Open Veterinary Journal, (2024), Vol. 14(6): 1370-1383
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Research Article
DOI: *10.5455/OVJ.2024.v14.i6.5*

Submitted: 14/02/2024 Accepted: 16/05/2024 Published: 30/06/2024

Pharmacological composition based on bacteriocin-nisin in experiments *in vitro* and *in vivo*

Maria Isakova*, Olga Oparina, Alexander Belousov and Yana Lysova

Federal State Budgetary Scientific Institution, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russian Federation

Abstract

Background: Antibiotic resistance is a global health problem related to the transmission of bacteria and genes between humans and animals. The development of new drugs with antimicrobial activity research is an urgent task of modern science.

Aim: The article presents data of *in vitro* and *in vivo* experiments on new pharmaceutical composition based on nisin.

Methods: The antimicrobial activity was studied on the mastitis pathogens. To identify microorganisms the Matrix-Assisted Lazer Desorption/Ionization Time-of-Flight (MALDI-TOF) (mass spectrometry) method was performed using. To determine sensitivity, the serial dilution method and the diffusion method were used. On laboratory animals, biochemical, hematological, and histological research methods were used. Female nonlinear white laboratory rats were used, which were divided into one control group and three experimental ones.

Results: "Duration" factor was statistically significant for the following indicators: hemoglobin, hematocrit, leukocytes, lymphocytes, erythrocyte sedimentation rate, and eosinophils. The "Dose" factor did not show significance for any indicator, which means that the effect was similar regardless of the dose chosen. When analyzing the biochemical indicators, significant differences were found in the "Duration" and "Dose" factors, in the direction of a decrease in the indicators of total protein, globulins, urea, and an increase in the concentration of alkaline phosphatase. When conducting histological studies in the first experimental group, it was established that there were no changes in the structural and functional units of the organs. In animals of the second experimental group, the presence of reversible pathological processes of a compensatory nature was noted. More profound changes in the structure of the studied organs were recorded in the third experimental group.

Conclusion: An *in vitro* study on cell cultures showed that the pharmacological composition has high antimicrobial activity against isolates from the mammary gland secretion of cows with mastitis. An *in vivo* study on laboratory animals showed that the developed composition belongs to the IV class of substances "low-hazard substances". Histological examination made it possible to select the safest dose of the pharmacological composition of no more than 500 mg/kg.

Keywords: Biochemical parameters, chronic toxicity, hematological parameters, Histological studies, Mastitis pathogens.

Introduction

Over the past two decades, the development of antimicrobial resistance as a result of the use of antibiotics in agriculture has become a serious global health problem due to the mass treatment of animals with antimicrobial drugs of critical importance to humans, e.g., third-generation cephalosporins and fluoroquinolones (Oliver *et al.*, 2011; Huemer *et al.*, 2020, Bhardwaj *et al.*, 2022). In dairy farming, mastitis is one of the important diseases, that leads to milk quality deterioration, a decrease in the dairy productivity of cows, and an increase in the culling of animals in the herd (Sharun *et al.*, 2021; Touza-Otero *et al.*, 2024). Antibiotics remain the drugs of choice in mastitis treatment, but the problem of their residues in milk and the development of resistance leads to many restrictions in animal husbandry (Krömker and Leimbach, 2017; Ruegg, 2018; El-Sayed and Kamel, 2021). As a result, scientists are exploring new therapeutic approaches to replace the use of antibiotics in mastitis treatment regimens (Sharun *et al.*, 2021; Kober *et al.*, 2022). Currently, the concept of mastitis control includes such methods: breeding of mastitis-resistant cows, development of new diagnostic and therapeutic methods, antimastitis vaccines, mammary gland disinfection products, use of communication technologies as educational tools, and the development of adapted protocols for drying off and feeding cows (Collado *et al.*, 2016; Penry, 2018; Rowe *et al.*, 2021; Esener *et al.*, 2021; Dzayee *et al.*, 2022;

***Corresponding Author:** Maria Isakova. Federal State Budgetary Scientific Institution, Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences, Russian Federation. Email: *Tmarya105@yandex.ru*

Kukeyeva *et al.*, 2023). As an alternative to the use of antibiotics, the effect and effectiveness of phytotherapy, nutraceuticals, bacteriocins, bacteriophages, phage lysines, and probiotics are being studied (Vázquez *et al.*, 2018; Lopes *et al.*, 2020; Zduńczyk *et al.*, 2020; Kober *et al.*, 2022; Raheel *et al.*, 2023). Bacteriocins are specific proteins that are produced by some bacteria that can suppress the vital activity of cells of other strains of the same species or related species of bacteria. One of the advantages of using bacteriocins is a decrease in the development of microbial resistance associated with the mechanism of action, which is based on damage to the structures of the bacterial cell, which leads to the death of the target cell (Zgheib *et al*., 2020). Nisin belongs to the first class of bacteriocins, and is a polypeptide lantibiotic formed by the microorganism *Streptococcus lactis*. Over the past two decades, due to the high level of activity against bacteria and low toxicity, the use of nisin has been extended to biomedical fields.

On the basis of the Institute of Organic Synthesis named after I.Ya. Postovsky Ural Branch of the Russian Academy of Sciences (Yekaterinburg, Russia), we have developed a pharmaceutical composition based on bacteriocin-nisin, silicon glycerolates, and boron bisglycerolates. Theoretical data on the study of the action mechanism of the components of the composition under study make it possible to use it in therapeutic regimens for the treatment of mastitis in cows (Shin *et al.*, 2016; Khonina *et al.*, 2020). The previously obtained results on antimicrobial activity against microorganism's reference strains provide the basis for further research of our pharmacological composition, as sensitivity to it was shown for *Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium, Escherichia coli, and Salmonella abony* (Isakova *et al.*, 2023).

The use of new preparations on highly productive cows is feasible only if their mechanism of action and specific effect on the functioning of organs, organ systems, and the whole organism are studied, which can be achieved through *in vitro* and *in vivo* studies.

The purpose of the work is to conduct *in vitro* and *in vivo* experiments to evaluate the possibility of using a new pharmaceutical composition based on nisin, silicon glycerolates, and boron bisglycerolates in mastitis treatment regimens. To achieve the goal, the following tasks were set to determine the antimicrobial activity of the pharmaceutical composition against isolated pathogens of mastitis and to establish the effect of the pharmaceutical composition on laboratory rats.

Materials and Methods

The research was carried out in the Department of Reproductive Biology and Neonatology, the Laboratory of Microbiological and Molecular Genetic Research Methods, the Laboratory of Immunology and Pathobiochemistry of the Ural Scientific Research Veterinary Institute, a structural unit of the Federal State Budgetary Scientific Institution Ural Federal Agrarian Research Center of the Ural Branch of the Russian Academy of Sciences with the support of a grant of the Russian Science Foundation No. 22-76-00009.

Substance under study

The pharmacological composition contains nisin as an active ingredient, and silicon glycerolates, boron bisglycerolates, and glycerin as excipients. NISAPLIN (Danisco, Great Britain), containing two components, was used as a source of nisin: nisin $C_{143}H_{230}N_{42}O_{37}S_7$ as an active substance—at least 10% by weight; sodium chloride NaCl as an inert carrier—the rest. The initial synthesis of silicon glycerolates is a reaction of transesterification of tetraethoxysilane $Si(OC_2H_5)_4$ with glycerin $C_3H_8O_3$ with the release of a theoretical amount of ethanol and the formation of a mixture of monomeric and oligomeric silicon glycerolates with a predominance (during the reaction in excess of glycerol) of the monomer—silicon tetrakisglycerolate, tetrakis (2,3-dihydroxypropoxy) silane $Si(-OCH_2-CH(OH)-CH_2OH)_4$. Boron bisglycerolates $H[B(C_3H_6O_3)_2]$ was obtained by esterification of boric acid H_3BO_3 with glycerin $C_3H_8O_3$ followed by removal of water in the form of azeotrope with toluene. The subsequent synthesis of compositions containing glycerohydrogels of silicon, boron, and nisin was carried out by the sol-gel method. The developed composition consists of silicon glycerolates in a 6-mole excess of glycerol $Si(C_3H_7O_3)_4{\cdot}6C_3H_8O_{38}$ – 3.0%, boron bisglycerolates $H[B(C_3H_6O_3)_2]$ – 2.0%, nisin – 0.3%, glycerin – 10%.

