



## Impact of 1,2,4-thio-triazole derivative-based liniment on morphological and immunological blood parameters of dogs suffering from dermatomycoses

V. M. Hunchak\*, V. P. Martynyshyn\*, B. V. Gutyj\*, A. V. Hunchak\*\*, O. M. Stefanyshyn\*\*, V. V. Parchenko\*\*\*

\*Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine

\*\*Institute of Animal Biology of NAAS, Lviv, Ukraine

\*\*\*Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine

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Stepan Gzhytskyi National  
University of Veterinary  
Medicine and  
Biotechnologies,  
Pekarska st., 50,  
Lviv, 79010, Ukraine.  
Tel.: +38-097-519-13-88.  
E-mail:  
doctorvethiv@ukr.net

Institute of Animal Biology  
of NAAS, V. Stus st., 38,  
Lviv, 79034, Ukraine.

Zaporizhzhia State  
Medical University,  
Maiakovskiyi avenue 26,  
Zaporizhzhia, 69035,  
Ukraine.

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The development of new preparations in soft medical form with antifungal effect and the ability to exert an antimicrobial effect on the secondary microflora accompanying the infection with accompanying desensitizing action is extremely relevant, as well as introduction of such drugs into veterinary medicine. The compound and compounding of the preparation with fungicidal and fungistatic effects for the treatment of dermatitides of dogs and cats caused by fungal skin infection are substantiated. The active agent of the developed medicine in the form of liniment was the S-derivative of triazole, and the base material was the standardized oil from milk thistle *Silybum marianum*. We determined the dynamics of the morphological parameters in blood of dogs with typical clinical skin pathology and its laboratory-confirmed fungal etiology. During the treatment of dogs with confirmed laboratory diagnosis “dermatophytosis”, the main medical preparation used was VetMikoDerm. It was heated on a water bath, gently applied and in thin layer was applied to the infected areas of skin 2–3 times a day over the period of treatment. Positive dynamics of recovery were seen already at 3–4th days of the treatment (skin hyperaemia, itching and signs of inflammation reduced). On the 10–14th days of the treatment, the signs of regeneration of skin and recovery of the coat were observed. In all cases without exception (regardless of the area of skin infected by fungus) in this period, no fungi were microscopically found in the scrapes. Use of VetMikoDerm preparation during the course of the treatment, against the background of inhibition of the inflammatory processes, relief from itch and gradual recovery of the epidermis and hair, also contributed to the activation of the functional condition of the liver (the activity of amino transferases decreased, while the concentration of urea increased). Minimization of the negative effect of the products of fungal intoxication, after use of the tested preparation for a medical purpose, led to recovery of the functional state of the cellular link of the immunity. The treatment effect of the newly-developed preparation must be related to the active substance with notable fungicidal effect. Thio-triazole derivative exerts its medical effect by inhibiting sterol biosynthesis and contributes to the decrease in the content of ergosterol necessary for proliferation and growth of cells of fungus. The oil of milk thistle used as base material for liniment provides not only high quality of medical form in the process of production and maintenance, and adds to the effect of the main substance. Due to flavonoids, vitamins, carotenoids, milk thistle improves the condition of the skin, contributes to its active regeneration, stimulates reparation processes and recovery of the epidermis and the coat. Against the background of the effective fungicidal effect, the newly-developed VetMikoDerm preparation in the form of liniment exerted anti-inflammatory, hepatoprotective, immunostimulating, wound-healing, regenerative and anti-itch effects.

**Keywords:** pharmacology; liniment; VetMikoDerm; *Silybum marianum*; dermatophytosis.

### Introduction

All species of pets, fur animals, camels, deer, tigers, rodents suffer from dermatoses. Humans also become infected with skin diseases. Dermatoses are broadly distributed all across the globe (officially recorded in 120 countries) and all the continents (Paterson, 2011). There is no doubt that the causes of skin diseases are various organisms (bacteria, viruses, fungi), ectoparasites (Acari, Siphonaptera, Phthiraptera, Ischnocera), autoimmune and endocrine disorders (Borysevych et al., 2000; Ivanov & Atamas, 2003; Bublyk et al., 2004).

