Hepatotoxic effect evaluation of probiotic medication of candidates for elemental status correction of animals based on research of biochemical blood indicators

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Abstract. The paper presents the data of possible application of probiotic medications, based on the bacteria of genus Bacillus as micronutrient medication for correction of mineral deficiency conditions that have the ability to accumulate metals, in order to deliver zinc-ions in the body of laboratory animals. The choice of the research objects of probiotic medications on the basis of bacteria Bacillus: Vetom 1.1 (the strain of B. subtilis 10641) and Vetom 3 (the strain of B. amyloliquefaciens 10642) is conditioned by the high depositing characteristics given by the microorganisms of essential elements, in particular zinc, from the different inorganic compounds. The evaluation of accumulation of bioavailable zinc forms was carried out on the laboratory rats as models. The inactivated biomass of bacteria, after long periodical cultivation in a liquid nutrient medium with zinc sulfate, was orally injected in their bodies. One of the main criteria for the development of medication candidates besides the evaluation of delivery effectiveness is the absence of pathological effects on the body of laboratory animals. To implement this task, we evaluated the hepatotoxic effect of applied medications on the basis of research of alanine transaminase, aspartate transaminase and alkaline phosphatase level in a blood serum throughout the experiment. The obtained data indicates the absence of pathological effects on hepatocytes, as the concentration of researched biochemical indicators was within the reference values throughout the experiment.

1. Introduction
Zinc is an essential element that is actively involved in various biological processes of different forms of life. It performs a regulatory function in the metabolism of proteins, fats, carbohydrates, hematopoiesis in the bodies of humans and animals [1, 2].

The consequence of mineral deficiency is annual increase of population disease rate. Zinc deficiency is a consequence considering not only unbalanced nutrition, but also the peculiarities of the geochemical zinc distribution, as well as a competition between elements. In the case of zinc, for example, the antagonist elements are cuprum, manganese, ferrum and cadmium, excessive content of which significantly affects the level of biological intake of zinc in the body. People with zinc deficiency often suffer from cold and infectious diseases as they have a lowered T-cell immune system [3].
Currently, there is a deficit of zinc consumption in almost all regions of Russian Federation. Despite the significant intake of zinc into the biosphere, there is a shortage of movable forms of zinc in the soils of most regions due to the activities of industrial companies. This situation leads to the lack of the microelement in the diet of citizens [4].

One of the possible options for the elemental status control of the bodies of animals and humans is the use of probiotic medications based on bacteria of genus Bacillus with high sorption characteristics [5, 6].

2. Materials and methods
2.1. The object of research
To carry out the experimental part we selected the following strains: B. subtilis 10641 (“Vetom 1.1”, the medication manufacturer – “Research center” Ltd. (Russia), B. amyloliquefaciens 10642 (“Vetom 3”, the medication manufacturer – “Research center” Ltd. (Russia). The essential elements were zinc sulfate (ZnSO4).

The rats of Wistar line were selected as a material, in the number of 96 specimens in their puberty age, i.e. the period when animals reach the maximum carcass weight.

2.2. Scheme of the experiment
To carry out the experiment research, the microorganisms were isolated from probiotic preparations. Then, they were cultured with zinc sulfate in a liquid nutrient medium. To prepare the saline solution of the researched metal, a sample was weighed based on calculations and dissolved in 100 ml of distilled water at a concentration of 0.05 M/ml. A metal saline solution and probiotic medications were added to the liquid nutrient medium with the following 24-hour cultivation in a thermostat at a temperature of 37 °C. After that the centrifugation was performed at 3000 rpm for 10 minutes (for the purpose of biomass deposition), followed by the removal of the supernatant liquid. The obtained biomass was placed in an autoclave (VC-31) and sterilized for 40 minutes to inactivate bacteria of genus Bacillus. The biomass was given per os to laboratory animals.

