

Original Research

Safety Assessment of a Medicinal Probiotic as an Adjunct in Tuberculosis Therapy: Implications for Pharmacy Practice

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Abstract

Objective: This study aimed to assess the acute and subacute toxicity of medicinal agent based on the association of probiotic lactic acid bacteria *Lactiseibacillus rhamnosus* 7n, *Lactiseibacillus parasasei* B-3/43, *Lactiseibacillus casei* 14d/13 and 1% alcohol extract of licorice (*Glycyrrhiza glabra*). **Method:** Laboratory mice and rats were selected for the study, and the medicinal agent was administered orally and intraperitoneally. **Result:** Based on the studies conducted on determination of acute toxicity with single oral administration to mice at doses of 25 mg/kg, 250 mg/kg, 2500 mg/kg and 5000 mg/kg and to rats at doses of 27.8 mg/kg, 278.0 mg/kg, 1111.2 mg/kg and 2222.4 mg/kg of weight, and with intraperitoneal administration to mice at doses of 2.5 mg/kg, 25.0 mg/kg, 250.0 mg/kg, 2500 mg/kg and to rats at doses of 2.8 mg/kg, 27.8 mg/kg, 278.0 mg/kg, 555.6 mg/kg of weight, it showed a favorable safety profile without signs of toxicity. When studying the subacute toxicity in laboratory mice at doses of 25.0 mg/kg, 250.0 mg/kg and 2500.0 mg/kg, no toxic effect on the body was revealed. **Conclusion:** The investigated substance can be classified as non-toxic/harmless. No biological reactivity or deaths were observed in animals during 14 days of acute toxicity assessment and 28 days of subacute toxicity assessment after administration of the tested solutions at various doses. The somatic state remained stable. Analysis of the body weight of animals showed no statistically significant differences between the experimental groups. In all groups, a positive increase in body weight was observed. A comparison of the absolute and relative masses of organs between the experimental groups showed no significant differences. Macroscopic examination revealed that anatomical location, morphological characteristics and appearance of the organs corresponded to the physiological norms characteristic of this species. The calculation of the average lethal dose (LD_{50}) is impossible due to the absence of fatal outcomes.

Keywords: Lactic acid bacteria; plants, medicinal; probiotics; toxicity

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INTRODUCTION

Probiotics are microorganisms that inhabit the mucous membranes of the body and are involved in maintaining its physiological functions, including digestive processes, regulation of the immune system, the state of the respiratory system, metabolic processes, and can also have a modulating effect on the central nervous system (CNS)¹⁻⁴. With sufficient abundance and species diversity, they form a balanced microbiome, which has a positive effect on the overall health of the host⁵.

Probiotic microorganisms are represented by many taxonomic groups, but the main and most commonly used are lactic acid bacteria (Lactic Acid Bacteria, LAB) and bifidobacteria (*Bifidobacterium* spp.)⁶. Currently, probiotic strains belonging to these groups officially have the status of "Generally Recognized as Safe" (GRAS) and widely used in the food industry and microbiology due to their immunomodulatory properties⁷. Probiotics in combination with prebiotics form synbiotic complexes that enhance the positive effects of each of the components^{8,9}. Such complexes may include extracts of medicinal plants containing a wide range of biologically active compounds. Licorice (*Glycyrrhiza glabra*) has long been known for its antitumor and antiviral properties. The roots of this plant are traditionally used to treat chronic inflammatory diseases^{10,11}. Due to the presence of biologically active compounds such as glycyrrhizic acid and glycyrrhetic acid, licorice roots have a



wide range of pharmacological properties, including antibacterial, antiviral, anti-inflammatory, antiarrhythmic, antioxidant and expectorant effects^{12,13,14}. They are used to develop new natural products with antiviral, antioxidant, anti-inflammatory, and cytotoxic effects, making them a valuable resource for the treatment of various diseases¹⁵⁻¹⁸.

Pharmacists play a key role in ensuring the safety and effectiveness of medicines, including probiotic drugs. Currently, their field of activity goes beyond the simple dispensing of medicines: they advise patients on the rational use of medicines, monitoring possible side effects and compliance with the treatment regimen, which increases the safety and effectiveness of treatment as a whole¹⁹. In addition, pharmacists are involved in the processes of pharmacovigilance and monitoring of drug safety, they collect data on adverse reactions and transfer them to national registries, thereby increasing the level of rational use of drugs²⁰. Data from clinical studies and reviews provide evidence that the involvement of pharmacists in TB treatment management processes improves adherence to therapy and treatment safety, reducing the incidence of complications, making them an integral part of practical pharmacy in this field²¹.

There is evidence in the literature that probiotics can have a clinically significant positive effect in the treatment of tuberculosis as an adjunctive therapy. Taking probiotics in conjunction with standard anti-tuberculosis chemotherapy helps to reduce adverse drug reactions, improve gastrointestinal tract function, and increase long-term treatment toleration. In addition, the antagonistic properties of probiotics, the ability to modulate the host immune response, support intestinal barrier function, and reduce systemic inflammation have been reported, which contributes to improved clinical outcomes in patients with tuberculosis²². Of particular interest is the combined use of probiotics with extracts of medicinal plants, in particular licorice (*Glycyrrhiza glabra*), which has anti-inflammatory, immunomodulatory and cytoprotective properties. It is assumed that such a combination can have a synergistic therapeutic effect, having an inhibitory effect on tuberculosis pathogens and having a positive effect on the tolerability of tuberculosis therapy and the general condition of the patient, which makes such combined drugs promising in the framework of complex treatment of tuberculosis.

Given that probiotics are widely available as over-the-counter drugs and their use in the treatment of various diseases, including tuberculosis, continues to expand, pharmacists should rely on preliminary evidence of safety and tolerability to form informed and informed recommendations to patients. Moreover, there is reliable evidence in the sources that the use of probiotics may be accompanied by various risks, for example, the likelihood of developing invasive infections (bacteremia, sepsis) in patients with immunodeficiency, transient disorders of the gastrointestinal tract, the development of D-lactate acidemia in people with short bowel syndrome, as well as possible horizontal transfer of antibiotic resistance genes between probiotic and opportunistic

microorganisms²³⁻³⁰. Preclinical toxicity studies such as the present study serve as an important source of evidence-based data that can be used by pharmacists in patient counseling, monitoring planning, and the selection of rational adjuvant therapy. Therefore, despite the safety of most probiotic strains, a set of studies aimed at assessing their safety, including possible toxicity, is required before introducing drugs based on them into pharmacological practice.

