



## **Study of the Influence of Local Strains of Lactobacillus on the Experimental Model of Ulcerative Colitis**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors GJK, NAE and DKN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GJK, BIT and BST managed the analyses of the study. Author BIT managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The article is devoted to one of the most important effects of local strains of lactic acid bacteria - the detection of anti-inflammatory action. For this purpose, studies were conducted on an experimental model of ulcerative colitis caused in experimental animals (mice). The data proving that the effect of various probiotic bacteria is unequal are presented. The ability of probiotic lactic acid bacteria in vitro and in vivo systems to influence the production of pro - and anti-inflammatory cytokines, to stimulate antimicrobial, anti-inflammatory, and protective effects have been shown. It is shown that it is necessary to study the specific effect of local lactobacillus strains on anti-inflammatory properties for a more adequate, effective selection of probiotic drugs in the treatment of intestinal ulcers. Data have been presented proving the positive effects of probiotic bacteria on the wound key.

**Keywords:** Probiotics; lactobacilli; intestinal microflora.

## 1. INTRODUCTION

Inflammatory bowel diseases (IBD), which include ulcerative colitis and Crohn's disease, are autoimmune, chronic, recurrent diseases and represent one of the most serious problems in modern gastroenterology and coloproctology [1,2]. According to the severity of the course, the frequency of complications, and mortality, these diseases occupy one of the leading places all over the world in the structure of diseases of the gastrointestinal tract. The study of the mechanisms of development of ulcerative colitis is an urgent medical and biological problem since its constant growth is observed throughout the world. So, according to foreign data, the incidence of UC ranges from 35-100 people for every 100,000 people, the prevalence reaches 505 per 100,000 people. [3,4]. The course of IBD leads to severe complications, the frequency of the formation of the pathological process at an early age has increased by 1.5 times, more severe extraintestinal manifestations have appeared, the development of resistance to traditional therapy is more often observed, early disability of patients and high mortality from these diseases requires the development of new therapeutic, and biotherapeutic approaches [5]. Inflammatory bowel disease is one of the factors that increase the risk of developing colorectal cancer. Inflammatory bowel diseases, due to the above features, have not only medical but also important social significance. The lack of a clear understanding of the etiology and pathogenesis of IBD leads to problems in the diagnosis and, to a greater extent, in the treatment of these diseases in the presence of a wide range of drugs used.

The chronic nature of IBD requires constant anti-relapse therapy, and during exacerbations, the use of drugs to induce remission. The main classes of drugs used to treat IBD are currently 5-aminosalicylic acid drugs, antibiotics, glucocorticosteroids, immunosuppressants, and monoclonal antibodies to tumor necrosis factor. Most of these drugs have pronounced side effects: impaired glucose tolerance, suppression of adrenal function, osteoporosis and ulcerative lesions of the stomach and duodenum, development of opportunistic infections, allergic or toxic reactions. Therefore, it seems very important, expedient, and extremely necessary to evaluate the effectiveness of a new and potentially safe class of drugs - probiotics, which

are living microorganisms that have a multidirectional beneficial effect on the functioning of the digestive system.

This problem is also of great interest to specialists in microbiology since the large intestine is a reservoir for many microorganisms and food antigens. Under physiological conditions, the epithelial barrier of the large intestine prevents the penetration of bacteria and toxic substances into the internal environment of the body and prevents the development of the inflammatory process. Violation of the structure and function of the epithelial barrier can lead to the development of some diseases of the colon, including ulcerative colitis [6].

To date, a modern hypothesis has been put forward, which presents the cause as a direct or indirect effect of a complex of genetic factors, local immunity, diet, and environmental factors, characterized by a change in the composition of the intestinal microbiota. Studies of the intestinal microflora of patients with inflammatory bowel diseases (IBD) have shown that the most typical causative agents of these diseases are: *Salmonella*, *Shigella*, *Campylobacter*, *Clostridium difficile*, *Escherichia coli* O157:H7, *Yersinia*, and some others. Numerous studies have demonstrated that there is a close relationship between microbiota composition and various aspects of host health, including physiological state, metabolism, and immunological response [7,8]. Summarizing many studies, it can be concluded that subpopulations of *E.coli* may play an important role in the pathogenesis of ulcerative colitis. It has been found that patients with Crohn's disease and ulcerative colitis have an increased concentration of *Enterobacteriaceae* and *Bacteroides* species [9]. Patients with IBD differ from healthy patients in that they have an altered composition of commensal gut bacteria and generally have an increased number of adhesive invasive *E.coli*, *Enterococci*, and an underestimate of *Bifidobacterium* and *Lactobacillus* species. To date, few microbiological studies have been carried out to identify inducers of IBD diseases and ways to inhibit these bacteria [10].