To implement the task of evaluation of prospective use of the microorganism probiotic strains as a regulatory factor of elemental status in the development of zinc deficiency, we formed 4 groups of animal analogs – 2 control and 2 experimental with 24 animals in each group. The first control group was on a mineral-deficient diet (K1). The second control group of intact animals was on the diet in accordance with the requirements of Russian regulations (the Order of the Ministry of Health of the USSR No. 163 of 10.03.1966). The results of the group were used as a criterion for physiological norm evaluation (K2). The experimental groups and the group K1 had a mineral diet for 20 days that leads to the development of zinc deficiency. B. subtilis 10641 (“Vetom 1.1”) was cultivated in a liquid nutrient medium with the excessive amount of ZnSO4 in dose of 01–1 ml per head. Then, after the process of cultivation the biomass of inactivated B. subtilis 10641 (“Vetom 1.1”) was given to the first experimental group (O1) within 10 days from the start of experiment. In the second experimental group (O2) B. amyloliquefaciens 10642 (Vetom-3) was used as the source of zinc-ions with a similar scheme of injection and dose. The research was carried out on the basis of comparative method of biological research, i.e. the animals were placed in the identical conditions and same timeline.

Experiments on the animals were carried out in accordance with the instructions, recommended by Russian regulations, 1987 and “The Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996). The laboratory animals were placed in the similar conditions. 2.3. Statistical processing
The collection of the material was carried out at intervals of five days (background study, the 5th day, the 10th day and the 15th day). Biochemical indicators of blood serum were determined using standard techniques. The obtained data were statistically processed using Student's t-test [7].

3. The results of research and its discussion
A complex evaluation of medication efficacy of the candidates for the zinc deficiency correction was made on the basis of inactivated probiotic strains. The process was carried out by means of determining the content of researched element in the tissues of animals, the study of hematological and biochemical indicators of blood. Intravital research of biochemical indicators allows the full evaluation of the impact of researched medications on the individual organs and organ systems as well as on the body as a whole. One of the main criteria for the research of medication candidates is the lack of toxic effect on the body. With this aim, we conducted the research of ALT, AST and alkaline phosphatase. It allows the evaluation of the degree of hepatotoxic effect of researched medications.

In the case of ALT’s results of the deficit control group in relation to the main indicators for the 5th, the 10th and the 15th day of the experiment, there was an increase of 10.5%, 13.25 and 12.6%. There was an increase of 1.2% on the 5th day of the experiment; a decrease of indicators was on the 10th and 15th day in the experimental group O2.

During the research of AST in the group of deficit control in relation to the main indicators on 5th, 10th and 15th days of the experiment, there was an increase of 3.36%, 1.3% and 1.5%. In the experimental group O2, there was an increase of 2.3% on the 10th day; a decrease of indicators occurred on the 15th day of the experiment.

During the research of alkaline phosphatase in the bodies of laboratory animals, it was stated that the applied medications “Vetom 1.1” and “Vetom 3” had no effect on the researched indicator throughout the experiment. In all groups this indicator did not exceed the referential value. However, it should be indicated that there was a slight increase in alkaline phosphatase on the 5th day in all experimental groups, followed by a decrease in the analyzed indicator by the 10th day of the experiment. In relation to the main indicators in the group of deficit control, there was an increase of 3.6% on the 5th day, 7.11% – on the 10th day, 0.76% – on the 15th day of the experiment. In relation to the main indicators, there was a slight increase on the 5th day in all experimental groups. This situation was recorded throughout the experiment.

The data analysis of biochemical blood test indicates a slight increase of the concentration of the researched indicators on the 5th day of the experiment. In our view, this phenomenon may be caused by the adaptive mechanisms of the bodies of laboratory animals to the injection of the high concentrations of bioavailable zinc, which considers high element concentrations as a xenobiotic factor. It should be mentioned that the increase of the researched indicators was within referential value and slightly exceeded the level of the intact animal group. To our opinion, it connected with the lack of cytopathic effect on hepatocytes. The hypothesis of the organism adaptation is confirmed by the reduction of concentration of all researched indicators to the level of intact animals on the 10th day of the experiment.

