



Study of radioprotective properties of chitosan at gamma-irradiation

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ABSTRACT

The objective of this study was the analysis of adaptogenic effects of chitosan at gamma-irradiation. The study of radioprotective properties of chitosan was carried out on male mice having the weight of 20-25 g, exposed to gamma-irradiation at the doses of up to 800 bar at the Gamma Panorama unit (radiation source – Cs¹³⁷, gamma dose rate – 14 R/min, duration of irradiation – 58.5 min). 4 experimental and 2 control groups with 7 animals in each have been formed. The medicine dose schedule, allowing to identify both the prophylactic effect and the therapeutic effect of chitosan, was used in the experiment. It was found out in experiments on white mice, with the purpose of determining 50% lethal dose (LD₅₀) that chitosan belongs to the substances of hazard class 4 because its LD₅₀ considerably exceeds 5000 mg/kg; at this, the toxicity of chitosan decreases parallel to its molecular weight decrease. The prophylactic and therapeutic effect of low-molecular chitosan (molecular weight 5-10 kDa) as at abdominal injection at the doses of 200 mg/kg of body weight. It has been demonstrated that the mice from the experimental group, I that received chitosan 3 days before the irradiation remained alive throughout the entire observation period (30 days). In the animals of II of II, III, IV experimental groups that received chitosan in 3, 9 and 22 days after the irradiation, the survival rate by the experiment end was 86, 43 and 29%, respectively, with the 100% death of animals in the control group I (irradiated animals without the use of chitosan) and full safety of animals in the control group II (non-irradiated animals).



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INTRODUCTION

As the result of the environment radioactive pollution, people and animals are exposed both to exter-

nal and internal irradiation. Internal irradiation is connected with the accumulation of active isotopes and heavy metals in food products of vegetable and animal origin, from where they enter a human body through a gastrointestinal tract with food (Yakobson *et al.*, 1998). The certain part of radio nuclides enter a body through the respiratory tracts and skin. In this context, the development of new efficient adaptogenic agents with poly functional properties, providing the body resistance to radiation and to numerous unfavorable environmental factors, remain relevant. In this context, creating biopharmaceuticals based on naturally biological active substances is considered prospective (Schettters *et al.*, 1990).

The scientific and practical interest to the use of chitosan grows annually. The more the scientists

learn about the properties of chitin and chitosan, the wider is the field of their practical application (Jaworski *et al.*, 2016). Innovative and unexpected directions of practical application appear every year. For the time being, the issues of using chitosan in food industry, as a para pharmaceutical agent for prophylactics and treatment of hypertensive disease, cardiovascular and gastrointestinal diseases; in agriculture, as a bio stimulant and as a means of fighting agricultural pests, are solved in the Russian Federation to the highest extent (Mammerickx *et al.*, 2010).

Achievements of Russian scientists and the foreign experience of obtaining the wide range of chitosan-based medicines prove the antiviral, antibacterial, antidote, anticoagulant, immune correction properties, lipopolysaccharide-binding and immune adjuvant activity of chitosan, which allows using it as an intestinal sorbent for the efficient protection from exo- and endotoxemia syndrome (Ruggiero and Bartlett, 2019).

Continuous environmental footprints caused considerable, and, in many areas, even critical pollution with heavy metals, radionuclides, oil products and other substances hazardous for wild-life and man. The base of durable radioactive pollutions is formed by long-living radionuclides, such as Cs¹³⁷, Sr⁹⁰, Zr⁹³, Zr⁹⁵, Ru¹⁰⁶, Ce¹⁴⁴, Sm¹⁵¹, Eu¹⁵⁴. As the result of the environment radioactive pollution, a man is exposed both to external and internal irradiation. At the integral action of radioactive products on human body, the need for their removal and detoxification arises (Ungar-Waron *et al.*, 1992, 1999; Konishi *et al.*, 2018).