Among the broad range of dermatitis-causing pathogens, a notable role belongs to the pathogenic fungi. In the system of the organic world, the latter take a significant part. They share features characteristic of both animals and plants. The fungi are close to animals in the peculiarities of nitrogen and carbon metabolisms, and chitin in the membrane of the latter (except oomycetes). Similarity of fungi to plants is the absorption of nutrients, and absence of swallowing (Bilaj, 1989). In dogs and

cats, dermatomycoses occur and easily spread due to violation of the the maintenance conditions of animals, therefore the main targets of the infection with fungi of *Trichophyton*, *Microsporum*, *Epidermophyton* genera are stray animals (Ruban, 2013; Kovalenko & Voronkova, 2018; Iovenko & Koval, 2019).

According to a number of scientists (Medvedev, 1999; Ruban, 2013), non-favourable weather conditions and damaged skin contribute to the manifestations of trichophytia or microsporia. The factors which conduce to the emergence of the skin pathology include the breed and age category of small pets most vulnerable to dermatomycoses.

An important role in the manifestation of virulent abilities of the pathogens of mycoses also belongs to a number of factors: decrease in the resistance of the macroorganism, hormone imbalance, impaired homeostasis, etiological factors, chronic course of viral, bacterial and parasitic diseases, etc. Disorders in the balance between the resistance of an animal's organism and virulence of microflora of the skin often provoke the development of skin pathology. During impaired homeostasis, the

homeostasis reduces in the organism in general and the skin, which provides a barrier function, in particular. At the same time, the microbial landscape of the skin changes with its consequent infection with pathogenic bacteria and fungi (Katunina, 2009; Mercer et al., 2013; Olszewska et al., 2016; Zhuk et al., 2017).

Usually, clinically such pathology is accompanied by dermatitides, exemas, itching, scratches, alopecia, seborrhea, ulcers (Luk'janovskij, 1995). However, most researchers and practitioners in the sphere of veterinary medicine state that the skin pathology and its derivatives should be considered not only a local process, but disease of the organism in general (Bublyk et al., 2004). At the same time, the condition of blood is important, which in many ways determines the natural resistance. Convincing evidence in the understanding of etiopathogenesis of dermatoses, including dermatophytoses, is provided by taking into the consideration the immunological disorders in the organism of animals, first of all in the cellular link of the immune system (Katunina, 2009; Zhuk et al., 2017).

Effective therapy of skin diseases in dogs means first of all use of a complex of preparations and should be directed at removal of all the factors which contribute to the development of dermatosis, and also the desensitization and activation of the trophic conditions of the infected area of the skin in combination with preparations of local action (Rosen, 1997; Del Rosso & Gupta, 2000).

Despite the large available resource of medical preparations against the skin pathologies in dogs, they, usually, cannot relieve dermatosis permanently. Most often, the disease obtains a chronic character with high probability of relapse (Borysevych et al., 2000; Ivanov & Atamas, 2003; Bublyk et al., 2004). Medical preparations for systematic treatment of dermatomycoses in dogs, according to Patterson (2011), are potent teratogens. In Ukraine, the market of veterinary preparations of local action in the forms of ointments, pastes, liniments, gels is mostly represented by preparations of foreign production. There is a very small amount of efficient domestic preparations with simultaneous antifungal, anti-inflammatory and wound-healing effects. Dermatomycoses in dogs and cats usually run their course with signs of itching, scratching the wounds and are characterized by secondary infection with microflora, complicating the course of skin pathology. Anti-inflammatory preparations with anti-itch effect in Ukraine have mostly steroid nature, and desensitizing ointments and liniments without hormone constituents are practically absent (Bilous et al., 2010). Therefore, the development of new preparations in soft medical form (ointments, pastes, liniments, creams, gels, etc) with antifungal action and property to exert antimicrobial action towards secondary microflora accompanying the infection, and at the same time exert a desensitizing effect, and introduction of such drugs to the practice of veterinary medicine are extremely relevant.

