in rabbits



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## ARTICLE INFO

## Keywords:

Na-,Ca-,Fe-polygalacturonate  
Pectin polysaccharides modification  
Acute toxicity  
Chronic toxicity  
Antianemic preparation

## ABSTRACT

The purpose of this study is the assessment of the acute and chronic toxicity of pharmacological substance sodium, calcium, iron-polygalacturonate (PG Na,Ca,Fe) in rabbits as one of the stages of preclinical studies. We studied an acute and chronic oral toxicity of PG Na,Ca,Fe, which stimulates the process of hemopoiesis, in male and female rabbits of the “Chinchilla”. According to the results of the study of acute toxicity of PG Na,Ca,Fe, treating with it the rabbits of both sexes in doses of 0.5–5 g/kg has no toxic effect (LD<sub>50</sub> greater than 5 g/kg). The histostructure of studied organs of animals, treated with preparations in a dose of 5 g/kg, did not differ from that of the animals of the control group. This study allow to classify PG Na,Ca,Fe as a preparation of the 6th class with respect to harmless drugs. An estimate of the chronic toxicity of PG Na,Ca,Fe at administration of preparation in the form of boluses to rabbits in doses 0.025, 0.262 and 0.5 g/kg of the body weight demonstrated that the general condition and behavior of animals did not differ from the norm. The data of hematological and biochemical studies of blood serum and urine, electrocardiographic studies, the study of the mass coefficients of the internal organs of the experimental rabbits, treated with PG Na,Ca,Fe in the mentioned doses for 60 days, compared to those obtained in the 30-day post-observation period, did not show significant changes with respect to the control and intact group of rabbits.

### 1. Introduction

In real life, consumers are exposed to complex mixtures of chemicals via food, water and commercial products consumption [1,2]. Acute and chronic toxicity evaluations for chemicals are necessary for choosing appropriate reference doses and regulatory limits, such as the acceptable daily intakes [3,4]. The classification, labelling and packaging (CLP) Regulation (Regulation 1272/2008/EC, 2015) gives the opportunity for Industry to perform animal testing in commercial mixtures as a last resort to prove a toxicological safety [3].

According to modern literature, studies on the use of oligo- and polysaccharides as therapeutic and prophylactic additives and the basis for medicines are intensively carried out in various countries. So, the well-known *Porphyra* (*Rhodophyta*) algae are an important source of food in many parts of the world. One of the main components of the *Porphyra* cell wall is the sulfated polysaccharide, which has antioxidant properties and is soluble in hot water. Based on this polysaccharide, the complex of iron (III) (liposome-PEG-PEI complex (LPPC)) has been synthesized, the physicochemical properties and the effect of anemia

inhibition at iron deficiency have been studied [5].

The preparation procedure and properties of iron (III) complexes with inulin are described in [6]. Inulin is a polyfructosan of vegetable origin, easily soluble in hot water and insoluble in cold water. The molecular weight of inulin is 5000–6000 Da. Complexes with Fe (III), obtained on the basis of functionalized inulin derivatives, demonstrated a good iron release profile under conditions, simulating those of the intestinal tract.

A large number of papers [7–9] is devoted to the study of the sorption properties and complexity of pectins and their derivatives. It has been shown that calcium-iron (III) polygalacturonate binds arsenic acid salts [10] and reduces the toxic effect of coffee acid [11]. In [12], iron polygalacturonates prepared on the basis of Grinsted XSS 100 pectin were proposed as a medicine for the treatment of anemia, the initial degree of pectin methylation was 59.4%. The data on the dependence of the Fe (II)/Fe (III) ratio in the final complex on the preparation conditions, in particular on the solution pH, are given in the paper.

The Fe (II)/Fe (III) ratio was studied by Mossbauer spectroscopy.

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<https://doi.org/10.1016/j.toxrep.2018.03.011>

Received 1 December 2017; Received in revised form 7 March 2018; Accepted 19 March 2018

Available online 21 March 2018

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Japanese authors [13,14] demonstrated a more pronounced increase in hemoglobin in rats fed by iron pectate compared to animals treated by inorganic ferrous iron in equivalent doses (13.5 mg/kg of diet).

In the A.E. Arbuzov Institute a method has been developed for the synthesis of water-soluble metal complexes based on citrus pectin, containing iron and other biogenic metals in a bioavailable form, and their biological activity is studied.

Among the obtained metal complexes of pectin polysaccharides with  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Ca}^{2+}$  ions the compounds with pronounced anti-anemic activity, increasing hemoglobin concentration and erythrocytes number and providing intensive restoration of hematological indices at blood loss, were revealed [15–17].

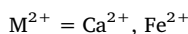
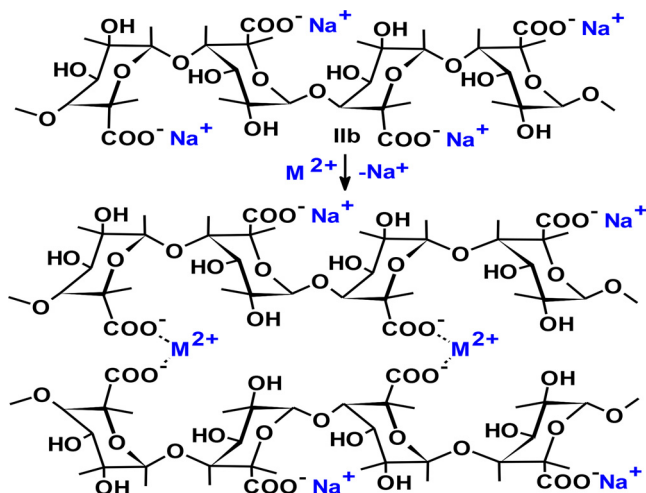
The object of research is a metal complex synthesized on the basis of citrus pectin with biogenic macro- and microelements (Ca, Fe) – sodium-, calcium-, iron-polygalacturonate, stimulating the process of hematopoiesis [18].

The purpose of this study is the assessment of the acute and chronic toxicity of sodium, calcium, iron-polygalacturonate in rabbits as one of the stages of preclinical studies of this pharmacological substance.

## 2. Materials and methods

### 2.1. Tested compound

The pharmacological substance sodium, calcium, iron-polygalacturonate (PG Na,Ca,Fe) (Structural Formula 1) was synthesized in the A.E. Arbuzov Institute of Organic and Physical Chemistry (Kazan). Synthesis and properties of this compound are described in the article [18].



**Formula 1**

### 2.2. Experimental animals

Experiments on acute and chronic toxicity were performed in rabbits of the “Chinchilla” breed of both sexes, provided by the cattery of Federal Center of Toxicological, Biological and Radiological Safety. The laboratory animals were adapted (kept in quarantine) before the start of the experiment for 14 days. The animals were kept in accordance with the rules of European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986) and the rules of laboratory practice (GLP standard) [19,20]. All animal experimentations and protocols were approved by the Local Ethics Committee of Federal Center of Toxicological, Biological and Radiological Safety (Protocol № 1 dated 22

**Table 1**

Toxicity of the pharmacological substance of sodium, calcium, iron-polygalacturonate at the introduction to rabbits.

Dose of PG Na,Ca,Fe, g/kg	0,50	1,00	2,00	3,00	4,00	5,00
Rabbits males						
The effect, lost (died)/total	0/3	0/3	0/3	0/3	0/3	0/3
Rabbits-females						
	0/3	0/3	0/3	0/3	0/3	0/3

November 2017).

Rabbits were kept in individual cages, without underlay, on lattice floors. The following conditions were maintained in the room with animals: air temperature 18–26 °C, relative humidity 30–70%, 100% ventilation without recirculation with air changing rate of 8–10 room volumes per hour, light mode was day/night. Animals had free access to water and feed. The drinking was done with filtered tap water, which was given ad libitum in standard autoclaved drinking bottles. For rabbits, a full feed for laboratory animals was used, that corresponds to GOST (State Standard) 50258-92 and is produced by “Laboratorkorm”.