There are publications in the literature on the definition of probiotic safety. Recent studies confirm that individual strains of *Lactocaseibacillus rhamnosus*, *Lactocaseibacillus paracasei* and *Lactocaseibacillus casei* have a favorable safety profile when administered orally. In the study by Kwon et al. (2024), a randomized placebo-controlled trial of the *Lactocaseibacillus rhamnosus* IDCC 3201 strain was conducted in patients with irritable bowel syndrome, which did not reveal serious toxicity or an increase in the incidence of severe adverse events, and in vivo studies and 90-day doxycycline tests in animals did not reveal systemic toxicity even at higher than recommended doses for humans³¹. Chen and colleagues (2024) conducted a comprehensive genotypic and phenotypic assessment of the *L. paracasei* NTU 101 strain and proved the absence of virulence genes and clinically significant antibiotic resistance³². Lee et al. (2024) investigated a new strain of *L. paracasei* IDCC 3401 and proved its favorable safety profile and suitability for use in probiotic preparations have been proven³³. Taken together, current publications support the conclusion that safety should be assessed at the level of a specific strain and that for each new product, a study should be conducted to determine potential toxicity.

The literature presents the results of a preclinical assessment of the acute and subacute toxicity of the probiotic agent AC-Probionorm, developed on the basis of lactic acid bacteria strains. The product is a dry powder in the form of a crystalline or porous mass of white or cream color, with a characteristic sour-milk odor and sweet-sour taste, containing a living mass of cells of lactic acid bacteria *Lactobacillus fermentum* 30 (1×10^9 CFU), *Lactobacillus cellobiosus* 36 (1×10^9 CFU). During the experiment, six groups of mice were formed, with five heads each. To determine acute toxicity, the studied medicinal agent was administered once in doses of 50 mg/kg, 300 mg/kg, 1000 mg/kg, 2000 mg/kg, 4000 mg/kg and 5001 mg/kg of animal body weight. After administration, the animals were monitored for signs of toxicity or death for 14 days. To assess subacute toxicity, the study was conducted on 30 rats weighing 180-200 g, which were divided into three groups of 10 animals: two experimental and one control. The animals of the experimental groups were repeatedly injected with the test agent on an empty stomach using a metal probe in a maximum allowable volume of 3 ml, while the rats of the control group were injected



Table 1. Average body weight of mice during the follow-up period after a single oral and intraperitoneal administration of the investigational medicinal agent in different doses, (M ± m)

Groups	Dose of medicinal agent, mg/kg	The average weight of animals before the start of the experiment with oral administration	The average weight of animals after 14 days with oral administration	Dose of medicinal agent, mg/kg	Average weight of animals before the start of the experiment with intraperitoneal administration	The average weight of animals after 14 days with intraperitoneal administration
1	25,0	23,06±0,31	25,42±0,81	2,5	23,20 ± 0,62	25,52 ± 0,77
2	250	23,5±0,57	26,08±0,73	25,0	23,08 ± 0,73	25,40 ± 0,65
3	2500	23,22±0,57	25,32±0,81	250,0	22,62 ± 0,54	25,42 ± 0,81
4	5000	22,82±0,63	24,88±0,97	2500	22,58 ± 0,43	25,84 ± 0,34

Note: the difference is significant in relation to the values of body weight before the start of the experiment ($p < 0.05$)

with an equivalent volume of water. During the entire observation period, there was no death of animals during the determination of acute and subacute toxicity, and no signs of intoxication were noted. Motor activity, coordination of movements and behavioral reactions remained within the normal range. Reactions to tactile, painful, sound, and light stimuli did not change. The intake of food and water was satisfactory. The studied medicinal agent did not have a negative effect on the dynamics of body weight gain, on the contrary, there was a positive dynamics of its increase. The condition of the skin and coat of the animals also remained within the physiological norm, which classifies the medicinal agent as a safe remedy. Repeated administration into the stomach also revealed no negative effects, and a safety comparison confirmed its comparability with the probiotic Fertal® (ESI srl, Italy)^{34,35}.

To determine the possible acute, subacute toxicity of a medicinal probiotic agent, we selected an association of lactic acid bacteria and alcohol licorice extract with the highest spectrum of biological activity against tuberculosis pathogens.

The purpose of this study is to determine the acute and subacute toxicity of oral and intraperitoneal administration of the investigational medicinal agent in various dosages in vivo in mice and rats with the subsequent prospect of its study and introduction into the dosage form.

MATERIALS AND METHODS

The object of research for the study of acute and subacute toxicity was a medicinal agent based on the association of probiotic lactic acid bacteria *Lactocaseibacillus rhamnosus* 7h, *Lactocaseibacillus parasasei* B-3/43, *Lactocaseibacillus casei* 14d/13 and extract of licorice (*Glycyrrhiza glabra*) in liquid form.

The medicinal agent is a liquid of opaque consistency, from light to dark brown in color, with a characteristic sour-milk odor. The agent is a certain amount (8.5×10^9 CFU/ml) of probiotic lactic acid bacteria in a culture medium with the addition of 1% alcohol extract of licorice. Nutrient medium components, g/ml: yeast extract - 0.005; meat extract - 0.01; glucose - 0.02; ammonium citric acid - 0.002; sodium acetic acid-0.005; potassium phosphoric acid 2-substituted - 0.002; magnesium sulfate 7-aqueous - 0.0002; manganese sulphate 4-aqueous - 0.00005; ammonium sulphate - 0.001.

The medicinal agent requires storage in a dry place protected from direct light, at a temperature from +2 °C to +8 °C, with a reliable shelf life of up to 5 months under these conditions.

The medicinal agent was transferred to the Laboratory of Pharmacology and Toxicology of the Scientific Center for Anti-Infective Drugs, Almaty, Republic of Kazakhstan for the determination of acute and subacute toxicity. During the determination of acute and subacute toxicity, the GLP principles were followed to the maximum. The study was conducted in accordance with Standard Operating Procedures (SOP) and Methodological Procedures (MPs).

