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Preclinical study of "Microfit" probiotic preparation influence on internals of laboratory rats

Abstract: Histologic research is one of the most reliable methods of diagnosis of pathologies of organs and tissues. This method allows estimating both macroscopic and microscopic structural changes in organs and tissues of animals. Data obtained in the course of the pathomorphological research have fundamental value for studying toxic influence of preparations at the stage of preclinical research. Results of histologic structure of internals and tissues of laboratory Sprague Dawley rats are presented in this article. As a result of experiments it has been established that no side effects have been revealed at preparation administration to animals. "Microfit" biological preparation exerted no negative impact on functional activity of internals of an organism of rats, caused no allergic reactions. The macroscopic research of internals of all experimental animals revealed no pathology after autopsy. Pathomorphological research of the main organs and tissues confirms safety of the structure of tissues. Following the research results, within one month (30 days) of administration of the biological preparation, it has been established that in case of course intragastric administration of "Microfit" preparation to white Sprague Dawley rats in a conditional-therapeutic dose (30 mg/kg) and a dose exceeding the conditional-therapeutic dose by 10 times (300 mg/kg), the preparation has no toxic effect on condition of internals and tissues.

Key words: biological preparation, toxicity, histologic research, pathomorphological research, Sprague Dawley rats, conditional-therapeutic dose

Introduction

In recent years, pro-biotic preparations even more often began to be applied in complex therapy of a number of pathological states proceeding against the background of violation of the composition of normal microflora of a human body [1; 2]. Oppressing growth of undesirable microorganisms, a probiotics creates conditions for development of normal intestinal microflora; provides colonizational resistance, carries out digestive, synthetic, immunomodulation, detoxication functions [3; 4].

Introduction of new preparations in clinical practice is feasible only on condition of detailed studying of their specific pharmacological activity and safety at the stage of experimental (preclinical) research. Preclinical research of safety of a preparation aims identification of the possible damaging action on an organism of experimental animals and assessment of their safety. The research allows to reveal organs and body tissues most sensitive to substances of the studied preparation, and to estimate tolerance to use of the studied preparation at laboratory animals [5-7]. Therefore, development of new preparations and confirmation of their efficiency and safety for humans remains a very relevant task in preparation.

At assessment of general toxic action of a new preparation, experiments for determination of chronic toxicity on laboratory animals are carried out, as they allow assuming further possibility of work with this preparation. Histologic research of internals and tissues of experimental animals is important for creation of new preparations. There can be various allergic reactions of an organism caused by administration of this or that preparation, reducing its efficiency and therapeutic action considerably [8; 9].

In this regard, the purpose of this research is studying of influence of "Microfit" probiotic biological preparation based on various strains of *Lactobacillus*, extract of buds of balsam poplar and adsorbing substance on the condition of internals and tissues of laboratory rats.

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Materials and methods

Preclinical histologic research on studying of chronic toxicity of "Microfit" biological preparation have been conducted in the laboratory of toxicology and pharmacology of RSE on the REU "National Center for Biotechnology" under the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan (CS MES RK). Experiments were carried out on white Sprague Dawley rats, with the initial weight of 180-240 g, received from the vivarium of RSE on the REU "National Center for Biotechnology" CS MES RK. 6 groups of rats have been created for experiments: two groups – control groups of males and females, the other four groups – experimental groups with 6 animals in each group.

"Microfit» preparation consists of lactic bacteria (*Lactobacillus casei, L.plantarum, L.sakei*), extract of buds of balsam poplar and an adsorbing substance (tagan sorbent).

"Microfit" biological preparation was administrated to rats intragastrically, daily, 7 times a week, in a conditional-therapeutic dose (30 mg/kg) and in the dose exceeding the conditional-therapeutic dose by 10 times (300 mg/kg) within 1 month. Animals receiving drinking water intragastrically in equivalent volume within 1 month served as control animals. Tests of "Microfit" biological preparation for administration taking into account the body weight of rats were prepared just before intragastric introduction for rats. For intragastric administration of "Microfit" biological preparation to laboratory rats, contents of a bottle were dissolved in drinking water. Control and experimental animals were managed in identical conditions.

The research was conducted according to the "Rules of carrying out preclinical research, medicobiological experiments and clinical tests in the Republic of Kazakhstan" [10]. The recommendations stated in "The guide to experimental (preclinical) studying of new pharmacological substances" [11; 12] were taken into account during the research. Treatment of animals complied with the ethical principles of good laboratory practice [13].