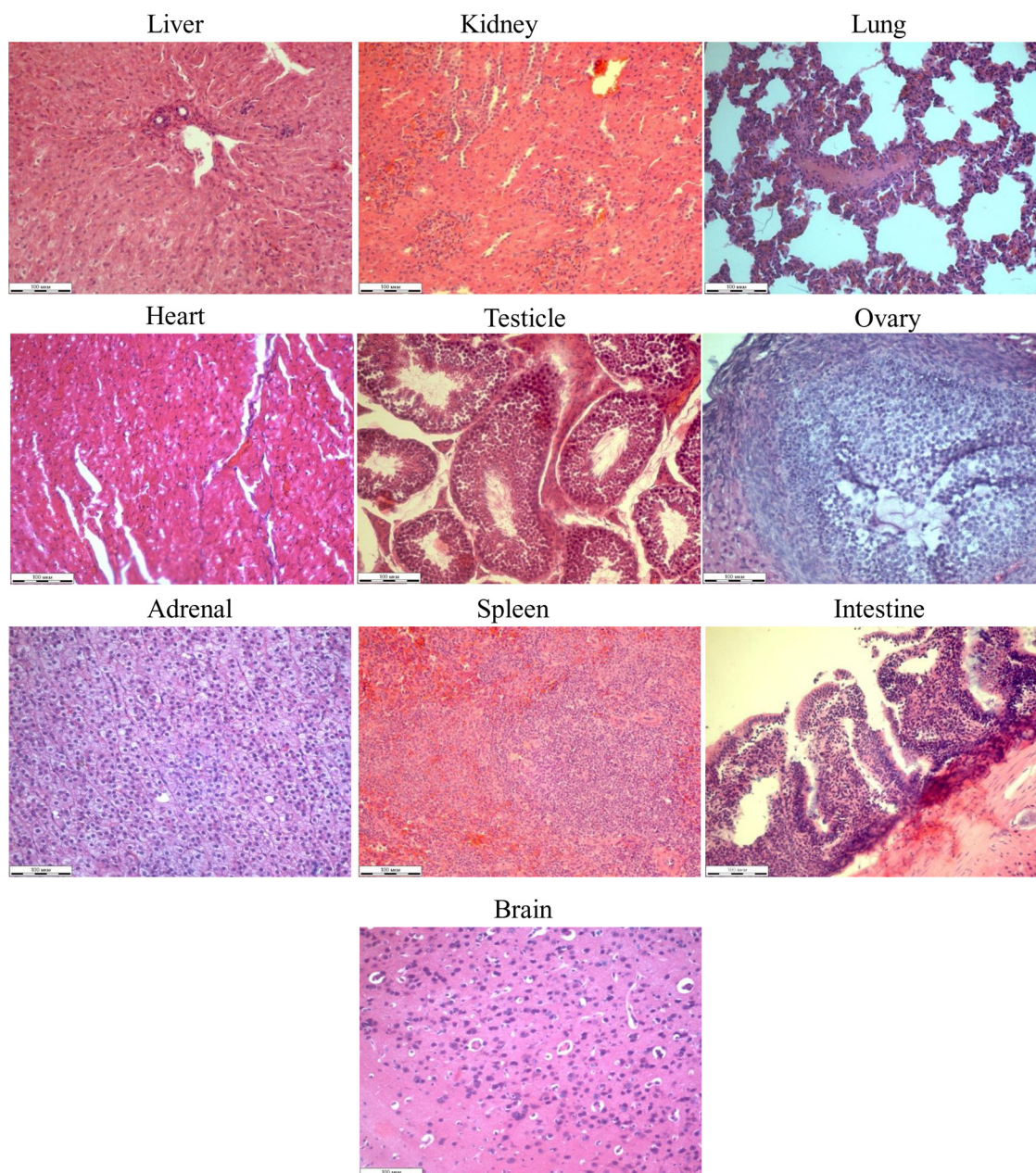
### 2.3. Evaluation of the acute toxicity of PG Na,Ca,Fe

To evaluate the acute toxicity, 6 groups of rabbits were formed, 6 individuals in each (3 males and 3 females) with a live body weight of 3.0–3.5 kg, according to method described in [21–23]. The effect of the drug was studied with intragastric administration in the form of boluses. To do this, the required amount of powder (for each animal in an individual dose) was weighed on scales and formed into a bolus with sterile distilled water. Administration was carried out once to animals deprived of food (for a period of not less than 8 h) with free access to water. The volume of the preparation administration was calculated individually for each animal, based on the body weight recorded just before the administration of the substance. The range of doses studied was 0.50-1-2-3-4-5 g/kg of body weight. Access to feed was resumed one hour after the preparation introduction. The distilled water which is the preparation solvent was injected intragastrically to control animals (3 males and 3 females of rabbits) with the help of a flexible probe. In addition, an intact group of rabbits (3 males and 3 females) that received a normal diet was formed. Observation of animals after the administration of the drug was carried out individually for 30 min, then at least once an hour for 4 h, then daily 1 time per day for 14 days. The list of recorded indicators: estimation of the general condition (integrated indicators: clinical picture, body weight, feed and water intake, mortality, signs of toxicity, rectal temperature); hematologic indices; pathomorphological studies.

### 2.4. Estimation of the chronic toxicity of PG Na,Ca,Fe

Three doses of the drug were tested – 1/10 of the maximum dose administered in the determination of  $\text{LD}_{50}$  (0.5 g/kg of body weight), then 1/10  $\text{LD}_{50}$ , the minimum therapeutic (prophylactic) dose (0.025 g/kg of body weight) and intermediate dose (0.262 g/kg of body weight), hereafter ID, according to [21,22,24]. For the experiment, 3 experimental groups of rabbits with an average mass of 2.0–2.2 kg were formed: group 1 – dose 1/10  $\text{LD}_{50}$  PG Na,Ca,Fe – 0.5 g/kg of body weight (6 males, 6 females); group 2 – therapeutic dose of PG Na,Ca,Fe – 0.025 g/kg of body weight (6 males, 6 females); group 3 – intermediate dose of PG Na,Ca,Fe – 0.262 g/kg of body weight (6 males, 6 females). In addition, 2 groups of control animals were formed 12 rabbits in each (6 males, 6 females): a group receiving solvent orally (distilled water) at a dose of 5 ml/kg and a group not receiving either drugs or a solvent (intact) (6 males, 6 females). PG Na,Ca,Fe was administered in the form of boluses.

The total duration of observation is 90 days (60 days of daily intragastric administration of the drug and 30 days of post-observation).



**Fig. 1.** Micromorphology of liver, kidney, lung, heart, spleen, adrenal, testes, ovaries, intestine and brain in rabbits of intact control group. Stained with Erlich hematoxylin and eosin. Zoom 150x, scale bar 100  $\mu$ m.

The general condition was assessed with a daily inspection of the animals. Weighing, rectal temperature measuring, water and feed intake were performed prior to the beginning of the experiment, every 10 days of the experiment and on the 30th, 60th and 90th day of the experiment.

Hematologic, biochemical studies of rabbits blood serum were carried out on the 30th, 60th and 90th days; the urinalysis was performed in 60 days and 90 days after the start of the study. Physiological studies (ECG in the second lead) were performed in 60 days after the start of the study.

### 2.5. Clinical and physiological studies

Clinical examination of each animal was carried out once before the administration of the preparation and later – daily. A detailed examination of the animal in the cage of the containing was performed.

They noted behavior (activity – increased or decreased), gait

(muscle tone, tremor, balancing), temperament (sluggishness, excitability, aggressiveness); appearance (exhaustion, obesity), the state of the wool cover (hair loss, wool sticking out, spotted, dull, smooth, shiny), eye condition (lachrymation, inflammation, corneal opacity), ears (inflammation, color – pallor or redness, secretion, sensitivity, twitching), limbs (color, swelling), teeth (color, loss); such physiological functions as breathing (nature, characteristics), excretion (color, consistency) were also evaluated.