The basic animal handling techniques were carried out according to:

SOP-PHT-001 Principles of Laboratory Animals Care

The selection of animals for the experiment and their observation is carried out according to:

SOP-PHT-021 Animals Observation

SOP-PHT-011 Veterinary Control of Animals

Randomization of animals is carried out according to:



Table 2. Average body weight of rats during the follow-up period after a single oral and intraperitoneal administration of the investigational medicinal agent in different doses, (M ± m)

Groups	Dose of medicinal agent, mg/kg	The average weight of animals before the start of the experiment with oral administration	The average weight of animals after 14 days with oral administration	Dose of medicinal agent, mg/kg	Average weight of animals before the start of the experiment with intraperitoneal administration	The average weight of animals after 14 days with intraperitoneal administration
1	27,8	187,46 ± 3,24	187,46 ± 3,24	2,8	188,10 ± 5,31	215,78 ± 3,37
2	278,0	189,50 ± 3,46	189,50 ± 3,46	27,8	193,32 ± 6,70	219,20 ± 1,39
3	1111,2	185,92 ± 4,35	185,92 ± 4,35	278,0	195,00 ± 5,31	220,94 ± 1,62
4	2222,4	188,22 ± 2,56	188,22 ± 2,56	555,6	194,4 ± 6,44	220,08 ± 1,69

Note: the difference is significant in relation to the values of body weight before the start of the experiment (p < 0.05)

SOP-PHT-003 Animal Randomization in the Experiment

Labeling of animals is carried out according to:

SOP-PHT-004 Marking of Animals Used in Research

The administration of the investigated substances is carried out according to:

SOP-PHT-009 Administration of Substances

Premedication and euthanasia are performed according to:

SOP-PHT-016 Rodent Analgesia, Anesthesia and Euthanasia

Cleaning and disinfection are carried out according to:

SOP-PHT-002 Cleaning and Disinfection of Laboratory Rooms and Animal Facility

SOP-PHT-007 Cleaning and Sterilization of Laboratory Equipment

SOP-GE-021 Waste Classes. Rules for Waste Collection and Disposal

An acute toxicity study was conducted to determine the tolerated, toxic, and lethal doses of a pharmacological substance and the causes of animal death. The parameters of acute toxicity are LD₅₀, i.e. the dose of the substance that causes the death of 50% of the animals in the observed group, and the determination of the toxicity class of the substance. The acute toxicity of the studied medicinal agent was assessed with oral and intraperitoneal single administration on two types of laboratory animals — mice weighing 22 g ± 10 % and rats weighing 180 g ± 10%.

The study was carried out according to the relevant documents³⁶⁻³⁹.

The experiment used mice and rats that had not previously been involved in research.

The animals were acclimatized for 5 days.

The pre-experimental veterinary examination of the animals was conducted in accordance with SOP-PHT-011 Veterinary Animal Control.

Mice for research were weighed before the start of the experiment and placed 5 animals in one cage according to the experimental groups.

The animals were kept at a temperature of (22 ± 2) °C, a relative humidity of 50-60%, and a natural light regime of 12:12.

Environmental conditions, nutrition, water, body weight control, veterinary control and medical examination were monitored in accordance with:

SOP-PHT-001 Principles of Laboratory Animals Care

SOP-PHT-011 Veterinary Control of Animals Health

SOP-PHT-022 Storage of Feed and Feeding Laboratory Animals

SOP-PHT-021 Animals Observation.

Identification/labeling of each animal was carried out according to

SOP-PHT-004.

During the study, the animals were on a full and balanced diet and had unlimited access to food and drinking water (ad libitum). medicinal agent solutions for oral administration in mice were prepared by diluting the initial drug solution with saline solution. Doses of 25 mg/kg, 250 mg/kg, 2500 mg/

Table 3. Body weight changes in mice with 28-day oral administration of the medicinal agent in different doses, (M ± m)						
Animal groups sex, doses		Average weight of animals, g				
		Day 1	Day 7	Day 14	Day 21	Day 29
Group (Gr) 1 – 25,0 mg / kg	♂	20,50 ± 0,47	25,48 ± 0,19	26,30 ± 0,16	27,78 ± 0,19	29,28 ± 0,19
	♀	20,40 ± 0,69	24,00 ± 0,16	24,50 ± 0,16	26,20 ± 0,16	27,90 ± 0,16
Gr 2-250.0 mg / kg	♂	20,46 ± 1,02	25,68 ± 0,19	26,58 ± 0,19	28,18 ± 0,19	29,78 ± 0,19
	♀	20,50 ± 0,47	24,28 ± 0,19	24,88 ± 0,19	26,68 ± 0,19	28,48 ± 0,19
Gr 3-2500.0 mg / kg	♂	20,20 ± 0,47	25,68 ± 0,33	26,58 ± 0,33	28,18 ± 0,33	29,88 ± 0,33
	♀	21,06 ± 0,92	24,40 ± 0,32	25,00 ± 0,32	26,80 ± 0,32	28,60 ± 0,32
Gr 4-Control	♂	20,30 ± 0,79	23,40 ± 0,79	26,42 ± 0,80	28,01 ± 0,79	29,60 ± 0,79
	♀	20,36 ± 0,38	23,36 ± 0,73	24,60 ± 0,22	26,88 ± 0,29	29,16 ± 0,54

Note: the difference is significant in relation to the control values ($p < 0.05$)

kg and 5000 mg/kg of the weight of the test animal were selected. The volume of administration is 1.0 ml for a dose of 5000.0 mg/kg (fractional) and 0.5 ml for all other doses.

To determine acute toxicity with intraperitoneal administration in mice, 2.5 mg/kg, 25.0 mg/kg, 250.0 mg/kg, and 2500 mg/kg doses of the medicinal agent were selected. Solutions for administration were prepared by diluting the starting agent with saline solution. The volume of administration was 1.0 ml for a dose of 5000.0 mg/kg (fractional) and 0.5 ml for all other doses.

To investigate acute toxicity after oral administration in rats, the following dosages were selected: 27.8 mg/kg, 278.0 mg/kg, 1111.2 mg/kg and 2222.4 mg/kg body weight. For a dose of 27.8 mg/kg, a solution of the test substance was prepared by diluting the initial agent with saline solution, the volume of administration was 0.5 ml. For a dose of 278.0 mg/kg, a 0.5 ml ready-made solution of the medicinal agent was used. For doses of 1111.2 mg/kg and 2222.4 mg/kg, the volume of administration of the initial drug solution was 2.0 ml (fractional) and 4.0 ml (fractional), respectively.