In 2016–2020, we substantiated the components and developed the compounding of new antifungal preparation in the form of 10% liniment with thio-triazole derivative (4-(5-(decilto)-4-methyl-4H-1,2,4-triazole-3-yl)methyl)morpholine) as active substance, and had standardized oil of milk thistle (*Silybum marianum*) as the base material. In the previous studies, we conducted a complex of pre-clinical surveys (the parameters of acute toxicity, chronic toxicity, cumulation, peculiarities of the action of the studied preparation on the skin (irritability, allergization) were determined, including parameters of its morphology during long term use), and the newly-developed preparation called VetMikoDerm was determined to be safe for animals and could be used as a preparation with local and reflex actions (Martynshyn et al., 2017, 2018; Shcherbyna et al., 2018).

The objective of this study was determining the effect of the newly-developed preparation VetMikoDerm in the form of liniment on the morphological and immunological parameters of blood, and also its therapeutic efficiency in dogs suffering from dermatomycosis.

## Materials and methods

Clinical and experimental studies on the effect of the VetMikoDerm preparation were performed in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). During the experimental part of the work, we gradually formed (by request to the veterinary clinic in Stry city of Lviv Oblast) two

groups of dogs according to the principle of analogues (usually mongrel animals of the age of 1–6 years, females and males) over the period from 01.03.2019 to 15.12.2019. The control group (C) in the amount of 12 individuals was clinically healthy, without notable signs of skin pathology, with dense and glossy hair. The experimental (E) group of dogs (24 individuals) had characteristic visually similar symptoms of skin damage. The extent of manifestation of the signs was different, but most characteristic manifestations of dermatosis in them was loss of gloss and dryness of the skin, spot boldness and diffusive loss of hairs, hyperemia, exudation and hyper pigmentation of the skin, itch, overlapping of scales of white or grey-white colour, sometimes with signs of dandruff. Infected animals were in the condition of continuous anxiety, irritation and tried to scratch or bite the infected region of the skin. Usually, at the initial stages of the development of this pathology, the infected area was humid and sweaty. Against this background, the animals were observed to have decrease in appetite, being weak and low-active, often having acute unpleasant smell. Several animals, against the background of strong itch, were seen to have edema of the soft tissues of the digits of the front and back limbs. The signs of the skin pathology were most often observed on the regions of the ears, snout, oral cavity, in skin folds, between the digits, on the lower surface of the neck, on the inner side of the thighs, in the anus area, etc.

The diagnosis was made with consideration of the anamnesis data characteristic for the clinical picture and after required laboratory assays.

Hematologic, biochemical and immunologic assays of blood were performed at the laboratory of Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies.

The blood for the survey was drawn from the vein of the forelimbs of the surveyed animals during the first examination at their admission to the clinic and in the end of the period of the treatment in case of complete recovery. From clinically healthy dogs, the blood was drawn in order to determine the studied parameters and their comparison to the infected animals subjected to the treatment with VetMikoDerm.

Hematologic assays were performed using VetAutoread hematological analyzer manufactured by IDECC Company, and biochemical (activity of aminotransferases, alkaline phosphatase, concentration of glucose, urea, creatinine) – IDECC Vet Test analyzer.

In whole blood, we determined the parameters of phagocytosis of neutrophils, the amount of T- and B-lymphocytes.

To determine etiologic factors which conduce to the development of dermatosis, we conducted the surveys of the skin (Vlisko, 2012). In dogs of the control group, the skin in the zone between the toes, lower surface of the neck or abdomen or the forelimb was washed and the water used for this purpose became the material for the microbiological assays. The water samples were taken from the entire surface of the wound using a Voles sterile applicator on a plastic stick with a test tube with screw cap and Amies medium. In order to prepare the necessary dilutions, we used sterile test tubes, to which the amount of 9 mL of sterile solution of sodium chloride was added. After preparing all the dilutions, using a pipette, we took 0.1 mL of the dilution from each test tube, and transported it in the Petri dishes with the corresponding elective medium. The dilution was rubbed using a sterile spatula until its complete absorption in the agar. The “inoculated” dishes were left on the table in the humidified box for 15 minutes and then kept in a thermostat at the temperature of 37 °C (meat peptone agar) and 38 °C – Sabouraud dextrose. The microorganisms were identified using the Bergey's Manual Trust. Furthermore, we microscopically inspected the biomaterial obtained using a sterile tooth brush using “brushing off” the infected hair, scales, dandruff, etc. The material was transferred to the microscope slides, 1–2 drops of mineral oil was added, and heated for several minutes. After adding 50% of aqueous solution of glycerine, it was covered by the microscope slide and inspected under the microscope for the presence of artrospors.

