



Influence of liposomal thiosulfonate drug on the blood parameters of cows suffering catarrhal mastitis

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Diseases of the mammary gland in cows are of multi-factor nature, which significantly complicates their control and treatment. One of the factors influencing development of mastitis is bacterial microflora. Because of limited use of antibiotics, development of novel alternative antimicrobial drugs is of great relevance. Therefore, the objective of the study was identifying the effect of an ethyl-thiosulfanilate-based liposomal drug on the hematological and biochemical parameters of blood of the cows suffering catarrhal mastitis. The animals were three times intramuscularly injected with the liposomal drug in the dose of 0.04 mL/kg of body weight. Blood from the cows was taken from the jugular vein on the day prior to administration of the drug, and also on the 3rd and 7th days after its application. During the bacterial study of the secretion of udder of sick cows, we collected 51 bacterial isolates, in which dominated *Staphylococcus aureus* (27.5%) and *Streptococcus* spp. (21.6%). In blood of the sick cows, we found higher number of leukocytes, increases in the concentrations of circulating immune complexes, lipid hydroperoxides, and ketone derivatives of oxidative modification of proteins. At the same time, we observed decreases in the number of lymphocytes and concentration of protein and the bactericidal activity of blood serum. Administration of the complex liposomal drug had a normalizing effect on the analyzed parameters of the cows. This was evidenced by 9.4% decrease in the total number of leukocytes, 18.5% decrease in the content of circulating immune complexes, 9.3% increase in lymphocytes, and 13.6% increase in the level of total protein. In the sick cows, the drug enhanced the bactericidal (by 35.4%) and lysozymic (by 36.3%) activities of blood serum, glutathione peroxidase activity, and increased the content of reduced glutathione. On the seventh day after injecting the drug into blood of the cows, we saw decrease in the level of intermediate and end products of lipid peroxidation and derivatives of oxidative modification of proteins. Therefore, the complex ethyl-thiosulfanilate-based liposomal drug promoted reduction of metabolic homeostasis of the organism, had positive effect on the activity of natural defense mechanisms in the organism and can be recommended for the treatment of cows with catarrhal mastitis.

Keywords: cattle; udder inflammation; leukocyte profile; resistance; products of lipid peroxidation.

Introduction

Currently, one of the priority directions of innovative development of animal farming is increasing the productivity of animals by introduction of modern methods of livestock management. Long stay of animals in closed premises (non-grazing maintenance), their high concentration in limited industrial areas, mechanization of productive processes, and various stress factors significantly decrease the level of natural resistance of animals to diseases. Among the commonest diseases observed in livestock against the background of the aforesaid negative factors, we should note a disease of the reproductive organs and the mammary gland, or mastitis (Wani et al., 2022). Numerous literature data suggest that mastitis in cows is the cause of substantial economic losses in the dairy sphere. In particular, the total global loss is assessed at four-five billion euros per year (Streefland & De Rooij, 2021, Wani et al., 2022). Mastitis-inflicted economic losses are first of all caused from decrease in production of milk, cost of treatment, and culling of animals (Hogeveen et al., 2011; Abdulsae & Kurtu, 2018; Romero et al., 2018). According to the data of many

authors, mastitis of cows affects 10% to 60% of a herd and is diagnosed in many countries of the world, including high-technology farms with good practices (Kurtyak et al., 2015; Gussmann et al., 2019).

Mastitis is a quite complex disease of lactating and dry cows, characterized by inflammation of the udder tissue as a result of physical damage, chemical irritation, or infection caused by pathogenic organisms. Complex etiology and multi-factor nature complicate the control of this disease.

Depending on noticeable features of a mammary-gland inflammation, there are designated clinical and subclinical mastitides. The latter causes no visible changes in the udder and quality of milk (Bagri et al., 2018), but notably affects its composition, mainly through increase in somatic cells (Shkromada et al., 2019). It has to be noted that the number of cows suffering subclinical mastitis is 3–5 times greater than the number of animals with the clinical forms of mastitis. A negative impact of mastitis is that even after a cow is healed, the function of its mammary gland does not always recover to its initial level and very often ends with atrophy and induration of affected quarters (Baidevlyatov & Baidevlyatova, 2019). High mastitis morbidity among productive dairy cows is due to a number

of factors. Usually, it occurs as an immune reaction to a bacterial invasion of the streak canal following impairments in protein and mineral metabolisms in the animal organism, decrease in non-specificity of the body's resistibility, and can also emerge as a result of chemical, mechanical, or thermal damage to the udder, for example, if the technology of machine milking is violated. Inflammatory processes can be promoted by oxidative stress with excessive accumulation of reactive oxygen species in the tissues of mammary gland, resulting from calving, early lactation, dysbalance in the energy value of diet (Sordillo, 2016; Shahid et al., 2017; Matukha et al., 2018; Sordillo, 2018; Suprovych et al., 2018).

A key role in the onset of udder inflammation is played by bacterial microflora. The etiological range of mastitis pathogens contains over 140 species and subspecies of potential pathogens that can cause mammary-gland inflammation. The main representatives of the conditionally pathogenic microflora isolated during cow mastitis are *Staphylococcus aureus* (Kukhtyn et al., 2021), *Streptococcus agalactiae*, *Streptococcus uberis*, coliform bacteria *Escherichia coli* and some other, less common mastitis pathogens (Kurtyak et al., 2015; Mazurenko et al., 2020; Abdi et al., 2021).

