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THE MAIN STAGES OF PHARMACEUTICAL DEVELOPMENT OF EMULGEL "PROBIOSKIN"

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The aim. *To conduct research on the pharmaceutical development of a complex preparation with probiotic "Probioskin" in the form of an emulgel for the treatment of infectious and inflammatory dermatological diseases.*

Methods. *Uniformity was determined by visual inspection of the test samples using an XSP-128 ULAB biological microscope. The study of the rheological properties of the samples was carried out using a Rheolab QC rheovisosimeter (Anton Paar, Austria) using a system of coaxial cylinders C-CC27/SS. Microbiological studies and biotesting on a biological model of ciliates were carried out in aseptic conditions of a laminar box (biological safety cabinet AC2-4E1 "Esco", Indonesia) of the Department of Biotechnology of the National University of Pharmacy (completely). Pharmacological studies (determination of the parameters of acute toxicity and anti-inflammatory properties on the model of acute exudative inflammation of the foot in rats caused by zymosan and carrageenan) were carried out on the basis of the Central Research Laboratory of the NUPh.*

Results. *On the basis of the complex of the carried out studies, the composition of the complex preparation for skin use "Probioskin" was substantiated. The analysis of the microbiological purity of the developed agent during the proposed shelf life of 12 months showed that the drug meets the requirements of the State Pharmacopoeia Monograph for cutaneous application in terms of the level of microbial contamination by foreign microflora. The complex of pharmacological studies carried out indicates that the drug "Probioskin" can be attributed to group 6 of class and classified as a "relatively harmless" agent. The study of the anti-inflammatory effect of the drug indicates that the drug exhibits moderate anti-inflammatory properties. Under the condition of zymosan inflammation, which is associated with the activation of leukotrienes as inflammatory mediators, the average antiexudative activity of the drug is 33 %. The drug has a moderate antiexudative effect under the condition of carrageenan edema, which is evidence of its effect on exudation processes mediated by prostaglandins. The mean AEA of the study drug was 24 %.*

Conclusions. *For the development of a soft preparation for skin use for the treatment of infectious and inflammatory dermatological diseases, the following components have been selected: active – lactobacilli, dexpanthenol, lactic acid; auxiliary – propylene glycol, peach oil, polysorbate-80, aristophlex, tocopherol, the concentration of which was substantiated on the basis of a complex of organoleptic, physicochemical, pharmacological, microbiological and biological studies. It has been experimentally established that the "Probioskin" emulgel meets the requirements of the SPhU in terms of the level of microbial contamination by extraneous microflora. Pharmacological studies allow the drug to be classified as "relatively harmless" with anti-inflammatory properties at a level not lower than the reference drug*

Keywords: *pharmaceutical development, probiotics, skin microbiome, dermatological diseases, emulgel, gelling agents*

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1. Introduction

An important problem of modern medicine and pharmacy is the treatment of dermatological skin diseases [1]. Depending on the cause and pathogenetic factor of dermatological disease, its severity and manifestations for treatment use drugs from different groups Anatomical Therapeutic Chemical (ATC) classification system – antibiotics and chemotherapeutic drugs, corticosteroids, antiseptics and disinfectants, antifungals, etc. [2, 3].

Microbiome maintenance and recovery is an important component of the treatment of dermatological diseases. But none of the drugs available on the domestic pharmaceutical market takes into account the need to maintain the skin microbiome, which plays a key role in skin function as a barrier against adverse environmental factors.

Given the importance of maintaining and restoring the skin microbiome in dermatological diseases and the lack of drugs on the Ukrainian pharmaceutical market that would take this factor into account, the development of effective skin products with probiotic component is an urgent task of modern medicine and pharmacy. These drugs will be promising for the treatment of infectious and inflammatory skin diseases – seborrheic dermatitis, rosacea, psoriasis, atopic dermatitis, as the main drug in mild forms or, depending on the disease, as a

concomitant during treatment and after to restore normal skin flora; with superficial skin lesions at risk of infection and inflammation: scratches, cuts, scratches, cracks, burns, abscesses, dermatitis.

In order to restore microbiomes, probiotics are used, which contain live microorganisms that benefit the health of the host when administered in sufficient quantities [4]. In this development, we selected as a probiotic component standard for many human microbiomes strains of bacteria of the genus Lactobacillus – non-pathogenic, non-toxigenic, gram-positive enzymatic microorganisms with the ability to produce lactic acid from carbohydrates and bactericidal and biologically active substances [5]. Probiotic strains of L. plantarum 8P-A3 and L. fermentum 90T-C4 are classic production strains that form the basis of a large number of domestic and foreign probiotics, mainly in the form of lyophilized biomass, used to treat dysbacteriosis of various etiologies in children and adults; are characterized by high antagonistic properties and specific activity and are safe for human use [6].

In the treatment of patients with infectious dermatoses use antibiotics (penicillins, cephalosporins, macrolides, tetracycline), which, in turn, further disrupt the natural microbiome of the skin [7]. But the presence of an antimicrobial component in complex therapy is desirable. It is also necessary to take into account the pH value in normal and skin pathologies. Deviations from normal skin acidity can be the cause or consequence of many serious barrier disorders. Therefore, to enhance the antimicrobial properties of the developed dosage form and reduce the alkalinity of the environment, which is characteristic of many dermatoses, as the drug component was selected lactic acid, which is a metabolic metabolite of the organism and can be considered as a biologically safe product. other antimicrobial substances [8].