In vitro studies

The study of antimicrobial activity was carried out in relation to isolated pathogens of mastitis in cows kept in agricultural organizations of the Sverdlovsk region with various technologies of maintenance and milking. The selection of mammary gland secretions was carried out in sterile containers immediately after milking of cows, after cleaning the side of an udder using an alcohol swab. During bacteriological and mycological studies cultures were taken from samples of mammary gland secretion from 60 cows with signs of mastitis. 10 µl of secretion was taken using a sterile calibrated loop and inoculated on nutrient media using the exhaustive inoculation method, nutrient media were used: 5% sheep blood agar (Columbia agar base, Bio-Rad, France); defibrinated sheep blood (E&O Laboratories, Scotland); yolk-salt agar (nutrient agar for the cultivation of microorganisms GRM-agar, FBUN State Research Center for Medical and Biology, Russia); chromogenic agar (UriSelect 4, Bio-Rad, France) and Sabouraud agar with 2% glucose and chloramphenicol (SIFIN diagnostics, Germany). The seeded Petri dishes were placed in a thermostat with aerobic conditions at a temperature of 37°C ± 1°C, then incubated in an atmosphere containing 5% CO_2. The cultures were incubated for up to 72 hours, with growth assessed after 24, 48, and 72 hours. Identification of colonies was carried out using MALDI-TOF mass spectrometry

(matrix-associated laser desorption ionization time-of-flight mass spectrometry) on a Vitek MS device (BioMerieux, France). To do this, the bacterial mass was applied to a spot slide, covered with 1 µl of matrix (α-cyano-3-hydroxycinnamic acid), dried at room temperature, then the mass spectra of ribosomal proteins were read by the device and compared with the database using Myla software. To determine sensitivity, the serial dilution method and the diffusion method were used.

Serial dilution method: For inoculation, a microbial suspension equivalent to 0.5 McFarland standard, diluted 100 times in MH nutrient broth, was used. 0.5 ml of inoculum was added to each tube containing 0.5 ml of the composition, and into one tube with 0.5 ml of nutrient broth without the composition ("negative" control). The final concentration of the microorganism in each tube was approximately $5 \cdot 10^8$ CFU/ml. The tubes were incubated in a normal atmosphere at a temperature of 35°C for 24 hours. To determine the presence of microorganism growth, the inoculated tubes were viewed in transmitted light. Culture growth in the presence of the composition was compared with a reference tube ("negative" control) containing the original inoculum. From the tubes that did not give visually visible growth, seeding was done on MPA (1 µl each).

Diffusion method: based on the penetration of the test substance into a solid nutrient medium and suppression of the growth of the microorganism culture. Suspensions of microorganisms at a concentration of 1.5 10^8 CFU/ml correspond to the McFarland turbidity standard of 0.5. For their preparation, a pure 24-hours culture of microorganisms was taken. To make the results representative, the experiments were carried out twice. The results were taken into account by measuring the diameter of zones of growth inhibition of microorganisms: up to 10 mm—resistant; 10–15 mm—intermediate sensitivity; 15–25 mm—sensitive.

The object of histological examination was micro-preparations of the liver, kidneys, spleen, hypogastric lymph node, bottom of the stomach, duodenum, pancreas, and thymus. The manufacture of micro-preparations was carried out by excision of a specific organ into pieces 3–5 mm thickness, fixation in a 10% solution of neutral buffered formalin, and tissue processing. After that, the pieces were enclosed in histological paraffin, and sections with a thickness of 3 microns (microtome - Microm HM450) were made. Then, dewaxing and staining in Carazzi hematoxylin and eosin were performed (10:2 minutes). Histopreparations were viewed using an Olympus BX 43 microscope (Olympus, Japan) with an ADF Professional 03 digital camera (ADF, USA).

In vivo studies

Female non-linear white laboratory rats (n = 40) were used, age at the beginning of the experiment was 8–9 weeks, and live weight—180–190 g. The choice of doses for the study of chronic toxicity was calculated from the maximum tolerated dose obtained in an earlier experiment on acute toxicity (5,000 mg/kg). As a result, the laboratory rats were divided into one control group and three experimental ones: 1—composition at a dose of 500 mg/kg (95 mg/head); 2—composition at a dose of 750 mg/ kg (142.5 mg/head); 3—composition at a dose of 1,000 mg/ kg (190 mg/head).

The animals were kept in accordance with the order of the Ministry of Health of the Russian Federation dated 04/01/2016 No. 199n "On approval of the Rules of good laboratory practice" and GOST 33216-2014 "Guidelines for the maintenance and care of laboratory animals. Rules for the maintenance and care of laboratory rodents and rabbits". For feeding, full-fledged granular compound feeds for laboratory animals were used in accordance with GOST 34566-2019 "Full-fledged compound feeds for laboratory animals. Technical specifications", manufactured at the Bogdanovich feed mill (Sverdlovsk region, Bogdanovich). The animals were watered from standard drinking bowls with tap water in accordance with GOST R 51232-98 "Drinking water. General requirements for the organization and methods of quality control". The animals were monitored daily according to the generally accepted scheme (Khabriev, 2005). Before the experiment, the animals underwent adaptation to the laboratory environment.

Manipulations with animals

During the experiment, the consumption of feed and water, the condition of the hair and mucous membranes, and behavioral reactions were considered. The weekly weighing was carried out on CAS SW-10 scales (South Korea) to study the increase in body weight. During the experiment, the developed composition was fed with water to laboratory rats for a 30-day period. Blood was taken from the animals for hematological and biochemical studies before and after the experiment. All manipulations with animals were carried out by personnel with veterinary education, appropriate qualifications, and experience of work with laboratory animals. At the end of the experiment, laboratory rats were euthanized, followed by autopsy, to conduct a macroscopic assessment of internal organs, followed by weighing and histological examination.

Hematological studies

Hematological studies were performed on an automatic veterinary hematology analyzer Abacus Junior Vet (Diatron, Austria) using standard reagents from the company (Diatron, Austria), the leukocyte formula was calculated in blood smears stained according to Romanowsky–Giemsa. The results were recorded visually using an Olympus BX 43 microscope (Olympus, Japan).

Biochemical studies

Biochemical studies were carried out using the Chem Well-2910 Combi analyzer (Awaveness Technology,

USA) and standard reagent kits (Vital Diagnostics Spb, Russia; DIALAB GmbH, Austria). Biochemical blood parameters were measured in native serum after blood centrifugation at 3,500 g (Laboratory clinical centrifuge OPN-3.02 "Dastan," Russia). The correctness of the measurements was confirmed by the control materials recommended by the reagent manufacturers.

Statistical analysis

During the statistical analysis of the data, calculations were performed using the program "Statistica 10.0." Methods of descriptive statistics, sample comparisons, and multidimensional exploratory analysis were also used. For all quantitative indicators, the average value was calculated with a 95% confidence interval, calculated using the nonparametric bootstrap technique (percentile method, n = 9999). The average values in the experiment were compared during a two-factor variance analysis with the factors: "Duration" (fixed, 2 gradations, correspond to the repetitions "1—the beginning of the experiment" and "2—the end of the experiment") and "Dose" (fixed, 3 gradations, correspond to concentrations "500 mg/kg", "750 mg/kg", "1,000 mg/kg"). To normalize the error distribution of the dispersion complex, all indicators were previously transformed using the Box–Cox transformation. To assess the effect of these same factors on the entire complex of hematological and biochemical parameters, a redundancy analysis (RDA) was used, combining the analysis of the main components and regression analysis. At the same time, to analyze the correlation matrix of the relationships between the indicators depending on the regressors ("Duration" and "Dose"), the data were previously transformed by Box–Cox and standardized by subtracting the average from each value, followed by dividing by the standard deviation. The statistical significance of the dependence of the complex of indicators on regressors was assessed in a randomization (n = 999) of the variance analysis. Calculations and graphical constructions were performed using PAST (v. 4.12) (Hammer *et al*., 2001; Yoshioka, 2002). The effects were considered statistically significant at $p < 0.05$ and insignificant - at $p > 0.10$.

Ethical approval

Studies on laboratory animals were conducted in accordance with the Guidelines for the experimental (preclinical) study of new pharmacological substances (Khabriev, 2005) and biological ethics principles set out in the European Convention for the Protection of Vertebrates Used for Experiments or Other Scientific Purposes and the Directive of the European Parliament and the Council of the European Union on the Protection of Animals Used for Scientific Purposes. The experiment involved the minimum possible number of laboratory animals to assess the reliable effect of the developed composition. The study was approved by the academic council Ural Scientific Research Veterinary Institute dated 17.10.2022.

Results

In an agricultural organization with tied housing systems and a stationary type of milking on a linear installation using an ADM-8 milking machine, mastitis in cows was caused by the following pathogens: *S. aureus, E. faecalis,* and *Enterobacter spp.* At the same time, *S. aureus* and *E. faecalis* were sensitive to the pharmaceutical composition in 100% of the samples. *Enterobacter spp.* showed sensitivity in 87.5% of cases. In an agricultural organization with a system of loose housing and milking of cows in a special milking barn using DeLaval equipment and a Herringbone milking machine, mastitis was caused by *S. aureus, E. faecalis, Enterococcus faecium, and E. coli. Staphylococcus aureus* was sensitive to the pharmaceutical composition in 77.8% of the samples, and intermediate sensitivity and resistance were observed in 11.1% of cases. *Enterococcus faecalis, Enterococcus faecium, and E. coli* showed 100% sensitivity (Fig. 1). During the study of the effect of the developed composition on laboratory animals, body weight was measured, as a result of which it was established that the dynamics of body weight gain in rats of the control and experimental groups had no statistically significant differences (Table 1). The absence of a negative effect with prolonged use of the developed composition was confirmed by the obtained mass coefficients of the internal organs of white rats, in which the differences between the control and experimental groups amounted to no more than 10% (Table 2).