As of now, there are several strategies of controlling and preventing mastitis of dairy animals, such as treatment with antibiotics or vaccination. However, most of them are either insufficiently acceptable or have a poor effect. For example, usage of antibiotics is restricted by the current requirements on quality of milk, which prohibit presence of antibiotic residues in it, because such milk can have a negative effect on human health, causing antibiotic resistance. Moreover, when treating livestock, antibiotics and products of their breakdown were found in manure, which is further distributed on soil, which in turn has an effect on diversity of soil bacteria and causes an unfavourable effect on the environment (Streefland & De Rooij, 2021).

It has to be noted that in many cases, course of the disease is complicated by inability of an animal to provide an effective defensive response to mastitis-cause microorganisms and to produce adequate reacting mechanism. Important negative factors enabling cow-udder bacterial infections survive are epithelial adhesion, which helps pathogenic organisms survive in the organism because of attachment to epithelial cells, phagocytosis of bacteria, and their ability to produce biofilm. This is accompanied by substantial genetic and further physiological changes in microorganisms, accompanied by loss of sensitivity to practically all classes of antibiotics (Streefland & De Rooij, 2021).

Therefore, because of the emergence and growing number of antibiotic-resistant pathogens, treatment of mastitis becomes more complicated and it remains one of the most harmful diseases of dairy cattle, which requires search for novel efficient therapeutic drugs with broad spectrum, high efficacy and action range, which provide timely treatment of a herd.

In this aspect of studies, an alternative to antibiotics is natural sulfonates and thiosulfates, isolated from plants of the *Allium* genus. Of those, the latter were the most promising for a practical application, in particular propyl propane thiosulfonate (PTSO; Fig. 1).

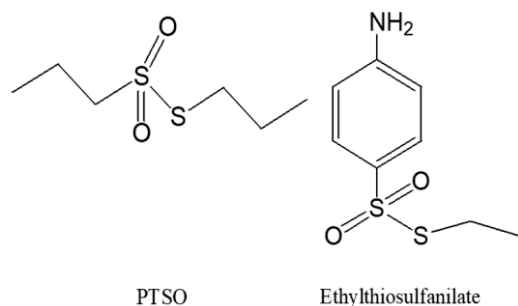


Fig. 1. Formulas of thiosulfonate compounds

Of compounds isolated from garlic or onion, PTSO gives the least unpleasant smell in milk (Abad et al., 2017; Streefland & De Rooij, 2021).

Propyl propane thiosulfonate exerts antimicrobial, antioxidant, metabolic, probiotic, anti-inflammatory and anti-tumour properties (Muscarella et al., 2007; Llana-Ruiz-Cabello et al., 2015; Zilbeyaz et al., 2021). At the same time however, it is a volatile substance, which complicates its broad

practical application. Therefore, efforts should be made to find a no less effective, but more convenient-to-use substance among synthetic thiosulfonates, which are also low-toxic and have a broad spectrum of biological action (Mampuy et al., 2019). A preliminary analysis of the literature data pertaining to the biological (antibacterial, antifungal, antioxidant, antiviral, anti-platelet, antiparasitic) activity and safety of synthetic thiosulfonates (Halenova et al., 2015; Oriabinska et al., 2017; Lubenets et al., 2019; Dmitryjuk et al., 2020; Kotyk et al., 2020; Liubas et al., 2022) suggests that a promising substance for treating mastitis is ethyl thiosulfanilate.

Therefore, the objective of our studies was to identify the influence of the developed liposomal drug based on ethyl thiosulfanilate on the hematological and biochemical parameters of blood and activity of natural factors of protection in the cows suffering catarrhal mastitis.

Materials and methods

The studies were carried out at one of the private enterprises in Kharkiv Oblast. All procedures with the animals were performed according to the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Experiments on Animals, adopted by the 1st National Congress of Bioethics (Kyiv, 2001), the Law of Ukraine on Protection of Animals from Abuse № 3447-IV (2006). The protocol of studies was approved by the Commission of Bioethical Expertise and was allowed in the higher-education institution the Podillia State University (Protocol No. 1 as of 02/08/2022,) and by the Main Management of State Food Service in Khmelnytskyi Oblast (Permit for Conducting Scientific Experiments, Testing using Experimental Animals as of 03/01/2022).

To study efficacy of the drug, we formed two groups of cows that had 2–3 lactation: control and experimental, 5–7 animals in each. The control group comprised clinically healthy animals, and the experimental was formed of randomly selected cows with catarrhal form of mastitis.

For the bacteriological analysis and identification of the main bacterial pathogens of mastitis, the biological material (secretion of an affected quarter of the udder) was collected in aseptic conditions into sterile bottles with screw caps. For cultivation and isolation of microorganisms, we used the following media: the BD Baird-Parker Agar (HiMedia, India) and salt-egg-yolk agar for staphylococci; 5% blood-salt agar and the Edward's agar (Biolife Italiana S.r.l.) for streptococci; the Endo agar and Levine agar (Farmaktyv) for enterobacteria (*Escherichia*, *Klebsiella* and other); and the *Pseudomonas* Isolation Agar (HiMedia) for *Pseudomonas aeruginosa*. The microorganisms were cultivated at 37 °C, and the results were evaluated after 24–48 h. Pure cultures were identified according to morphological, tinctorial, cultural, and biochemical properties using the Bergey's Manual of Systematic Bacteriology (Vos et al., 2011).

Cows of the experimental group were three times intramuscularly injected with the ethyl-thiosulfanilate-based liposomal drug in the dose of 0.04 mg/kg of body weight of the animals (with 1.25% ETC content). The interval between the administrations was 24 h. The animals of the control group were given an equivalent dose of sodium chloride.

Ethyl ether thiosulfoacid was synthesized at the Department of the Technology of Biologically Active Compounds, Pharmaceutics and Biotechnologies of the Lviv Polytechnic National University (Lubenets et al., 2006).