The new mild preparation for skin application also offers the introduction of dexpanthenol, which belongs to the group of "skin" vitamins, used as a dermatoprotector due to regenerating, anti-inflammatory, reparative action. The use of dexpanthenol together with a probiotic component in one dosage form is justified due to the physiological needs of lactobacilli: in addition to carbohydrates, lactobacilli need various growth factors for their development, including pantothenic acid and its derivatives.

The aim. To conduct research on the pharmaceutical development of a complex preparation with probiotic "Probioskin" in the form of an emulgel for the treatment of infectious and inflammatory dermatological diseases.

2. Planning (methodology) of the research

Treatment of dermatological skin diseases is most often carried out with mild drugs for skin application, due to the need for local action of the drug and ensuring a certain amount on the skin surface [9].

The therapeutic activity of topical drugs depends on a number of interrelated factors, the main of which is the activity of active substances, as well as their interaction with the carrier base, which provides full pharmacological activity [10]. That is why the main task in creating a new drug is to choose effective and safe active substances and excipients that would fully affect the treatment of the disease.

Also important in the development of soft drugs is the scientific and experimental justification for choosing the base-carrier of active substances, because only the right combination of active ingredients with the base could provide the necessary action of the drug, appropriate technological and consumer qualities of the drug, makes it possible to regulate the release and bioavailability of active substances [11].

Microbiological stability of drugs is a mandatory component of their quality, so at the stage of pharmaceutical development it should be considered to ensure microbiological purity [12].

One of the stages of preclinical study of a new pharmaceutical drug is the study of its biological properties, based on which it is possible to assess the spectrum of action of the drug, its advantages over existing analogues and the possible presence of toxic effects [13].

To solve the tasks we have developed a research plan consisting of the following successive stages:

1. Selection and justification of the concentration of active components.
2. Substantiation of the qualitative composition and quantitative content of excipients.
3. Determining the type and composition of the base of a mild preparation for topical use.
4. Analysis of the microbiological purity of the developed drug during the proposed shelf life.
5. Conducting preclinical studies of the developed drug.

3. Materials and methods

The objects of the study are samples of emulgel with selected active substances – lactobacilli Lactobacillus fermentum or Lactobacillus plantarum, which form the basis of the drug "Lactobacterin" (Biopharma, Ukraine), in the form of lyophilized biomass, dexpanthenol (Merck KGaA, Germany) and lactic acid (Surfachem Group Ltd, UK). Following excipients were used – Aristoflex AVC (Slariant Surfactants, Switzerland), propylene glycol (Dow Chemical Company, Germany), peach oil (Surfachem Group Ltd, UK), polysorbate-80 (Merck KGaA, Germany), which were substantiated by previous studies [14]. Emulgel samples were made in the laboratory. An aqueous concentrate was first prepared by dissolving dexpanthenol in propylene glycol and mixing with lactic acid solution and purified water. Next, a gelling agent was added to the aqueous concentrate, left to swell, and stirred to form a homogeneous gel. The oil concentrate of the probiotic component was prepared by adding lyophilized biomass of lactobacilli to polysorbate-80, stirred until a homogeneous liquid mass, then tocopherol and peach oil were added with constant stirring. The oil concentrate was introduced into the gel base to obtain an emulgel.

The homogeneity of the experimental samples was determined by visual inspection on a glass slide for the absence of visible particles, foreign inclusions, signs of physical instability: aggregation, coalescence, coagulation of particles. The XSP-128 ULAB biological micro-

scope was used to visually observe the homogeneity of the samples.

Colloidal stability was determined by keeping the samples in a water bath at 45±2 °C for 20 min followed by centrifugation for 5 min. The sample was considered stable if no stratification was observed in the tubes after centrifugation.

Thermal stability was determined by keeping the samples sequentially in a thermostat at (40±2) °C, in a refrigerator at (10±2) °C, at room temperature in the absence of delamination.

The rheological (structural-mechanical) properties of the samples were studied using a Rheolab QC rheoviscometer (Anton Paar, Austria) using the C-CC27/SS coaxial cylinder system. The Rheolab QC rheometer is equipped with RheoPlus software.

Microbiological research (study of the possibility of joint use of lactobacilli with active and auxiliary substances in one dosage form, antimicrobial properties of components and drug, identification and quantification of lactobacilli, microbiological purity) and biotesting on biological models of ciliates were performed in aseptic conditions of laminar box (biosafety cabinet AC2-4E1 “Esco”, Indonesia) at the Department of Biotechnology, National University of Pharmacy.

Identification of lactobacilli and the number of living cells as critical indicators of the preparation containing live microorganisms was carried out at each stage of the study. Bacterioscopic control was performed by viewing smears prepared from samples of dissolved drug and Gram-stained. Bacteriological control – by sowing on a thick medium MRS-4 and liquid medium MRS-1. The number of live lactobacilli (in co-cultivation of lactobacilli with the components of the drug at the stages of development and in the finished product) was determined by surface seeding on Petri dishes with a dense medium MRS-4; after 48 h of incubation at (37±1) °C, colonies grown on the surface of the medium were counted and the number of live lactobacilli, expressed in CFU (colloid-forming units), was calculated in 1 ml of the preparation.