To search for dependencies in the obtained hematological data by examining the significance of differences, a variance analysis was used, which showed that the effect of the "Duration" factor was statistically significant for the following indicators: hemoglobin, hematocrit, leukocytes, lymphocytes, erythrocyte sedimentation rate (ESR), and eosinophils. At the beginning of the experiment, hemoglobin in animals of the control and all experimental groups was 161±5.13, 155±5.86, 152±4.36, and 164±4.59 g/l, respectively. At the end of the 30-day experiment period, a slight decrease (within the physiological normal value) of this indicator was observed in all groups: in the control group to 156±3.42 g/l, in the experimental groups—145 ± 4.16, 150 ± 3.67, and 146 ± 4.21 g/l, respectively (Table 3). With regard to the hematocrit index, before the start of the experiment, a slight increase was found in the control group by 2.8%, in the first and third experimental groups by 0.3% and 3.7%. At the end of the experiment period, this indicator in all groups was within the limits of physiological values. The ESR before the use of the pharmaceutical composition in the control, second, and third experimental groups was lower than the reference value by 0.50 mm/hour. At the end of the experiment period, this indicator in all groups was included in the range of the reference normal value. The indicators of leukocytes and lymphocytes at the beginning of the experiment

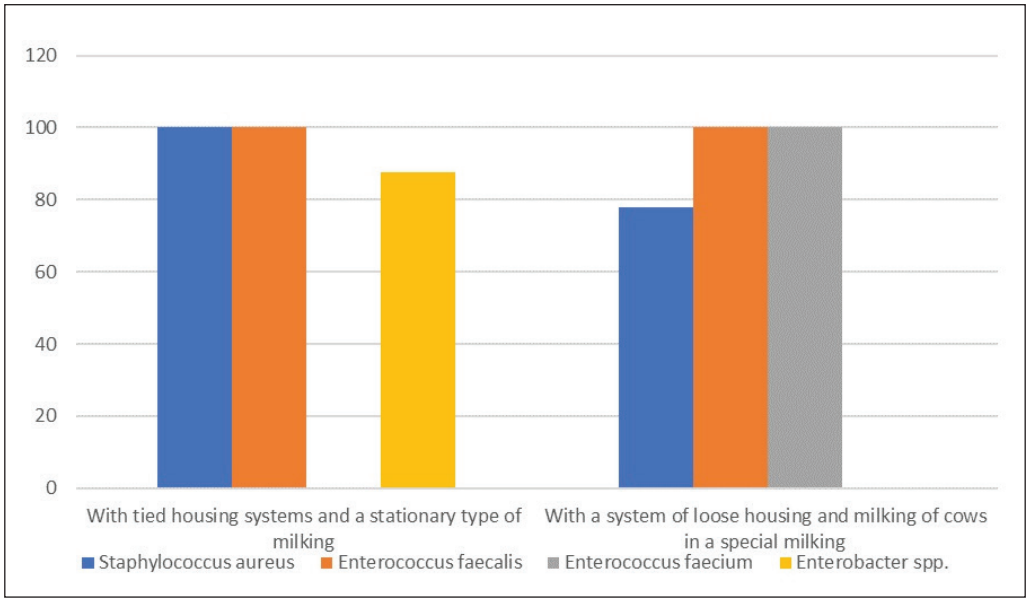

Fig. 1. Sensitivity of isolated causative agents of cow mastitis to the used pharmacological composition with different technologies for keeping and milking animals (n = 60).

Table 1. Body weight of white laboratory rats in the study of chronic toxicity of the studied composition (n = 40).

Weighing period	Group of animals			
	Control group, g	Experimental group 1, g	Experimental group 2, g	Experimental group 3, g
Before experience	186.04 ± 14.72	185.72 ± 10.09	180.14 ± 12.23	187.24 ± 13.45
In 1 week	198.92 ± 9.83	196.26 ± 13.47	189.52 ± 10.07	196.91 ± 12.20
In 2 week	207.33 ± 20.68	208.19 ± 17.82	197.48 ± 18.22	204.46 ± 11.62
In 3 week	215.52 ± 14.14	217.56 ± 15.39	208.27 ± 14.64	213.34 ± 16.35
In 4 week	223.41 ± 17.21	225.44 ± 12.04	217.31 ± 11.28	222.59 ± 10.18

Table 2. Mass coefficients of the internal organs of white rats in the study of chronic toxicity of the composition under study (n = 40).

Group of animals	Animal weight, g	Mass coefficients of internal organs				
		Heart	Lung	Liver	Kidneys	Spleen
Control	223.41 ± 17.21	0.52 ± 0.11	0.70 ± 0.11	6.06 ± 0.38	0.84 ± 0.03	0.22 ± 0.08
Experimental group 1	225.44 ± 12.04	0.47 ± 0.09	0.69 ± 0.24	5.71 ± 0.63	0.82 ± 0.11	0.19 ± 0.04
Experimental group 2	217.31 ± 11.28	0.47 ± 0.03	0.68 ± 0.09	5.59 ± 0.38	0.83 ± 0.03	0.21 ± 0.02
Experimental group 3	222.59 ± 10.18	0.51 ± 0.06	0.65 ± 0.10	5.61 ± 0.06	0.79 ± 0.08	0.21 ± 0.04

and at its completion in all groups were within the physiological values. Nevertheless, in the first and second experimental groups, an increase in leukocytes was noted by 1.5 times, in the third experimental group by 1.8 times, while in the control group, a decrease in this indicator was found by 1.1 times. Upon completion of the application of the pharmaceutical composition, an increase in lymphocytes was observed: in the control group by 1.2 times, in the first and third experimental groups, this increase was 1.4 and 1.5, respectively, the maximum increase of 1.7 times was observed in the second experimental group.

Since the experiment was carried out in two repetitions, the characteristic of this factor effect is distributed

Table 3. The average value of hematological parameters of blood of white laboratory rats (n = 40).

Indicators			Normal value	Control group		Experienced group 1		Experienced group 2		Experienced group 3	
				Factor "Term"							
				1	2	1	2	1	2	1	2
Erythrocyte, 10^{12}/l			5.3–10	9.76 ± 0.75	9.05 ± 0.29	10.11 ± 0.47	8.66 ± 0.58	9.72 ± 0.95	8.87 ± 0.24	10.36 ± 0.90	8.55 ± 0.81
Hemoglobin, g/l			140–180	161 ± 5.13	156 ± 3.42	155 ± 5.86	145 ± 4.16	152 ± 4.36	150 ± 3.67	164 ± 4.59	146 ± 4.21
Hematocrit, %			35–52	54.83 ± 7.02	51.68 ± 1.79	52.3 ± 2.67	47.54 ± 3.44	51.96 ± 6.26	48.77 ± 1.36*	55.65 ± 4.7	48.48 ± 4.02
Platelets, 10^9/l			500–1370	1258.00 ± 449.6	959.25 ± 76.36	754.00 ± 313.95	890.00 ± 71.61	722.00 ± 401.45	843.50 ± 47.51*	1084.00 ± 58.53	891.75 ± 112.23
ESR, mm/h			1–2	0.50 ± 0.01	1.00 ± 0.08	0.75 ± 0.35	1.20 ± 1.02	0.50 ± 0.25	1.00 ± 0.01	0.67 ± 0.29	1.20 ± 0.40
Leukocyte, 10^9/l			2.1–19.5	12.01 ± 3.80	10.64 ± 1.13	7.77 ± 0.73	11.96 ± 0.52	9.19 ± 1.39	14.50 ± 3.20	6.03 ± 0.83	10.76 ± 1.77
Lymphocyte, 10^9/l			2.0–14.1	6.04 ± 0.29	7.17 ± 1.33	5.42 ± 0.13	7.49 ± 1.02	5.51 ± 0.49	9.54 ± 1.61	3.86 ± 0.56	5.75 ± 0.47
Differential White Blood Cell Count, %	Neutrophil	juvenile	0–1	0	0	0	0	0	0	0	0
		rod nuclear cell	1–4	1.67 ± 0.56	2.00 ± 1.41	1.00 ± 0.05	2.00 ± 0.02	1.33 ± 0.58	2.50 ± 1.00	1.00 ± 0.01	3.50 ± 1.91
		segmentated cell	20–35	24.33 ± 5.69	18.75 ± 3.20	18.33 ± 7.50	29.50 ± 5.00**	29.67 ± 10.02	21.00 ± 6.98	27.00 ± 4.36	34.75 ± 3.40**
	Lymphocyte		55–75	69.33 ± 4.93	73.00 ± 8.76	76.33 ± 6.51	62.75 ± 7.18	64.67 ± 11.5	69.00 ± 5.60	68.33 ± 4.73	55.75 ± 5.05**
	Monocytes		1–5	3.00 ± 1.73	0	1.33 ± 1.15	0.50 ± 0.50	1.33 ± 1.03	1.00 ± 0.41	0.67 ± 1.15	0.75 ± 0.96
	Basophil		0–1	0.33 ± 0.28	0	0.33 ± 0.28	0	0.32 ± 0.10	0.50 ± 0.28	0.30 ± 0.05	0.50 ± 0.28
	Eosinophils		1–5	1.33 ± 1.15	6.25 ± 5.73	2.33 ± 0.58	5.25 ± 3.20	2.67 ± 1.15	6.00 ± 1.83	2.67 ± 1.15	4.75 ± 1.50