Considering that the basis of pathogenesis is an imbalance in the microbiome of the gastrointestinal tract and intestinal immunity, the use of probiotics as an adjunct to standard anti-

inflammatory and immunosuppressive therapy has its expediency and validity.

Based on the above, it can be said that one of the main reasons for the development of inflammatory processes in the intestine is defects in the immunological properties of the body's defense, in particular during the transition to a chronic form. It is known that with the manifestation of pathogenicity by microorganisms, a genetically determined cytokine cascade is triggered, and the sluggish nature of inflammation or the transition to a chronic type is due to an inadequate formation of inflammatory effector cells (neutrophils and macrophages), as well as an imbalance between the ratio of pro-inflammatory and anti-inflammatory cytokines [6,11]. Thus, immunological disorders that cause inflammation are associated with cytokines, which are regulatory proteins that form a universal network of mediators' characteristic of both the immune system and cells of other organs and tissues. All cellular events take place under the control of this class of regulatory proteins: proliferation, differentiation, apoptosis, and specialized functional activity of cells [12,13,14]. Also, cytokines differing in biochemical and biological properties affect the functional activity of cells participating in the reactions of innate and acquired immunity [13,14,15]. The regulation of the body's defense reactions by cytokines occurs not only within the immune system but also by organizing defense reactions at the level of the whole organism due to the regulation of almost all aspects of the development of inflammation and the immune response [6,15,16,17].

With a decrease in the body's defense reactions, the use of probiotics, taking into account their biological properties, contributes to the maintenance of the body, ensures the prevention and treatment of inflammatory processes. Besides, many probiotic strains exhibit anti-inflammatory properties through their action on various immune cells, reduce the secretion of pro-inflammatory cytokines, and lead to the induction of anti-inflammatory cytokines. It has been proven that the use of the multi-species probiotic VSL # 3 prevents the recurrence of postoperative infection in patients. For the treatment of active ulcerative colitis, as well as for maintenance therapy, clinical efficacy data is most effective for VSL # 3 and *Escherichia coli* Nissle 1917. investigated the regulatory effect of garidizan from the wild poppy *Artemisia frigida* Willd on intestinal microbiota dysbiosis in a

mouse model of ulcerative colitis and studied the possible mechanism of the therapeutic effect of garidizan on UC. It has been noted that the therapeutic effect of garidizan on ulcerative colitis is associated with a direct effect on the *Lachnospiraceae* family of bacteria [18].

The aim of this study is to screen local probiotic lactobacilli strains with antimicrobial activity against dominant harmful microorganisms that cause inflammatory diseases of the gastrointestinal tract and to develop new methods in the prevention and treatment of inflammatory and ulcerative diseases of the human gastrointestinal tract.

## 2. MATERIALS AND METHODS

Strains of probiotic cultures were isolated from ecologically clean products (cheese, feta cheese, pickles, and tarragon plant). Were identified in the sanitary and hygienic laboratory under the Ministry of Health of the Republic of Uzbekistan by mass spectrometry (MALDI TOF) [19].

Scientific research was carried out on experimental animals - 48 sexually mature male and female mice weighing 21-26 g, according to the requirements of the Pharmacological Committee of the Republic of Uzbekistan from 2000. The experimental animals were previously quarantined for two weeks at room temperature 18-25°C and with natural light. In the course of the experiment, the qualitative and quantitative indicators of stool, motor activity, the state of the coat were determined in mice, and their death was separately recorded. After the administration of the drugs, a visual examination was carried out, including an assessment of the state of the mucous membrane of the colon and determination of the number of ulcerative lesions. A prerequisite for this study was the measurement of the bodyweight of experimental animals on the third, sixth, and ninth days after taking the drugs.

The creation of an experimental model of ulcerative colitis was carried out according to the method of Fitzpatrick et al. [20]. with a single introduction of 4% acetic acid.