Doses of 2.8 mg/kg, 27.8 mg/kg, 278.0 mg/kg, and 555.6 mg/kg of body weight were selected for intraperitoneal administration in rats. Working solutions for doses of 2.8 mg/kg and 27.8 mg/kg were prepared by diluting the initial medicinal agent with saline solution, the volume of administration was 0.5 ml. For a dose of 278.0 mg/kg, the medicinal agent was used in a ready-made form in a volume of 0.5 ml. For a dose of 555.6 mg/kg, the volume of administration was 1.0 ml of the initial agent solution.

When determining the acute toxicity of a medicinal probiotic agent in in vivo experiments, 4 experimental groups of 5 animals each were formed. After a single oral administration of various doses of the investigational agent to determine acute toxicity, animals were monitored for mortality or toxic signs on the first day every two hours during working hours, and in the following days every 24 hours.

All animals were monitored for 14 days after administration of the solutions of the studied medicinal probiotic agent. During this period, an assessment of possible signs of acute toxicity caused by the action of the test substance was carried out. Clinical manifestations of toxic effects were recorded as they appeared, indicating the time of onset, severity, and duration. Regular animal monitoring was carried out to prevent cases of deaths of the studied animals due to cannibalism and tissue autolysis.

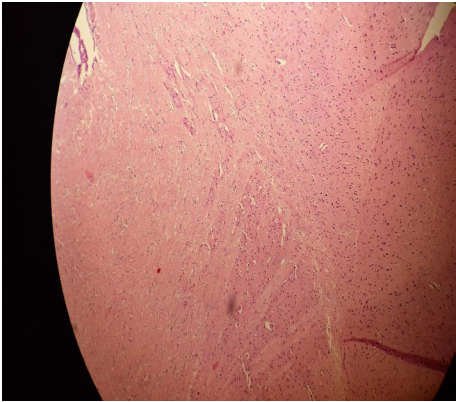
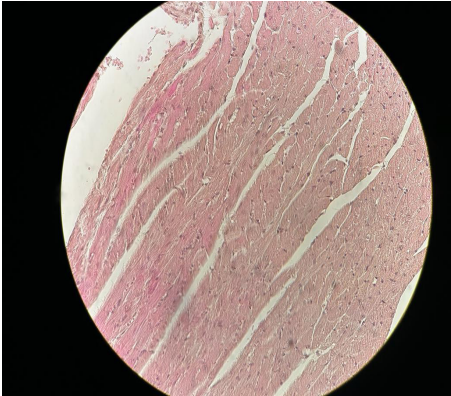
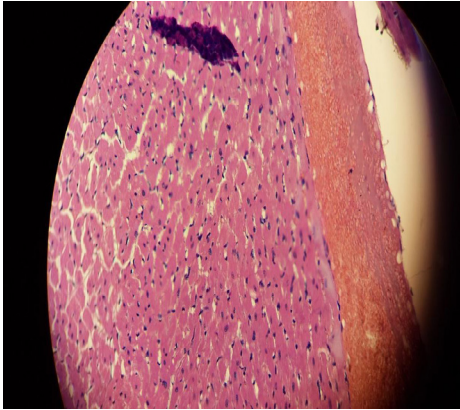
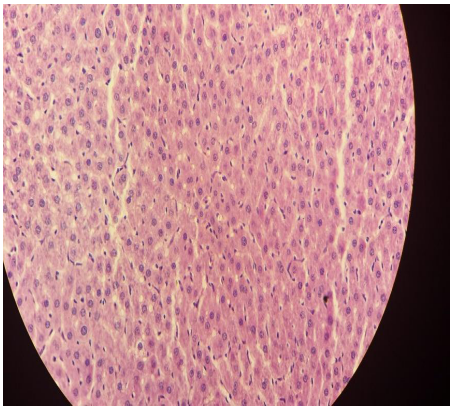
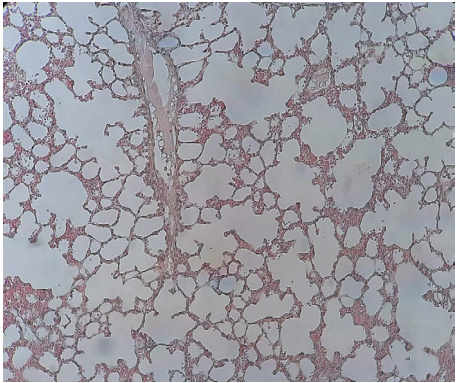
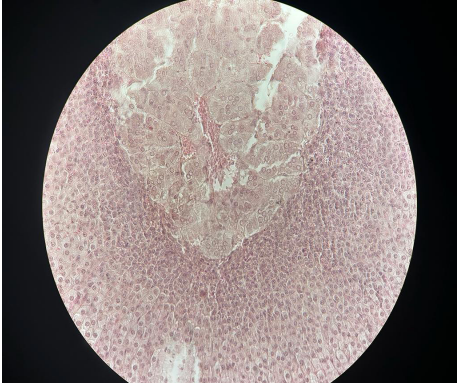
The body weight of the animals was measured immediately before dosing and then weekly throughout the observation period.