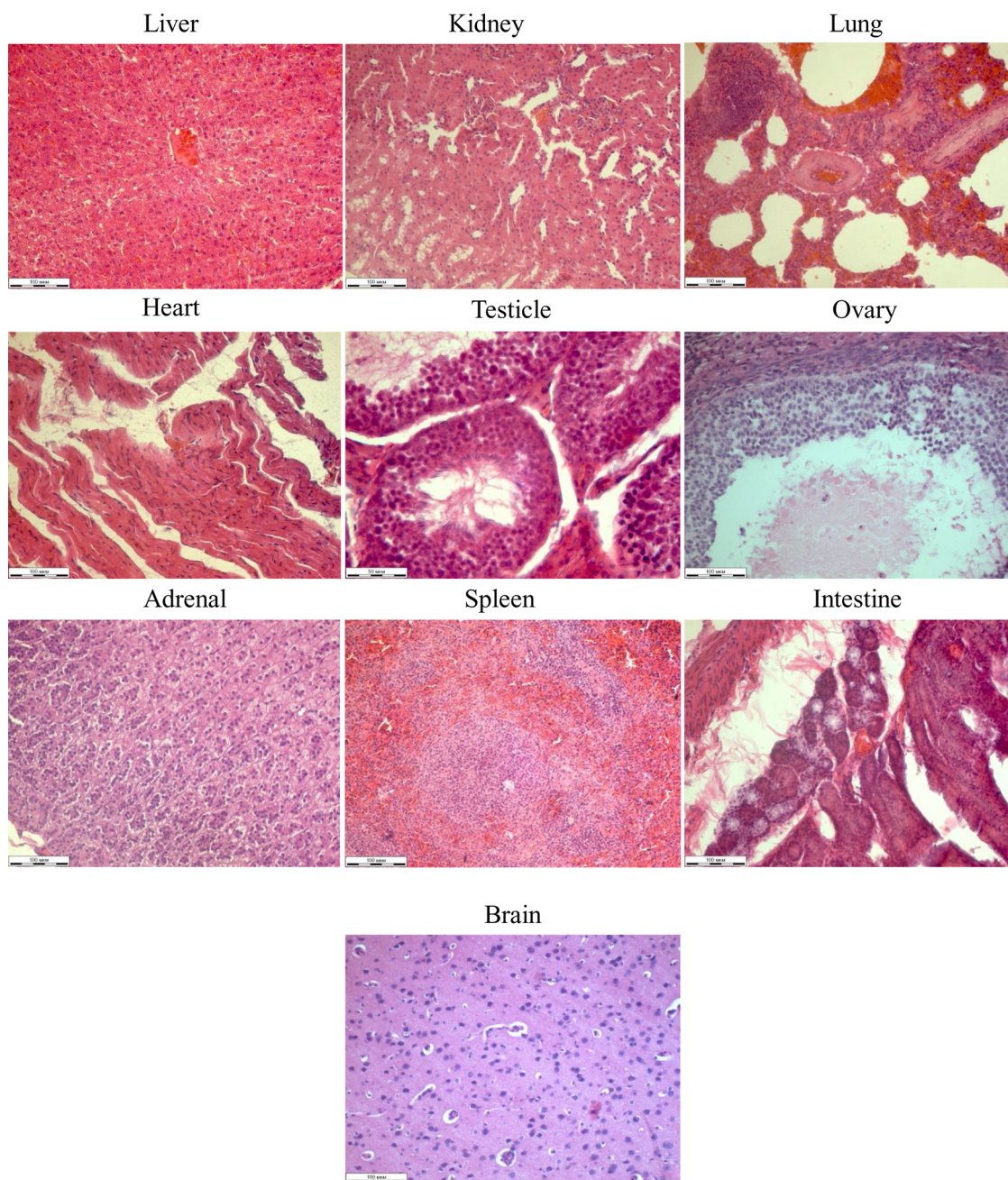
The animals were weighed on a ‘Newton’ scale, and the rectal temperature was recorded using a medical thermometer “Little Doctor”.

### 2.6. Hematologic studies

Blood for the study of hematologic parameters was taken from the rabbits margin ear vein. EDTAK<sub>3</sub> was used for hematological studies as an anticoagulant.

Hematologic parameters (leukocytes, lymphocytes, monocytes,





**Fig. 2.** Micromorphology of liver, kidney, lung, heart, spleen, adrenal, testes, ovaries, intestine and brain in rabbits of control group. Stained with Erlich hematoxylin and eosin. Zoom 150x, scale bar 100  $\mu$ m.

granulocytes, erythrocytes, hemoglobin, average erythrocyte volume, mean hemoglobin content in erythrocyte, mean hemoglobin concentration in one erythrocyte, platelets, thrombocyte) were studied using the Mythic 18 hematology analyzer.

### 2.7. Biochemical studies of serum of rabbits

Determination of biochemical parameters (total protein, glucose, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total calcium) was performed on a biochemical analyzer ARD-200 using special sets of Chronolab reagents.

### 2.8. Urine examination

The determination of urine indicators was performed using LabStrip

URINALISIS test strips (manufactured by West-und-Handless GmbH, Austria).

### 2.9. Pathomorphological studies

The pathomorphological study included necropsy, macroscopic examination, weighing of internal organs, microscopic studies (histopathology).

### 2.10. Histological examination of internals (viscera)

The viscera and tissues were fixed, dehydrated and impregnated with paraffin. The slices 45  $\mu$ m thick were made from paraffin blocks. Cuts were stained using standard methods with hematoxylin-eosin and examined with a Leica light microscope.



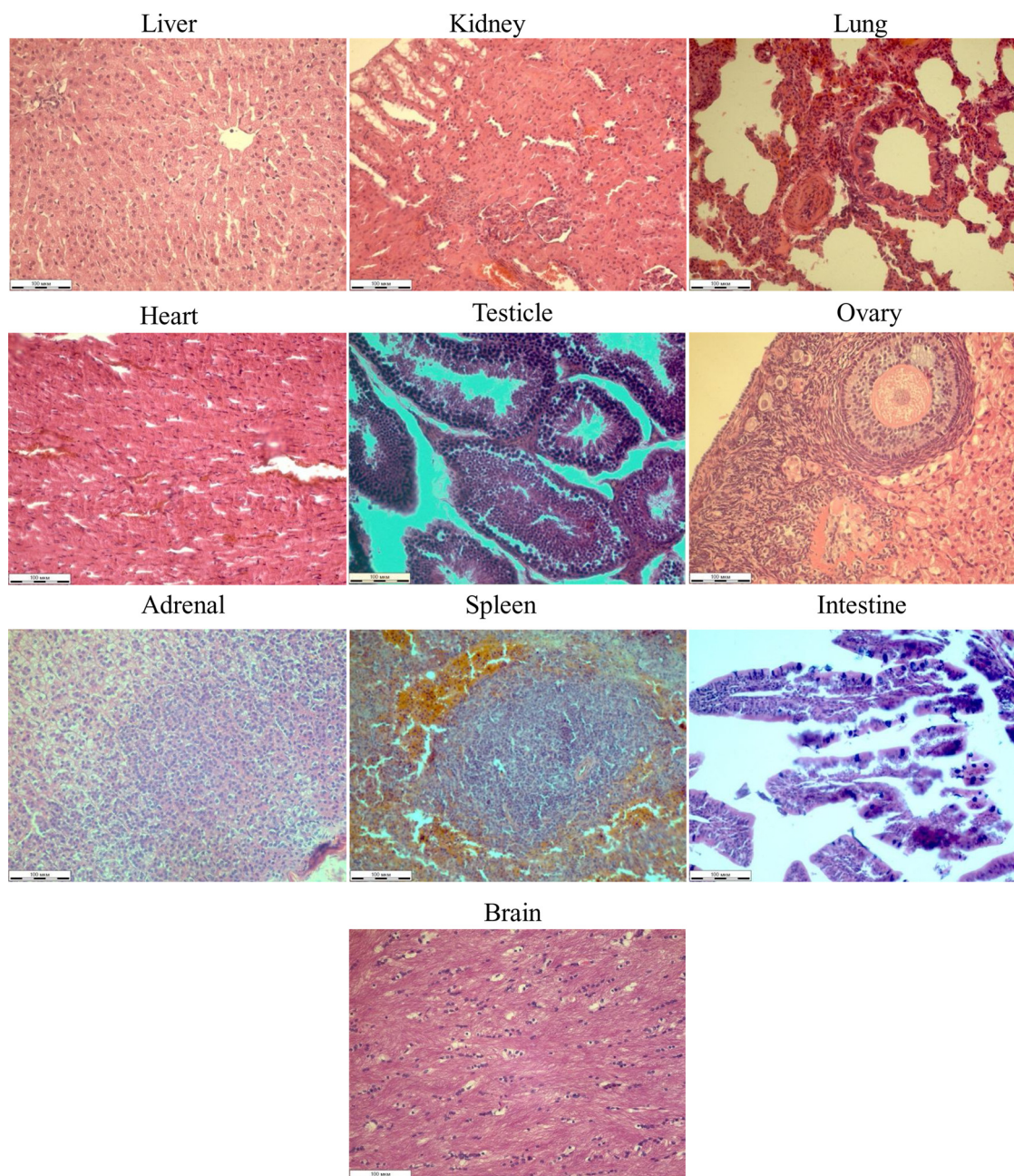


Fig. 3. Micromorphology of liver, kidney, lung, heart, spleen, adrenal, testes, ovaries, intestine and brain in rabbits of the animals group received a pharmacological substance at a dose of 5000 mg/kg. Stained with Erlich hematoxylin and eosin. Zoom 150 $\times$ , scale bar 100  $\mu$ m.