Upon termination of administration of "Microfit" biological preparation (in one month from the beginning of administration of the biological preparation), they removed internals of animals. For a microscopic research, they took heart, lungs, liver, spleen, kidneys, ovary/testicle. Internals from 3 animals from each of the studied groups were taken for carrying out a histologic research. The microscopy of tissue structures of internals was carried out on Axioskop 40 light-optical microscope, Carl Zeiss, Germany, at increase in 200. Coloring method: hematoxylin and eosine.

Statistical processing of the results was carried out with the use of "Statistica 6.0" software package, Microsoft Excel 97. Distributions were described by average (M) and a mean square deviation (SD) for all animals in the group. Intergroup differences were estimated against the nonparametric criterion Mann-Whitney U-test [14; 15].

Experiments were made according to the "Rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" [16].

Results and their discussion

In experiments on studying of chronic toxicity of "Microfit" biological preparation, heart, lungs, liver, spleen, kidneys, ovary/testicle were taken for a histologic research. After autopsy, macroscopically the internals had a usual arrangement, without pathology. The research of tissue structures of internals (heart, lungs, liver, spleen, kidneys, ovary/testicle) was conducted.

Microscopically, the tissue of a lung of a male rat from the control group is normal. The structure of tissue is kept, without pathological changes. Lumen of alveoluses is normal. Interalveolar partitions are plethoric, with diapedetic hemorrhages. Walls of bronchial tubes and bronchioles are of different caliber, normal. Integumentary epithelium with a focal desquamation. Peribronchial lymph nodes of normal structure (A).

The structure of lung tissue of a male rat receiving preparation in a dose of 30 mg/kg is kept, without pathology. Lumen of alveoluses is free. Interalveolar partitions are slightly edematous, plethoric, with single diapedetic hemorrhages. Walls of bronchial tubes and bronchioles are of different caliber, normal. Integumentary epithelium with centers of proliferation and a desquamation. In the lumen of a part of bronchioles – desquamated cells of epithelium (B).

The structure of lung tissue of a male rat receiving preparation in a dose of 300 mg/kg is kept, without pathology and anomalies. Lumen of alveoluses is free. Interalveolar partitions are slightly edematous, plethoric, with single diapedetic hemorrhages. Walls of bronchial tubes and bronchioles are of different caliber, normal. Integumentary epithelium with centers of proliferation and desquamation. In a lumen of a part of bronchioles – desquamated cells of epithelium (C). Histologic research of structures of lung tissues of male rats are presented on the Figure 1.

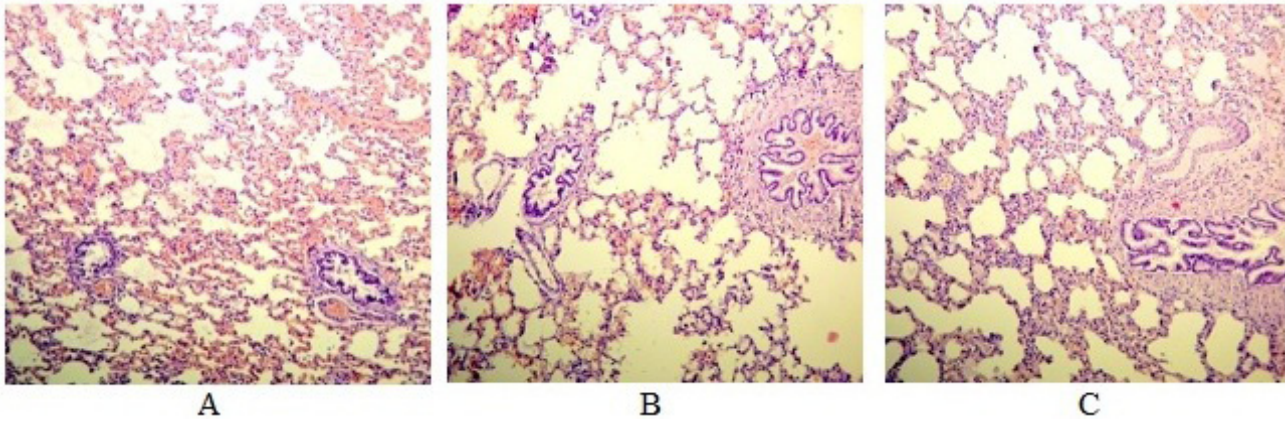

Figure 1 – Histologic structure of lung tissue of a male rat: A – control group;
B – influence of preparation in a dose of 30 mg/kg;
C – influence of preparation in a dose of 300 mg/kg. Coloring by hematoxylin and eosine.