Antimicrobial activity of ethyl thiosulfanilate was studied using the method of diffusion in agar (well diffusion method) in *in vitro* conditions in relation to 5 museum strains of the test microorganisms: *Staphylococcus aureus* ATCC 25923 (F-49), *S. epidermidis* 191, *Klebsiella pneumoniae* 43, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATC 27853 (F-51). To prepare the resulted solution with 0.1 g/mL concentration of the studied compound, we used dimexide, because sediment settled in the physiological solution. Further dilutions were made using physiological solution. Obtained dilutions of the studied compound were placed on sterile disks, 0.02 mL on each.

Standardization of the study conditions was achieved by 10 mm thickness of medium in the Petri dishes and 6 mm diameter of a well in it, using suspension of a daily culture of the test microorganism for inoculation. At the same time, we used 0.5 microbial load according to McFar-

land, which was identified using a Densimat densitometer (manufactured by BioMerieux, France). After inoculating suspension of a test strain on a solid growth medium, the wells were filled with solutions of the studied substance. Then, the Petri dishes were placed in a thermostat for 24 h at the temperature of 37 °C. Then, we assessed the results by presence of growth inhibition zones of the test strains, which were clearly seen around the “wells”, by measuring the diameters of those zones in millimeters. Depending on diameters of growth inhibition zones of the studied microorganisms around the well, strains can be classified to sensitive, moderately resistant or resistant. A strain was considered sensitive to a sample if the diameter exceeded 25 mm, sensitive if the diameter equaled 15–25 mm, and low-sensitive in case it was 8–14 mm.

The liposomal drug was manufactured in the immunological laboratory of the Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine. It contains a combination of substances with antimicrobial action and complementing compounds, with the following ratios of the components per 10 cm³ of the drug: ethyl thiosulfanilate – 90–110 mg, vitamin A – 160–170 IU, vitamin D₃ – 220–230 IU, vitamin E – 0.2–0.4 mg, lecithin – 20.0–30.0 mg, twin – 0.04–0.06 cm³, water for injection up to 10 cm³. The indicated mixture was mixed and dispersed on a UZDN-1 ultrasound disperser at 22 kHz frequency for 2–3 minutes until a thin emulsion.

The material for the study was the blood of animals, taken a day prior to the administration and on the third and seventh days after injecting the drug. Hematological parameters of blood were studied according to the generally accepted methods. Concentration of hemoglobin was measured by the hemiglobincyanide method, erythrocytes and leukocytes were counted in Goryaev’s chamber. The leukogram was analyzed by the widely used method in Romanowsky-Giemsa-stained blood smears under immerse system of microscope, by differential count of 100 leukocytes.

The activity of humoral link of non-specific resistance of the body was identified according to circulating immune complexes (CIC), bactericidal, and lysozymic activity of blood serum. Total protein, content of lipid-peroxidation products (lipid hydroperoxides and TBA-active products), oxidation modification of proteins (aldehyde OMP370 and ketone OMP430 derivatives), and the activity of enzymes of antioxidation defense, such as catalyze, superoxide dismutase (SOD), glutathione peroxidase, – according to the generally accepted methods described in the manual (Vlizlo et al., 2012).

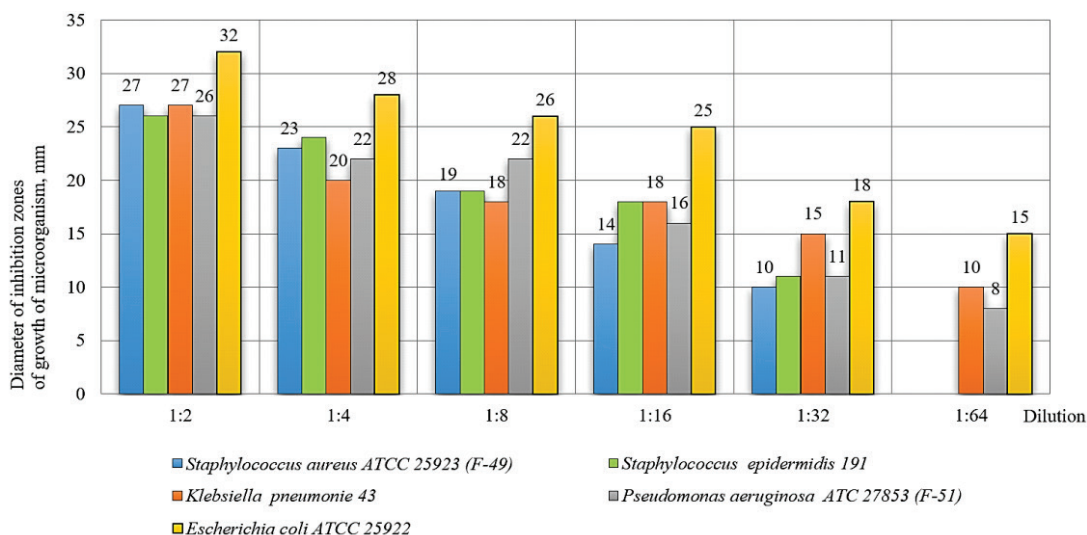


Fig. 3. Antimicrobial activity of various concentrations of ethyl thiosulfanilate towards the microorganisms of reference strains

Prospects of usage of the studied liposomal thiosulfonate drug for treatment of bovine mastitis were preliminary evaluated by determining the antimicrobial activity of ethyl thiosulfanilate (Fig. 3). Results of the studies illustrate the activity of the drug towards the indicated strains of microorganisms. Their sensitivity remained until 1:16 dilution, indicating the prospects of antibacterial liposomal drug.