The object of research was the local strains of lactobacilli: *Lactobacillus casei* K7 / 3, *Lactobacillus plantarum* ET-2, *Lactobacillus plantarum* AB-1, *Lactobacillus plantarum* CO-1 and Mix - a composition of four probiotics, developed by researchers of the Laboratory of

Microbiology and Biotechnology of Probiotics of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan. Local strains of lactobacilli have a wide spectrum of microbial action [21,22].

The *Lactobacillus casei* K7/3 strain is patented as an antimicrobial agent, the synthesis of exopolysaccharides of local strains of lactobacilli has been studied [2,17,23].

To assess the effectiveness of the effect of strains of lactobacilli, the animals were divided into 6 groups: group 1 - control, animals with model pathology, group 2 - animals receiving K7 / 3 at a dose of 1 ml per mouse, group 3 - animals receiving ET-2 at a dose of 1 ml per mouse, group 4, animals receiving AB-1 at a dose of 1 ml per mouse, group 5 animals receiving CO-1 at a dose of 1 ml per mouse, group 6 animals received the association Mix at a dose of 1 ml per mouse and the control group without pathology.

To study the effect of probiotics on the immune system, as well as to determine its effectiveness in immunosuppressive therapy for IBD, immunological studies were carried out based on the method for determining the concentration of cytokines, TNF $\alpha$  and interleukin-10 (IL-10) in blood serum using IL-10 as an example). Thus, interleukin-10 in mice was determined in the test samples (blood serum) by the method of enzyme-linked immunosorbent assay. This method is carried out using the «IFA-4IL» kit based on the «sandwich» - a variant of the enzyme-linked immunosorbent assay. For research, we used two monoclonal antibodies with different epitope specificity to IL-10, one of which is immobilized on the solid phase (inner surface of the wells), the other is conjugated with peroxidase. At the first stage of the analysis, IL-10 contained in the calibration and test samples bind to antibodies immobilized on the inner surface of the wells. At the second stage of the analysis, immobilized IL-10 interacts with a conjugate of second antibodies -peroxidase. The amount of bound conjugate is directly proportional to the amount of IL-10 in the test sample.

During incubation with the substrate mixture, the solution stains in the wells. The degree of color is directly proportional to the amount of bound labeled antibodies. After measuring the optical density of the solution in the wells, the concentration of IL-4 in the samples to be

determined was calculated based on the calibration curve.

The method for the determination of cytokines by enzyme-linked immunosorbent assay was carried out using test systems (developed by ZOO VEKTOR-BEST, Russia, Novosibirsk), which is based on the "sandwich" method of solid-phase immune enzyme immunoassay using horseradish peroxidase as an indicator enzyme. After the completion of the main stages of work, 10-15 minutes before the end of incubation, a solution of the substrate-chromogenic mixture was prepared. The wells of the plate were thoroughly washed: added washing solution (300  $\mu$ l), then by adding distilled water were removed by shaking (the procedure was repeated 3-5 times). Then, 200  $\mu$ l of the substrate-chromogenic mixture solution was added to all wells of the plate. Incubation was carried out for 20 minutes at room temperature in the dark. The reaction was stopped by adding 50  $\mu$ l of 1N sulfuric acid solution. The results, determining the activity of bound peroxidase, were recorded using an automatic photometer for microplates at a wavelength of 492 nm, establishing zero absorbance for the wells with a standard without the detected cytokine in solution. To quantify the results of the study, a commercial computer program "Microplate manager" was used, which reflects the dependence of optical density on concentration for a standard antigen and allows one to compare the samples under study. The sensitivity of this method corresponded to 5-30 pg/ml.

The experiments were carried out in triplicate, the mean estimate and standard error (SE) were calculated using Microsoft Excel (Microsoft Corporation, USA). The deviation of the obtained results  $P \leq 0.05$  from the control values was analyzed by the ANOVA program.

### 3. RESULTS AND DISCUSSION

When exposed to experimental animals in various strains of lactobacilli, the following results were revealed: in animals of the control group, the regeneration processes were slower than in other groups tested with local strains, as evidenced by changes in the affected areas of the mucous membrane of the large intestine and the area of ulcers; From the second day to the sixth day, in experimental mice induced by ulcerative colitis, diarrhea with an admixture of blood was observed. On the third day, the death of mice was noted in all experimental groups -

65%, except for the control group (without pathology). Within 36 hours after the reproduction of acute colitis, there was a decrease in the motor activity of the animals, a reckless gait and painful posture, the coat was disheveled and dull. On the tenth day, the animals had similar external signs with the control group, that is, the coat was smooth, clean, active gait, and the character of the stool returned to normal.