Studies of the antimicrobial action of lactic acid were performed by microbiological methods described in SPhU 2.0, Vol. 1, Articles 2.6.12, 2.6.13 and SPhU 2.2, Article 5.1.3, antimicrobial action of the drug – SPhU 2.5, Articles 2.6.36, 2.6.38, using as test strains: Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 885-653, Aspergillus brasiliensis ATCC 16404. A working suspension of microorganisms containing about 100 CFU/ml was prepared peptone. Preparation of test samples was performed by placing 1 g of substance in a measuring vessel and bringing to 10 ml of the prepared working suspension of the test microorganism. From the prepared samples were sown 1 ml in Petri dishes with appropriate nutrient medium. Saburo-dextrose agar with chloramphenicol was used to determine the number of yeasts and molds. Mannitol salt agar and cetrimide agar were used to determine the number of Staphylococcus aureus and Pseudomonas aeruginosa, respectively. The cultures were incubated at (33±1) °C (bacteria) and (23±1) °C (fungi). To determine the initial microbial load, the study was performed similarly except for the introduction of samples. The criterion for assessing the preservative effect was the reduction in the number of viable cells of test microorganisms in the samples for a certain period of time after their contamination.

Microbiological tests to determine the number of contaminating microorganisms were performed according to SPhU 2.5, Article 2.6.36, for testing for certain types of microorganisms – according to SPhU 2.5, Article 2.6.38 by surface seeding. The antimicrobial activity of the drug was eliminated by increasing the volume of the diluent. Checking the suitability of the method for determining the total number of viable cells is to compare the results of counting the number of test microorganisms obtained in the presence of the test drug and on control inoculations. The suitability of the method for determining the total number of viable microorganisms was tested for studied samples of the drug in a 1:100 dilution with a solvent buffer solution of sodium chloride and petone pH=7.0 with 5 % polysorbate-80, 0.5 % lecithin and 0.1 % histidine hydrochloride. To determine the number of contaminating aerobic microorganisms (AMCC) used a thick nutrient medium without sugar (nutrient agar with 9 % sodium chloride), on which during incubation under aerobic conditions at a temperature of 30 °C to 35 °C 72 h lactobacilli grow slowly as tiny colonies, which makes it easy to detect contaminating microorganisms, which are characterized by higher growth rates and larger colonies. Saburo-dextrose agar with chloramphenicol to inhibit bacterial growth was used to determine the number of yeast and mold contaminants (YMCC). Mannitol-salt agar and cetrimide agar, respectively, were used to test Staphylococcus aureus and Pseudomonas aeruginosa for certain species. The importance of testing for the presence of these types of microorganisms was assessed in the light of the use of the drug (mild drug for dermal use).

Pharmacological studies (determination of the parameters of acute toxicity and anti-inflammatory properties in the model of acute exudative foot inflammation in rats caused by zymosan and carrageenan) were performed on the basis of the Central Research Laboratory of NUPh. The studies were performed in rats (females/males). Animals were treated in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. In a study of anti-inflammatory activity in a model of acute exudative foot inflammation in rats caused by zymosan and carrageenan, “Pantestin-Darnitsa” gel was used as a comparison drug. The research was conducted in accordance with the guidelines [15] and Orders of the Ministry of Health of Ukraine No. 944 of 14.12.2009 and No. 95 of 16.02.2009 in compliance with the requirements of Good Laboratory Practice (GLP) [16].

Biotesting (study of antioxidant properties) was performed on a biological model of Paramecium caudatum ciliates, which were grown in Lozina-Lozynskogo nutrient medium at a temperature of 20–26 °C. Live yeast Rhadotorula gracilis with the addition of wheat flour was

used to feed the paramecium. The experiment evaluated the effect of samples on the duration of the period of activity of ciliates in the environment with the addition of toxic substances (acute experiment). As toxicants used: 1 % solution of hydrogen peroxide, which in vitro decomposes to peroxide radicals and damages mainly the lipid part of the membrane; 14 % ethyl alcohol, which damages the membrane structure [17].

Statistical processing of research results was performed in accordance with the requirements of SPhU 2.3, Article 5.3. The results of the obtained data were statistically calculated by calculating the mean values and their standard errors (*M m*), or maximum and minimum values (M_{min}÷M_{max}). and non-parametric methods of analysis (Kraskel-Wallis test, Mann-Whitney test) according to the nature of the distribution, the significance level $p<0.05$ was adopted, and the standard STATISTICA 6 software package was used.

4. Results

In probiotics, the optimal number of lactobacilli responsible for the manifestation of positive mechanisms of action of these microorganisms is 10^7–10^9 CFU (colony forming units)/ml (or CFU/g) [18]. This is the range we focused on when creating the drug. The final choice of the number of cells in the composition of the tool was determined by co-cultivation of lactobacilli with other active ingredients.