The sensitivity of microorganisms and fungi to the action of VetMikoDerm preparation was determined using disk-diffusive method (Vlisko, 2012). To the sterile Himedia disks standardized according to ISO 9001 2015, ISO 13485 2012 WHO GMP, we added corresponding amounts of the studied preparations. For active growth of fungi in the experiment, we prepared growth agar (Sabouraud agar with pH not lower

than 6.0), on which a "lawn" of strains of microbial culture was put, identified in the previous water sample from the infected skin area. Petri dishes with pure cultures were dried and then put onto the disks with the tested preparations. After 70–72 h of their incubation in the thermostat (28 °C), we measured the growth inhibition zones of the cultures around the disks with antifungal substances and assessed the sensitivity of dermatophytes to the action of the surveyed preparation.

The extent of the positive effect of the surveyed preparations on the animals with signs of dermatomycosis was determined according to the evaluation of the condition of the skin, relief from itching and scratching of the tissues. At the same time, such indicators were important as the period during which we observed no eschars, ulcers, scales, split end hair; how characteristic were the signs of the course of the inflammatory process; beginning and complete healing of the wound and recovery of hair cover, etc.

The data were analyzed using Statistica 6.0 program (StatSoft Inc., USA). The data are presented in tables as  $x \pm SD$  ( $x \pm$  standard error). The differences between the values in the control and experimental groups were determined using ANOVA, where the differences were considered reliable at  $P < 0.05$  (taking into consideration the Bonferroni correction).

## Results

We determined the dynamics of morphological parameters in blood of dogs with characteristic clinical skin pathologies and laboratory confirmation of the fungal etiology. We determined that the number of erythrocytes, concentration of hemoglobin, hematocrit volume in the blood of the infected dogs compared to the control had no significant deviations (Table 1).

**Table 1**

The effect of VetMikoDerm on the morphological parameters of blood in dogs suffering from dermatosis ( $x \pm SD$ ,  $n = 24$ )

Parameters	Control	Experimental group	
		after admission to the clinic	after complete recovery (after 14–24 days of treatment)
Erythrocytes, $10^{12}/L$	$6.3 \pm 0.4$	$6.0 \pm 0.6$	$6.2 \pm 0.4$
Hemoglobin, g/L	$117.6 \pm 2.1$	$109.4 \pm 3.6$	$122.4 \pm 3.1$
Hematocrit, %	$42.3 \pm 1.9$	$40.2 \pm 1.7$	$42.0 \pm 1.4$
Mean corpuscular volume, $\mu m^3$	$67.1 \pm 2.7$	$67.0 \pm 3.9$	$67.8 \pm 4.5$
Mean corpuscular hemoglobin, pg	$18.7 \pm 1.0$	$18.2 \pm 1.8$	$19.8 \pm 1.0$
Mean corpuscular hemoglobin concentration, g/100 mL	$27.8 \pm 1.9$	$27.2 \pm 1.5$	$29.1 \pm 1.1$

Note: in this and the following tables statistically reliable differences were considered compared with the control group: \* –  $P < 0.05$ ; \*\* –  $P < 0.01$ ; \*\*\* –  $P < 0.001$ .

At the same time, we should note the tendency towards the changes in particular indices in blood of dogs subjected to the treatment with VetMikoDerm. Therefore, mean corpuscular hemoglobin concentration (MCHC) in the animals of the experimental group was 4.7% higher compared with clinically healthy dogs and 7.0% with parameters of the infected dogs after submission to the clinic.

The general tendency of leukocytes in blood of dogs of the experimental group (E) was within the range of physiological values, though compared with the clinically healthy animals this tended to increase (Table 2). In seven animals (29.2%), in which the area of the fungal skin infection was large (50–60%), the number of leukocytes was 18.2–21.2% higher than the control. In the infected animals, the leukogram in blood significantly changed. Against the background of neutrophilic leukocytosis, the percentages of stab granulocytes (by 10.8%) and eosinophils (by 24.6%) increased. The concentration of stab neutrophils and basophils was respectively 58.3% and 57.1% lower than in clinically healthy animals. Distinctive was also the tendency towards decrease in blood lymphocytes. The dynamics of the blood parameters of dogs after complete healing of the wounds and recovery of the coat after 2–3 week treatment with VetMikoDerm preparation did not significantly change

either. It should be noted that the most functionally active neutrophils with segmented nuclei in the animals' blood reduced compared with the parameter of the animals after admission to the clinic, though was slightly higher than in the control.