4. Conclusion

Summarizing the results, obtained in the experiments in vivo, the perspectivity of using probiotic strains of microorganisms should be indicated as the candidate medications for the correction of mineral deficiency, in particular, of zinc deficiency. The selection criterion of microorganism-producer of essential bioavailable element is the research of their sorption depositing characteristics which, according to the literature data, has a two-phase nature. The first phase is characterized by the biosorption of elements by cellular wall components and do not involve the energy state of the cell. The second phase is characterized by the energy-dependent intracellular accumulation, occurring with the participation of ion-transporter enzymes [9-11].

A number of authors stated that the most important mechanisms. The first mechanism is the exclusion of heavy metals by the permeability of the cell barrier and their immobilization outside the cell by bacterial metabolites. The second one is the detoxification of ions of toxic metals that enter the cytoplasm under the effect of various chemiosmotic or energy-dependent systems, as well as mechanisms of intracellular sequestration involving low-molecular proteins, such as cysteine-rich metallothioneins [12].
To summarize the above, it can be indicated that one of the possible options for the regulation of the elemental status of the bodies of animals and humans is the usage of biotic medications on the basis of waste products of transient microflora representatives with high sorption characteristics [3].

The results of the biochemical study of the blood of experimental animals to evaluate the hepatotoxic effect of researched medication candidates indicates the lack of their negative impact on the body. This fact ensures about the prospects of research on the development of the use of nutraceutical micronutrients, based on probiotic strains in the system of elemental status correction of the macroorganism in the geochemical conditions, causing the development of mineral deficiency.

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References
[1] Khanafari A, Eshghdoost S and Mashinchian A 2008 Removal of Lead and Chromium from Aqueous Solution by Bacillus circulans Iranian J. of Environmental Health, Sci. and Engineer. 53 195–200
[2] Skalny A A, Karganov M Yu, Skalnyi A V, Fomina M A and Nikonov A A 2017 The effect of zinc supplementation on the zinc and selenium status in exercise J. of Trace Elements in Medicine and Biology 41(1) 39
[3] Waihung L N and Lau M H F 2007 Peter Biosorption and desorption of copper (II) ions by Bacillus sp Appl. Biochemistry and Biotechnology 107 (3) 581–91
[4] Yilmaz E 2003 Ince Metal tolerance and biosorption capacity of Bacillus circulans strain EB1 Res. in Microbiol. 154 (6) 409–15
[5] Skalnyi A V, Salnikova E V, Kvan O V, Sizentsov A N and Salnikov I A 2016 Research of interrelation of zinc bioaccumulation in food products and the human body in the region of Orenburg J. of Orenburg State University 10 79–81
[6] Kvan O, Gavrish I, Lebedev S, Korotkova A, Miroshnikova E, Bykov A, Serdaeva V and Davydoval N 2018 Effect of probiotics on the basis of Bacillus subtilis and Bifidobacterium longum on the biochemical parameters of the animal organism Environmental Sci. and Pollution Res. 25 (3) 2175–83
[7] Sizentsov A N, Kvan O V, Notova S V and Galchenko T A 2014 The effectiveness of probiotic medications usage based on bacteria of genus Bacillus in the system of ferrum delivery J. of Recovery Medicine 2 66–75
[8] Sizentsov A N, Galchenko T A and Martynovich U I 2013 Accumulation of heavy metals by probiotic medications based on bacteria of genus Bacillus in vitro conditions J. of Kazan State Academy of Veterinary Medicine named after N.E. Bauman 216 303–7
[9] Trevors J T, Oddie K M and Belliveau B H 2009 Metal resistance in bacteria FEMS Microbiology Reviews 1(1) 39–54
[10] Sheyda E., Sipaylova O., Kvan O., Notova S., Nesterov D., Rusakova E., Kosyan D., Duskaev G. 2014 Functional properties of antimicrobial peptides extracted from hens’ platelets. Life Science Journal 11(9).25 180-184
[11] Duskaev G.K., Rakhatmulin S.G., Kazachkova N.M., Sheida Y.V., Mikolaychik I.N., Morozova L.A. and B.H. Galiev. Effect of the combined action of Quercus cortex extract and probiotic substances on the immunity and productivity of broiler chickens. Veterinary World, 11(10): 1416-1422.
[12] Lanieva O 2009 Mechanisms of bacteria resistance to heavy metals J. of Microbiological 71(6) 54–65