According to Table 1. when treated with the tested strains of *Lactobacillus plantarum* AB-1, *Lactobacillus casei* K7 / 3, Mix in appropriate doses, there is an increase in weight compared to the control group without pathology and the tested strains, both control with pathology and *Lactobacillus plantarum* ET-2 and CO-1 strains.

Also, a test screening was carried out within 10 days. Based on induction, which was caused by the introduction of 4% acetic acid, the development of pronounced destructive changes in the mucous membrane of the colon with edema, hyperemia, and hemorrhages was observed.

As a result of experimental studies presented in Table 2. it was found that in animals receiving

Mix - a composition of four probiotics, the area of ulcers decreased 3.15 times faster than in the control (without treatment) group, where the degree of damage to the mucous membrane decreased in 2.05 times.

Treatment with the tested strains showed that with the introduction of *Lactobacillus casei* K7 / 3, the area of ulcers decreased 2.19 times, *Lactobacillus plantarum* ET-2 - 1.89 times, *Lactobacillus plantarum* AB-1 2.07 times, *Lactobacillus plantarum* CO-1 1.81 times compared with the control group. When studying the state of the mucous membrane of the large intestine, it was found that among the groups receiving local strains there was an improvement in the state of the mucous membrane in 1,57; 1,33; 1,65, and 1,20 times higher compared to the control group.

According to the results presented in Tables 1. and 2. when treating with local strains of all studied drugs, the most pronounced anti-inflammatory properties in an experimental model of ulcerative colitis in a comparative aspect have an association - a composition of four probiotics.

**Table 1. Weight of animal in different days of treatment**

Group	Weight of animals			
	Initial (gr)	3-day treatment	6-day treatment	10-day treatment
Control group without pathology	20±1.7	20.6±1.3	21±1.1	23±1.5
Control group with pathology	24±1.3	16±1.5	18±1.7	18,5±1.3
<i>Lactobacillus casei</i> K7/3	20±1.5	18±1.8	18±1.3	19,5±1.6
<i>Lactobacillus plantarum</i> ЭТ-2	21±1.3	16±1.4	17±1.5	17,5±1.2
<i>Lactobacillus plantarum</i> АБ-1	23±1.4	21±1.1	23±1.3	24±1.6
<i>Lactobacillus plantarum</i> СО-1	21±1.5	16±1.3	17±1.5	17,5±1.2
Mix - a composition of four probiotics	25±1.7	20±1.3	23±1.5	24±1.7

**Table 2. Mucosal lesions and Ulcer area for different treatment group**

Group	Dose	Mucosal lesions, points	Ulcer area, mm <sup>2</sup>
Controlgroup (notreatment)	-	5.5±0.25	135.04±24.53
<i>Lactobacillus casei</i> K7/3	0.5 ml / per mouse	3.50±0.04*	61.66 ±24.16*
<i>Lactobacillus plantarum</i> ЭТ-2	0.5 ml / per mouse	4.13±0.17	71.53±23,14
<i>Lactobacillus plantarum</i> АБ-1	0.5 ml / per mouse	3.33±0.12*	65.10 ±21.03*
<i>Lactobacillus plantarum</i> СО-1	0.5 ml / per mouse	4.57±0.15	74.71±21.07
Mix - a composition of four probiotics	0.5 ml / per mouse	2.67±0.33*	42.75±5.05*

\* — deviation is significant compared to control ( $P < 0.05$ )

The results of studying the effect of local strains of lactobacilli on the production of pro-inflammatory and anti-inflammatory cytokines showed the following. Cytokines primarily regulate the development of local defense reactions in tissues with the participation of various types of blood cells, endothelium, connective tissue, and epithelium [19,24]. Synthesis of a complex of proinflammatory cytokines that stimulate most of the further events in the development of the inflammatory response and ensure the correct expansion of the activation of various cell types are involved in the maintenance and regulation of inflammation. At the tissue level, cytokines are responsible for the development of the inflammatory process, then for the processes of tissue regeneration, in general for the development of systemic inflammation, which determines their wide range of biological activity. Thus, cytokines are conductors that form and regulate the entire complex of protective reactions of the body during the introduction of pathogens [25,11].