*-significant criteria at the level of $p < 0.05$; **- significant criteria at the level of $p < 0.01$.

along one axis. The latter was statistically significant in the randomization variant of the variance analysis ($p < 0.001$) and explained 14.9% of the total variability of the set of hematological parameters. According to the projections of vectors and indicator points on this axis, it can be seen that in the first period laboratory animals were characterized by higher concentrations of erythrocytes, hematocrit, and hemoglobin and by low values of ESR and the absolute number of lymphocytes. At the end of the experiment, the situation was reversed. We believe that changes in these indicators, depending on the "Duration" factor, may be associated with errors in the preanalytical stage, connected with stress factors and compensatory reactions that occur during the experiment period and during the blood sampling manipulation. Since these indicators were within the reference values for the animal species used in the experiment (McClure, 1999; Sorokina *et al*., 2019), it can be concluded that the developed composition has no effect on the indicators of the qualitative and quantitative composition of blood elements, as well as hematopoiesis. The "Dose" factor did not show significance for any indicator, which means that the effect was similar regardless of the dose chosen, the most significant effects were noted for leukocytes ($p = 0.097$) and the absolute number of lymphocytes ($p = 0.095$).

During the analysis of variance of the biochemical profile parameters of animals of the experimental groups, during the 30-day period of administration of the developed composition, indicators were found with minimal and statistically insignificant changes, since none of the main factors (duration, dose) were significant for aspartate aminotransferase and creatinine. When analyzing the indicators, significant differences were found in the "Duration" and "Dose" factors, in the

direction of a decrease in the indicators of total protein, globulins, and urea. On the contrary, an increase in the concentration in animal serum was observed in terms of alkaline phosphatase ($F_{(1/30)}$=23.67; $p < 0.001$). In the ordination diagram, this is demonstrated by placing the labels of these indicators (Creatinine, Urea) near the origin (Fig. 2).

Thus, at the end of the 30-day period of pharmaceutical composition use in animals of the control and experimental groups, a decrease in the total protein index was found to be 1.1 times compared with the values at the beginning of the experiment. When analyzing the "Dose" factor in laboratory rats of all experimental groups, a decrease in this indicator was observed compared with animals of the control group to 65.5, 65.8, and 64.0 g/l. A similar downward trend was recorded with respect to the globulin index: thus, the decrease set by the "Duration" factor in animals of the control group was 14.9%, in the first experimental group—14.0%, the maximum decrease was established in the second and third experimental groups Sorokina—16.9% and 18.9%, respectively. The decrease set by the "Dose" factor in animals of the experimental groups compared with the control group was 8.4%, 8.1%, and 11.7%, respectively. The minimum decrease in urea levels determined by the "Dose" factor was established in the first experimental group (4.40 mmol/l), in the second and third experimental groups, a decrease in the studied indicator was observed to the level of 3.50 and 2.63 mmol/l, respectively. A decrease in the urea index, considering the "Duration" factor, was established as 1.3 times in the control and third experimental groups, 1.4 times in the second experimental group; in the first experimental group, a slight increase in the urea index by 0.17 mmol/l was observed.

The dependence of the alkaline phosphatase index on the "Duration" regressor was established. While the maximum increase of this indicator to the level of 368 and 344 U/l, was observed in the second and third experimental groups, respectively, that received the composition at dosages of 750 and 1,000 mg/kg. Nevertheless, in these groups, several animals were identified that stand out from the total samples with a sufficiently high indicator level (Table 4).

Due to the established changes in the indicators described above in all groups of animals with respect to the "Duration" factor and the revealed variations in numerical data on a number of indicators, it is not possible to fully reflect the effect of the pharmaceutical composition on functional changes in the work of internal organs (Jacobson-Kram and Keller, 2006; Voitenko *et al*., 2020).

In this regard, for a more detailed assessment of the morphofunctional state of the organs of laboratory animals against the background of the use of a new pharmaceutical composition, we studied histological micro-preparations.

During microscopy, it was established that the morphological structure of the studied organs of rats of the control and first experimental groups corresponded to physiological parameters, no changes in the structural and functional units of the organs were revealed (Fig. 3A–D).

When studying histological preparations from animals of the second experimental group, the presence of reversible pathological processes of a compensatory nature was noted. Activation of malpighian bodies, β-cells of Langerhans islets, and Kupffer cells was observed, which can lead to excessive formation of lymphocytes in the spleen, increased insulin secretion in the pancreas, and a decrease in the phagocytic activity of hepatic stellate and endothelial cells, and as a result, the development of functional and immunological disorders in the organism (Fig. 4A–C). Vascular hyperemia was registered in the liver (Fig. 4D), which can contribute to metabolic disorders in tissues, the development of dystrophic and inflammatory processes, and as a result, the proliferation of connective tissue elements.

As a result of the response to the introduction of a foreign substance in the intestine, the symptoms of

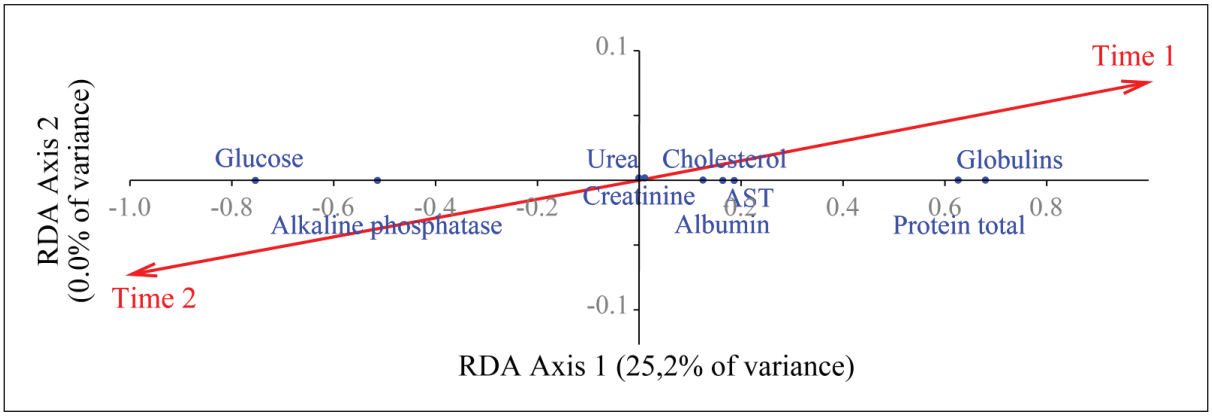

Fig. 2. Biochemical parameters in the space of the first two canonical axes of RDA, set by the "Duration" factors.

Table 4. Two-factor analysis of biochemical parameters of blood serum of white laboratory rats (n = 40).