The large number of mineral, synthetic and organic sorbents, specific in relation to heavy metals and radionuclides, is known. Natural polymers hold the special place among organic sorbents. They include chitosan, the N-desacetylated form of chitosan, and the main structural component of teguments of crustaceans and insects, and fungal cell walls. Chitin and chitosan contain several functional groups – hydroxylic, carbonylic, amino-, acetyl amide groups and oxygen bridges, so the mechanism of heavy metals sorption with these polymers has a rather complex nature (Khudhair *et al.*, 2016). Depending on the conditions, it may include complex formation and superficial adsorption, however, the most investigators now tend to believe that the chelated complex formation, conditioned by high electron donor ability of nitrogen and oxygen atoms, prevails the most. Due to this, chitin sorbents have a surprisingly wide range of sorbed elements. On practice, they are ions of all metals, except alkali and

alkali-earth. All the information listed above served as the basis for chitosan testing as a radioprotector.

MATERIALS AND METHODS

High toxicity of chitosan samples was determined by the value of 50% lethal dose (LD₅₀), in experiments on nonlinear white mice at the age of 2-2.5 months with the weight of 18-22 g, from which, groups were formed, each containing 10 animals. Chitosan medications with the molecular weight (MW) of 5-10 kDa, 10-20 kDa, 20-50 kDa, 50-100 kDa, were introduced IP as a single dose in the volume of 0.5 cm³ in the form of 2% gel. Control animals were introduced the acetic acid aqueous solution with pH 5.8. After the test medications injection, the animals have been observed for 2 weeks. Appearance and behaviour of the animals, condition of the hair coat and visible mucous coats, attitude to feed, mobility, breathing rate, terms of animal's death or their recovery were taken into account. LD₅₀ for chitosan medications by Kerber method.

The study of radioprotective properties of chitosan was carried out on male mice with the weight of 20-25 g, exposed to gamma-irradiation at the doses of up to 800 bar at the Gamma Panorama unit (radiation source – Cs¹³⁷, gamma dose rate – 14 R/min, duration of irradiation – 58.5 min). 4 experimental and 2 control groups with 7 animals in each have been formed. The medicine dose schedule, allowing to identify both the prophylactic effect and the therapeutic effect of chitosan, was used in the experiment. Control I – irradiated animals, Control II – animals were not irradiated. Chitosan was introduced IP (MW 5-10 kDa) at the dose of 200 mg/kg of body weight to animals of experimental groups: Experimental group I – 3 days before the irradiation, II, III and IV experimental groups in 3, 9 and 22 days after the irradiation, respectively. Terms of chitosan introduction depend on radiation disease stages. The observation period was 30 days.