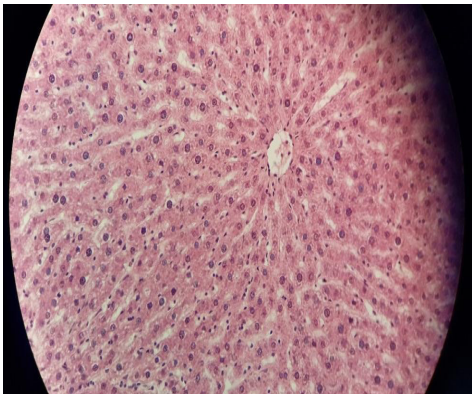
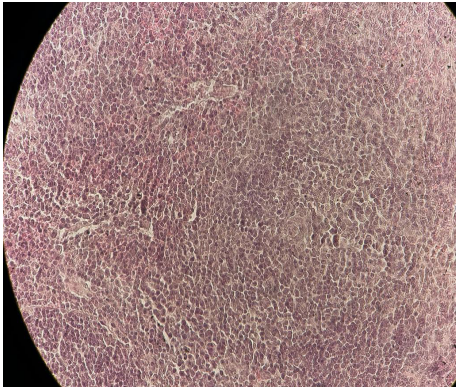
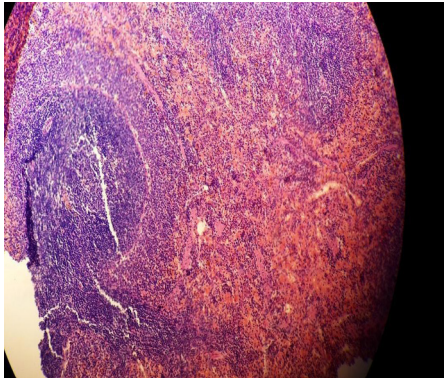
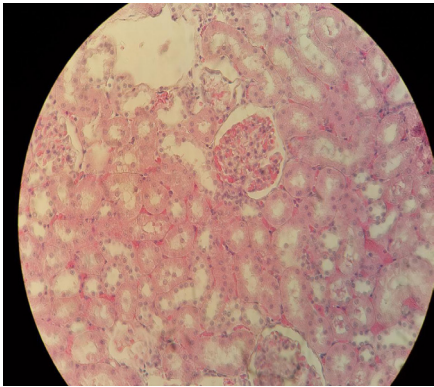
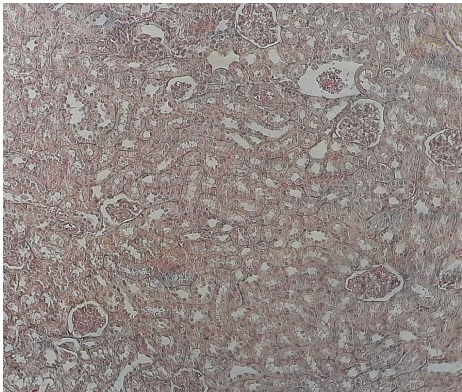
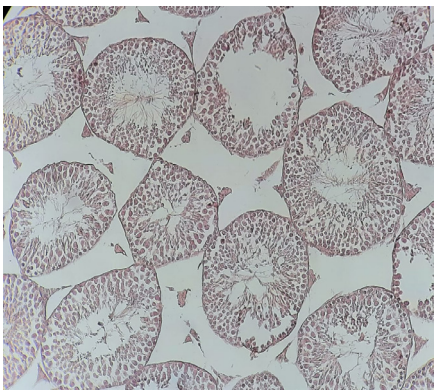
After 14 days of observation (on day 15), the animals were euthanized following isoflurane anesthesia (SOP-PHT-016), and autopsy and macroscopic examination were performed. Internal organs were weighed: liver, lungs, spleen, kidneys, and heart. Their relative weight coefficients are calculated and statistical processing of digital data is carried out.

Macroscopic analysis was performed according to SOP-PHT-019 Macroscopic research of laboratory animal's internals. During macroscopy, the condition of the external integuments, physiological openings, regional lymph nodes, internal organs and tissues was assessed.

After the autopsy, internal organs (liver, lungs, spleen, kidneys, and heart) were weighed. Organs were weighed in animals of all groups during planned necropsy. Paired organs were weighed together. In addition to the absolute weight of organs, the percentage of organ weight to body weight (relative weight coefficients) was determined and statistical processing of digital data was carried out.

The evaluation of the results of the conducted studies after a single oral administration of solutions of the studied medicinal agent to mice in doses of 25.0 mg/kg; 250.0 mg/kg; 2500.0 mg/kg and 5000.0 mg/kg of body weight was carried out based on the following criteria:

Organ name	Control group	Experimental group
Brain	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>
Heart	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>
Lungs	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>

<p>Liver</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>
<p>The spleen</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>
<p>Kidneys</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×40 objective, ×10 ocular</p>

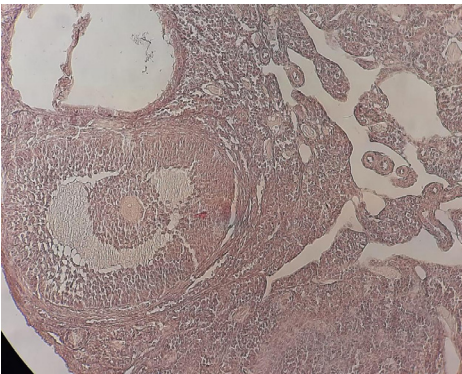
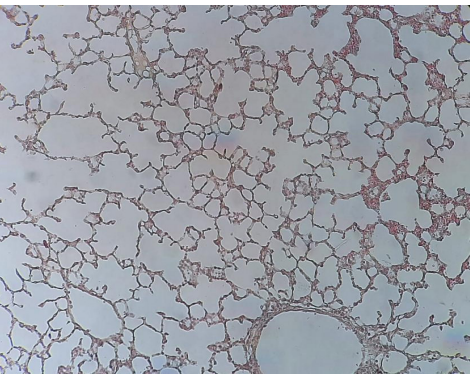
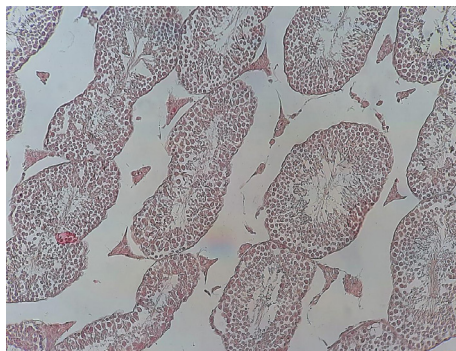
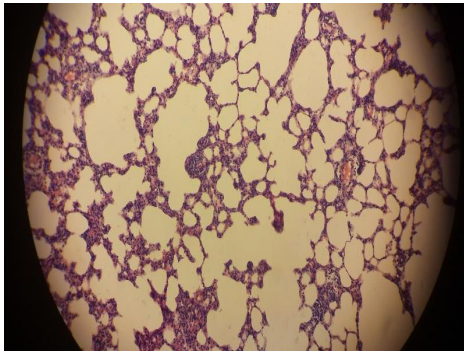
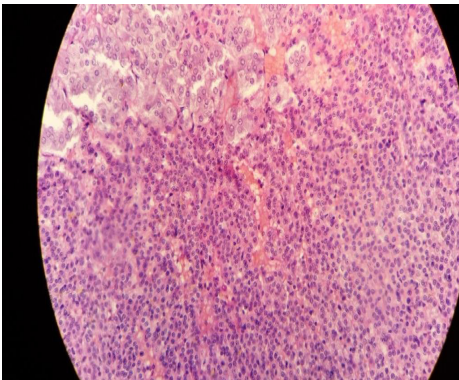
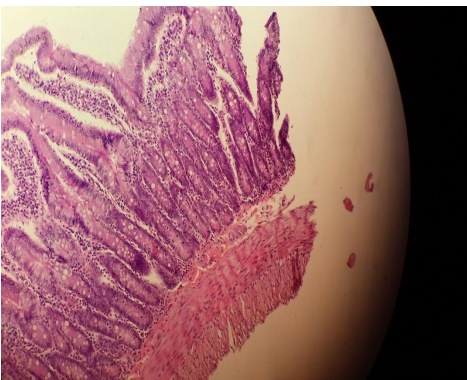
The ovaries	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>
Testicles	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>
The adrenal glands	 <p>Magnification: ×20 objective, ×10 ocular</p>	 <p>Magnification: ×20 objective, ×10 ocular</p>