Factor "duration"	Factor "dose"			Control group
	Experimental group 1 *Middle value [Min;Max]*	Experimental group 2 *Middle value [Min;Max]*	Experimental group 3 *Middle value [Min;Max]*	*Middle value [Min;Max]*
Total protein, g/l; Normal value: 61-82				
1	71.8 [67.2; 78.3]	75.1 [73.1; 76.9]	74.1 [69.3; 81.7]	74.0 [69.1; 79.0]
2	65.5 [62.0; 70.5]	65.8 [64.3; 67.3]	64.0 [61.4; 65.5]	70.2 [68.5; 72.2]
Albumin, g/l; Normal value: 27–38				
1	29.8 [29.4; 30.3]	31.4 [30.2; 32.4]	31.2 [29.3; 32.9]	27.7 [26.4; 28.6]
2	29.4 [28.8; 30.2]	29.6 [28.7; 30.3]	29.2 [28.4; 30.0]	30.8 [39.8; 32.0]
Globulins, g/l; Normal value: 32.8–42.5				
1	42.0 [37.8; 48.0]	43.6 [41.4; 45.0]	42.9 [38.5; 50.2]	46.3 [42.6; 50.4]
2	36.1 [33.2; 40.2]	36.2[35.6; 37.2]	34.8 [33.0; 36.2]	39.4 [38.7; 40.2]
AST, U/l; Normal value: 60–223				
1	132 [122; 140]	130 [119; 151]	120[102; 131	121 [92; 140]
2	112 [98; 125]	127[116; 141]	132 [121; 143]	111 [98; 123]
Glucose, mmol/l; Normal value: 3.27-11.56				
1	2.67 [2.60; 2.80]	2.97 [2.90; 3.10]	2.90 [2.50; 3.20]	3.47 [3.00; 4.40]
2	4.45 [4.00; 4.90]	4.48 [4.20; 4.75]	4.35 [4.05; 4.65]	3.75 [3.13; 4.15]
Creatinine, mmol/l; Normal value : 44.3–84.5				
1	38.2 [36.7; 39.0]	36.3 [28.9; 43.7]	41.6 [29.7; 59.7]	49.3 [36.3; 72.6]
2	43.6 [34.9; 58.9]	34.2 [28.1; 39.9]	41.3 [32.1; 50.6]	34.0 [20.3; 52.3]
Urea, mmol/l; Normal value: 3.10–7.77				
1	4.23 [3.90; 4.60]	4.90 [4.40; 5.40]	3.50 [2.70; 4.40]	5.70 [3.90; 7.50]
2	4.40 [3.28; 5.38]	3.50[2.90; 4.35]	2.63 [1.80 3.63]	4.43 [3.80 5.05]
Alkaline phosphatase, U/l; Normal value: 79–287				
1	161 [115; 184]	193 [137; 233]	221 [177; 253]	199 [134; 290]
2	252 [196; 334]	368 [289; 448]	344 [299; 390]	242 [218; 268]
Cholesterol, mmol/l; Normal value: 1.13–2.91				
1	1.80 [1.80; 1.80]	1.93 [1.80; 2.20]	2.50 [2.10; 3.00]	1.67 [1.10; 2.00]
2	1.65 [1.35; 1.95]	2.00 [1.78; 2.23]	1.68 [1.48; 1.85]	1.88 [1.60; 2.13]

enterocolitis were determined, manifested by catarrhal inflammation of the mucous membrane (Fig. 4E).

Deeper changes in the structure of the studied organs were registered in the third experimental group receiving the maximum dose of the composition. Thus, in the liver, against the background of moderate fatty degeneration of hepatocytes, the erasure of the beam-radial structure of the lobules was established, leading to disturbances from metabolic processes (Fig. 5A). In some organ areas, hepatocyte death was observed, associated with the presence of foci of necrosis, which causes a disorder of the detoxification function of the liver (Fig. 5B). A histological complex of focal glomerulitis was observed in the kidneys, characterized by proliferation within the renal glomerulus lobule. Mesangial cells and infiltrates located in peri-glomerular area were registered in the renal interstitial tissue (Fig. 5C). An increase in the urinary space of the glomeruli was established. In all the studied micro-preparations, an increase in inflammatory proliferation of the connective tissue stroma and blood vessel walls was noted. Eosinophilic catarrh is detected in the intestines (Fig. 5D).

Discussion

Modern methods of treating mastitis in cows are based on the use of broad-spectrum antibiotics (Alfonseca-Silv *et al.*, 2021; Dong *et al.*, 2022; Sigmund *et al.*, 2023). Resistance to these drugs is increasing, which makes them practically useless and requires the development of new approaches to both prevention and treatment of mastitis (Roy *et al.*, 2016; Han *et al.*,

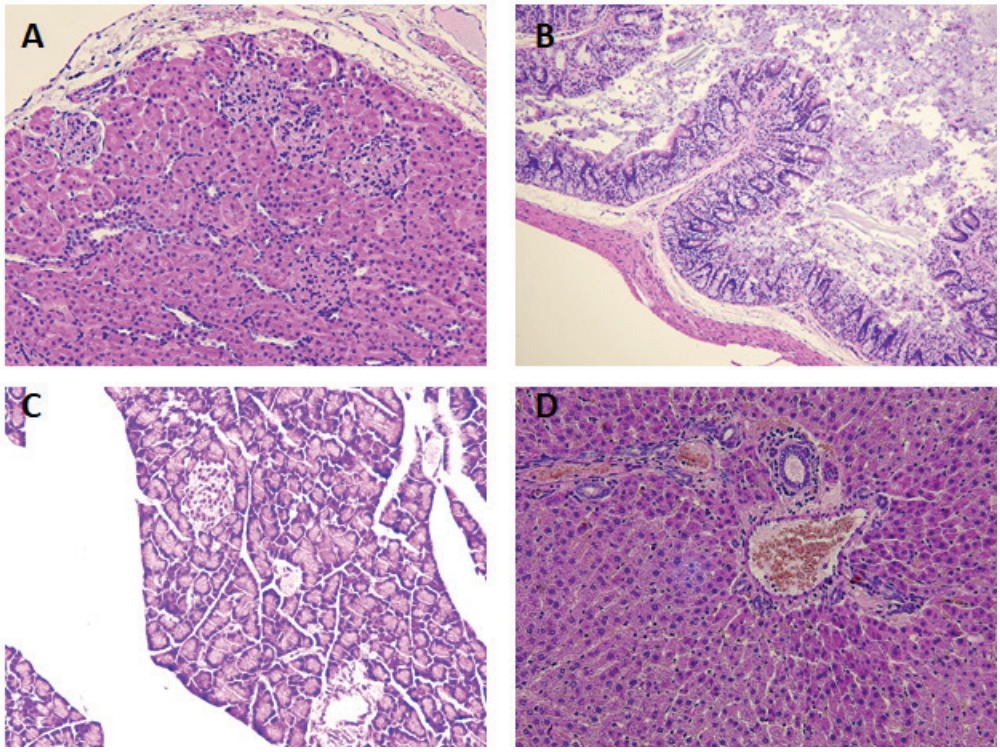

Fig. 3. Histological structure of organs of laboratory rats, experimental group 1. (A) Kidney without pathological process. (B) Intestines without pathological process. (C) Pancreatic islets without pathological process. (D) Moderate blood supply to the liver vessels.

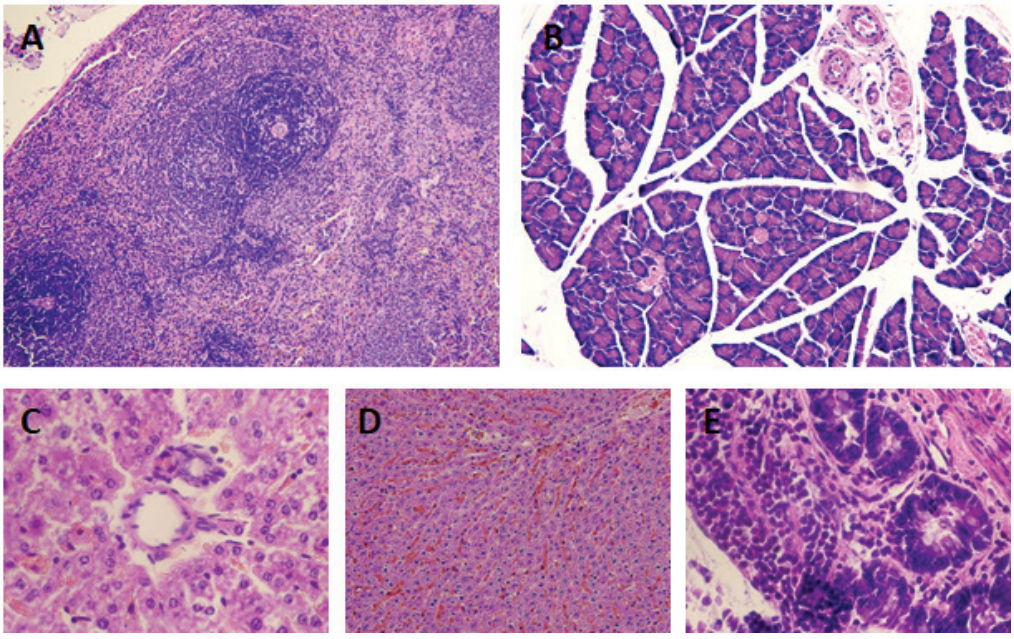

Fig. 4. Histological structure of organs of laboratory rats, experimental group 2. (A) Additional lymphoid follicles in the spleen. (B) Activation of pancreatic islets cells. (C) Activation of Kupffer cells (stellate reticuloendotheliocytes of the liver). (D) Hyperemia of the liver vessels. (E) Catarrh of the intestines.