The choice of lactic acid concentration was performed according to the method of evaluating the effectiveness of antimicrobial preservatives. The results of this series of experiments showed that lactic acid in the range of studied concentrations (0.2–1.0 %) has antimicrobial and antifungal properties. The most pronounced antimicrobial properties against bacterial cultures (Staphylococcus aureus, Pseudomonas aeruginosa) and fungal cultures (Candida albicans, Aspergillus brasiliensis) were for concentrations of 0.8 % and 1.0 %, but no significant difference was observed between them. After 2 days of cultivation, the logarithm of the reduction in the number of viable cells of Staphylococcus aureus and Pseudomonas aeruginosa was 3.15 and 2.37, respectively; after 7 days – 3.85 and 3.12; after 14 and 28 days – were not detected. For cells of the fungi Candida albicans and Aspergillus brasiliensis, respectively, after 2 days the logarithm of the decrease in the number of viable cells was 1.33 and 1.27; after 7 days – 2.60 and 3.09, after 14 days – 3.67 for Candida albicans and were not detected for Aspergillus brasiliensis, after 28 days – were not detected for both cultures. Considering these results and the results of studies of the combined use of lactobacilli with different contents of lactic acid to create the drug stopped at a concentration of 0.8 % with the number of lactobacilli 10^9 CFU/g.

Substantiation of dexpanthenol concentration was performed by co-cultivation of lactobacilli with the substance in concentrations at concentrations of 1.0, 2.5 and 5.0 %. Our previous studies [19] showed that co-cultivation of lactobacilli with dexpanthenol showed a slight increase in viable cells at 1 % component (after 48 h of cultivation the number of viable cells was (3.90±0.12) 10^9 CFU/ml), and significant increase at 2.5 % (number of viable cells was 4.66±0.13) 10^9 CFU/ml) and 5 % (number of viable cells was 4.70±0.26) 10^9 CFU/ml). Thus, we see that dexpanthenol in a concentration of 2.5 to 5.0 % is an effective growth factor for lactobacilli. The final choice of dexpanthenol concentration was made by substantiating the tocopherol concentration and was specified during pharmacological studies in the study of anti-inflammatory activity of the developed drug.

An antioxidant, tocopherol, was used to protect microorganisms from oxygen and prevent oxidation of drug components. The choice of tocopherol concentration was made using a biological object – a culture of Parametium caudatum cells. In this series of experiments, samples of emulgel with different tocopherol content of 0.5 %, 1 %, 1.5 % were prepared. The choice of tocopherol concentration was made in parallel with the refinement of dexpanthenol concentration (among concentrations from 2.5 to 5.0 % in 0.5 % increments).

The effect of experimental basic solutions of samples with different concentrations of dexpanthenol and tocopherol on the duration of preservation of motor activity of parameters after the addition of cellular poisons are shown in Table 1.

Table 1

The effect of samples on the duration of preservation of motor activity of parameters after the addition of cellular toxins

The concentration of tocopherol in the basic solution of the sample	Duration of motor activity of paramecium, min	
	in 14 % solution of ethanol	in 1 % hydrogen peroxide solution
Dexpanthenol 2,5 %		
0.5 %	3.95±0.21	2.05±0.15
1 %	4.07±0.13	2.48±0.07
1.5 %	4.95±0.23	2.65±0.14
Dexpanthenol 3.0 %		
0.5 %	4.87±0.21	3.46±0.13
1 %	5.52±0.10	4.75±0.21
1.5 %	6.38±0.09	4.03±0.16
Dexpanthenol 3.5 %		
0.5 %	5.10±0.12	3.81±0.15
1 %	6.05±0.20	5.54±0.13
1.5 %	7.30±0.18	3.42±0.10
Dexpanthenol 4 %		
0.5 %	5.14±0.15	4.97±0.06
1 %	6.57±0.25	6.03±0.30
1.5 %	7.35±0.23	3.31±0.15
Dexpanthenol 4.5 %		
0.5 %	5.20±0.11	5.03±0.09
1 %	6.64±0.18	6.10±0.15
1.5 %	7.40±0.20	3.40±0.19
Dexpanthenol 5 %		
0.5 %	5.27±0.25	5.12±0.13
1 %	6.78±0.13	6.15±0.21
1.5 %	7.44±0.08	3.43±0.10
Control (Dexpanthenol 0 %)		
–	3.15±0.25	1.87±0.22

Note: n=5, P=95 %

Exposure to tocopherol and dexpanthenol sample solutions prolonged the period of ciliate activity under the influence of poisons compared to control, the best results were observed with the addition of 1 % tocopherol at a dexpanthenol concentration of 4 % as the best in this experiment. With increasing dexpanthenol content from 2.5 % to 4 %, the protective effect of the samples increased intensively. Subsequent increases in the concentration of dexpanthenol to 5 % slightly increased the time of motor activity of microorganisms.