Figure 1. Histostructure of the internal organs of experimental animals (color: hematoxylin-eosin)

- Animal survival/mortality during the entire follow-up period of 14 days;
- Body weight of the animals receiving the test solutions;
- Somatic indicators;
- Macroscopic description of internal organs;
- Weight coefficients of internal organs.

To study subacute toxicity, solutions of the probiotic agent under study were administered orally to laboratory mice for 28 days at doses of 25.0 mg/kg, 250.0 mg/kg and 2500.0 mg/kg. For oral administration, solutions for doses of 25.0 and 250.0 mg/kg were prepared by diluting the initial solution with 0.9% saline solution, and the initial solution was used for a dose of 2500.0 mg/kg. The volume of administration is 0.5 ml for all doses. Purified water in a volume of 0.5 ml was used as a control. The study used white outbred mature laboratory mice in the amount of 40 heads (10 individuals of both sexes for each dose and for the negative control group), weighing $22 \text{ g} \pm 10\%$.

Animals were monitored for mortality or toxic signs on a daily basis. All animals were monitored for 28 days of oral administration of the investigational agent and the control solution. During the entire period of the experiment, laboratory animals were monitored daily to assess signs of toxic effects of the test substance, including general behavior, survival, and condition in the area of administration. The registration of somatic indicators was carried out by changing external signs, such as behavior, condition of the coat and color of the mucous membranes, with the data recorded in the primary documentation. The body weight of the animals was determined before the start of the experiment and then weekly in grams throughout the entire observation period. Daily feed intake was calculated from the difference in the weight of the feeders before and 24 hours after feeding.

Histological preparations of internal organs were stained with hematoxylin-eosin according to the standard procedure. Microscopic examination was performed using direct light microscopes Leica DM1000 and ZEISS Axio Scope A1 with magnification of the lens $\times 10$, $\times 20$, $\times 40$, $\times 100$ and eyepiece $\times 10$ (total magnification $\times 100$, $\times 200$, $\times 400$, $\times 1000$).

The necessary calculations were carried out using standard Microsoft Excel 2007 programs, and the arithmetic mean and standard deviation (StD) were calculated for all numerical data. In the presence of animal mortality, LD_{50} is calculated using the least squares method using probit analysis.

RESULTS

When studying the acute toxicity of a medicinal probiotic agent during the observation period (14 days) after administration of the studied doses of agent solutions, no animal deaths were

recorded. During observation, a satisfactory condition was noted in mice receiving the test solutions when administered orally at doses of 25 mg/kg, 250 mg/kg, 2500 mg/kg and when administered intraperitoneally at doses of 2.5 mg/kg, 25.0 mg/kg, 250.0 mg/kg weight, and in rats receiving oral solutions at doses of 27.8 mg/kg, 278.0 mg/kg, 1111.2 mg/kg and 2222.4 mg/kg and when administered intraperitoneally at doses of 2.8 mg/kg, 27.8 mg/kg, 278.0 mg/kg, 555.6 mg/kg weight. There were no signs of toxic poisoning, biological reactivity, or other abnormalities in the appearance and behavior of the animals. The somatic condition remained stable, with no pronounced changes (condition of the coat, coloration of the oral mucosa, and other signs).

The analysis of animal body weight showed no statistically significant differences between the experimental groups (Table 1 and Table 2).

In all groups, a positive increase in body weight was observed, corresponding to the physiological norm (with oral administration to mice from 2.06 to 2.58 g, with intraperitoneal administration - from 2.32 to 3.26 g; with oral administration to rats - from 19.48 to 24.18 g, with intraperitoneal administration to rats - from 25.68 to 27.68 g). A comparison of the absolute masses of internal organs and their relative weight coefficients between different experimental groups did not reveal significant differences.

According to the results of a macroscopic examination following planned autopsy, the anatomical location, morphological characteristics and appearance of the internal organs corresponded to the physiological norms characteristic of this species. No pathological changes were found.

On physical examination, the mucous membranes are moist and pink, the physiological openings are clean, without signs of pathology. Subcutaneous lymph nodes are not enlarged during skin separation. Upon autopsy, the anatomical location of the internal organs, their appearance and morphological features corresponded to the norm typical for this biological species; no pathological changes were recorded.

The heart had a characteristic structure; the left ventricle with a cross-section was of normal size. The aorta was well visualized, its lumen is free. The lungs were uniformly colored, with no signs of seals or emphysematous changes. The spleen in all the studied groups was oblong in shape, dark cherry in color, with moderate density; when cut, the tissue did not leave a scrape on the instrument. The liver is a uniform dark red color, the surface is smooth, without signs of tumor growth. The lobular structure of the organ was preserved, and there was no scraping during the incision. The buds are typically bean-shaped, with a smooth, shiny, brown surface; the capsule is thin, transparent, and easily separated. A dark cortical zone was clearly visible in the longitudinal section; the renal pelvis was not dilated and remained free. The organs of the reproductive system are not visually altered. The oral cavity is free, the incisors are of

normal shape, without deformations.

Histological studies were not performed, since macroscopy of animals receiving single oral solutions of the studied medicinal agent in the studied doses did not reveal visible pathological changes in internal organs.

According to the results of the study of acute toxicity of a medicinal probiotic agent with a single oral and intraperitoneal administration to mice, the average lethal dose of LD₅₀ could not be calculated due to the absence of death of animals at all doses used. The toxicity class is defined as a non-toxic substance.