Clinical symptoms of catarrhal mastitis had their peculiarities, determined by etiological factor, course of the process, and individual peculiarities

Differences between values of the control and experimental groups were determined using Tukey’s test, where the differences were considered statistically significant at $P < 0.05$.

Results

During the bacteriological study of 20 samples of secretion of the quarter of cow udder with an inflammatory process, we obtained 51 isolates of bacteria. The microbial landscape of mastitis pathogens was represented by Gram-positive (*Staphylococcus* spp., *Streptococcus* spp.) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) microorganisms (Fig. 2).

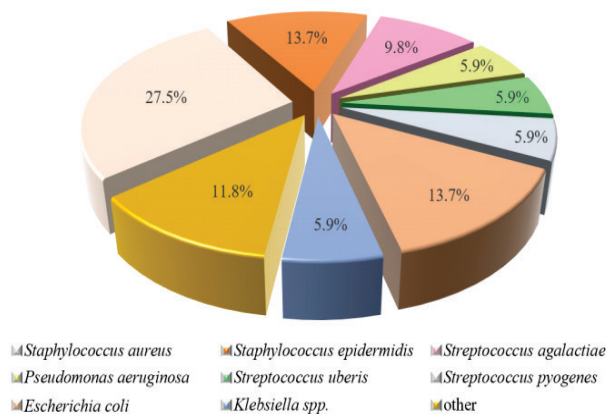


Fig. 2. Microbial profile of the udder secretion of the cows with catarrhal mastitis

Specific weight in etiology of the disease belonged to staphylococci (41.2%). Streptococci were isolated almost twice as rarely (21.6%). With the similar frequency, *Escherichia coli* and *Klebsiella pneumoniae* (21.5% in total) were isolated. In most cases, there dominated association of microorganisms (89.5%) and only in 10.5% of the cases did we observe a monoculture (*Staphylococcus aureus*). The rarest bacteria we found were *Pseudomonas aeruginosa* and *Streptococcus uberis* (5.9% each), isolated only in associations with other pathogens.

of the body. Pathological process was accompanied by the spread of inflammatory reaction with notable catarrhal exudation. General condition of the animals was satisfactory. During palpation of the affected quarter of the udder, we saw heightened local temperature, slight soreness, and densification in the tissues. Milk secretion decreased. From the affected part of the mammary gland, we yielded watery milk with admixture of clusters and flakes of casein. The research we performed revealed that catarrhal mastitis of the cows led to changes in hematological profile (Table 1).

In particular, in contrast to the clinically healthy cows, blood of the sick cows was found to have a tendency towards decrease in hemoglobin and erythrocytes. At the same time, three days following the treatment, changes in hemoglobin concentration in experimental-group animals – compared with the control – were expressed to a higher degree ($P < 0.05$). Those data indicate inhibiting effect of disease factors on oxygen-transporting function of blood.

It has to be noted that blood of the sick animals contained 56.6% ($P < 0.001$) more leukocytes prior to injection of the drug than blood of the control group, suggesting notable leukocytosis. At the same time, concentration of total protein in blood serum of those cows was 1.96% ($P < 0.05$) lower than in the control.

Parenteral administration of the ethyl-sulfanilate-based drug to experimental-group animals was accompanied by a normalizing effect on the mentioned blood parameters. This was evidenced by absence of significant changes in the number of leukocytes in the blood of the sick cows already on the third day of the experiment, and on the seventh day after beginning of the treatment, the overall number of leukocytes in the blood of experimental-group cows declined by 9.4% ($P < 0.05$), compared with the period prior to the drug administration. At the same time, on the third

day of the experiment, the content of overall protein in the blood serum increased by 4.1% ($P < 0.05$), compared with the level observed on the first day of the experiment; on the seventh day, the total-protein content increased by 13.6% ($P < 0.05$).

Results of the blood leukogram, presented in Table 2, demonstrate a pronounced lymphopenia in blood of the mastitis-suffering cows. On the first day of the treatment and three days following, the number of lymphocytes in blood was respectively lower by 10.1 ($P < 0.01$) and 7.01 ($P < 0.05$) than in the clinically healthy animals. By contrast, the number of eosinophils in blood of the sick cows was higher than in the control group during all study periods ($P < 0.001$): by 1.63 prior to administration of the drug, by 1.56 three days later, and 1.20 seven days later. Similar changes were also recorded when studying the number of neutrophilic granulocytes in the blood of the sick cows, especially band neutrophils. In particular, their number was 2.2 and 1.6 and 1.2 ($P < 0.05$) higher than in the control on the first day of the treatment and on the third and seventh days after, respectively. The indicated changes in the ratio of individual forms of leukocytes in blood of the sick cows occurred against the background of increasing number of monocytes, especially during the period before injecting the studied liposomal drug.

Table 1
Hematological parameters and content of total protein in blood of the cows ($\bar{x} \pm SE$; $n = 5$)

Parameters	Group of animals	Period of study		
		prior to injecting the drug	3 rd day after the treatment started	7 th day after the treatment started
Hemoglobin, g/L	control	97.4 ± 1.1 ^a	101.5 ± 1.1 ^b	101.6 ± 1.3 ^b
	ethyl thiosulfanilate	95.8 ± 1.8 ^a	98.1 ± 2.1 ^a	103.0 ± 1.1 ^b
Erythrocytes, 10 ¹² /L	control	6.75 ± 0.11 ^a	6.61 ± 0.15 ^a	6.66 ± 0.12 ^a
	ethyl thiosulfanilate	5.99 ± 0.15 ^b	6.43 ± 0.12 ^{ab}	6.29 ± 0.16 ^{ab}
Leukocytes, 10 ⁹ /L	control	6.93 ± 0.44 ^a	7.55 ± 0.17 ^b	7.41 ± 0.71 ^b
	ethyl thiosulfanilate	10.91 ± 0.72 ^b	10.45 ± 0.93 ^b	9.83 ± 0.61 ^b
Total protein, g/L	control	69.1 ± 1.1 ^a	71.2 ± 0.4 ^a	71.8 ± 1.1 ^a
	ethyl thiosulfanilate	67.8 ± 0.8 ^a	70.5 ± 0.2 ^{ab}	77.0 ± 0.2 ^b

Note: different letters indicate samplings that are significantly different between each other by the indicated characteristics of blood of the animals of experimental and control groups prior to injection of the drug, and on the 3rd and 7th days after the injection, respectively, according to the results of the Tukey test with the Bonferroni's correction.

