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

S.T. Minzanova[a,*], V.A. Milyukov[a], A.V. Krayushkina[a], D.M. Arkhipova[a], A.B. Vyshtakalyuk[a,*], L.G. Mironova[a], V.F. Mironov[a], K. Kh. Papunidi[b], E.I. Semenov[b], I.R. Kadikov[b], O.G. Sinyashin[a]

[a] A.E. Arbuzov Institute of Organic and Physical Chemistry of Kazan Scientific Center of Russian Academ of Sciences, Arbuzov str. 8, Kazan, 420088, Russia
[b] Federal Center of Toxicological, Biological and Radiological Safety, Russia

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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_{50} 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 (Rhodephyta)* 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 complexability 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.

* Corresponding authors.
E-mail addresses: minzanova@iopc.ru (S.T. Minzanova), alex.vysh@mail.ru (A.B. Vyshtakalyuk).

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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 Co^{2+}, Cu^{2+}, Fe^{2+}, and 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].

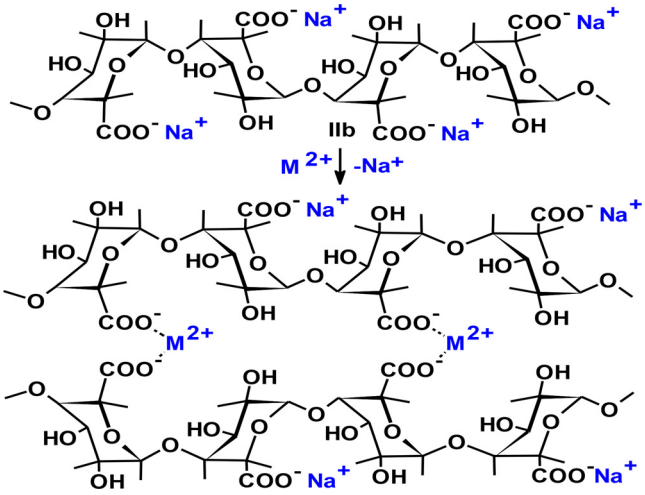
The object of research is a metal complex synthesized on the basis of citrus pectin with biogenic macro- and microelements (Ca, Fe) – sodium-, calcium-, iron-polygalacturonate, stimulating the process of hematopoiesis [18].

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].



$M^{2+} = Ca^{2+}, Fe^{2+}$

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 November 2017).

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

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 adlibitum 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 LD_{50} (0.5 g/kg of body weight), then 1/10 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 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).