### 2.11. Statistical analysis

For all quantitative data, the group mean (M), the standard error of the mean (SEM) or the standard deviation (SD) were calculated. The probability of differences in the mean values in the groups was determined using the t-Student test. Differences were considered valid at a significance level of  $p < 0.05$ .

## 3. Results

### 3.1. The study of acute toxicity

As shown in Table 1, no lethal effects were achieved at intragastric administration of the pharmacological substance sodium, calcium, iron-polygalacturonate to rabbit females and rabbit males at doses of 0.5–5 g/kg.

A detailed examination of an animal was performed in the cage of the containing on a standard open area. The behavior of rabbits of all groups of animals that received intragastric administration of the drug, as well as muscle tone, did not differ from that of the behavior of intact animals throughout the experiment (from the day of administration of the drug to the last day of observation). The animals were non-aggressive, the sluggishness of the animals was not recorded, the appearance and condition of the coat was not different from those of intact animals.

Hair loss, patchiness or dullness of the fur was not observed, the fur was smooth and shiny. Lachrymation, inflammation, clouding of the cornea was not noted. Inflammation of the ears, ear secretion, twitching of the ears was not observed, edema of the limbs and changes in the skin of the limbs and ears were not noted. There was no change in the color of the teeth or their loss.

Physiological functions: the character of the breathing was smooth,

**Table 2**

The effect of PG Na,Ca,Fe intragastric administration on the rabbits body weight, kg ( $M \pm m$ ,  $n = 6$ ).

Group of animals, form and dose of the drug	Term of the study			
	Background	30th day	60th day	90th day
<b>Male (M)</b>				
Control Distilled water, 5 ml/kg)	2,07 ± 0,03	2,59 ± 0,08	2,87 ± 0,08	3,15 ± 0,2
Intact	1,92 ± 0,06	2,59 ± 0,05	2,92 ± 0,1	3,2 ± 0,2
1/10 LD50, 0,5 g/kg	2,18 ± 0,04	2,75 ± 0,07	3,05 ± 0,08	3,10 ± 0,2
ID, 0,262 g/kg	2,16 ± 0,09	2,68 ± 0,12	3,1 ± 0,13	3,35 ± 0,4
TD, 0,025 g/kg	2,14 ± 0,05	2,41 ± 0,07	3,1 ± 0,09	3,28 ± 0,2
<b>Female (F)</b>				
Control (solvent, 5 ml/kg)	2,02 ± 0,03	2,54 ± 0,1	2,8 ± 0,12	3,18 ± 0,3
Intact	2,09 ± 0,1	2,65 ± 0,09	2,99 ± 0,13	3,3 ± 0,4
1/10 LD50, 0,5 g/kg	2,17 ± 0,05	2,87 ± 0,1	2,95 ± 0,1	3,04 ± 0,3
ID, 0,262 g/kg	2,25 ± 0,10	2,83 ± 0,12	3,16 ± 0,11	3,35 ± 0,3
TD, 0,025 g/kg	2,02 ± 0,08	2,43 ± 0,19	2,74 ± 0,2	3,06 ± 0,4

Note: ID – intermediate dose, TD – therapeutic dose. There are no significant differences between test and control at 95% probability level.

without any peculiarities, there was no pathologic salivation, the urination was natural, the color of the urine was natural, the excretion was normal and of natural consistency, except for the first days after the administration – it was darker, a dose-dependent effect was noted (in animals with a greater dosage of the drug, the color of the excreta was darker), which is due to the composition of the drug (it contains ferrous iron, part of which, oxidized to trivalent, causes darkening of the excreta). Sexual differences in the clinical picture were not detected.

No significant changes were observed in body weight of rabbits injected with a pharmacological substance in any of the compared groups with respect to the values in the control group. There were no significant changes in rectal temperature with respect to the values in

**Table 3**

Influence of PG Na,Ca,Fe intragastric administration on the hematological indices of male rabbits, ( $M \pm m$ ,  $n = 6$ ).

Term of the study	Hematological index	Test groups, dose				
		Intact animals	Control	0,5 g/kg (1/10 LD <sub>50</sub> )	0,025 g/kg (TD)	0,262 g/kg (ID)
<b>30th day</b>	Leukocytes, *10 <sup>9</sup> /l	5,7 ± 0,64	8,3 ± 0,51	7,3 ± 0,49	9,1 ± 0,51	8,1 ± 0,53
	Lymphocytes, %	54,5 ± 1,38	56,8 ± 1,92	62,6 ± 1,54	47,1 ± 1,32	63,0 ± 1,69
	Monocytes, %	1,0 ± 0,05	1,3 ± 0,07	1,6 ± 0,07	1,3 ± 0,05	1,2 ± 0,08
	Granulocytes, %	44,5 ± 1,55	41,9 ± 1,63	35,8 ± 1,27	51,6 ± 1,14	35,8 ± 1,31
	Erythrocytes, *10 <sup>12</sup> /l	5,38 ± 0,13	5,13 ± 0,16	5,17 ± 0,15	5,13 ± 0,09	5,14 ± 0,14
	Hematocrit, %	31,4 ± 0,48	32,7 ± 0,55	30,0 ± 0,74	31,2 ± 0,32	31,9 ± 0,66
	Hemoglobin, g/l	118 ± 4,1	123 ± 4,3	114,6 ± 2,3	117,1 ± 2,7	121,3 ± 2,7
	The average content of hemoglobin in the erythrocyte, pg	21,9 ± 0,52	24,0 ± 0,58	22,0 ± 0,34	22,8 ± 0,36	23,5 ± 0,42
	Platelets, *10 <sup>9</sup> /l	196,5 ± 50,3	282,4 ± 67,4	305,4 ± 37,4	300,6 ± 44,3	308,1 ± 45,9
	<b>60th day</b>	Leukocytes, *10 <sup>9</sup> /l	6,9 ± 0,5	9,3 ± 0,56	12,0 ± 0,56	8,8 ± 0,74
Lymphocytes, %		63,1 ± 1,25	64,5 ± 1,8	47,2 ± 1,6	61,6 ± 1,2	73,4 ± 1,3
Monocytes, %		1,3 ± 0,06	1,0 ± 0,08	1,0 ± 0,08	1,1 ± 0,08	0,8 ± 0,07
Granulocytes, %		35,6 ± 1,47	34,5 ± 1,5	51,8 ± 1,1	37,3 ± 1,12	25,8 ± 1,44
Erythrocytes, *10 <sup>12</sup> /l		5,71 ± 0,14	5,3 ± 0,12	5,22 ± 0,2	4,76 ± 0,11	6,13 ± 0,15
Hematocrit, %		33,7 ± 0,52	34,0 ± 0,62	29,7 ± 0,63	30,2 ± 0,33	31,2 ± 0,7
Hemoglobin, g/l		123,1 ± 4,3	120,3 ± 4,6	110,0 ± 2,6	108,6 ± 2,9	113,4 ± 2,9
The average content of hemoglobin in the erythrocyte, pg		21,5 ± 0,56	22,6 ± 0,62	21,1 ± 0,36	22,7 ± 0,32	18,4 ± 0,43
Platelets, *10 <sup>9</sup> /l		213,8 ± 43,7	297,6 ± 41,3	306,0 ± 27,2	349,3 ± 38,5	185,0 ± 42,5
<b>90th day</b>		Leukocytes, *10 <sup>9</sup> /l	12,8 ± 1,2	9,9 ± 1,1	9,2 ± 1,1	11,5 ± 1,6
	Lymphocytes, %	50,1 ± 2,5	73,0 ± 3,1	61,7 ± 2,4	61,6 ± 2,4	61,2 ± 2,5
	Monocytes, %	1,1 ± 0,14	1,2 ± 0,17	2,0 ± 0,11	1,4 ± 0,11	1,6 ± 0,17
	Granulocytes, %	48,8 ± 2,6	25,8 ± 2,0	36,3 ± 1,7	37,0 ± 2,2	37,2 ± 2,7
	Erythrocytes, *10 <sup>12</sup> /l	6,27 ± 0,24	6,32 ± 0,21	6,08 ± 0,21	6,02 ± 0,3	6,43 ± 0,4
	Hematocrit, %	35,2 ± 1,0	40,3 ± 1,3	36,1 ± 1,2	37,1 ± 0,8	36,2 ± 1,6
	Hemoglobin, g/l	121,0 ± 7,7	137,0 ± 9,1	131,0 ± 5,2	132,2 ± 5,7	129,3 ± 6,0
	The average content of hemoglobin in the erythrocyte, pg	19,3 ± 1,1	21,7 ± 1,2	21,6 ± 0,8	21,9 ± 0,8	20,1 ± 0,9
	Platelets, *10 <sup>9</sup> /l	398,0 ± 53,6	283,0 ± 53,8	395,4 ± 52,5	407,4 ± 56,3	312,4 ± 57,3

Note: There are no significant differences between test and control at 95% probability level.

the control group in any of the compared groups.