The introduction of lactobacilli into the soft preparation was provided in the form of lyophilized biomass of strains of L. fermentum and L. plantarum, which are the basis of the probiotic preparation "Lactobacterin". Lyophilized biomass of lactobacilli is a hygroscopic, fine substance, which is proposed to be administered through natural vegetable oils, which due to their affinity for skin lipids have a positive effect on lipid metabolism in tissues, restore skin barrier functions and have emollient properties. Almond, peach, apricot, olive, corn and sunflower oils are most often used to prepare dosage forms. But the direct introduction of lyophilized biomass of lactobacilli in oil showed uneven distribution, accumulation of crystals and the formation of large conglomerates (Fig. 1), so we proposed the introduction of probiotic component in a mild dosage form by pre-dissolving it in polysorbate-80, which in addition to emulsifying properties, are a source of fatty acids required for the metabolism of lactobacilli [20]. Due to the low volume mass of the lyophilized substance of lactobacilli, the concentration of polysorbate-80 below 3 % led to incomplete dissolution of biomass, and at a concentration of 3 % uniform distribution of particles was observed (Fig. 2, *a*).

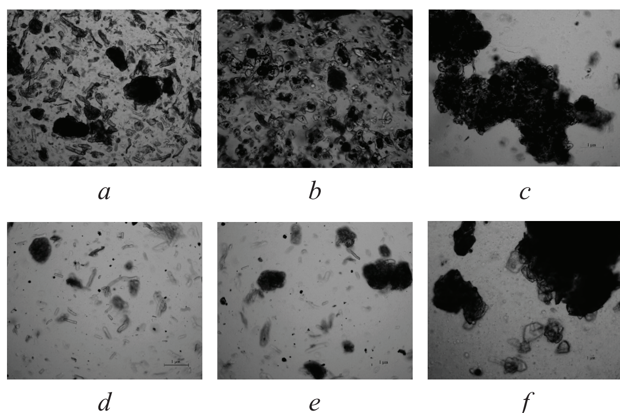

Fig. 1. Microscopic analysis of the suspension of lyophilized biomass of lactobacilli in oils: *a* – peach; *b* – almond; *c* – corn; *d* – olive; *e* – sunflower; *f* – apricot

This method showed high survival rates of lactobacilli (the number of living cells in the control and with 3 % polysorbate-80 was (3.858±0.47) 10^9 CFU/ml and (3.992±0.342) 10^9 CFU/ml, respectively), and such indicators such as system stability and uniform particle distribution in oil concentrate were best when using peach oil at a minimum concentration of 10 % (Fig. 2, *b*), which was sufficient for emulgel samples to withstand studies on thermal stability and colloidal stability.

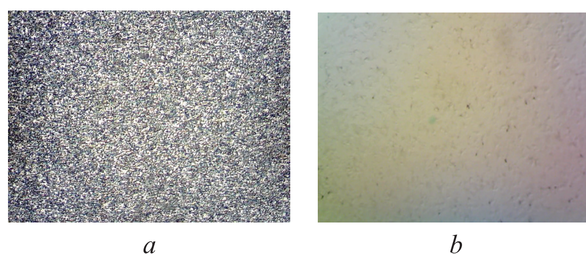

Fig. 2. Microscopic analysis of the suspension of lyophilized biomass of lactobacilli: *a* – in polysorbate-80; *b* – in a system with polysorbate-80 and peach oil

Propylene glycol, in which dexpanthenol was dissolved, was added to the samples to give the mild dosage form high consumer properties and to prevent it from drying out. Drying of samples with different concentrations of propylene glycol (5, 10, 15 % (increase in concentration led to dilution of samples)) was determined by the difference between the initial mass of the sample and the mass of the sample during 24 h of storage at 25 °C. The percentage of moisture loss in the sample with a concentration of propylene glycol of 5 % was 3.6 %; increasing the concentration of propylene glycol to 10 % and 15 % in the samples reduces the degree of its drying and is 2.6 % and 2.5 %, respectively. Substantiation of propylene glycol concentration was also performed on the main critical parameter in this series of experiments – the number of viable bacteria: in the cultivation of lactobacilli with the addition of propylene glycol 5 % and 10 % there was a slight decrease in viable bacteria compared to control – a decrease of 1.19 and 2.35 times respectively, when adding 15 % – 6.08 times. Thus, increasing the concentration of propylene glycol to 15 % and above is impractical.

Given the characteristics of different types of bases used as carriers of active substances, the need to protect bacterial cells from the action of water and maintain their viability in the composition of the drug, we proposed to use an emulgel base. Based on previously conducted organoleptic, physicochemical, microbiological and rheological studies among structural agents – hydroxyethylcellulose, sodium alginate, Sepiplus 400, Carbopol 934, Aristoflex AVC focused on the latter [13]. The study of the properties of the emulsifier, the basis of which, in addition to the structure former Aristoflex AVC, included excipients in selected concentrations – propylene glycol – 10 %, peach oil – 10 %, polysorbate-80 – 3 %, showed acceptable rheological and osmotic characteristics [13].

The next step was to determine the optimal concentration of the structure former. The concentration of Aristoflex AVC in the samples ranged from 1 % to 2.5 % with a step of 0.5 %, the criterion for selection was rheological indicators of stability (Fig. 3).