When studying subacute toxicity after administration of solutions of the tested probiotic agent to mice at doses of 25.0 mg/kg; 250.0 mg/kg; 2500.0 mg/kg of body weight, no toxic effects were observed during the entire observation period of 28 days: mortality, behavioral disorders, and changes in feed intake. The somatic state remained stable, with no marked changes. All animals were in a normal condition throughout the entire study and recovery period. All the animals showed a normal level of arousal when they were captured, a reaction to touch, visual and auditory reactions, and a reaction to tail pinching. The average values of the strength of motor activity of males and females from all groups receiving medicinal agent solutions in different doses were comparable with the control group. There were no general toxic effects: deaths, acute toxic reactions such as ataxia, hypersalivation, or seizures.

Analysis of the body weight of mice showed no statistically significant differences between the experimental groups. In all groups, a positive increase in body weight was observed, corresponding to the physiological norm (in males - from 8.78 to 9.68 g; in females – from 7.50 to 8.80 g) (Table 3).

A comparison of the absolute masses of internal organs and their relative weight coefficients between different experimental groups did not reveal significant differences.

According to the results of a macroscopic examination after planned autopsy, the anatomical location, morphological characteristics and appearance of the internal organs corresponded to the physiological norms characteristic of this species. No pathological changes were found.

Morphofunctional changes were not detected. An external examination of animals receiving oral medication revealed no visual changes in the assessment of physique, palpation of the coat, skin, mucous membranes (mouth, eyes, conjunctiva, external genitalia and anus). The coat is clean, there are no foci of alopecia. The mucous membranes of the eyes, nose and mouth are typically pale pink in color, and there is no discharge. The teeth are properly ground. The ear canals are clean. Vibrissae are typical. The anal and genital areas are unchanged. The skin is free of signs of inflammation and phlegmon, edema, hematomas and lumpy nodules in the subcutaneous

tissue. The location of the internal organs is typical. There is no fluid in the abdominal and thoracic cavities. The lymph nodes (inguinal, bronchial, axillary, cervical, mandibular) are not enlarged. The prostate gland is unchanged. The diaphragm dome is unchanged. The thymus gland has a typical location, unchanged, soft, grayish-white in color, without features. The muscles of the heart are dark red in color, homogeneous without damage, no hemorrhages were detected. The aorta is clearly visualized. The transverse incision at the level of the left ventricle did not show an increase in its walls. The lung tissue is airy, the fragment quickly floats up, the individual lobes are full-blooded. During the incision, light, slightly foaming contents flowed out of the lumens of the large bronchi. A gallbladder with intense yellow contents. The liver is dark cherry-colored, homogeneous, and leaves no marks on the scalpel blade. No signs of hepatomegaly or venous fullness were found. The mesenteric lymph node is unchanged. The spleen was dark red in color, without signs of hemorrhage, not ulcerated, and left no marks on the scalpel blade when cut longitudinally. The buds are smooth, smooth, and dark brown in color. The boundary between the cerebral and cortical layers in the sagittal section is clearly differentiated, the cortical substance prevails over the cerebral. The adrenal glands are cream-colored and rounded above the upper pole of the kidney. The serous membranes of the stomach and intestines are smooth and shiny, the Peyer's patches are unchanged. There are no foci of necrosis or additional formations in the pancreas. When the incision is made along the large curvature of the stomach, food remains (feed) are noted, without the smell of rotting. The folded structure of the mucosa is preserved, ulcers are absent. A cross-section of the duodenum results in a homogeneous yellow chyme. Fecal matter is formed in the large intestine. The organs of the reproductive system are unchanged: the testicles are lowered and the ovaries have no characteristic surface nervousness. The uterine cavity is free, and the uterine horns are passable when probed. The brain is light gray, typical, vessels are visualized. Cranial cavity without foci of pathological hematopoiesis. The location of the internal organs is correct/typical, there was no evidence of any solderage, or a sharp increase or decrease in size.

Histological analysis (in the maximum-dose group and the control group) confirmed the absence of signs of degeneration, necrosis, or inflammation in the tissues of the cardiorespiratory system, liver, kidneys, spleen, brain, and reproductive organs. Figure 1 shows histological preparations of the internal organs of mice in the control group compared with the experimental group, which received the medicinal agent at a maximum dose of 2500 mg/kg for 28 days.

According to histological data, compensatory adaptive immunomodulatory reactions without signs of acute damage with certain functional signs of activation of the adrenal cortex and a transient inflammatory reaction in the renal tissue and mild reactive changes in the liver and heart are noted in the studied organs of the experimental group.

Hematological parameters (hemoglobin, hematocrit, erythrocytes, average volume of erythrocytes, leukocytes, lymphocytes, monocytes, granulocytes, eosinophils, platelets, and ESR) were within the physiological norms for this animal species. Blood biochemical parameters (aspartate aminotransferase, alanine aminotransferase, bilirubin, glucose, total protein, urea, creatinine, alkaline phosphatase, cholesterol) showed no statistically significant differences in males and females compared with the control group. The parameters of the general urinalysis were without significant changes between groups and gender.

Oral administration of the medicinal agent based on the association of probiotic lactic acid bacteria *L. rhamnosus* 7h, *L. paracasei* B-3/43, *L. casei* 14d/13 and 1% alcohol extract of licorice *Glycyrrhiza glabra* in the studied dosages of 25.0 mg/kg, 250.0 mg/kg and 2500.0 mg/kg demonstrates a favorable safety profile, without signs of subacute toxicity.

According to the results, the investigational medicinal probiotic agent based on the association of probiotic lactic acid bacteria and alcohol extract of licorice with oral and intraperitoneal administration in mice and rats in the above doses can be classified as non-toxic / harmless in accordance with the modified classification system of the Organization for Economic Cooperation and Development (OECD) according to the Order of the Ministry of Health of the Republic of Kazakhstan dated February 4, 2021.

DISCUSSION

Studies were conducted to determine the acute and subacute toxicity of the medicinal agent based on the association of probiotic lactic acid bacteria *L. rhamnosus* 7h, *L. paracasei* B-3/43, *L. casei* 14d/13 and 1% alcohol extract of licorice *Glycyrrhiza glabra*.