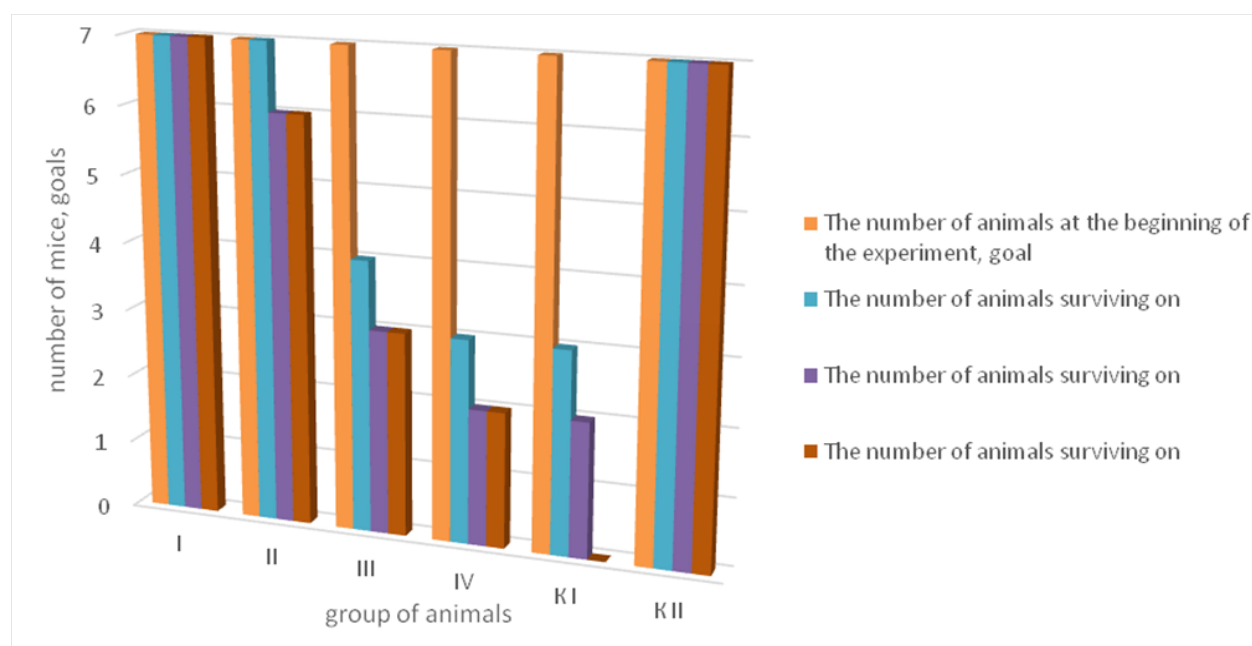
RESULTS AND DISCUSSION

Studies of several authors demonstrate the radioprotective effect of medications based on high-molecular chitosan at the introduction of radioactive isotopes to animals. However, the most hazardous effect at the living body is caused by ionizing irradiation. For this reason, we have been studying the radioprotective properties of chitosan on laboratory animals, exposed to gamma-irradiation. Radioprotector is a chemical substance protecting a body from ionizing radiation.

The optimal molecular weight of chitosan was

Table 1: Results of determining the 50% lethal dose of chitosan on white mice

Molecular weight 5-10 kDa							
Dose, mg/kg	2500	5000	10000	20000	40000	80000	LD ₅₀ mg/kg
Survived	10	10	10	9	5	0	19216,
died %	0	0	0	1	5	10	4
Molecular weight 10-20 kDa							
Dose, mg/kg	1250	2500	5000	10000	20000	40000	LD ₅₀ mg/kg
Survived	10	10	10	9	8	0	17417,4
died %	0	0	0	1	2	10	
Molecular weight 20-50 kDa							
Dose, mg/kg	1250	2500	5000	10000	20000	40000	LD ₅₀ mg/kg
Survived	10	10	9	9	5	0	16348,1
died %	0	0	1	1	5	10	
Molecular weight 50-100 kDa							
Dose, mg/kg	625	1250	2500	5000	10000	20000	LD ₅₀ mg/kg
Survived	10	10	9	8	4	0	15137,2
died %	0	0	1	2	6	10	

**Figure 1: Results of the study of radioprotective properties of chitosan**

selected based on the comparative evaluation of parameters of acute toxicity of chitosan with the molecular weight of 5-10 kDa, 10-20 kDa, 20-50 kDa and 50-100 kDa (Table 1).

Based on the evidence found it may be concluded that chitosan belongs to the substances of hazard class 4 because of its 50% lethal dose (LD_{50}) considerably exceeds 5000 mg/kg; at the same time, so far as the molecular weight of chitosan decreases, its toxicity decreases, too.

Chitosan with the molecular weight of 5-10 kDa was selected as a radioprotector, because such low-molecular polymer has the lower toxicity, penetrates into blood and, consequently, into all zoo blasts, more efficiently and rapidly. The results of studies carried out are given in the Figure 1.

It is seen from the data given in the Figure 1 that the mice of experimental group I that received chitosan medication 3 days before the irradiation, remained alive throughout the observation (30 days), while the animals of II, III and IV experimental groups demonstrated the survival rate by the experiment end of 86, 43 and 29%, respectively, with the 100% death of animals in the control group I and full safety of animals in the control group II.

It was shown that the prophylactic oral introduction of chitosan prior to single irradiation of gamma-irradiation causes the body radio resistivity condition to develop.

CONCLUSIONS

Due to the number of its chemical composition features, chitosan with the molecular weight of 5-10 kDa is capable of binding radionuclides and of excreting them from the body in the most efficient way. As the result of the study conducted, the positive prophylactic and therapeutic effect at the use of low-molecular chitosan on mice, exposed to gamma-irradiation, was obtained. The results obtained supplement the scientific validity of chitosan as an adaptogenic biologically active substance. The detected adaptogenic effects of chitosan in the conditions of radiation disease simulation open a prospect of its use with the purpose of creating the macro organism homeostasis protection system at vital activity in the conditions of unfavorable environmental factors.

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