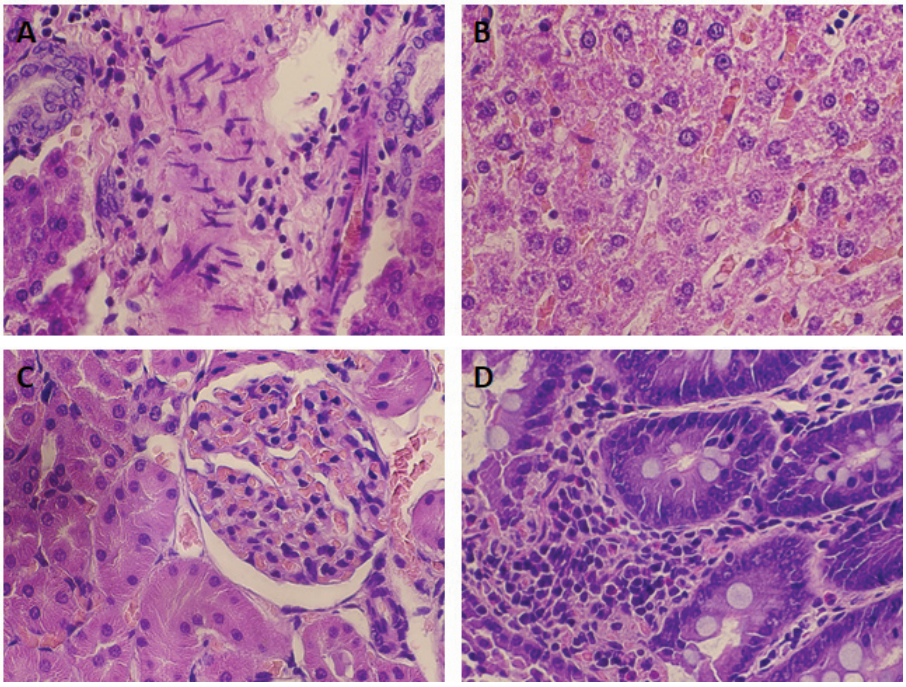

Fig. 5. Histological structure of organs of laboratory rats, experimental group 3. (A) Foci of cirrhosis of the liver. (B) Foci of necrosis in the liver. (C) Periglomerular infiltrates in the kidneys. (D) Infiltration of intestinal walls with eosinophils.

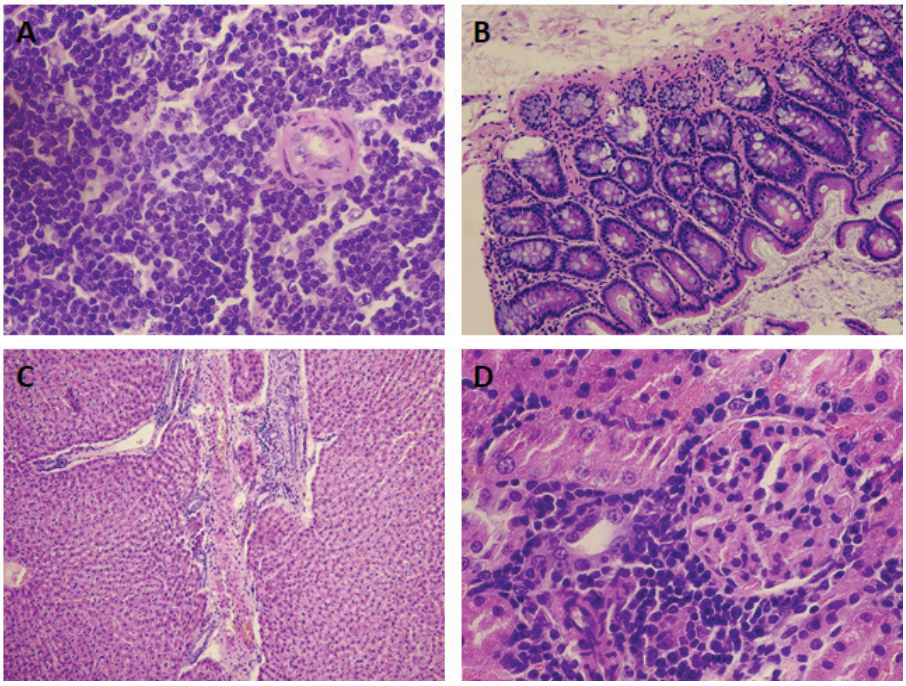

Fig. 6. Pathological changes in organs of laboratory rats, experimental group 3. (A) Hyperplasia of splenic follicles. (B) Hypersecretion in the intestines. (C) Foci of cirrhosis in the liver. (D) Periglomerular infiltrates in the kidneys.

2022; Vollenweider *et al.*, 2023). In the last decade, the potential of bacteriocins as next-generation therapeutics against drug-resistant bacteria has been explored (Pircalabioru *et al.*, 2021; Flynn J. *et al.*, 2022; Khan *et al.*, 2023). Bacteriocins from lactic acid bacteria are being tested as agents against bacterial and

viral infections; which can inhibit biofilm synthesis (Fernandes and Jobby, 2022; Heinzinger *et al.*, 2023; Daba and Elkhateeb, 2024). A number of studies have established high antimicrobial activity of bacteriocin-nisin against several species of *staphylococci*, including *S. aureus*, *Staphylococcus epidermidis, Staphylococcus haemolyticus,* and *Staphylococcus saprophyticus* (Cao *et al.*, 2007; Bennett *et al.*, 2021; Nguyen-Tra Le *et al.*, 2022), as well as methicillin-resistant *S. aureus* with multiple resistance to many antibiotics (Wang *et al.*, 2023). There are studies on clinical populations of Streptococcus agalactiae isolates that demonstrate levels of sensitivity to nisin (Hayes *et al.*, 2019). In studies by Pérez–Ibarreche *et al.* (2021), nisin was used to effectively reduce biofilm formation in Streptococcus uberis strains that cause bovine mastitis. The use of bacteriocin-nisin, which has antimicrobial activity against the main pathogens of mastitis, opens up the possibility of its use as an alternative to antibiotics (Cao *et al.*, 2007; Kitazaki *et al.*, 2017; Bennett *et al.*, 2022). In some studies, the high antimicrobial activity of bacteriocin-nisin against *S. aureus* was established (Cao *et al.*, 2007; Bennett S. *et al.*, 2021; Nguyen-Tra Le *et al.*, 2022). Our research data confirm the results of Cao *et al.* (2007), Bennett S. *et al.* (2021), and Nguyen-Tra Le *et al.* (2022) on the expediency of including nisin in mastitis treatment regimens. However, according to some literature sources, it has been established that the use of bacteriocins of any class can lead to the development of resistance (Draper *et al.*, 2015; Bastos *et al.*, 2015). There are studies that have shown that the best option for using bacteriocins in the treatment of infectious diseases may be to combine them with other substances that exhibit antimicrobial activity (Mathur *et al.*, 2017). Therefore, we have selected silicon glycerolates and boron bisglycerolates. In humans and animals, silicon as a chemical compound helps to remove toxic substances from cells, strengthens the protective functions of tissues, and helps reduce inflammation in cells, which determines its choice as a penetrant. The ability of silicon to concentrate in certain organs is known, as well as to stimulate the growth of connective and epithelial tissue (Boguszewska-Czubara, 2011; Keith, 2013; Farooq and Dietz, 2015; Vapirov *et al.*, 2017; Sunita *et al.*, 2022). Boron is a conditionally essential trace element, its compounds have an anti-inflammatory effect, and normalize metabolic processes in cells and tissues (Khaliq *et al.*, 2018; Abdelnour *et al.*, 2018). The compounds have a high transcutaneous conductivity of drugs, which makes it possible to reduce the dose of active ingredients while maintaining high efficacy and also have a reparative and regenerating effect, which makes it possible to use various therapeutic regimens (Chupakhin *et al.*, 2017). In this study, experiments were conducted *in vitro* and *in vivo* on a pharmacological composition based on nisin, with the addition of silicon glycerolates and boron bisglycerolates. Our studies of the developed composition have shown the presence of antimicrobial activity against *S. aureus*, *E. faecalis*, *Enterococcus faecium*, *E. coli*, and Enterobacter spp. Since the discovery of bacteriocins, researchers have been primarily focused on determining their antimicrobial activity. However, for the clinical use of bacteriocins as antimicrobials, it is necessary to study their clinical efficacy, bioavailability, and safety. Safety studies have shown that bacteriocins have low toxicity and hemolytic activity (Soltani *et al.*, 2021; Guryanova, 2023). For example, the cytotoxicity of nisin is several times higher than the minimum inhibitory concentration (Shin *et al.*, 2016; Thanjavur *et al.*, 2022). Conducting studies on chronic toxicity made it possible to establish the effect of the pharmaceutical composition on the body of laboratory rats to establish a safe dose for further research on highly productive cows. Thus, at a dose of the developed composition of 500 mg/kg, no processes dangerous to the vital activity of animals were detected, and the structure of the organs corresponded to anatomical parameters. A study of the organs of animals receiving the developed composition at a dose of 750 mg/kg revealed reversible compensatory changes, e.g., in the liver and spleen, indicating regeneration processes and protective and adaptive reactions of the body. In the third experimental group, where the dose of the studied composition was 1,000 mg/kg, a number of pathological changes were registered in the intestines, liver, kidneys, and spleen of laboratory rats (Fig. 6A–C), the processes were regarded as reversible pathological, on the verge of irreversible—changes characteristic of circulatory disorders in the vessels of the microcirculatory bed in the liver and kidneys, which can lead to decompensation, as a result of which the dose is characterized as critical. Our results coincide with previous studies; compared with other drugs, bacteriocins, including nizin have a number of advantages, such as antimicrobial activity (Bennett S. *et al.*, 2021; Nguyen-Tra Le *et al.*, 2022, Wang *et al.*, 2023) and low toxicity (Soltani *et al.*, 2021; Guryanova, 2023).