Considering that in ulcerative colitis, numerous mechanisms of tissue and cellular damage are involved, the main role is still assigned to the violation of the barrier function of the intestinal mucosa, as well as its ability to restore [7,4,26]. It was found that through defects in the intestinal mucosa, bacterial agents are introduced, which trigger a cascade of inflammatory and immune reactions. In this regard, it was found that the content of cytokines in the peripheral blood is in direct proportion to the timing of the exacerbation of the disease, and reflects the dynamics of the pathological process.

By the above, to assess the severity and predict the course of the disease in the dynamics of the development of pathology, the concentration of one of the most striking and basic pro-inflammatory (TNF $\alpha$ ) and one of the anti-inflammatory cytokines (IL-10) in the blood serum of laboratory animals with an experimental model of an ulcer was determined. The studies were carried out on the 6th and 9th days of ulcer correction.

The study of tumor necrosis factor-alpha (FNO $\alpha$ , TNF $\alpha$ ), cachexin, which is a pleiotropic pro-inflammatory cytokine that performs regulatory and effector functions in the immune response showed that they are secreted by macrophages, T- and B-lymphocytes, neutrophils, stimulate inflammation, activate and damage cells, causing fever (pyrogen). The name of the factor is due to

the ability to inhibit the growth of certain tumors, and its increased concentration can cause toxic shock. Previously, it was found that TNF $\alpha$  leads to an increase in protein production in the acute phase of inflammation, is a chemoattractant of macrophages and Langerhans cells, a stimulator of angiogenesis, a potential activator of monocytes, as well as phagocytosis and production of free radicals [6,13,15,27,2].

The results of immunological studies have shown that after 3 days of experimental correction of the ulcer, the content of TNF $\alpha$  increases at the earliest stages of exacerbation (Fig. 1).

Besides, during the research it was revealed that the maximum concentration of TNF $\alpha$  was observed at the height of the disease in the groups with control pathology, the excess was observed almost 3 times ( $36.8 \pm 4.66$ ,  $P < 0.001$ ), K7 / 3 - 2.8 times ( $34.5 \pm 1.48$ ,  $P < 0.001$ ), ET-2 - 2.6 times ( $32.8 \pm 1.49$ ,  $P < 0.001$ ), AB-1 - 2.4 times ( $30, 2 \pm 1.45$ ,  $P < 0.001$ ), CO-1- 2.7 times ( $33.8 \pm 1.42$ ,  $P < 0.001$ ), Mix - 1.9 times ( $24.2 \pm 1.17$ ,  $P < 0.01$ ), while the TNF $\alpha$  content in the control group of healthy mice corresponded to  $12.3 \pm 1.41$ . The statistical reliability identified during the study and the results obtained can predict and even indicate possible prolonged chronic inflammation.

After 6 days of experimental correction of the ulcer, a gradual decrease in the concentration of TNF $\alpha$  was found in all experimental groups that received lactobacilli strains in appropriate doses, compared to the control group with pathology.

So, on the 9th day, the cytokine level in groups K7 / 3, ET-2, and CO-1 were 1.2 times lower ( $P < 0.001$ ), AB-1 - 1.5 times lower ( $23.3 \pm 1, 84$ ,  $P < 0.001$ ), Mix - 1.7 times ( $20.5 \pm 1.34$ ,  $P < 0.01$ ), while the concentration of tumor necrosis factor in the control group of healthy mice was stable and remained at a certain level ( $12.3 \pm 1.41$ ).

According to the literature data, an increased level of TNF $\alpha$  is observed in the blood serum and in other pathologies, such as pulmonary dysfunction, abnormalities in the normal course of pregnancy, oncological diseases, bronchial asthma, as well as during the period of exacerbation of diseases of the gastrointestinal tract and during the post-traumatic state, which was also confirmed in our studies [18,24,9,28,29,30].

The next stage of research was devoted to the determination of the concentration of the anti-

inflammatory cytokine IL-10 (Fig. 2). Thus, the cytokine IL-10, possessing many anti-inflammatory properties, including the ability to suppress a fever, is a key regulator of the immune response [11].IL-10 produced by T cells (Th2) can be considered as an antagonist of many cytokines. IL-10 in animals was produced by Th2 cells (including humans), some types of

regulatory T cells, cytotoxic T lymphocytes, monocytes/macrophages, dendritic and mast cells, B lymphocytes. Thus, IL-10 inhibits the production of IFN by Th1 cells. Also, it inhibits the proliferative response of T cells to antigens and mitogens and inhibits the secretion of TNF and other cytokines by activated monocytes [9,19,31].