### 3.1.1. Influence of PG Na,Ca,Fe on the histopathology of internal organs

Morphological examination of liver, kidney, lung, heart, spleen and adrenal, testes and ovaries, intestine and brain of control and intact rabbits is performed and results are compared with those for the group of animals received a pharmacological substance at a dose of 5000 mg/kg (Figs. 1–3).

**Liver:** In the liver cuts, the vessels are dilated, unevenly filled with erythrocytes and erythromass. The lobular structure is preserved, the veins in the center of the lobules are thin, their lumens are enlarged, contain red blood cells. Hepatocytes are eosinophilic in color, contain one nucleus, and rare cells are with two nuclei. Some hepatocytes have a light cytoplasm (Figs. 1 and 3). Kupffer cells are thin, there are only few.

**The kidneys:** In the kidneys of the intact group of animals, the vessels are dilated, filled with erythromass. In the cuts, a capsule covering the kidney consisting of connective tissue fibers. is visualized. In the cortical layer, glomeruli are visible, which have an almost oval shape. The glomeruli are surrounded by a capsule with a narrow space. The proximal tubules are composed of a cubic epithelium. The boundaries between its cells are not clear. Single tubules are with epithelium desquamated by 1/2. Distal tubules are with more planar epithelial cells. In the medulla, the tubular epithelium is light and low (Fig. 1). In the control group (Fig. 2) as well as in the group treated with PG Na,Ca,Fe at a dose of 5000 mg/kg (Fig. 3), a similar picture of the kidneys structural and morphological organization was observed.

**Lungs:** In the lungs of the intact group animals the blood supply is moderate. Lungs have a spongy structure. Alveoli are separated from each other by interalveolar septa. Some alveoli are dilated with interruptions of the septa. In bronchi of different caliber and some alveoli, erythromasses are determined. The epithelium of the bronchial mucosa is a single-layer, multi-row cylindrical ciliated. Single goblet cells are defined. In the lumens of some vessels, voids of round and oval shape

**Table 4**

Influence of PG Na,Ca,Fe intragastric administration on the hematological indices of female rabbits, (M ± m, n = 6).

Term of the study	Hematological index	Test groups, dose				
		Intact animals	Control	0,5 g/kg (1/10 LD <sub>50</sub> )	0,025 g/kg (TD)	0,262 g/kg (ID)
<b>30th day</b>	Leukocytes, *10 <sup>9</sup> /l	9,0 ± 0,59	7,1 ± 0,44	10,9 ± 0,38	7,3 ± 0,41	10,5 ± 0,41
	Lymphocytes, %	53,9 ± 1,95	47,7 ± 1,57	63,1 ± 1,45	39,5 ± 1,55	48,7 ± 1,53
	Monocytes,%	1,8 ± 0,07	1,0 ± 0,06	1,4 ± 0,05	0,9 ± 0,06	1,6 ± 0,06
	Granulocytes,%	44,3 ± 1,93	51,3 ± 1,48	35,5 ± 1,14	59,6 ± 1,18	49,7 ± 1,12
	Erythrocytes, *10 <sup>12</sup> /l	5,00 ± 0,15	5,01 ± 0,09	4,84 ± 0,06	5,15 ± 0,05	4,77 ± 0,116
	Hematocrit, %	30,3 ± 0,56	28,9 ± 0,64	29,1 ± 0,21	29,7 ± 0,28	31,6 ± 0,43
	Hemoglobin, g/l	116 ± 3,4	108 ± 3,7	111,3 ± 3,1	114,2 ± 2,3	120,2 ± 3,4
	The average content of hemoglobin in the erythrocyte, pg	23,2 ± 0,46	21,6 ± 0,38	22,1 ± 0,47	22,1 ± 0,65	25,2 ± 0,52
	Platelets, *10 <sup>9</sup> /l	349,1 ± 41,8	267,3 ± 38,5	354,2 ± 35,6	310,7 ± 88,1	242,8 ± 65,3
<b>60th day</b>	Leukocytes, *10 <sup>9</sup> /l	7,6 ± 0,7	8,4 ± 0,47	10,6 ± 0,45	10,9 ± 0,52	9,7 ± 0,44
	Lymphocytes, %	67,9 ± 1,76	70,0 ± 1,5	34,6 ± 1,2	69,3 ± 1,4	51,3 ± 1,6
	Monocytes,%	1,2 ± 0,08	2,4 ± 0,07	0,9 ± 0,06	1,2 ± 0,09	1,5 ± 0,06
	Granulocytes,%	30,9 ± 1,82	27,6 ± 1,6	64,5 ± 1,3	29,5 ± 1,15	47,2 ± 1,18
	Erythrocytes, *10 <sup>12</sup> /l	5,69 ± 0,17	4,17 ± 0,08	5,17 ± 0,09	4,28 ± 0,07	5,2 ± 0,13
	Hematocrit, %	33,5 ± 0,57	27,8 ± 0,58	31,3 ± 0,34	27,1 ± 0,31	29,5 ± 0,48
	Hemoglobin, g/l	120,8 ± 3,8	97,3 ± 3,9	117,2 ± 3,7	98,4 ± 2,6	107,3 ± 3,1
	The average content of hemoglobin in the erythrocyte, pg	21,1 ± 0,51	23,3 ± 0,43	22,6 ± 0,41	22,9 ± 0,48	20,6 ± 0,47
	Platelets, *10 <sup>9</sup> /l	272,4 ± 33,4	205,7 ± 22,8	379,8 ± 30,3	270,8 ± 64,7	309,2 ± 35,7
<b>90th day</b>	Leukocytes, *10 <sup>9</sup> /l	12,7 ± 1,3	9,2 ± 1,2	13,6 ± 0,9	13,4 ± 1,7	7,7 ± 0,9
	Lymphocytes, %	48,6 ± 2,8	62,7 ± 2,8	36,7 ± 2,1	38,3 ± 2,5	62,3 ± 2,8
	Monocytes,%	1,7 ± 0,22	1,4 ± 0,19	1,1 ± 0,09	1,4 ± 0,12	1,8 ± 0,15
	Granulocytes,%	49,7 ± 2,9	35,9 ± 1,8	62,2 ± 1,9	60,3 ± 2,1	35,9 ± 2,6
	Erythrocytes, *10 <sup>12</sup> /l	5,15 ± 0,3	5,57 ± 0,17	6,06 ± 0,12	5,14 ± 0,16	6,54 ± 0,3
	Hematocrit, %	33,7 ± 0,9	35,3 ± 1,2	35,8 ± 0,8	32,3 ± 0,8	36,9 ± 1,1
	Hemoglobin, g/l	118,0 ± 8,4	122,0 ± 6,6	131,0 ± 6,4	114,8 ± 5,4	131,0 ± 6,3
	The average content of hemoglobin in the erythrocyte, pg	22,9 ± 1,1	21,9 ± 1,1	22,6 ± 0,9	22,2 ± 1,2	20,0 ± 0,9
	Platelets, *10 <sup>9</sup> /l	389,0 ± 45,2	389,0 ± 64,3	452,5 ± 65,6	448,5 ± 71,9	299,0 ± 48,5

Note: There are no significant differences between test and control at 95% probability level.

**Table 5**

Influence of PG Na,Ca,Fe intragastric administration on the biochemical parameters of blood serum of rabbits, (M ± m, n = 6).