Structural and mechanical parameters of the samples increase with increasing concentration of Aristoflex AVC polymer to 2.0 %. The sample with a concentration of Aristoflex AVC 2.5 % is almost on the same level with the sample with a concentration of 2.0 %. An explanation for this may be the probable solvation threshold of the

polymer at which the molecule is not fully untwisted due to the highly structured dispersion medium. Evidence of a highly structured dispersion medium is the delay in the restoration of the structure, i.e., on the rheograms between the ascending and descending curves there is a certain space, which is called the area of the hysteresis loop. Thus, the use of Aristoflex AVC at a concentration of 2.0 % is justified by the set of features in this development as the basis of the carrier.

The results of a complex of organoleptic, physicochemical, pharmacological, microbiological and biological studies were used to develop the composition and technology of the drug.

An important step in the biological testing of the drug is the analysis of microbiological purity to determine compliance with the requirements of SPhU [22].

In determining the criteria for the acceptability of microbiological purity for a new drug for dermal use with probiotics focused on SPhU 2.4, Article 5.1.4 and SPhU 2.5 of the general monograph "Living biotherapeutic drugs for human use": the number of contaminating aerobic microorganisms (AAMS) should not exceed 10^2 CFU/g or CFU/ml; the number of contaminating yeasts and molds (YMCC) – 10^1 CFU/g or CFU/ml; certain species of microorganisms (Staphylococcus aureus and Pseudomonas aeruginosa) in 1 g or 1 ml are absent.

According to the requirements of SPhU, the sterility of nutrient media, solvent, growth properties of nutrient media and the suitability of the method of determining the total number of viable cells were initially tested. The control in determining the growth qualities of the environment is a standard environment with guaranteed growth properties, which correctly manifests the quantitative and qualitative growth of microorganisms (morphology of colonies). Prior to the start of the study, the growth properties of nutrient media were tested. The nutrient media corresponded to growth properties and passed the sterility test, and the test microorganisms met the taxonomic characteristics – the morphology of the colonies on the media and the morphology of the cells under microscopy were typical for the strain. Based on the test, the method of surface seeding with dilution using a typical neutralizing solvent, suitable for determining the number of microorganisms in the samples (counted only the test microorganism used as inoculum).

During long-term storage under normal conditions at room temperature, control tests were performed every 3 months for 15 months of storage (the proposed shelf life of the drug under development is 1 year, which is generally acceptable for drugs containing live microorganisms). The results of microbiological purity of the samples during 15 months of storage are given in Table 2.

Table 2

The results of testing the drug "Probioskin" for microbiological purity

Expiration date	Total amount, CFU/ml			
	AAMC	YMCC	Staphylococcus aureus	Pseudomonas aeruginosa
Immediately after manufacture	less than 100	less than 10	Absent	Absent
Storage for 12 months	less than 100	less than 10	Absent	Absent
Storage for 15 months	less than 100	less than 10	Absent	Absent

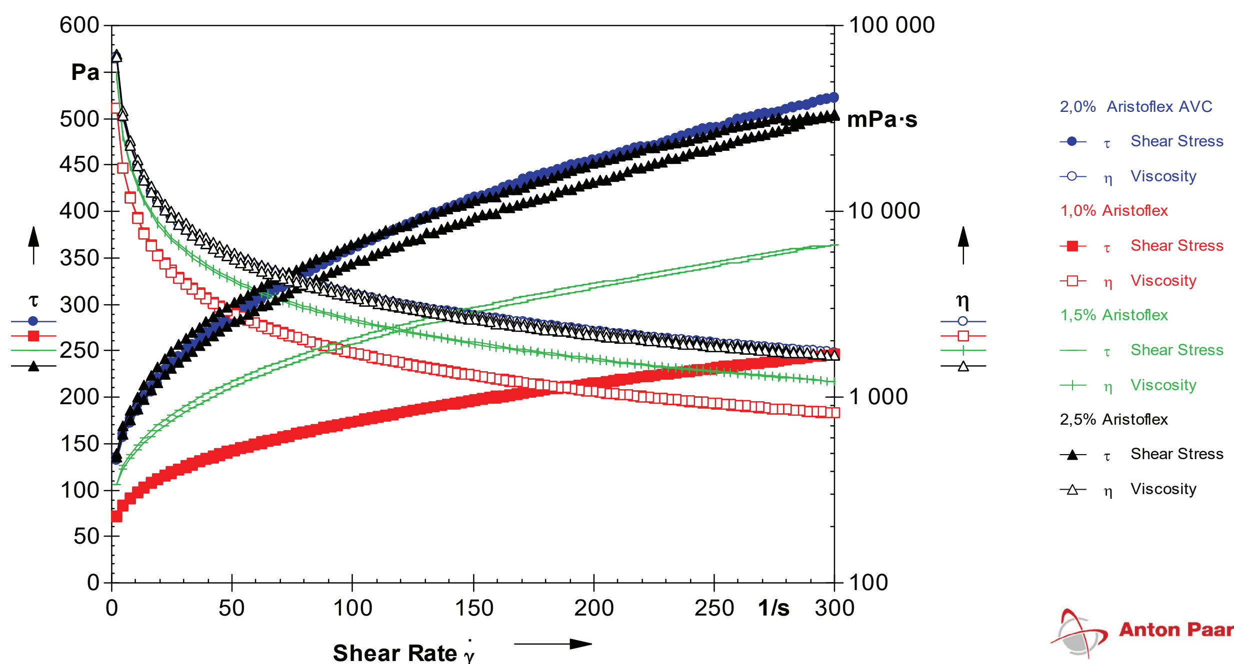

Fig. 3. Dependence of shear stress (τ, Pa) and structural viscosity (η, mPa·s) on the shear rate gradient of the studied samples

Tests of microbiological purity (Table 2) of the gel sample showed that the number of contaminating aerobic microorganisms (AAMS) is not more than 10^2 CFU/g, the number of contaminating yeast and mold fungi (YMCC) is not more than 10 CFU/g, microorganisms of the family Staphylococcus aureus and Pseudomonas aeruginosa in 1.0 g are absent, which meets the requirements of SPhU.