To study the acute toxicity of a medicinal probiotic agent, the study was conducted on two types of animals (mice and rats) and with two methods of administration (oral and intraperitoneal). The following doses were selected for the administration of the test agent: for oral administration to mice - 25 mg/kg, 250 mg/kg, 2500 mg/kg, 5000 mg/kg; for rats - 27.8 mg/kg, 278.0 mg/kg, 1111.2 mg/kg, 2222.4 mg/kg; for intraperitoneal administration to mice - 2.5 mg/kg, 25.0 mg/kg, 250.0 mg/kg, 2500 mg/kg; for rats - 2.8 mg/kg, 27.8 mg/kg, 278.0 mg/kg, 555.6 mg/kg weight. There were 4 groups of animals in each experimental line, with 5 heads each. After administration of the medicinal agent, the animals were observed for 14 days. During the observation period, biological reactivity and death of the animals were not noted, there were no signs of toxic damage, abnormalities in appearance and behavior of the animals. After 14 days, all the animals were euthanized and autopsied. Macroscopic examination revealed no abnormalities in the location, appearance, or other parameters of the internal organs, and no pathological changes

were found. There was no significant difference between the weight indices of the internal organs and the relative weight coefficients of the mice of the experimental groups. Based on the conducted studies, the medicinal agent in the indicated doses was determined to be a non-toxic substance. The results obtained are consistent with a study that evaluated the toxicity of the probiotic strain *Bacillus* sp. DU-106 in vivo in laboratory mice. As a result of the conducted studies on the definition of acute toxicity with the oral method of administration, it was found that a dose of 1.0×10^{12} CFU / kg body weight did not cause signs of acute toxicity in mice, therefore this dose is considered safe for humans. There were also no differences in body weight and blood levels of D-lactic acid between the control and experimental groups. No cases of fatal outcome of the test animals were found⁴⁰.

According to the results of a study on laboratory mice of subacute toxicity of a probiotic agent in doses of 25.0 mg/kg, 250.0 mg/kg and 2500.0 mg/kg, it was found that it does not have a toxic effect on the body with 28-day use. At the same time, there was no death of mice in the experimental groups, and no biological reactivity of the organism was detected. The weight indicators and body weight gain corresponded to the physiological norm, and no changes in the relative weight coefficients of the animals' internal organs were detected. Hematological and biochemical parameters of blood and urine correspond to this type of animal. Macroscopic examination showed that administration of a medicinal probiotic for 28 days at a maximum dose of 2500.0 mg/kg did not cause structural changes in the internal organs of mice during histological examination. Similar results are known from studies on laboratory mice of acute and subacute toxicity of lyophilizate of a medicinal probiotic agent from the most active strains of lactic acid and propionic acid bacteria: *L. plantarum* 2b/A-6 + *L. brevis* B-3/A-26 + *L. acidophilus* 27w/60 + *P. shermanii* 8. The authors of this study concluded that with a single oral administration of medicinal probiotic agent solutions in doses of 300 mg/kg; 2000 mg/kg; 4000 mg/kg and 5001 mg/kg of body weight, this agent can be classified as non-toxic. Also, when determining subacute toxicity at doses of 5.0×10^6 CFU; 10.0×10^6 CFU; 15.0×10^6 CFU for 28 days, this agent does not have a toxic effect on the body of the examined animals⁴¹.

The data obtained demonstrate the absence of signs of toxic effects, which indicates a favorable safety profile. The positive effect on animal body weight indicates a good tolerance of the drug, which is especially important for patients receiving long-term and severe TB therapy.

From the point of view of pharmaceutical practice, the results of the study are of direct practical importance, since they provide pharmacists with reliable data on the safety and tolerability of the drug. Since probiotics are widely available drugs and their use in the treatment of various diseases, including tuberculosis, continues to expand, pharmacists should rely on preliminary evidence of safety and tolerability in order to form informed



and informed recommendations to patients. These data allow pharmacists to assess the risk–benefit ratio, participate in the selection of rational adjuvant therapy and monitor the tolerability of treatment, especially in patients with weakened immune systems.

The doses used in the study significantly exceed the estimated therapeutic levels for humans, which provides a sufficient margin of safety. The principle of safety assessment is not so much a direct calculation of the doses of the drug per person, but rather a demonstration that the drug is safe even at high doses, which creates the basis for further research. However, additional studies, including pharmacokinetics, evaluation of efficacy and tolerability in humans, are needed to be implemented in clinical practice. Before being introduced into clinical practice, it is necessary to consistently expand preclinical safety data to support rational use, confirm the safety profile and tolerability of drugs in clinical trials (phases I–III), and develop an application and monitoring protocol⁴².

These data highlight the pharmacist’s key role in the subsequent stages: patient consultation, assessment of tolerability and side effects, adjustment of therapy, and participation in the rational introduction of a new drug. Thus, the present study provides preclinical evidence of safety and tolerability, which can serve as a basis for the informed use of probiotic drugs as auxiliary components of tuberculosis therapy and for informed decision-making by pharmacists.

CONCLUSION

Thus, in the course of this study, the absence of acute and subacute toxicity of a medicinal probiotic agent was proved based on the association of probiotic lactic acid bacteria *Lactiseibacillus rhamnosus* 7h, *Lactiseibacillus parasaei* B-3/43, *Lactiseibacillus casei* 14d/13 and 1% alcohol extract of licorice *Glycyrrhiza glabra*. Oral and intraperitoneal administration of the medicinal agent was not accompanied by hematological, histochemical and physiological disorders among the tested laboratory animals.

The results obtained are consistent with data from similar studies. Therefore, based on the results of preclinical trials and, in particular, on the absence of toxicity, in the future it is possible to conduct clinical trials and use this probiotic agent as a medicinal and prophylactic drug in the complex therapy of tuberculosis.

AUTHOR’S CONTRIBUTION

Irina Ratnikova: Conceptualization, Methodology, Supervision, Project Administration, Funding Acquisition

Amankeldi Sadanov: Supervision, Project Administration, Conceptualization, Funding Acquisition

Baiken Baimakhanova: Validation, Supervision, Project Administration

Erik Shorabayev: Supervision, Project Administration, Resources

Saltanat Orazymbet: Formal Analysis, Data Curation, Supervision

Elmira Namaz: Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization

Kairat Turgenbayev: Software, Validation, Formal Analysis

Nurshat Utegenova: Investigation, Data Curation, Visualization

Aknur Turgumbayeva: Software, Investigation, Visualization

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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