Table 2
Leukogram of blood of the cows (%; $\bar{x} \pm SE$, $n = 5$)

Parameters	Groups of animals	Period of study		
		prior to injecting the drug	3 rd day after the treatment started	7 th day after the treatment started
Basophils	control	0.771 ± 0.213 ^a	0.823 ± 0.421 ^b	0.720 ± 0.125 ^b
	ethyl thiosulfanilate	0.822 ± 0.294 ^b	0.674 ± 0.413 ^b	0.842 ± 0.342 ^b
Eosinophils	control	3.84 ± 0.70 ^a	4.12 ± 0.86 ^b	4.65 ± 1.03 ^b
	ethyl thiosulfanilate	6.23 ± 0.98 ^b	6.44 ± 1.11 ^a	5.52 ± 0.98 ^b
Band neutrophils	control	4.02 ± 0.51 ^a	3.31 ± 0.43 ^b	3.32 ± 0.43 ^b
	ethyl thiosulfanilate	8.94 ± 1.25 ^b	5.45 ± 0.48 ^b	4.13 ± 0.38 ^a
Segmented-nucleus neutrophils	control	30.3 ± 1.1 ^a	29.9 ± 1.4 ^a	26.3 ± 1.6 ^b
	ethyl thiosulfanilate	33.2 ± 1.2 ^a	32.3 ± 1.3 ^{ab}	27.0 ± 1.3 ^b
Lymphocytes	control	57.2 ± 1.1 ^a	56.3 ± 0.9 ^a	55.1 ± 1.0 ^a
	ethyl thiosulfanilate	47.1 ± 1.2 ^a	49.3 ± 1.2 ^a	51.5 ± 1.0 ^a
Monocytes	control	3.02 ± 0.47 ^a	3.10 ± 0.54 ^a	3.50 ± 0.55 ^b
	ethyl thiosulfanilate	4.21 ± 0.59 ^b	4.11 ± 0.58 ^a	3.72 ± 0.52 ^b

Note: see Table 1.

Therefore, the blood of the cows suffering catarrhal mastitis was observed to have a simple regenerative shift of nucleus leftward. At the same time, increase in the number of eosinophils can be explained by sensitizing properties of the microorganisms, especially staphylococci. At the same time, increase in the number of monocytes could perhaps be associated with compensatory reaction in response to deficiency of other immune-competent cells during the infection and activation of defensive abilities of the body.

Injection of the liposomal drug to experimental-group cows promoted a rehabilitating effect on the ratio of individual forms of leukocytes in the blood. This was evidenced by increase in the number of lymphocytes, decrease in the number of eosinophils and segmented-nucleus neutrophils in blood of the sick cows, especially on the seventh day of the experiment, compared with those parameters before the treatment.

For a multifaceted evaluation of the condition of organism, we studied the systems that are responsible for stress and resistance. Humoral factors of natural resistance include bactericidal and lysozymic activities of

blood serum, and also circulating immune complexes (Table 3). When analyzing the parameters of humoral link of non-specific defense factors in animals with the clinical form of mastitis, we noted a significantly lower level of bactericidal activity of blood serum, compared with its values in the clinically healthy animals. This indicates that inflammatory processes of the bovine mammary gland were accompanied by inhibition of protective abilities of the body, first of all natural defensive factors. At the same time, we saw 11.6% ($P < 0.05$) increase in the concentration of circulating immune complexes (CICs). Their level in blood was an important indicator of non-specific immunological defense of the body. A high CIC level in blood serum was observed during inflammatory and autoimmune processes.

As is known, an inflammatory process in cow entails changes in the immunobiological reactions that are directed at neutralization and elimination of the alien antigen. Formation of circulating immune complexes is a physiological mechanism of defending the animal organism, which influences the activation of cellular and humoral immunities, thereby elimina-

ting exogenous and endogenous pathogens. A high level of circulating immune complexes in blood serum is found during inflammatory processes.

The conducted studies revealed that administration of the ethylthiosulfanilate-based liposomal drug to experimental-group cows had a positive effect on the activity of humoral link of natural defense mechanisms. This was evidenced by absence of significant differences in the studied parameters of natural resistance in experimental-group cows, as compared with the control already on the third day of the experiment. At the same time, on the seventh day since the start of treatment, in experimental-group cows, parameters of bactericidal and lysozymic activity of blood serum increased respectively by 12.4 ($P < 0.001$) and 10% ($P < 0.001$), the content of circulating immune complexes dropped 18.5% ($P < 0.01$), compared with the period prior to administration of the drug.

The results revealed decrease in antigen load on the organism of the sick cows and the normalizing influence on the activity of humoral defense factors, produced by the tested liposomal drug.

The condition of the immune system of a body to a high degree depends on its metabolic homeostasis and efficiency of antioxidant defense mechanisms. The udder tissue of cow during lactation quite often experiences impaired homeostasis, conditioned by post-labour complications and accumulations of free radicals.