Term of the study	Group of animals, index	Test groups, dose				
		Intact animals	Control	0,5 g/kg (1/10 LD <sub>50</sub> )	0,025 g/kg (TD)	0,262 g/kg (ID)
<b>30th day</b>	<b>Male</b>					
	Total protein, g/l	75,3 ± 4,3	74,8 ± 3,6	75,1 ± 6,1	69,5 ± 4,6	74,3 ± 4,2
	ALT, units/l	84,3 ± 17,6	72,1 ± 14,4	64,7 ± 6,9	80,4 ± 13,6	75,6 ± 11,6
	AST, units/l	47,1 ± 5,1	47,7 ± 11,5	47,7 ± 11,5	44,5 ± 7,8	48,5 ± 7,2
	Glucose, mmol/l	8,5 ± 0,8	8,3 ± 0,7	8,3 ± 0,6	8,1 ± 0,7	8,8 ± 0,7
	<b>Female</b>					
	Total protein, g/l	78,3 ± 3,7	68,3 ± 4,3	71,0 ± 3,6	72,8 ± 3,7	76,6 ± 3,9
	ALT, units/l	76,1 ± 15,1	68,5 ± 10,1	58,7 ± 5,7	82,6 ± 12,5	77,4 ± 12,7
	AST, units/l	47,3 ± 8,3	47,6 ± 3,8	46,5 ± 6,4	49,3 ± 5,6	46,7 ± 9,5
	Glucose, mmol/l	7,7 ± 0,4	8,2 ± 0,6	8,1 ± 0,5	8,4 ± 0,4	7,9 ± 0,6
<b>60th day</b>	<b>Male</b>					
	Total protein, g/l	76,8 ± 4,9	75,6 ± 4,1	72,6 ± 5,7	68,6 ± 4,9	71,5 ± 5,6
	ALT, units/l	81,6 ± 12,4	79,5 ± 11,6	72,2 ± 11,5	65,8 ± 13,4	76,5 ± 12,4
	AST, units/l	48,9 ± 6,8	47,6 ± 10,2	48,6 ± 5,5	46,3 ± 8,9	48,6 ± 6,4
	Glucose, mmol/l	8,4 ± 0,7	8,4 ± 0,5	8,1 ± 0,4	8,0 ± 0,9	8,5 ± 0,6
	<b>Female</b>					
	Total protein, g/l	77,4 ± 4,5	71,4 ± 4,8	77,3 ± 4,3	70,1 ± 3,5	74,9 ± 4,2
	ALT, units/l	79,8 ± 13,6	83,7 ± 14,6	67,5 ± 9,5	79,6 ± 12,5	77,4 ± 11,7
	AST, units/l	46,7 ± 7,5	46,2 ± 9,4	49,4 ± 6,3	49,5 ± 4,5	46,8 ± 7,8
	Glucose, mmol/l	7,9 ± 0,6	8,1 ± 0,5	7,4 ± 0,6	7,6 ± 0,6	7,6 ± 0,8
<b>90th day</b>	<b>Male</b>					
	Total protein, g/l	78,7 ± 6,8	77,9 ± 5,8	73,4 ± 7,3	73,2 ± 6,7	73,7 ± 8,1
	ALT, units/l	81,4 ± 14,6	68,8 ± 13,7	76,8 ± 9,6	81,4 ± 14,7	73,4 ± 13,4
	AST, units/l	46,6 ± 5,1	47,5 ± 5,8	48,5 ± 8,4	48,5 ± 7,4	46,8 ± 6,2
	Glucose, mmol/l	8,5 ± 0,9	8,7 ± 0,6	8,4 ± 0,8	8,5 ± 0,9	8,7 ± 0,8
	<b>Female</b>					
	Total protein, g/l	78,5 ± 7,1	73,7 ± 7,3	78,2 ± 6,5	72,5 ± 5,8	76,6 ± 5,7
	ALT, units/l	63,5 ± 13,2	76,4 ± 12,6	65,5 ± 7,2	86,6 ± 15,1	78,7 ± 11,5
	AST, units/l	47,8 ± 7,2	48,4 ± 7,2	49,3 ± 4,5	46,8 ± 5,6	49,5 ± 7,8
	Glucose, mmol/l	8,1 ± 0,8	8,4 ± 0,7	8,6 ± 0,7	8,9 ± 0,8	8,7 ± 0,9

Note: There are no significant differences between test and control at 95% probability level.



**Table 6**

The effect of PG Na,Ca,Fe intragastric administration on the heart rate and ECG of rabbits (M ± m).

Group of animals, index	Test groups, dose				
	Intact animals	Control	0,5 g/kg (1/10 LD <sub>50</sub> )	0,025 g/kg (TD)	0,262 g/kg (ID)
<b>Male</b>					
Heart rate, beats/min	218,0 ± 14,3	216,0 ± 12,4	217,0 ± 10,9	211,0 ± 15,1	221,0 ± 13,6
P, mV	0,07 ± 0,02	0,1 ± 0,02	0,1 ± 0,02	0,07 ± 0,02	0,08 ± 0,02
R, mV	0,1 ± 0,07	0,12 ± 0,06	0,2 ± 0,05	0,2 ± 0,06	0,13 ± 0,05
S, mV	-0,03 ± 0,02	-0,05 ± 0,02	-0,05 ± 0,01	-0,05 ± 0,02	-0,03 ± 0,02
T, mB	-0,01 ± 0,06	-0,03 ± 0,07	-0,03 ± 0,05	-0,02 ± 0,04	-0,04 ± 0,05
PQ, ms	0,07 ± 0,01	0,05 ± 0,01	0,05 ± 0,01	0,06 ± 0,01	0,06 ± 0,01
QT, ms	0,11 ± 0,02	0,12 ± 0,02	0,11 ± 0,02	0,12 ± 0,02	0,1 ± 0,02
<b>Female</b>					
Heart rate, beats/min	212,0 ± 11,4	221,0 ± 13,2	219,0 ± 14,2	219,0 ± 13,7	210,0 ± 10,8
P, mV	0,09 ± 0,02	0,08 ± 0,02	0,09 ± 0,02	0,09 ± 0,02	0,07 ± 0,02
R, mV	0,2 ± 0,08	0,16 ± 0,07	0,2 ± 0,06	0,2 ± 0,06	0,17 ± 0,06
S, mV	-0,05 ± 0,01	-0,05 ± 0,02	-0,03 ± 0,01	-0,03 ± 0,02	-0,04 ± 0,02
T, mB	-0,05 ± 0,03	-0,04 ± 0,02	-0,05 ± 0,04	-0,03 ± 0,02	-0,03 ± 0,04
PQ, ms	0,05 ± 0,01	0,05 ± 0,01	0,06 ± 0,01	0,04 ± 0,01	0,04 ± 0,01
QT, ms	0,12 ± 0,02	0,13 ± 0,02	0,13 ± 0,02	0,1 ± 0,02	0,11 ± 0,02

Note: There are no significant differences between test and control at 95% probability level.

are found (Fig. 1). In the control group (Fig. 2) as well as in the group treated with PG Na,Ca,Fe at a dose of 5000 mg/kg (Fig. 3), a similar pattern of structural and morphological organization of the lungs was observed.

**Heart:** In the heart of the animals of the intact group (Fig. 1), cardiomyocytes oriented transversely and longitudinally, are eosinophilic with basophilic nuclei. The structure of the cells is preserved. Connective tissue layers are thin, eosinophilic. Vessels are dilated, with erythromass in the lumens, others are empty. In the control group (Fig. 2), as well as in the group given PG Na,Ca,Fe at a dose of 5000 mg/kg (Fig. 3), no differences were found compared to the intact group.

**Testicles:** In the testes, the blood supply is reduced, the structure without singularities (Fig. 1). Convoluted seminiferous tubules are determined. On the basal membrane the spermatogenic cells are disposed in a continuous layer. Small spermatogonia of round shape is defined. In the control group (Fig. 2) as well as in the group given PG Na,Ca,Fe at a dose of 5000 mg/kg (Fig. 3), no differences from the intact group were detected.

**Ovaries:** No difference in the micromorphology of the ovaries between the intact, control groups and the group administered with PG Na,Ca,Fe (Figs. 1–3) was detected. In the ovaries blood supply is reduced. Clusters of primordial follicles under the belly are determined. Primary follicles, as well as follicles with oocytes at different stages of maturation are visible.

**Adrenal:** The adrenal gland is moderately filled with blood, all layers are present in the cut. Glomerular, fascicular and reticular layers are without features, no differences between the groups were detected (Figs. 1–3).

**Spleen:** The spleen is covered with a capsule, from which trabeculae stretch. Numerous red blood cells are in the red pulp. Venous sinuses are dilated, contain erythrocytes. Follicles are with numerous lymphocytes, single macrophages are visible. In T-dependent zones, a small number of lymphocytes are observed. No differences between the groups were detected (Figs. 1–3).

**Intestines:** In the wall of the small intestine the blood supply is reduced. The mucous layer is covered with a single-layered cylindrical limbic epithelium. Crypts are well distinguishable, free. Submucous, muscular serous layers are anemic without features, no differences between groups is observed in histological structure (Figs. 1–3).