An important stage of development is the study of pharmacological research. The effect of the developed drug "Probioskin" on the body of experimental animals was evaluated in dynamics for 2 weeks. Determined body weight (initial state, 3, 7 and 14 days), assessed the general condition of animals after emulgel application (appearance, respiration, salivation, urination and defecation) [23]. Survival and clinical signs, skin condition (erythema, necrosis, scab, edema) were observed for 14 days (Table 3).

Table 3

The effect of emulgel "Probioskin" on the body weight (g) of rats, n=6 ($M \pm m$)

Group of animals	Dynamics of observation			
	Initial condition	3 days	7 days	14 days
males				
Intact control	212±2.8	217±2.8	225±3.9*	235±5.2*
Emulgel	210±3.4	214±2.4	222±3.3*	229±2.4*
females				
Intact control	167±3.1	175±3.4	180±2.9*	188±3.1*
Emulgel	168±3.1	173±2.8	178±3.1*	189±3.0*

*Note: * – differences are statistically significant relative to baseline (Newman-Keyles test); n is the number of animals in the group*

Dermal application of emulgel at a maximum dose of 22610 mg/kg did not cause a negative reaction of the body to the weight of animals compared with the group of intact animals, a positive increase in both males and females of the group showed statistically significant deviations from baseline. No effect on the gastrointestinal mucosa was detected.

According to many researchers [3–9], one of the mechanisms of action of skin probiotics is antimicrobial and anti-inflammatory. The study of the antimicrobial action of samples of emulgel "Probioskin" and control samples (control 1 – emulgel of the developed composition without the addition of probiotic component, control 2 – working suspension of the microorganism without samples) was performed on standard test strains of microorganisms, counting only test microorganisms as an inoculant. The results of this series of experiments are given in Table 4.

It was found that the test samples of the developed drug and control 1 (emulgel without probiotic component) inhibited the growth of test cultures of microorganisms. The number of S. aureus cells compared to control 2 was 2.8 times less for control 1 and was not detected for the developed drug, the number of P. aeruginosa was 2.1 and 7.3 times less for control 2 and the drug, respectively. A similar trend was observed for fungi: the number of C. albicans was 2.5 and 7.4 times less for control 2 and the drug, respectively, A. brasiliensis – 2.6 and 3.7, respectively. Moreover, in relation to all test cultures there is a statistically significantly greater antimicrobial effect of the developed drug compared to control 1. It should be noted that at the end of the observation period (28 days of cultivation) cells of bacteria and fungi were not detected. These results confirm the combination of antimicrobial action of lactic acid and lactobacilli.

To expand the understanding of the mechanism of action of the developed drug, a study of anti-inflammatory activity in a model of acute exudative foot inflammation in rats caused by zymosan (Table 5) and carrageenan (Table 6). "Pantestin-Darnitsa" gel with a dexpanthenol content of 5 % was used as a comparison drug.

Table 4

Determination of antimicrobial activity of emulgel "Probioskin"

Sample	Test strain of the microorganism			
	Staphylococcus aureus ATCC 6538	Pseudomonas aeruginosa ATCC 9027	Candida albicans ATCC 885-653	Aspergillus brasiliensis ATCC 16404
	Number of cells, CFU/ml			
Emulgel "Probioskin"	Not detected	12.0±0.9	11.0±0.6	15.0±0.6
Control 1	26.0±6.5	41.0±4.2	32.0±4.1	21.0±2.5
Control 2	74.0±2.7	88.0±6.9	81.0±5.5	55.0±7.1

Note: n=5, P=95 %, Not detected – viable cells of test microorganisms were not detected

Analysis of the obtained data shows that in the time interval of 0.5–1 h there is a statistically significant difference in the severity of the anti-exudative component of inflammation (ΔV) in the group to which the study drug was applied ($p<0.05$). In the time interval from 2 to 3 hours, he showed statistically significant anti-exudative action only in the last hour, the average AEA is 33 %.

In animals treated with "Pantestin-Darnitsa" gel, statistically significant suppression of foot volume increase by 59 %, 39 % and 40 % was observed 0.5, 1 and 3 hours after phlogogen administration compared to untreated animals, respectively, the average AEA is 38 %.