Table 3

Parameters of humoral link of non-specific resistance of the cows ($\bar{x} \pm SE$, $n = 5$)

Parameters	Group of animals	Period of study		
		prior to injecting the drug	3 rd day after the treatment begun	7 th day after the treatment begun
Bactericidal activity of blood serum, %	control	42.3 ± 1.1 ^a	41.1 ± 1.2 ^a	46.1 ± 1.4 ^b
	ethyl thiosulfanilate	35.1 ± 1.0 ^b	39.1 ± 1.0 ^a	47.4 ± 1.2 ^b
Lysozymic activity of blood serum, %	control	31.0 ± 0.6 ^a	34.0 ± 1.5 ^b	35.8 ± 1.3 ^b
	ethyl thiosulfanilate	27.5 ± 1.6 ^b	30.8 ± 1.3 ^{ab}	37.5 ± 0.7 ^b
Circulating immune complexes, mmol/L	control	77.5 ± 0.7 ^a	75.7 ± 0.7 ^a	74.0 ± 1.2 ^a
	ethyl thiosulfanilate	86.5 ± 2.7 ^b	73.5 ± 1.0 ^b	70.5 ± 1.6 ^b

Note: see Table 1.

Table 4

Concentration of lipid peroxidation products in blood plasma of the cows ($\bar{x} \pm SE$, $n = 5$)

Parameters	Group of animals	Period of study		
		before injecting the drug	3 rd day after start of the treatment	7 th day after start of the treatment
Malondialdehyde, $\mu\text{mol/L}$	control	5.96 ± 0.09 ^a	5.89 ± 0.07 ^a	5.87 ± 0.08 ^a
	ethyl thiosulfanilate	6.16 ± 0.19 ^a	6.17 ± 0.29 ^a	5.75 ± 0.18 ^b
Lipid hydroperoxides, E480/mL	control	1.45 ± 0.03 ^a	1.50 ± 0.03 ^a	1.48 ± 0.05 ^a
	ethyl thiosulfanilate	1.75 ± 0.06 ^b	1.65 ± 0.06 ^a	1.31 ± 0.08 ^b

Note: see Table 1.

Injecting the animals with the liposomal drug caused inhibiting effect on the intensity of LPO processes. This was seen in absence of significant changes in their concentration already on the third day after the treatment had begun. The mastitis cows were observed to have significant 31.6% ($P < 0.01$) decrease in the content of lipid hydroperoxides on the seventh day.

Administration of the liposomal drug promoted decrease in the content of TBA-active products and lipid hydroperoxides in experimental-group cows, indicating its normalizing effect on the intensity of lipid-peroxidation processes. Thus, components of the drug weakened the lipid peroxidation processes, and its liposomal form exerted a prolonged action. This can be due to detoxifying and antioxidant properties of ethyl thiosulfanilate and complex of fat-solving vitamins, which contain the studied agent. Reaction of the indicated compounds with lipid peroxides is likely responsible for their ability to decrease the level of lipid peroxidation.

Similar changes were seen while examining the intensity of oxidative modification of proteins (Table 5). Concentrations of aldehyde and ketone derivatives of oxidative modification of proteins in cows of the experimental group was higher than in the control during all stages of the study, except for the group of cows with mastitis on the seventh day after administration of the liposomal drug. This group was observed to have significant 24.6% ($P < 0.05$) decrease in the content of ketone derivatives. The number of aldehyde derivatives of oxidative modification of proteins at the beginning of the study was much higher in animals of the experimental group, by 27.4% ($P < 0.05$), and changed insignificantly after administration of the drug. Peroxidative damages to the cellular structures are prevented by the antioxidant system that regulates the reactions of lipid

As a contagious disease, mastitis is known to intensify lipids peroxidation and accumulation of active oxygen species, which in some cases functions as a protective mechanism fending off pathogens. Therefore, study of changes in metabolic homeostasis of lactating cows is important for evaluation of their physiological condition. Influx of toxic compounds from primary inflammation sites through the lymphatic and blood-circulating system to the tissues and organs of an animal body causes generalization of endotoxiosis. Early detection of biochemical markers of endogenous intoxication, accumulated during pathological processes, can be a prognostic factor of disease development. A commonly used criterion for identification of the degree of endogenous intoxication of a body is the level of products of lipid peroxidation (LPO). The conducted studies revealed that inflammatory processes in the mammary gland in experimental-group animals were accompanied by increased intensity of LPO processes. Those changes were to a higher degree expressed in the concentration of intermediate LPO products. In particular, concentration of lipids in blood plasma in experimental-group cows prior to injection of the drug was 20.7% ($P < 0.01$) higher than in control-group animals (Table 4).

Similar, but less pronounced, changes were seen in the content of end products of LPO – TBA-active products (malondialdehyde – MDA).

peroxidation in membranes, controls the content of active oxygen species, free radicals, and end metabolism products. In the conditions of physiological homeostasis, reactive oxygen species are effectively neutralized by mechanisms of cellular defense – enzymatic (superoxide dismutase, catalase, glutathione peroxidase, hemoxidase-1, redox proteins) or non-enzymatic (ascorbic acid, tocopherol, carotenoids, glutathione, selenium) antioxidants (Mavangira et al., 2015; Franco et al., 2017). Our studies of blood of cows with catarrhal mastitis revealed the tendency towards decrease in the activity of antioxidant-system enzymes, such as catalase and superoxide dismutase (Table 6).