Conclusion

An *in vitro* study on cell cultures showed that the pharmacological composition has high antimicrobial activity against isolates from the mammary gland secretion of cows with mastitis such as: *S. aureus, E. faecalis, Enterococcus faecium, E. coli,* and *Enterobacter spp*. The results obtained during *in vivo* experiments on laboratory animals indicate that the developed composition belongs to the IV class of substances "low-hazard substances" and can be recommended for clinical trials on highly productive animals. The data obtained during histological examination make it possible to choose the safest dose of a pharmacological composition of no more than 500 mg/kg. When using a dose of 750 mg/kg, changes in blood metabolic parameters were established, the

studied histological preparations indicated the presence of reversible compensatory processes, as a result of which it is necessary to use this dosage with caution in animals with severe liver and kidney dysfunction. When using a critical dose of 1000 mg/kg, deeper changes in organs were observed, established during histological examination.

Acknowledgment

The authors express Ural Scientific Research Veterinary Institute gratitude for the opportunity to conduct the study. The authors are grateful to the candidate of biological sciences Nokhrin D.Yu. for assistance with statistical analysis.

Conflict of interest

All authors declare that there is no conflict of interest.

Authors' contributions

The concept and design of the study, collection and processing of material, statistical processing: Maria Isakova. Conducting research: Olga Oparina, Alexander Belousov, Yana Lysova.

Funding

The study was carried out with the support of grant of the Russian Science Foundation No. 22-76-00009.

Data availability

All data supporting the findings of this study are available within the manuscript.

References

Abdelnour, S.A., Abd El-Hack, M.E., Swelum, A.A., Perillo, A. and Losacco, C. 2018.The vital roles of boron in animal health and production: a comprehensive review. J. Trace Elem. Med. Biol. 50, 296–304.

Alfonseca-Silva, E., Cruz-Villa, J.C., Gutiérrez, L. and Sumano, H. 2021. Successful treatment of recurrent subclinical mastitis in cows caused by enrofloxacin resistant bacteria by means of the sequential intramammary infusion of enrofloxacin HCl-2H(2)O and ceftiofur HCl: a clinical trial. J. Vet. Sci. 22(6), e78.

Bastos, M.C., Coelho, M.L. and Santos, O.C. 2015. Resistance to bacteriocins produced by Gram-positive bacteria. Microbiology (Reading). 161(Pt 4), 683–700.

Bennett, S., Said, L.B., Lacasse, P., Malouin, F. and Fliss, I. 2021. Susceptibility to Nisin, bactofencin, pediocin and reuterin of multidrug resistant *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* Causing Bovine Mastitis. Antibiotics (Basel). 10(11), 1418.

Bennett, S., Fliss, I., Ben Said, L., Malouin, F. and Lacasse, P. 2022. Efficacy of bacteriocin-based formula for reducing *Staphylococci, Streptococci*, and total bacterial counts on teat skin of dairy cows. J. Dairy Sci. 105(5), 4498–4507.

Bhardwaj, S., Mehra, P., Dhanjal, D.S., Sharma, P., Sharma, V., Singh, R., Nepovimova, E., Chopra, C. and Kuča, K. 2022. Antibiotics and Antibiotic resistance- flipsides of the same Coin. Curr. Pharm. 28(28), 2312–2329.

Boguszewska-Czubara, A. 2011. Silicon in medicine and therapy. J. Elementol. 16(3), 489–497.

Cao, L.T., Wu, J.Q., Xie, F., Hu, S.H. and Mo, Y. 2007. Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. J. Dairy Sci. 90(8), 3980–3985.

Chupakhin, O.N., Khonina, T.G., Kungurov, N.V., Zilberberg, N.V., Evstigneeva, N.P., Kohan, M.M., Polishchuk, A.I., Shadrina, E.V., Larchenko, E.Yu., Larionov, L.P. and Karabanalov, M.S. 2017. Silicon boron containing glycerohydrogel, which has wound healing, regenerating and antimicrobial activity. Proc Acad Sci. The series is chemical 3, 558–563.

Collado, R., Prenafeta, A., González-González, L., Pérez-Pons, J.A. and Sitjà, M. 2016. Probing vaccine antigens against bovine mastitis caused by *Streptococcus uberis*. Vaccine. 34(33), 3848–3854.

Daba, G.M. and Elkhateeb, W.A. 2024. Ribosomally synthesized bacteriocins of lactic acid bacteria: Simplicity yet having wide potentials—a review. Int. J. Biol. Macromol. 256(Pt 1), 128325.

Dong, L., Meng, L., Liu, H., Wu, H., Schroyen, M., Zheng, N. and Wang, J. 2022. Effect of cephalosporin treatment on the microbiota and antibiotic resistance genes in feces of dairy cows with clinical mastitis. Antibiotics (Basel). 11(1), 117.

Draper, L.A., Cotter, P.D., Hill, C. and Ross, R.P. 2015. Lantibiotic resistance. Microbiol Mol. Biol. Rev. 79(2), 171–191.

Dzayee, S.A., Khudhur, P.K., Mahmood, A., Markov, A., Maseleno, A. and Ebrahimpour Gorji, A. 2022. Computational design of a new multi-epitope vaccine using immunoinformatics approach against mastitis disease. Anim. Biotechnol. 33(6), 1359–1370.

El-Sayed, A. and Kamel, M. 2021. Bovine mastitis prevention and control in the post-antibiotic era. Trop. Anim. Health Prod. 53(2), 236.

Esener, N., Maciel-Guerra, A., Giebel, K., Lea, D., Green, M.J., Bradley, A.J. and Dottorini, T. 2021. Mass spectrometry and machine learning for the accurate diagnosis of benzylpenicillin and multidrug resistance of *Staphylococcus aureus* in bovine mastitis. PLoS Comput. Biol. 17(6), e1009108.

Farooq, M.A. and Dietz, K.-J. 2015. Silicon as versatile player in plant and human biology: overlooked and poorly understood. Front. Plant Sci. 6, 1–14.

Fernandes, A. and Jobby, R. 2022. Bacteriocins from lactic acid bacteria and their potential clinical applications. Appl. Biochem. Biotechnol. 194(10), 4377–4399.

Flynn, J., Ryan, A. and Hudson, S.P. 2022. Synergistic antimicrobial interactions of nisin A with