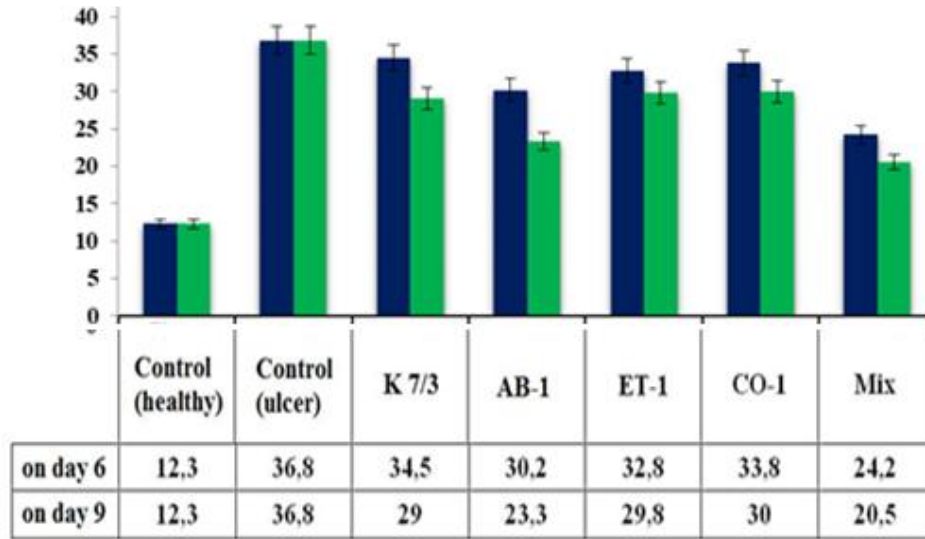


Fig. 1. TNFα content after 6 and 9 days of experimental correction of the ulcer

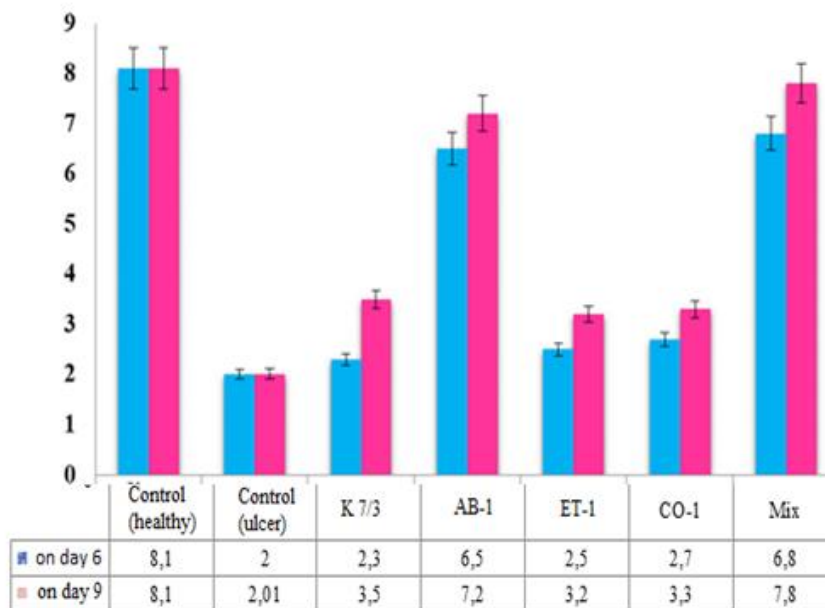


Fig. 2. The content of IL-10 after 6 and 9 days of experimental correction of the ulcer

It became known that IL-10 promotes the development of humoral immune response, serves as a synergist of IL-4 when acting on B cells, protects them from apoptosis, enhances their proliferation and differentiation into antibody-forming cells, and also enhances the synthesis of IgM and IgA [13,14,24]. At the same time, an excess of IL-10 can lead to a decrease in the defenses against infection, thereby leading to the development of chronic infections [15,32].