**Brain:** In the brain, the blood filling is uneven. Some vessels contain erythromass, some are empty. The cortical cells are arranged according to the layers, the structure is well determined. In the structure of the brain tissue, there was no difference between the groups (Figs. 1–3).

### 3.2. Chronic toxicity study

#### 3.2.1. Influence of PG Na,Ca,Fe on the dynamics of rabbit body weight

During the period of the study a statistically significant change in the body weight of rabbits injected with a pharmacological substance for 60 days was not found in comparison with the indices of intact and control animals (Table 2).

No significant changes were also detected in rectal temperature and behavior in any of the compared groups relative to the values in the control group.

#### 3.2.2. Influence of PG Na,Ca,Fe on hematologic indices

Data of hematological studies are presented in Table 3 for rabbits-males, in Table 4 – for rabbits-females. No significant changes relative to the control group ( $p < 0.05$ ) were revealed.

#### 3.2.3. The influence of PG Na,Ca,Fe on some biochemical indices

Table 5 presents the results of biochemical studies of serum from experimental rabbits. There were no significant changes relative to the control and intact group of rabbits ( $p < 0.05$ ).

#### 3.2.4. Influence of PG Na,Ca,Fe on the cardiovascular activity of rabbits

The effect of the investigated drug on the character of the electrocardiogram in rabbits on the 60th day was studied, the results are shown in Table 6. No significant changes were observed with respect to the control group ( $p < 0.05$ ).

#### 3.2.5. Influence of PG Na,Ca,Fe on the urine test of the experimental rabbits

The urine test data for the experimental rabbits are presented in Table 7. No significant changes were observed with respect to the control group ( $p < 0.05$ ).

#### 3.2.6. Influence of PG Na,Ca,Fe on the mass coefficients of rabbit organs

The effect of the introduction of the preparation of the pharmacological substance of sodium, calcium, iron-polygalacturonate on the mass coefficients of organs is studied, and the analysis of the mass coefficients for the groups of animals taking sodium-, calcium-, iron-polygalacturonate, solvent, and intact animals ( $p < 0.05$ ). The results are presented in Table 8 for male rabbits, and in Table 9 – for female rabbits.

## 4. Discussion

Safety pharmacology is an essential part of the drug development



**Table 7**  
Effect of acute intravenous administration of PG Na,Ca,Fe on the biochemical parameters of urine of rabbits ( $M \pm m$ ,  $n = 6$ ).

Term of the study	Group of animals, urine indicator	Test groups, dose				
		Intact animals	Control	0,5 g/kg (1/10 LD <sub>50</sub> )	0,025 g/kg (TD)	0,262 g/kg (ID)
60th day	<b>Male</b>					
	Bilirubin, mmol/l	50	50	50	50	50
	Urobilinogen, mmol/l	35	35	35	35	35
	Ketones, mmol/l	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5
	Ascorbic acid, mmol/l	< 0,6	< 0,6	< 0,6	< 0,6	< 0,6
	Glucose, mmol/l	Odp.	Otp.	Otp.	Otp.	Otp.
	Protein, g/l	0,3	0,3	0,3	0,3	0,3
	Erythrocytes/μl	< 5	< 5	< 5	< 5	< 5
	pH	8,5	8,5	8,5	8,5	8,5
	Nitrites, mg/dl	< 0,05	< 0,05	Otp.	< 0,05	< 0,05
	Leukocytes/μl	< 10	< 10	< 10	< 10	< 10
	Specific density	1,010	1,010	1,010	1,010	1,010
	<b>Female</b>					
	Bilirubin, mmol/l	50	50	50	50	50
	Urobilinogen, mmol/l	35	35	35	35	35
Ketones, mmol/l	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	
Ascorbic acid, mmol/l	< 0,6	< 0,6	< 0,6	< 0,6	< 0,6	
Glucose, mmol/l	Otp.	Otp.	Otp.	Otp.	Otp.	
Protein, g/l	0,3	0,3	0,3	0,3	0,3	
Erythrocytes/μl	< 5	< 5	< 5	< 5	< 5	
pH	8,5	8,5	8,5	8,5	8,5	
Nitrites, mg/dl	< 0,05	< 0,05	Otp.	< 0,05	< 0,05	
Leukocytes/μl	< 10	< 10	< 10	< 10	< 10	
Specific density	1,010	1,010	1,010	1,010	1,010	
90th day	<b>Male</b>					
	Bilirubin, mmol/l	50	50	50	50	50
	Urobilinogen, mmol/l	35	35	35	35	35
	Ketones, mmol/l	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5
	Ascorbic acid, mmol/l	< 0,6	< 0,6	< 0,6	< 0,6	< 0,6
	Glucose, mmol/l	Otp.	Otp.	Otp.	Otp.	Otp.
	Protein, g/l	0,3	0,3	0,3	0,3	0,3
	Erythrocytes/μl	< 5	< 5	< 5	< 5	< 5
	pH	8,5	8,5	8,5	8,5	8,5
	Nitrites, mg/dl	< 0,05	< 0,05	Otp.	< 0,05	< 0,05
	Leukocytes/μl	< 10	< 10	< 10	< 10	< 10
	Specific density	1,010	1,010	1,010	1,010	1,010
	<b>Female</b>					
	Bilirubin, mmol/l	50	50	50	50	50
	Urobilinogen, mmol/l	35	35	35	35	35
Ketones, mmol/l	< 0,5	< 0,5	< 0,5	< 0,5	< 0,5	
Ascorbic acid, mmol/l	< 0,6	< 0,6	< 0,6	< 0,6	< 0,6	
Glucose, mmol/l	Otp.	Otp.	Otp.	Otp.	Otp.	
Protein, g/l	0,3	0,3	0,3	0,3	0,3	
Erythrocytes/μl	< 5	< 5	< 5	< 5	< 5	
pH	8,5	8,5	8,5	8,5	8,5	
Nitrites, mg/dl	< 0,05	< 0,05	Otp.	< 0,05	< 0,05	
Leukocytes/μl	< 10	< 10	< 10	< 10	< 10	
Specific density	1,010	1,010	1,010	1,010	1,010	

Note: There is no reliable difference between experience and control at 95% probability level.

process that is aimed at the identification and prediction of adverse effects prior to clinical trials [25]. According to the results of the study of acute toxicity of PG Na,Ca,Fe, treating with it the rabbits of both

sexes in doses of 0.5–5 g/kg has no toxic effect. The maximum administered dose 5 mg/kg exceeded 100 times the therapeutic human dose. The results of the acute toxicity study (LD<sub>50</sub> greater than 5 g/kg) allow to classify sodium, calcium, iron-polygalacturonate as a preparation of the 6th class with respect to harmless drugs according to the Hodge and Sterner classification [26].

During the study of statistically significant changes in the body weight of rabbits injected with a pharmacological substance for 60 days, no specific features in the increase in body weight in males or female rabbits were recorded in comparison with the indices for animals in intact and control groups. There were no abrupt changes – no increase or decrease in body weight, i.e. the increase in body weight was gradual (Table 2). Also, there were no significant changes in rectal temperature and behavior relative to the values in the control group in any of the compared groups.

Changes in the hematological pattern of males and females were recorded: there were some differences in such indices as the number of leukocytes and erythrocytes in the blood of rabbits, insignificant fluctuations in the percentage ratio of lymphocytes, monocytes and granulocytes were detected. However, these fluctuations in the indices were statistically unreliable ( $p < 0.05$ ) and indicate the absence of oppression of hematopoiesis under the influence of the drug (Tables 3 and 4). Under the influence of the test preparation, the metal complex with biogenic macro- and microelements (Ca, Fe) – sodium-, calcium-, iron-polygalacturonate, synthesized on the basis of citrus pectin, a tendency to increase the content of erythrocytes and hemoglobin was manifested. This observation is naturally consistent with its anti-anemic effect, which, we believe, will show a more pronounced stimulating hematopoiesis action in case of anemia.

Minor changes in biochemical parameters of blood serum of males and females were recorded (Table 5): a tendency was observed to increase the content of total protein in all groups, which fully agrees with the increase in body weight of experimental and control animals. In addition, fluctuations in the glucose content, activity of the aspartate aminotransferase and alanine aminotransferase enzymes were recorded, however, no significant changes were observed with respect to the control and intact groups of rabbits ( $p < 0.05$ ).