**Table 2**

Leukogram of blood of dogs with skin pathology under the impact of liniment of VetMikoDerm ( $x \pm SD$ ,  $n = 24$ )

Parameters	Control	Experimental group	
		after admission to clinic	after complete recovery (after 14–21 days of treatment)
Leukocytes, $10^9/L$	$14.4 \pm 0.9$	$15.6 \pm 0.7$	$15.0 \pm 0.5$
Leukogram, %			
Basophils	$1.1 \pm 0.1$	$0.7 \pm 0.1^{**}$	$1.0 \pm 0.2$
Eosinophils	$5.7 \pm 0.4$	$7.1 \pm 0.4^*$	$6.1 \pm 0.5$
Neutrophils:			
young	–	–	–
stab	$5.7 \pm 0.9$	$3.6 \pm 0.5^*$	$5.8 \pm 0.7$
with segmented nuclei	$49.8 \pm 1.0$	$55.2 \pm 2.0^*$	$50.5 \pm 4.1$
Lymphocytes	$33.4 \pm 1.0$	$31.4 \pm 1.2$	$32.5 \pm 1.2$
Monocytes	$4.8 \pm 0.2$	$3.5 \pm 0.4^{**}$	$4.2 \pm 0.4$

Decrease of the percentage of neutrophils with segmented nuclei and eosinophils in the blood of dogs with microsporidia during the treatment with VetMikoDerm preparation against the background of increase in the level of lymphocytes (by 20%) is obviously the result of decrease in the allergenic and sensitizing processes on the skin and characterizes the dominant role of these forms of leukocytes in the provision of adaptive reactions of the organism to the action of exo- and endogenic factors. With assays of particular biochemical parameters of blood serum, we determined that in the dogs of the experimental group, probable increase in the activity of enzymes alanine-aminotransferase (by 32.2%) and alkaline phosphatase by 16.7% occurred. At the same time, the concentration of urea was 22.9% lower than in the animals of the control group (Table 3).

**Table 3**

Dynamics of biochemical parameters of blood of dogs during the treatment of dermatophytoses with VetMikoDerm ( $x \pm SD$ ,  $n = 24$ )

Parameters	Control	Experimental group	
		after admission to clinic	after complete recovery (after 14–21 day of treatment)
Alanine-aminotransferase, U/L	$38.4 \pm 1.2$	$50.8 \pm 3.7^{**}$	$41.4 \pm 2.9$
Aspartate-aminotransferase, U/L	$18.1 \pm 1.4$	$20.2 \pm 2.1$	$17.4 \pm 1.9$
De Ritis Coefficient (AsAT/AlAT)	0.47	0.40	0.42
Alkaline phosphatase, U/L	$94.8 \pm 3.7$	$110.6 \pm 4.8^*$	$100.2 \pm 3.9$
Glucose, mmol/L	$5.4 \pm 0.8$	$4.7 \pm 0.5$	$4.9 \pm 0.8$
Creatinine, $\mu mol/L$	$126.6 \pm 5.6$	$138.4 \pm 7.2$	$130.4 \pm 6.7$
Urea, mmol/L	$7.40 \pm 0.23$	$6.02 \pm 0.48^*$	$6.80 \pm 0.50$

The levels of glucose and creatinine in blood serum of dogs with notable dermatomycosis did not significantly differ from the parameters of animals of the control group. At the same time, the dogs with broad area of the inflammatory process on the skin (two dogs, 50–60% of the skin infected) and one dog with deep wounds against the background of itching and deep scratches had even more distinctive dynamics of the studied parameters. They were also observed to have increased activity of aspartate aminotransferase, decrease in de Ritis coefficient, and the level of urea in blood was lower compared with the clinically healthy animals.