Structure of heart tissue of a male rat from the control group and male rats from the group receiving "Microfit" in doses of 30 mg/kg and 300 mg/kg without deviation and pathology. Muscle fibers of auricles, ventricles and partitions are normal. Contracting and conveying cardiomyocytes are slightly edematous. Peri-muscularly – hypostasis, single erythrocytes. Vessels with the phenomena of uneven plethora. Lumen of coronal arteries is empty (Figure 2; A, B, C).

The structure of spleen tissue of a male rat from the control group and male rats from the group receiving "Microfit" in doses of 30 mg/kg and 300 mg/kg is normal and without pathology. Lymphoid follicles are normal, lymphocytes surround the central arteries in the form of "couplings". In reticular stroma, there are focal hemorrhages. In microhaemo-circulation vessels, there is uneven plethora. In the capsule there is a circulatory disturbance in the form of diapedetic hemorrhages (Figure 3; A, B, C).

The structure of liver tissue of control male rat is not damaged. The frame structure of hepatocytes is kept, moderated plethora of central veins and vessels of portal tracts. Portal tracts are small, insignificant lymphohysteocytic infiltration. The number of Kupffer and Ito cells in sinusoids is not changed. Phenomenon of uneven plethora in vessels. Normal cholangioles (Figure 4; A).

Lumen of central veins of liver of male rats receiving preparation in doses of 30 mg/kg and 300 mg/kg is slightly expanded. Hepatic segments, trabeculas and beams of usual structure. Periportal tracts and interlobular intervals are slightly expanded due to poor hypostasis. The number of Kupffer Ito cells in sinusoids is not changed, plethora is noted. Phenomenon of uneven plethora in vessels. Cholangioles of usual structure (Figure 4; B, C).

Structure of tissue of kidneys of a male rat from the control group and male rats receiving preparation in doses of 30 mg/kg and 300 mg/kg are kept, normal, without pathology and deviations. Renal glomerulus are equal, located evenly on the whole cortical layer. Intraglomerullar anses capillaires are unevenly plethoric. Lumen of proximal and distal tubules is free. Poor hypostasis in stroma. Diapedetic hemorrhages in the capsule. Epithelium of kidney pelvis with centers of desquamation (Figure 5; A, B, C).

Structure of testicle tissue of male rat from the control group and male rats receiving preparation in doses of 30 mg/kg and 300 mg/kg are not damaged, without pathological changes. Tissue of a testic is presented by numerous segments with existence of multiple equal testicular tubules containing homogeneous eosinophylic mass and squamous cells of epithelium in the lumen. Tubules are covered from within by the epithelio-spermatogenous layer located on the basal membrane with existence of 4-5 layers. The epithelio-spermatogenous layer is presented by two cellular differona: spermatogenous and supporting cells. Numerous Sertoli cells and interstitial Leydiga cells (Figure 6; A, B, C) are visible.

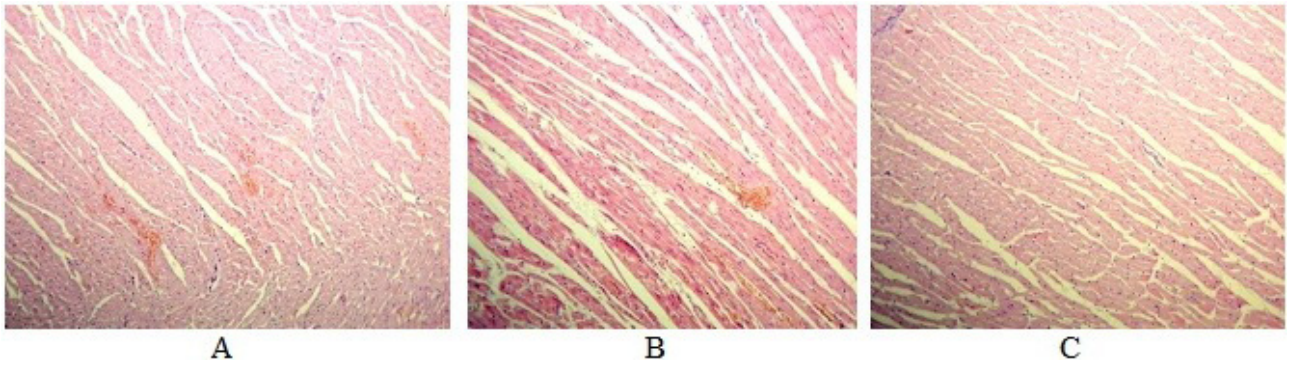

Figure 2 – Histologic structure of myocardium tissue of a male rat: A – control group;
B – influence of preparation in a dose of 30 mg/kg;
C – influence of preparation in a dose of 300 mg/kg. Coloring by hematoxylin and eosine.