Table 5

Dynamics of antiexudative action of "Probioskin" emulgel and "Pantestin-Darnitsa" gel on the model of acute exudative foot inflammation in rats caused by zymosan ($M \pm m$), n=6

Group of animals		Dynamics of inflammation, hours			
		0.5	1	2	3
Positive control	ΔV	0.70±0.04	0.58±0.06	0.50±0.11	0.68±0.07
emulgel "Probioskin"	ΔV	0.45±0.08*	0.35±0.07	0.41±0.10	0.41±0.09
	AEA, %	35	39	18	39
"Pantestin-Darnitsa" gel	ΔV	0.28±0.07*	0.35±0.06*	0.43±0.05	0.41±0.04*
	AEA, %	59	39	13	40

*Note: * – differences are statistically significant relative to the group of positive control, p<0.05*

Table 6

Dynamics of antiexudative action of "Probioskin" emulgel in comparison with "Pantestin-Darnitsa" gel in the model of foot inflammation in rats caused by carrageenan ($M \pm m$), n=8

Experimental conditions		Dynamics of inflammation, hours				
		1	2	3	4	5
Positive control	ΔV, ml	0.41±0.03	0.98±0.04	1.28±0.05	1.39±0.06	1.43±0.05
"Probioskin" emulgel	ΔV, ml	0.39±0.05	0.71±0.07 *	0.84±0.06 *	0.98±0.04 *	1.11±0.09 *
	AEA, %	7	27	35	29	23
"Pantestin-Darnitsa" gel	ΔV, ml	0.32±0.04	0.62±0.05 *	0.93±0.11 *	0.98±0.10 *	1.05±0.14 *
	AEA, %	23	37	28	29	27

*Note: * – differences are statistically significant for the group of positive control, p<0.05; AEA – antiexudative activity (%); ΔV is the difference between the volume of the swollen and non-swollen paw (ml)*

According to the obtained data, the application of the study drug on the paw of animals led to a statistically significant reduction in paw edema from 2 to 5 hours of observation compared with the positive control. The maximum value of AEA drug reached 3 hours – 35 %. The average AEA of the study drug was 24 %. Analyzing the activity of the emulgel "Probioskin" with the comparison drug gel "Pantestin-Darnitsa" it can be stated that the studied drug activity was not inferior. The anti-exudative activity of Pantestin-Darnitsa gel was 29 %.

5. Discussion

Based on a set of studies, the composition of a complex preparation for skin application "Probioskin" was substantiated (Table 7).

Table 7

The composition of the drug for skin application "Probioskin"

Substances	Content, g
Dexpanthenol	4.0
Lactic acid	0.8
Lactobacilli	$1 \cdot 10^9$ CFU/g
Propylene glycol	10.0
Peach oil	10.0
Polysorbate-80	3.0
Aristoflex AVC	2.0
Tocopherol	1.0
Purified water	up to 100.0

Analysis of the microbiological purity of the developed drug "Probioskin" during the proposed shelf life of 12 months showed that the level of microbial contamination by foreign microflora meets the requirements of SPhU for drugs for dermal use.

A set of pharmacological studies shows that the drug "Probioskin" when applied dermally does not lead to the death of animals at a dose of 22610 mg/kg, this allows it to be classified as group 6 and classified as a "relatively harmless" agent (LD_{50}>22610 mg/kg) [23]. Dermal application at a maximum dose of 22610 mg/kg does not cause a negative reaction of the body to the weight of animals compared to the group of intact animals, a positive increase is evidenced by statistically significant conclusions about the initial state.

The study of the anti-inflammatory effect of the drug shows that the drug exhibits moderate anti-inflammatory properties. In the case of zymosan inflammation, which is associated with the activation of leukotrienes as mediators of inflammation, the average antiexudative activity of the drug is 33 %. The drug has a moderate anti-exudative effect in the presence of carrageenan edema, which is evidence of its effect on exudation processes mediated by prostaglandins. The average AEA of the study drug was 24 %.

Thus, our studies showed that the developed drug "Probioskin" with a concentration of dexpanthenol 4 % in anti-inflammatory activity was not inferior to the comparison drug "Pantestin-Darnitsa" with a concentration of dexpanthenol 5 %, due to one of the mechanisms of action of the probiotic component.

Study limitations. Studies have not studied changes in the microbial "landscape" of the skin when using the drug and the immunostimulatory activity of the emulgel "Probioskin", which could potentially have the drug due to the action of the probiotic component.

Prospects for further research. The next stage of the research is the validation of the technological process and its scaling in production conditions.

6. Conclusions

The following components have been selected for the development of a mild preparation for skin application for the treatment of infectious and inflammatory dermatological diseases and the restoration of the skin microbiome: active – lactobacilli, dexpanthenol, lactic acid; excipients – propylene glycol, peach oil, polysorbate-80, aristoflex, tocopherol, the concentration of which was justified on the basis of a set of organoleptic, physicochemical, pharmacotechnological, microbiological and biological studies. The expediency of creating a new drug with probiotic in the form of emulgel, which provides the possibility of introducing components with different properties into the composition of the tool, high rates of viable cells and stability of the tool. It has been experimentally established that "Probioskin" emulgel meets the requirements of SPhU for preparations for dermal application in terms of the level of microbial contamination by foreign mi-

croflora. Microbiological studies have established high antimicrobial properties of the drug. Pharmacological studies allow to classify the drug as "relatively harmless" with anti-inflammatory properties at a level not lower than the comparison drug.

Conflict of interests

The authors declare that they have no conflicts of interest.

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