Use of VetMikoDerm preparation throughout the course of the treatment, up to a point, against the background of inhibition of inflammatory processes, relief from itching, and gradual recovery of the epidermis and hair, contributed to the activation of the functional condition of the liver.

The study revealed that in dogs admitted to clinic with signs of dermatomycosis, the phagocytic activity of neutrophils reliably decreased (by 16.6%) and the percentage of B-lymphocytes and the total con-

tent of immunoglobulins were higher by 46.9% and 8.5% respectively than the parameters of clinically healthy dogs, which obviously is the result of humoral response to the action of allergens of fungal and bacterial nature. The defense reaction of the organism, share of T-lymphocytes in the blood of dogs of the experimental group likely had a tendency towards increase. However, to a larger extent, it increased due to T-suppressors (Table 4).

**Table 4**

The effect of VetMikoDerm preparation on immunological parameters of blood of dogs infected with dermatophytes ( $x \pm SD$ ,  $n = 24$ )

Parameters	Control	Experimental group	
		after admission to clinic	after complete recovery (after 14–21 day of treatment)
Phagocytic activity, %	36.2 ± 1.7	30.2 ± 2.1*	35.7 ± 1.3
Phagocytic number, mk/cell	6.2 ± 0.7	6.4 ± 0.3	6.5 ± 0.7
Phagocytic index, %	30.6 ± 1.3	26.6 ± 2.1	27.7 ± 0.9
T-lymphocytes, %	35.3 ± 2.5	38.4 ± 1.7	38.0 ± 1.4
T-helpers	22.0 ± 1.3	20.2 ± 1.5	24.0 ± 1.3
T-suppressors	13.2 ± 0.9	17.6 ± 1.1	14.1 ± 1.7
B-lymphocytes	7.9 ± 0.7	11.6 ± 0.9**	8.8 ± 0.7
Immunoglobulins, mg%		560.1 ±	
	516.4 ± 11.5	12.2*	552.8 ± 8.9*

Therefore, minimization of the effect of products of fungal intoxication after treatment using liniment VetMikoDerm led to gradual recovery of the immune condition.

During the studies on the inoculations from the skin of animals of the control group, there were isolated small colonies of conditionally pathogenic microflora which was mostly represented by the association of microorganisms, particularly *Proteus* spp., *Escherichia coli* and *Klebsiella pneumoniae*. The pattern of bacteriological and especially fungal contamination in the affected regions of the skin of dogs was different and had its own peculiarities. Therefore, in three dogs (12.5%), there were found separate colonies of *Staphylococcus aureus*, and *Enterobacter* in two dogs (8.3%). At the same time, 18 animals (75%), against the background of single colonies of conditionally pathogenic microflora, were observed to have fungi of *Trichophyton* and *Candida* genera.

## Discussion

The dermatophyte nature of the skin infection in most animals was evidenced in the results of the microscopy of the biomaterial (affected hair, scales, dead tissues). Large spindle-shaped exospores of fungi were found on the hyphae of mycelium. According to the characteristic positions of macroconidium and spores described by Lebedko (2003), the fungi of *Trichophyton* (thin and smooth surface, hyphae of mycelium were straight with septa and lay between the hairs), *Microsporium* genera (mycelium was ramified, the present spores were scattered across the field of the examination, the surface of microconidia was thick and thorny) were identified in the scrapes. The present arthrospores which under the microscope appeared as fragmentation of hyphae to separate cells are most characteristic of yeast-like fungi (Ruban, 2013).

According to the determination of the susceptibility of the culture of fungi to the impact of the tested preparations, the growth inhibition of the zone of the fungal preparation produced by VetMikoDerm was 10–13 mm in 12 animals (50.0%), 7–8 mm in 8 (33.3%), 3–4 mm in two (8.3%), and 0 mm in two (8.3%).