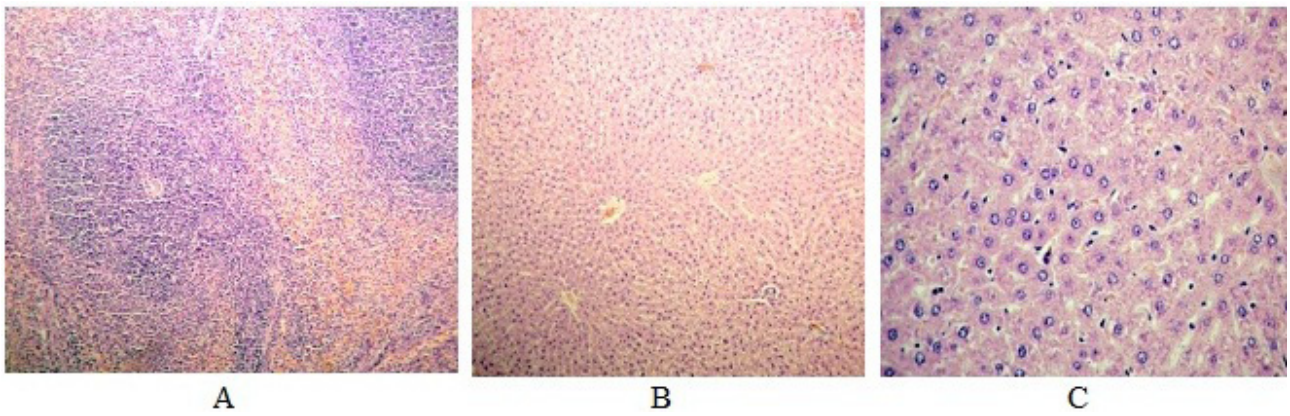

Figure 3 – Histologic structure of spleen of a male rat: A – control group;
B – influence of preparation in a dose of 30 mg/kg;
C – influence of preparation in a dose of 300 mg/kg. Coloring by hematoxylin and eosine.

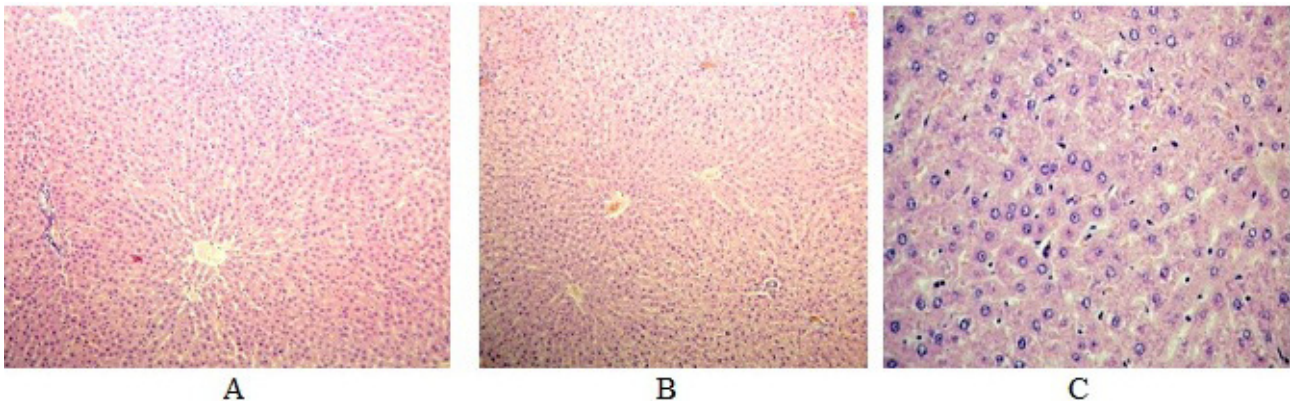

Figure 4 – Histologic structure of liver of a male rat: A – control group;
B – influence of preparation in a dose of 30 mg/kg;
C – influence of preparation in a dose of 300 mg/kg. Coloring by hematoxylin and eosine.