biopolymers and solubilising agents for oral drug delivery. Eur. J. Pharm. Biopharm. 171, 29–38.
Guryanova, S.V. 2023. Immunomodulation, bioavailability and safety of bacteriocins. immunomodulation, bioavailability and safety of bacteriocins. Life 13(7), 1521.
Hammer, Ø., Harper, D.A.T. and Ryan, P.D. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica. 1, 1–9.
Han, G., Zhang, B., Lu, B., Luo, Z., Zhang, J., Wang, Y., Luo, Y., Yang, Z., Shen, L., Yu, S., Cao, S. and Yao, X. 2022. Molecular typing and prevalence of antibiotic resistance and virulence genes in *Streptococcus agalactiae* isolated from Chinese dairy cows with clinical mastitis. PLoS One. 17(5), e0268262.
Hayes, K., Field, D., Hill, C., O'Halloran, F. and Cotter, L.J. 2019. A novel bioengineered derivative of nisin displays enhanced antimicrobial activity against clinical *Streptococcus agalactiae* isolates. Glob. Antimicrob. Resist. 19, 14–21.
Heinzinger, L.R., Pugh, A.R., Wagner, J.A. and Otto, M. 2023. Evaluating the translational potential of bacteriocins as an alternative treatment for *Staphylococcus aureus* infections in animals and humans. Antibiotics (Basel). 12(8), 1256.
Huemer, M., Mairpady Shambat, S., Brugger, S.D. and Zinkernagel, A.S. 2020. Antibiotic resistance and persistence-Implications for human health and treatment perspectives. EMBO Rep. 21(12), e51034.
Isakova, M.N., Lysova, Ya. Yu. and Khonina, T.G. 2023. Development of new medicinal compositions based on bacteriocin-nisin, with subsequent assessment of their antimicrobial activity. Vet. Med. 7, 43–49.
Jacobson-Kram, D. and Keller, K.A. 2006. Toxicological testing handbook: principles, applications, and data interpretation. USA: Informa Healthcare.
Keith, R.M. 2013. Silicon: the health benefits of a metalloid. Met Ions Life Sci. 13:451–73.
Khabriev R.U. 2005. Guide to experimental (preclinical) study of new pharmacological substances "Publishing House "Medicine", pp: 832.
Khaliq, H., Juming, Z. and Ke-Mei, P. 2018. The physiological role boron on health. Biol. Trace Elem. Res. 186(1), 31–51.
Khan, F., Singh, P., Joshi, A.S., Tabassum, N., Jeong, G.J., Bamunuarachchi, N.I., Mijakovic, I. and Kim, Y.M. 2023. Multiple potential strategies for the application of nisin and derivatives. Crit. Rev. Microbiol. 49(5), 628–657.
Khonina, T.G., Kungurov, N.V., Zilberberg, N.V., Evstigneeva, N.P., Kokhan, M.M., Polishchuk, A.I., Shadrina, E.V., Nikitina, E.Yu., Permikin, V.V. and Chupakhin, O.N. 2020. Structural features and antimicrobial activity of hydrogels obtained by the sol–gel method from silicon, zinc, and boron glycerolates. J. Sol-Gel Sci. Technol. 3, 682–692.
Kitazaki, K., Koga, S., Nagatoshi, K., Kuwano, K., Zendo, T., Nakayama, J., Sonomoto, K., Ano, H. and Katamoto, H. 2017. *In vitro* synergistic activities of cefazolin and nisin A against mastitis pathogens. J. Vet. Med. Sci. 79(9), 1472–1479.
Kober, A., Saha, S., Islam, M.A., Rajoka, M.R, Fukuyama, K., Aso, H., Villena, J. and Kitazawa, H. 2022. Immunomodulatory Effects of probiotics: a novel preventive approach for the control of bovine mastitis. Microorganisms. 10(11), 2255.
Krömker, V. and Leimbach, S. 2017. Mastitis treatment-reduction in antibiotic usage in dairy cows. Reprod. Domest. Anim. 3, 21–29.
Kukeyeva, A., Abdrakhmanov, T., Yeszhanova, G., Bakisheva, Z. and Kemeshov, Z. 2023. The use of a homeopathic preparation in the treatment of subclinical form of mastitis in cows. Open Vet. J. 13(8), 991–1002.Lopes, T.S., Fontoura, P.S., Oliveira, A., Rizzo, F.A., Silveira, S. and Streck, A.F. 2020. Use of plant extracts and essential oils in the control of bovine mastitis. Res. Vet. Sci. 131, 186–193.
Mathur, H., Des, F., Rea, M.C., Cotter, P.D., Hill, C. and Ross, R.P. 2017. Bacteriocin-antimicrobial synergy: a medical and food perspective. Front. Microbiol. 8, 1205.
McClure, D.E. 1999. Clinical pathology and sample collection in the laboratory rodent. Vet. Clin. North Am. Exot. Anim. Pract. 2(3), 565–569.
Nguyen-Tra Le, M., Kawada-Matsuo, M. and Komatsuzawa, H. 2022. Efficiency of antimicrobial peptides against multidrug-resistant *Staphylococcal* Pathogens. Front. Microbiol. 13, 930629.
Oliver, S.P., Murinda, S.E. and Jayarao, B.M. 2011. Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. Foodborne Pathog. Dis. 8(3), 337–355.
Penry, J.F. 2018 Mastitis control in automatic milking systems. Vet. Clin. North Am. Food Anim. Pract. 34(3), 439–456.
Pérez-Ibarreche, M., Field, D., Ross, R.P. and Hill, C. 2021. A bioengineered nisin derivative to control *Streptococcus uberis* Biofilms. Appl. Environ. Microbiol. 87(16), e0039121.
Pircalabioru, G.G., Popa, L.I., Marutescu, L., Gheorghe, I., Popa, M., Czobor Barbu, I., Cristescu, R. and Chifiriuc, M.C. 2021. Bacteriocins in the Era of antibiotic resistance: rising to the challenge. Pharmaceutics. 13(2), 196.
Raheel, I., Mohammed, A.N. and Mohamed, A.A. 2023. The efficacy of bacteriocins against biofilm-producing bacteria causing bovine clinical mastitis in dairy farms: a new strategy. Curr. Microbiol. 80(7), 229.
Rowe, S.M., Vasquez, A.K., Godden, S.M., Nydam, D.V., Royster, E., Timmerman, J. and Boyle, M. 2021. Evaluation of four predictive algorithms for

intramammary infection status in late-lactation cows. J. Dairy Sci. 104(10), 11035–11046.

Roy, S., Riley, M.A. and Crabb, J.H. 2016. Treating bovine mastitis with nisin: a model for the use of protein antimicrobials in veterinary medicine. In book: The Bacteriocins: Current Knowledge and Future Prospects. Poole, UK: Caister Academic Press, pp: 127–140.

Ruegg, P.L. 2018. Making antibiotic treatment decisions for clinical mastitis. Vet. Clin. North Am. Food Anim. Pract. 34(3), 413–425.

Sharun, K., Dhama, K., Tiwari, R., Gugjoo, M.B., Iqbal Yatoo, M., Patel, S.K., Pathak, M., Karthik, K., Khurana, S.K., Singh, R., Puvvala, B., Amarpal Singh, R., Singh, K.P. and Chaicumpa, W. 2021. Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review. Vet. Q. 41(1), 107–136.

Shin, J.M., Gwak, J.W., Kamarajan, P., Fenno, J.C., Rickard, A.H. and Kapila, Y.L. 2016. Biomedical applications of nisin. J. Appl. Microbiol. 120(6), 1449–1465.

Sigmund, M., Egger-Danner, C., Firth, C.L., Obritzhauser, W., Roch, F.F., Conrady, B. and Wittek, T. 2023.The effect of antibiotic versus no treatment at dry-off on udder health and milk yield in subsequent lactation: a retrospective analysis of Austrian health recording data from dairy herds. J. Dairy Sci. 106(1), 452–461.

Soltani, S., Hammami, R., Cotter, P.D., Rebuffat, S., Said, L.B., Gaudreau, H., Bédard, F., Biron, E., Drider, D. and Fliss, I. 2021. Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations. FEMS Microbiol. Rev. 45(1), fuaa039.

Sorokina, A.V., Alekseeva, S.V., Eremina, N.V. and Durnev, A.D. 2019. Experience in conducting clinical and laboratory studies in preclinical assessment of drug safety (part 1: hematological studies). Bull Sci Center Expertise Med Prod. 9(3), 97–206.

Sunita, A., Sonal, V., Robina, A., Pankaj, K. and Anjana, V. 2022. Surprising benefits of silicon in plants and human be. World J. Biol. Pharm. Health Sci. 12(3), 359–364.

Thanjavur, N., Sangubotla, R., Lakshmi, B.A., Rayi, R., Mekala, C.D., Reddy, A.S. and Viswanath, B. 2022. Evaluating the antimicrobial and apoptogenic properties of bacteriocin (nisin) produced by *Lactococcus lactis*. Process. Biochem. 122, 76–86.

Touza-Otero, L., Landin, M. and Diaz-Rodriguez, P. 2024. Fighting antibiotic resistance in the local management of bovine mastitis. Biomed. Pharmacother. 170, 115967.

Vapirov, V.V., Feoktistov, V.M., Venskovich, A.A. and Vapirova, N.V. 2017. On the behavior of silicon and its biological role in nature Scientific notes of Petrozavodsk State University. Physico-Chem Biol. 2(163), 95–102.

Vázquez, R., García, E. and García, P. 2018. Phage lysins for fighting bacterial respiratory infections: a new generation of antimicrobials. Front. Immunol. Sec. Microbial. Immunol. 9, 1–12.

Voitenko, N.G., Makarova, M.N. and Zueva, A.A. 2020. Variability of biochemical blood parameters and establishment of reference intervals in preclinical studies. Post 1: Rats. Lab. Anim. Sci. Res. 1, 47–53.

Vollenweider, A., Corti, S., Hochreutener, M., Biner, B., Stephan, R. and Bleul, U. 2023. Mastitis pathogens and antibiotic resistance in beef cows in Switzerland. Schweiz. Arch. Tierheilkd. 165(1), 39–51.

Wang, J., Ma, X., Li, J., Shi, L., Liu, L., Hou, X., Jiang, S., Li, P., Lv, J., Han, L., Cheng, Y. and Han, B. 2023. The synergistic antimicrobial effect and mechanism of nisin and oxacillin against methicillin-resistant *Staphylococcus aureus*. Int. J. Mol. Sci. 24(7), 6697.

Yoshioka, K. 2002. KyPlot—a user-oriented tool for statistical data analysis and visualization. Computational Statistics. 3, 425–437.

Zduńczyk, S. and Janowski, T. 2020. Bacteriophages and associated endolysins in therapy and prevention of mastitis and metritis in cows: Current knowledge. Anim. Reprod. Sci. 218, 106504.

Zgheib, H., Drider, D. and Belguesmia, Y. 2020. Broadening and enhancing bacteriocins activities by association with bioactive substances. Int. J. Environ. Res. Public Health. 17(21), 7835.