It should be noted that the AST and ALT enzymes activity indices, which are markers of hepatotoxicity, indicate that there is no toxic effect of the drug on the liver. As is known, the protective function of the liver is especially important when exposed to toxic substances, and this organ performs the crucial role of decontamination of toxic substances from the intestine. Absence of hepatotoxic effect is especially important for antianemic drugs, for which, in accordance with the features of the pharmacological group of drugs, long-term use is required.

From the data presented in Table 7, it is seen that in all groups of animals, irrespective of the test dose or sex of animals, biochemical indices of urine – bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, nitrite ions, leukocytes, pH and specific density, were almost identical and corresponded to the physiological norms of healthy animals. There were no significant changes relative to the control group ( $p < 0.05$ ). The obtained results testify to the absence of nephro- and hepatotoxic effects of the studied drug.

The study of the values of the organs mass indices (Table 8 and 9) revealed no significant differences between groups of animals that received sodium-, calcium-, iron-polygalacturonate or a solvent, and intact animals ( $p < 0.05$ ). Body weight and the mass factors of internal organs were within acceptable limits and indicated the absence of the drug toxicity effect on the condition of internal organs. This is also confirmed by the results of the histological examination of internal organs when the animals were given the maximum dose of the drug.

Thus, according to the results of the study of the chronic toxicity of the sodium-, calcium-, iron-polygalacturonate pharmacological substance, administration of it to the rabbits of both sexes in doses of 0.025–0.5 g/kg does not negatively affect the hematological blood

**Table 8**  
Influence of introduction of PG Na,Ca,Fe on the male rabbit organs mass ratios (g/kg) (M ± m).

Term of the study	Index (organ)	Test groups, dose				
		Intact animals	Control	0,5 g/kg (1/10 LD <sub>50</sub> )	0,262 g/kg (ID)	0,025 g/kg (TD)
60th day	Liver	27,2 ± 3,15	28,4 ± 2,6	26,4 ± 3,3	26,8 ± 2,5	29,6 ± 3,6
	Kidneys	2,6 ± 0,06	2,7 ± 0,07	2,6 ± 0,07	2,5 ± 0,05	2,7 ± 0,08
	Lungs	4,8 ± 1,16	4,9 ± 1,0	4,9 ± 1,0	4,9 ± 0,9	4,7 ± 1,1
	Heart	2,4 ± 0,21	2,3 ± 0,16	2,5 ± 0,18	2,3 ± 0,19	2,5 ± 0,2
	Spleen	0,47 ± 0,08	0,51 ± 0,06	0,52 ± 0,09	0,51 ± 0,09	0,43 ± 0,06
	Adrenals	0,06 ± 0,01	0,05 ± 0,01	0,06 ± 0,01	0,06 ± 0,01	0,06 ± 0,01
	Ovaries/ testicles	1,6 ± 0,18	1,8 ± 0,2	1,8 ± 0,18	1,9 ± 0,25	1,5 ± 0,2
90th day	Liver	28,7 ± 2,9	29,1 ± 3,0	27,8 ± 2,6	27,4 ± 2,8	27,5 ± 3,1
	Kidneys	2,7 ± 0,07	2,6 ± 0,08	2,5 ± 0,08	2,6 ± 0,09	2,6 ± 0,07
	Lungs	5,2 ± 1,2	4,7 ± 0,7	4,8 ± 1,1	4,6 ± 0,6	4,8 ± 0,7
	Heart	2,5 ± 0,16	2,2 ± 0,2	2,4 ± 0,15	2,4 ± 0,14	2,3 ± 0,21
	Spleen	0,52 ± 0,09	0,46 ± 0,07	0,48 ± 0,07	0,46 ± 0,07	0,48 ± 0,07
	Adrenals	0,05 ± 0,01	0,05 ± 0,01	0,05 ± 0,01	0,06 ± 0,01	0,06 ± 0,01
	Ovaries/ testicles	1,8 ± 0,2	1,9 ± 0,16	1,7 ± 0,15	1,8 ± 0,19	1,9 ± 0,13

Note: There are no significant differences between animals from experience and control groups at 95% probability level.

**Table 9**  
Influence of introduction of PG Na,Ca,Fe on the female rabbit organs mass ratios (g/kg) (M ± m).

Term of the study	Index (organ)	Test groups, dose				
		Intact animals	Control	0,5 g/kg (1/10 LD <sub>50</sub> )	0,262 g/kg (ID)	0,025 g/kg (TD)
60th day	Liver	24,2 ± 2,7	25,7 ± 2,8	25,3 ± 2,4	23,9 ± 2,3	26,3 ± 2,9
	Kidneys	2,6 ± 0,08	2,7 ± 0,07	2,7 ± 0,06	2,6 ± 0,08	2,7 ± 0,06
	Lungs	4,87 ± 0,6	4,7 ± 0,8	4,6 ± 0,8	4,7 ± 0,8	4,5 ± 0,8
	Heart	2,2 ± 0,15	2,1 ± 0,23	2,3 ± 0,16	2,2 ± 0,13	2,1 ± 0,2
	Spleen	0,6 ± 0,07	0,49 ± 0,09	0,63 ± 0,08	0,57 ± 0,08	0,46 ± 0,05
	Adrenals	0,05 ± 0,01	0,05 ± 0,01	0,06 ± 0,01	0,06 ± 0,01	0,05 ± 0,01
	Ovaries/ testicles	0,06 ± 0,02	0,06 ± 0,02	0,07 ± 0,02	0,06 ± 0,02	0,08 ± 0,03
90th day	Liver	26,4 ± 2,5	27,8 ± 2,9	26,9 ± 2,8	25,8 ± 2,5	28,1 ± 2,5
	Kidneys	2,5 ± 0,06	2,5 ± 0,09	2,5 ± 0,07	2,5 ± 0,04	2,7 ± 0,09
	Lungs	5,0 ± 0,9	4,9 ± 0,6	4,7 ± 0,9	4,5 ± 0,7	4,6 ± 0,9
	Heart	2,4 ± 0,19	2,3 ± 0,14	2,5 ± 0,18	2,3 ± 0,19	2,2 ± 0,16
	Spleen	0,55 ± 0,06	0,42 ± 0,1	0,59 ± 0,09	0,47 ± 0,05	0,49 ± 0,09
	Adrenals	0,06 ± 0,01	0,06 ± 0,01	0,06 ± 0,01	0,06 ± 0,01	0,05 ± 0,01
	Ovaries/ testicles	0,05 ± 0,02	0,07 ± 0,03	0,06 ± 0,02	0,07 ± 0,01	0,06 ± 0,02

Note: There are no significant differences between animals from experience and control groups at 95% probability level.

indices, biochemical parameters of blood serum and urine, cardiovascular system and weight of organs. The maximum administered dose 0.5 mg/kg during 90 days exceeded 10 times the therapeutic human dose.

The studies of this work were performed at a high modern level, which is confirmed by data of contemporary literature describing similar researches [1–4,27,28]. The observed results are very important because they demonstrate the toxicology safety of sodium-, calcium-, iron-polygalacturonate and allow to recommend this compound for further clinical trials as antianemic preparation.

## 5. Conclusion

According to the results of the study of the acute toxicity of sodium-, calcium-, iron-polygalacturonate pharmacological substance, no toxic effect, leading to death, was detected at administration of the substance to rabbits of both sexes in doses of 0.5–5 g/kg. Histological examination of viscera did not reveal pathological changes, the histostructure of animals, treated with preparations in a dose of 5000 mg/kg, did not differ from that of the animals of the control group. The results of the study allow us to classify sodium-, calcium-, iron-polygalacturonate as a preparation of the VI class (of relatively harmless compounds).

An estimate of the chronic toxicity of sodium-, calcium-, iron-polygalacturonate pharmacological substance at intragastric administration of preparation in the form of boluses to rabbits of both sexes

bolus in doses 0.025, 0.262 and 0.5 g/kg of the body weight demonstrated that the general condition and behavior of animals did not differ from the norm.

The data of hematological and biochemical studies of blood serum and urine, electrocardiographic studies, the study of the mass coefficients of the internal organs of the experimental rabbits, treated with pharmacological substance in the mentioned doses for 60 days, compared to those obtained in the 30-day post-observation period, did not show significant changes with respect to the control and intact group of rabbits.

In conclusion, the pharmacologic substance sodium-, calcium, iron-polygalacturonate, given orally to female and male rabbits, did not produce both acute and chronic toxicities.

## Conflicts of interest

All authors disclose the absence of potential conflicts of interest.

## Acknowledgment

The authors are grateful to Ph.D. O. Fominykh for technical assistance.

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