At the same time, parenteral administration of the complex liposomal drug to the cows produced a 30.2% spike in glutathione-peroxidase activity in erythrocytes and 17.9% ($P < 0.01$) increase in reduced glutathione on the seventh day after the treatment begun. Results of the studies revealed that catarrhal mastitis in cows increased the intensities of LOP and OMP and decreased the activity of enzymes of the antioxidant defense system, leading to oxidative stress. At the same time, we observed deviations in hematological parameters of the body and decline of the immune function, especially the natural defense factors. We saw a rehabilitating effect of components of the studied drug on the activity of the indicated systems in the cows suffering catarrhal mastitis.

Discussion

Bacteriological analysis of milk from cows with catarrhal-mastitis revealed that 27.5% of the total number of isolated microorganisms were *Staphylococcus aureus* and 21.6% were *Streptococcus* spp. The high level

of isolation of staphylococci can be associated with the fact that their main reservoir in the body is the skin of the animal's udder. There is an opinion that mastitis caused by *Staphylococcus* infection is conditioned by absence of proper hygiene on farms, bad health of animals, and absence of proper attention to the health of the mammary gland specifically, etc. (Sulejmani

et al., 2016). According to the data Horiuk et al. (2018), in 26.6% of the cows suffering mastitis of streptococcus etiology, the skin of the udder teats was infected by the same microorganisms. Therefore, cows with streptococcus and *Staphylococcus* mastitides are the main reservoir and source of infection for a healthy herd.

Table 5

Content of aldehyde and ketone derivative of oxidative modification of proteins in blood serum of the cows ($x \pm SE$, $n = 5$)

Parameter	Group of animals	Study period		
		before injecting the drug	3 rd day after starting the treatment	7 th day after starting the treatment
Aldehyde derivatives of oxidative modification of proteins	control	22.1 ± 1.9 ^a	20.2 ± 1.2 ^a	24.6 ± 2.0 ^a
	ethyl thiosulfanilate	28.2 ± 1.5 ^b	26.5 ± 2.4 ^a	27.5 ± 2.1 ^b
Ketone derivatives of oxidative modification of proteins	control	35.9 ± 2.0 ^a	34.2 ± 2.5 ^a	36.2 ± 2.1 ^a
	ethyl thiosulfanilate	42.2 ± 2.3 ^b	37.6 ± 3.1 ^{ab}	33.2 ± 2.1 ^b

Note: see Table 1.

Table 6

Activity of enzymes of antioxidant defense and concentration of reduced glutathione in blood of the cows ($x \pm SE$, $n = 5$)

Parameters	Group of animals	Study period		
		before injecting the drug	3 rd day after starting the treatment	7 th day after starting the treatment
Catalase, UE/mL	control	2.45 ± 0.25 ^a	2.24 ± 0.09 ^b	2.35 ± 0.12 ^a
	ethyl thiosulfanilate	1.98 ± 0.45 ^b	2.16 ± 0.08 ^b	2.85 ± 0.04 ^b
Superoxide dismutase, U/mL	control	23.1 ± 3.7 ^a	22.9 ± 1.1 ^a	23.2 ± 2.8 ^a
	ethyl thiosulfanilate	19.6 ± 2.0 ^b	22.2 ± 2.7 ^{ab}	24.9 ± 2.1 ^a
Glutathione peroxidase in erythrocytes, nmol of glutathione/min x mg of protein	control	23.8 ± 1.3 ^a	23.4 ± 1.3 ^a	24.8 ± 1.3 ^a
	ethyl thiosulfanilate	20.9 ± 0.8 ^b	21.1 ± 1.0 ^b	27.3 ± 0.9 ^b
Reduced glutathione in erythrocytes, μmol/L	control	0.443 ± 0.022 ^a	0.423 ± 0.016 ^b	0.431 ± 0.021 ^a
	ethyl thiosulfanilate	0.390 ± 0.014 ^b	0.412 ± 0.014 ^b	0.462 ± 0.014 ^b

Note: see Table 1.

Presence of *Pseudomonas aeruginosa* in 5.9% of the samples indicated complication of the treatment because according to our observations and the data of Dantas et al. (2014) *Pseudomonas aeruginosa* quite often displays resistance to most antibacterial drugs because of its ability to prevent them from invading the cell through the external membrane, or actively remove them from the cell in case of invasion.

The traditional antibiotic therapy of mastitis is not always effective. Moreover, the milk of such cows can be found to contain antibiotic residues, which affect the quality of milk in a negative way and can also lead to development of resistance to them among humans. Therefore, the search for effective and low-toxic antibacterial drugs is an extremely relevant task (Tomanić et al., 2023).

Over the recent decades, there has been an active search for alternative-to-antibiotics innovative drugs containing systems of oriented transport and distribution of active pharmaceutical ingredients across the tissues. Those include liposomal drugs that have a number of advantages: they prolong the action of an administered pharmaceutical drug in the body; alter the pharmacokinetics of drugs, significantly increase their pharmaceutical efficacy; protect medical compounds from being ruined; protect healthy cells from toxic action of drugs (El Bayoumi & Torchilin, 2010; Polkovnikova, 2022).

Liposomal drugs are clinically used for an array of medical needs when treating cancer, fungal and viral diseases, in vaccines, photodynamic therapy, radiofrequency ablation, and local analgesia and anesthesia (Bulbake et al., 2017; Wang & Grainger, 2019).

In our studies, we used the ethyl-sulfanilate-based liposomal drug. This compound is known to be a synthetic analogue of natural antibiotic allicin and is characterized by powerful antimicrobial, anti-inflammatory, biocide, antioxidant, and immune-modulating properties.

A study by Oriabinska et al. (2017) was focused on the action of ethyl thiosulfanilate towards the conditionally pathogenic fungi *Candida tropicalis*. It was demonstrated that the drug in subfungicidal concentration (125 μm/mg) exerted effects on some aspects of anabolism and energy metabolism of the fungi: inhibited endogenous respiration and decreased the pool of nucleic acids. In addition, changes were found in lipogenesis of the *C. tropicalis*.