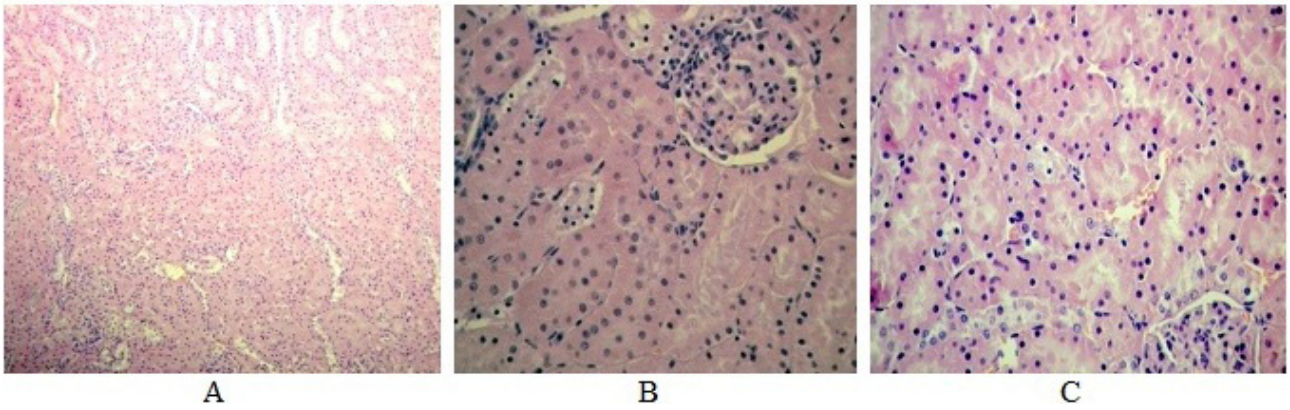

Figure 5 – Histologic structure of kidneys of a male rat: A – control group;
B – influence of preparation in a dose of 30 mg/kg;
C – influence of preparation in a dose of 300 mg/kg. Coloring by hematoxylin and eosine.

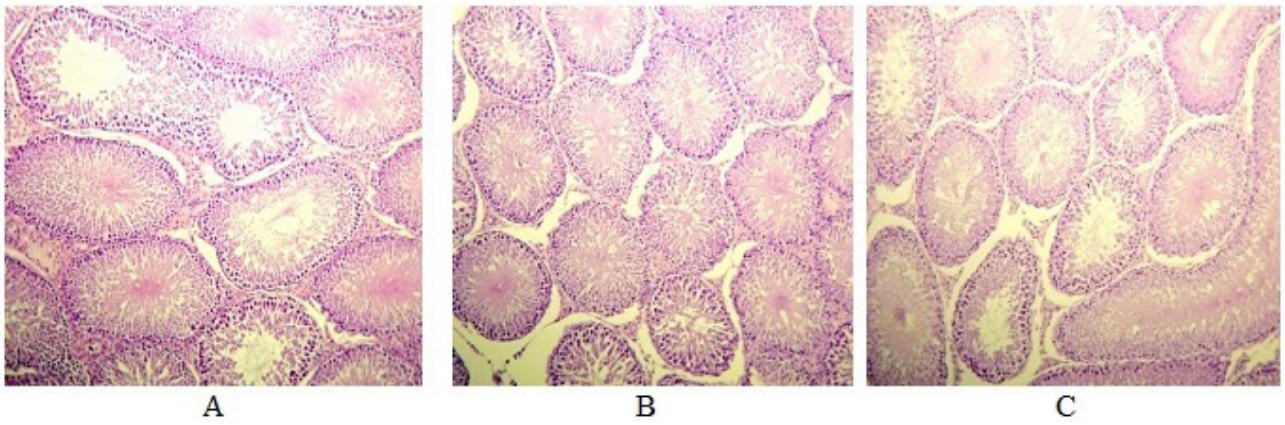

Figure 6 – Histologic structure of testicle of a male rat: A – control group;
B – influence of preparation in a dose of 30 mg/kg;
C – influence of preparation in a dose of 300 mg/kg. Coloring by hematoxylin and eosine.

Conclusion

As a result of studying of chronic toxicity of «Microfit» combined preparation, it has been established that its administration does not cause death of rats, does not damage the general condition of internals and tissues. At macroscopic research of heart, liver, kidneys, lungs, spleen, testicles/ovaries, no degenerate changes have been revealed at administration of two levels of doses (30 mg/kg and 300 mg/kg) of «Microfit» biological preparation. Macroscopically, all organs and tissues had the color, sizes and structure corresponding to reference values for this species of animals. The Patho-morphological research of the main organs and tissues confirms safety of the structure of tissues.

Thus, at course intragastric administration during 1 month in doses of 30 mg/kg and 300 mg/kg, the studied preparation exerts no impact on the state and morphology of internals and tissues of animals. Therefore, the results the patho-morphological of research have confirmed lack of toxic injuries of the vitals and tissues connected with administration of «Microfit» preparation.

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