After the diagnosis was confirmed, particularly regarding the identification of the fungal nature of the skin infection, we conducted a complex of measures to provide efficient treatment of the animals. The new VetMikoDerm preparation we have developed was used as the main drug. It was heated in a water bath (35–50 °C) for 10–20 s, lightly sprinkled and was thinly applied to the infected skin regions 2–3 times per day during 10–14 days, in severe cases (three animals had the area of the skin taken over by fungus measuring over 60%) up to one month. Moreover, in case of severe itch, we additionally gave tablets of Apovel preparation of Eksekan sugar cubes. In case of zones of sweat or heightened moisture in the infected region, we used antibacterial powder (streptocide, iodoform or xeroform and zinc oxide). Two dogs admitted to the clinic with signs of

biting of the skin were additionally treated with Chemi-sprey preparation. Then, liniment was applied to the skin only after 2–3 h. In the process of the treatment, the positive dynamics in the recovery of animals were seen on the 3–4th days after the treatment (reddening of the skin and signs of inflammation decreased). On the 10–14th days of the treatment, notable signs of the skin regeneration and recovery of the hair coat were observed. As with the animals with large (by the area) infections of the skin (over 50%), the course of their treatment was longer and the complete restoration of the hair could be seen after 28–30 days. In all cases without exception (depending on the area of the infection), on the 14–21st days of the study, no fungi in the scrapes were found.

Therefore, we can state that the newly-developed VetMikoDerm preparation for the external application has notable healing effect on the dogs suffering from dermatomycosis. Its action obviously should be related to the presence of antifungal substance in liniment, active substance of which is 1,2,4-thio-triazole derivative. For the azoles of this group, characteristic feature in the mechanism of the fungicidal action is inhibition of the enzymes which take part in the biosynthesis of the sterols of the cells, decrease the content of ergosterol, contribution to the accumulation of anomalous sterols, thus damaging the structure of the cellular membrane of fungus. Cells of fungi need ergosterol for proliferation, and its decrease is probably the reason of inhibition of the growth of cells (Vashchenko et al., 2009).

Soft medical forms, apart from the biologically active substance – the main carrier of the medical effect, contain additional substances which in combination with the active agent result in an efficient and safe drug (Bilous et al., 2010; Zazharskyi et al., 2019). As the base for the liniments of the veterinary purposes, plant oils are used. During the substantiation of the compounding and production of VetMikoDerm preparation, as the additional substance, we used the oil from milk thistle.

The immunostimulating effect of the milk thistle is exerted by flavonoids grouped under the general name of Silymarin (Zhao et al., 2000; Khariv et al., 2016; Martyshuk et al., 2016). It is a mixture of three isomers: silichristin, silidianin, silibin (Fraschini et al., 2002; Kim et al., 2003; Hamza & Al-Harbi, 2015). The greatest pharmacological effect is displayed by silibin (Kren & Walterova, 2005). The highest amount of flavolignans is concentrated in the seed coat of milk thistle (7.0%) and only 0.12% is in the seed itself (Ismaili et al., 2016). At the same time, not only is it important the fact that as a dispersal system it provides high quality of the preparation in the process of production and maintenance, but also its property to increase the effect of remedium cardinale. Due to the presence of flavolignans, vitamins, particularly vitamin E in the oil, milk thistle improves the condition of the skin, contributes to its active regeneration, stimulates the reparative processes, renewal of the epidermis and the coat (Katiyar et al., 2011; Sati et al., 2016; Karimi et al., 2018). According to some reports (Wu et al., 2015; Khariv et al., 2017), active substance in the content of *Silybum marianum* normalizes the activity of the sebaceous glands. Non-saturated fatty acids, as constituents of the oil, contribute to effective removal of rash, dandruff from the skin, prevent the enlarging of the pores, exhibit anti-itch effect, etc.

In spite of the fact that during the fungal skin infection in dogs the main clinical symptoms are associated with its impaired functional and morphological condition, the use of liniment made on the base of the oil from milk thistle not only has practical but special therapeutic significance.

## Conclusion

VetMikoDerm preparation used in the scheme of treatment of dogs suffering from fungal dermatoses has a positive effect on blood circulation, exhibits hepatoprotective and immunostimulating effects. Against the background of fungicidal effect, the preparation in the form of liniment displays anti-inflammatory, wound-healing, regenerative and anti-itch effects. In cases of severe fungal infections of the skin, complete recovery with restoration of the coat occurred on 10–14th days.

The perspective of the further research. Determining pharmacodynamics and pharmacokinetics of the newly-developed preparation.

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