Results of the studies of ethyl thiosulfanilate on the condition of the pro/antioxidant system in the liver, blood, and the kidneys of laboratory rats revealed that exposure (application, injections) to ethyl sulfanilate in the dose of 100 mg/kg of the body weight per day for 14 days resulted in

decrease in the concentration of lipid hydroperoxides in the liver of the rats subject to Cr(VI) (Kotyč et al., 2020). Ethyl thiosulfanilate alone and in a combination with Cr(VI) activated glutathione reductase and increased the concentration of reduced glutathione in blood erythrocytes of the rats and also enhanced the superoxide-dismutase activity and decreased the catalase activity (Kotyč et al., 2019). Preliminary processing with ethyl thiosulfanilate for 14 days in the dose of 100 mg/kg decreased ($P < 0.05$) the intensity of Cr(VI)-induced processes of peroxidation of lipids and proteins in the kidneys of the rats. At the same time, preliminary effect of the drug also prevented exhaustion of the general pool of reduced glutathione ($P < 0.05$) after 14 days of the action of potassium bichromate (Kotyč & Iskra, 2021).

There are other studies of liposomal drugs with antimicrobial, anti-inflammatory and antioxidant properties. Milk thistle (*Silybum marianum* (L.) Gaertn.) preparations have a particularly pronounced antioxidant activity (Gutyj et al., 2022; Lieschova & Brygadyrenko, 2023). Therefore, Lipointensyl (liposomal drug based on interferone and *S. marianum*), given to the Black Spotted dairy bulls in the conditions of cadmium and plumbum load, inhibited the lipid-peroxidation processes. Components of the drug reduced the concentrations of primary and end products of the LOP, in particular the level of conjugated dienes by 22% and TBA-active products by 20%. Intramuscular injection of the liposomal drug to bulls of the experimental group enhanced the antioxidant defense of their organism. On the 30th day of the experiment, reduced glutathione in blood of experimental-group animals increased by 9.8%. The study of enzymatic link of glutathione system revealed that in the blood of the animals that had received the Lipointenzyl liposomal drug, the activities of glutathione peroxidase and glutathione reductase increased by 24.0% and 27.7% respectively by the 30th day of the experiment (Gutyj et al., 2022).

When studying the leukocyte profile of blood from cows with catarrhal mastitis, we observed significant increase in numbers of leukocytes and eosinophiles against the background of decrease in lymphocytes. Obviously, any effect on the animal organism and a response reaction to it causes structural damage to the tissues and physiological systems of the organisms. At the same time, the main role in the support of homeostasis is played by the immune system. Also, decrease occurred in the activity of cellular and humoral factors of non-specific resistance: bactericidal and lysozymic activities of blood serum. Against this background, conditionally pathogenic microflora activates, especially staphylococci, which cause delayed-type hypersensitivity, which contributes to the transition of the

diseases to a chronic form. Those data are consistent with the results of the study (Kibebew, 2017).

The main tasks of the immune system are identification and elimination of the alien agents in the organism. One way of removing an antigen is formation of an antigen-antibody immune complex. Exo- and endogenous antigens in the organism with respective antibodies can form circulating immune complexes. It is believed that a respective level of immune complexes should be in the blood constantly, realizing the physiological processes of homeostasis support. Moreover, this indicates adequate reaction of the organism to an external influence (Ezzat Alnakip et al., 2014).

Level of the CICs was used as an indicator of activation of humoral link of the immune system. In blood serum of the cows suffering catarrhal mastitis, the CIC level was significantly higher than in the clinically healthy cows. A large amount of pathogenic CICs indicates sensitization of the body and ineffective functioning of the systems that control the CIC level (first of all the systems of complement and phagocytes). Formation of CICs in the body is a component of a normal immune response. Immune complexes are permanently present in blood plasma of healthy animals in small amounts and are removed from the body by cells of mononuclear system of phagocytosis. However, in some cases, this mechanism malfunctions, leaving immune complexes circulating in blood for a long time, resulting in their pathogenic effect on the body. The level of CIC formation reflects the phases of development of humoral immune response to the inflammatory process and shows the interrelation with the cellular course of disease: the level of circulating immune complexes can be normal during an onset of disease, high during development and manifestation of symptoms, and normalizing during a recovery (Zhelavskiy & Dmytriv, 2018). When treating the cows suffering from catarrhal mastitis using the liposomal drug, we recorded decrease in the level of circulating immune complexes and enhancement of natural defense mechanisms. This can explain neutralization and elimination of CICs. The obtained results suggest inhibition of defensive capacities of the cows with catarrhal mastitis. Similar results were obtained by other researchers when using drugs based on ether oils from medicinal plants (Lopes et al., 2020).

Conclusion

Intramuscular injection of the ethyl-thiosulfanilate-based drug positively influenced the hematological and biochemical blood parameters of the cows diagnosed with catarrhal mastitis. On the seventh day of the experiment, in the blood of the sick animals, there occurred decrease in leukocytes, increase in total protein, and enhancements in bactericidal and lizozyme activities. On the 7th day after injecting the drug, those animals were observed to have reduced concentrations of end and intermediate products of lipid peroxidation and also derivatives of oxidative modification of proteins, while the glutathione peroxidase activity and the content of reduced glutathione in blood erythrocytes increased, compared with the initial level that had been recorded prior to administration of the drug. Content of circulating immune complexes dropped significantly, demonstrating positive effects of the drug components on the activity of natural defense mechanisms.

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The authors declare no conflict of interests.

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