The results of the studies after 3 days of correction of the experimental model of ulcerative colitis showed the following values: the concentration of the anti-inflammatory cytokine IL-10 in the blood serum was relatively low in the groups with control pathology - 4 times ( $2.00 \pm 0.68$ ,  $P < 0.001$ ), K7 / 3 - 3.5 times ( $2.3 \pm 0.56$ ,  $P < 0.001$ ), ET-2 - 3.2 times ( $2.5 \pm 0.67$ ,  $P < 0.001$ ), AB -1 - 1.2 times ( $6.5 \pm 0.56$ ,  $P < 0.001$ ), CO-1- 3 times ( $2.7 \pm 0.56$ ,  $P < 0.001$ ), Mix - 1.2 times ( $6.8 \pm 0.83$ ,  $P < 0.01$ ), while the content of healthy mice in the control group in the norm corresponded to  $8.1 \pm 0.87$  pg / ml.

Analysis of the level of IL-10 after 6 days of correction of the experimental model in the blood serum showed positive changes in the groups with oral administration of the above strains of lactobacilli. Thus, in the K7 / 3, ET-2 and CO-1 groups, the concentration increased by an average of 1.6 times ( $P < 0.001$ ), AB-1 - 3.6 times ( $P < 0.001$ ), and in the Mix group - 3.9 times ( $P < 0.01$ ), while in the control group with pathology it corresponded to  $2.01 \pm 0.68$  pg/ml.

The results of the analysis of the literature data on the sharp increase in the production of pro-inflammatory cytokines in the wound focus in the

first hours and days after the modeling of ulcerative colitis were confirmed in our studies. This indicates the stimulation of the development of the inflammatory response. In the early phase, TNF $\alpha$  and other pro-inflammatory cytokines are expressed by neutrophils, then by macrophages and cells of the regenerating epidermis. As the acute phase and inflammatory phenomena decrease, the repair processes begin to intensify, therefore, the concentration of IL-10 increases. IL-10 is present in high concentrations in wound fluid. Expression and production of IL-10 are detected in wounds from the moment of wounding and throughout the healing time. The main sources of IL-10 are epidermal cells and mononuclear cells infiltrating the wound focus - macrophages and lymphocytes. IL-10 inhibits infiltration by neutrophils and macrophages, reduces overexpression of chemokines and pro-inflammatory cytokines in the wound.

Normally, the largest number of *lactobacilli*, including *Lactobacillus casei* and *Lactobacillus plantarum*, is found in the large intestine. Directly contacting with enterocytes, lactobacilli stimulate the defense mechanisms of the human body, including an increase in the rate of regeneration of the mucous membrane, activate phagocytosis, as well as the synthesis of lysozyme, interferons, and cytokines [7,4,24]. Local inflammation and wound healing are associated not only with the influx of leukocytes - neutrophils, macrophages, lymphocytes, mast cells - into the wound focus but also with a properly selected complex therapy of various strains of lactobacilli. Apparently, for the successful resolution of inflammation and, ultimately, wound healing, the balance between stimulating and inhibiting factors in the wound focus is of great importance.



**Fig. 3. The state of the intestines of experimental animals (mice) with ulcerative colitis**





**Fig. 4. The state of the intestines of experimental animals after treatment with lactobacilli (treatment for 10 days)**

#### 4. CONCLUSIONS

Thus, Mix - a composition of four local strains of probiotics that have pronounced therapeutic properties, is one of the effective drugs for the correction of disorders caused by ulcerative lesions of the mucous membrane of the large intestine. Based on the results of the test screening. Mix - a composition of four probiotics also showed high activity compared to the strains *Lactobacillus casei* K7 / 3, *Lactobacillus plantarum* ET-2, *Lactobacillus plantarum* AB-1, *Lactobacillus plantarum* CO-1, which confirms its expediency for use in cases of ulcerative colitis, provides an effective opportunity conservatively treat the disease. Besides, in the course of the research, local strains showed long-term preservation of their biochemical activity, depending on external factors (the composition of the nutrient medium, temperature, etc.), which contributed to the sustainable viability of the population of microorganisms, its practical value.

According to the results of immunological studies, it can be concluded that a change in the cytokine profile is one of the criteria for the effectiveness and appropriateness of corrective therapy with the corresponding strains of lactobacilli of domestic production.

Consequently, the effectiveness and safety of various bacterial preparations used for therapeutic and prophylactic purposes in the pathology of ulcerative colitis studied on experimental models have been confirmed and this direction of research has prospects for further experiments. Such studies will help to identify possible new therapeutic methods of correction aimed at healing and rapid recovery of the body after suffering from ulcerative colitis.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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