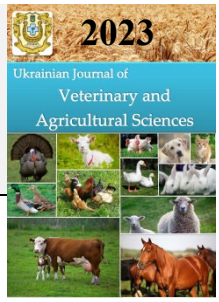
Ukrainian Journal of
Veterinary and Agricultural Sciences
http://ujvas.com.ua

The Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv

original article | UDC 619.615.5 | doi: 10.32718/ujvas6-1.06

Volume 6
Number 1



The dermal toxicity of an antiseptic preparation as determined by the different research methods

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Article info
Received 06.02.2023
Received in revised form 06.03.2023
Accepted 07.03.2023

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Abstract

The article presents the results of the study of acute and subacute skin toxicity of an antiseptic ointment based on ichthyol. The research was conducted using the classic method and according to the methodology of the Organization for Economic Cooperation and Development (OECD No. 410). The study of the dermal toxicity of the product under study by the classic method showed that long-term use of the drug in animals of the experimental groups did not cause significant changes in the concentration of hemoglobin, the number of erythrocytes, leukocytes, the value of hematocrit, the average concentration of hemoglobin in the erythrocyte (MCHC), the average volume of the erythrocyte (MCV), the average content of hemoglobin in the erythrocyte (MCH) and the number of platelets, the content of total protein, the level of creatinine and the activity of AST. The study of skin toxicity of the product under study according to the OECD method No. 410 showed that the number of erythrocytes increased in the animals of the I, II, and III research groups, respectively, by – 10.7 ($P < 0.01$), 6.0 ($P < 0.05$), and 7.5 % ($P < 0.05$). At the same time, the concentration of hemoglobin did not change. In addition, in the animals of the I and II experimental groups, a tendency towards an increase in hematocrit was established. In the animals of the I, II, and III experimental groups, changes in the erythrocyte index were noted; in particular, a probable decrease in the average content of hemoglobin in the erythrocyte (MCH) was established, respectively by – 5.2 ($P < 0.05$), 5.2 ($P < 0.01$) and 7.1 % ($P < 0.001$) of the average erythrocyte volume (MCV), respectively by – 6.9 ($P < 0.01$), 5.5 % ($P < 0.01$) and 7.2 % ($P < 0.001$). The use of the drug in a therapeutic dose caused a slight decrease in the level of total protein, urea, and creatinine against a slight increase in the activity of AST and ALT. At the same time, the animals of the II and III research groups, which received the drug under investigation at five times and ten times the therapeutic dose, noted an increase in the level of urea, respectively, by – 3.3 and 8.5 %, and creatinine, respectively, by – 1.9 and 8.0 % ($P < 0.05$), the activity of AST, respectively, by – 2.3 and 3.2 %, and ALT, respectively, by – 10.4 ($P < 0.05$) and 13.4 % ($P < 0.05$) compared to the values of animals of the control group.

Keywords: laboratory rats; acute; subacute dermal toxicity; OECD test № 410; hematological indicators; biochemical indicators.

Citation:
Kushnir, V. I. (2023). The dermal toxicity of an antiseptic preparation as determined by the different research methods. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 6(1), 40–44.

1. Introduction

Mild medicinal forms, particularly ointments, are widely used for the local treatment of skin diseases in humans and animals. Such pharmacological groups of drugs allow the introduction of hydrophilic and hydrophobic biologically active substances of different natures and properties into the composition of the medicinal product, which affects the active substance's release, increasing the effectiveness and safety of medicines (Tsulun et al., 2014; Koller & Bauer, 2022; Kang et al., 2022).

However, with the introduction of antibiotics into medical practice, attention to such agents has significantly decreased, and the scope of their application has significantly decreased (Kravchenko et al., 2016). In addition, the insufficient effectiveness of many ointments is associated with the composition and ointment base, which are only sometimes sufficiently effective in treating purulent wounds (Petronic-Rosic, 2022).

With this in mind, in medical practice, great attention is paid to developing and introducing antiseptic drugs that do not have a toxic effect on macroorganisms or a high bactericidal effect. This is because the resistance of microorganisms to such drugs develops more slowly than antibiotics, and allergic reactions occur much less often (Blatun, 2002; Maksimovskij et al., 2010; Eltwisy et al., 2022; Di Lodovico et al., 2022; Kukhtyn et al., 2022). At the same time, one of the essential stages in developing safe, effective, and competitive medicines is the study of their toxicological parameters (Karbovskyi et al., 2016; Gutyj et al., 2017; Todoriuk et al., 2018; Varcholyak & Gutyi, 2019; Karpenko et al., 2022; Martyshuk et al., 2022).

In the European community, preclinical studies are conducted following EU Regulations (Council Regulation (EC) No 440/2008) and the recommendations of the Organization for Economic Cooperation and Development (OECD), which allows for a comprehensive assessment of the investigated substance according to the degree of its danger and potential impact on the body of animals and the environment (OECD, 2017). However, in the practice of veterinary medicine in Ukraine, there is no clearly described methodology for determining the toxicity of drugs for long-term dermal application. Such studies are conducted according to a method that does not allow for a thoroughly assessing of the toxicity of drugs. In particular, when evaluating different ways of determining skin toxicity during long-term use of a wound-healing ointment (made based on betamethasone dipropionate, gentamicin sulfate, and clotrimazole), other toxicological effects were established (Kushnir et al., 2022).

The aim of the research was in a comparative aspect to determine the acute and subacute toxicity of the drug for dermal application using the national (classical) method and the method according to OECD No. 410 (OECD, 1981). An antiseptic ointment based on ichthyol was a marker for the comparative assessment of the two methods.

2. Materials and methods

Determination of the acute dermal toxicity of the drug under study was carried out by the classic method on white Wistar rats 3–4 months old, with body weight 220–240 g. In a tentative experiment, the drug was applied once to the skin in the dose range of 50, 500, 2500, and 5000 mg/kg of body weight, while three animals were used for each dose. In the extensive experiment, three groups of white rats (control and two experimental), six animals in each, were formed according to the principle of analogs. The animals of the I (control) group were treated with liquid paraffin (the base of the drug), the animals of the II research group were treated with the drug at a dose of 2500 mg/kg, and the animals of the III group were treated with 5000 mg/kg of body weight. The drug was applied once to the skin of animals.

According to the OECD test No. 402 (OECD, 2017), healthy, young animals with a body weight of 220–240 g were used when determining acute skin toxicity by an alternative method. The studied agent was applied to the prepared skin surface and kept in contact with a porous gauze bandage and a non-irritating tape for 24 hours. Determination of acute skin toxicity was carried out in two stages. In the first stage, the studied drug was used in 200, 1000, and 2000 mg/kg of body weight, using one animal for each dose. In the second stage, the studied drug was administered to two animals at a dose of 2000 mg/kg of weight to confirm the classification.

When determining acute skin toxicity by classical and alternative methods, a section (at least 10 % of the total body surface area) was excised from the dorsal surface of the body 24 hours before applying the test agent. Healthy animals with intact skin, without existing cracks, cuts, and redness, were selected for the experiment.

After applying the drug, laboratory animals were observed for 14 days. At the same time, the following indicators were taken into account: general condition, the behavior of animals, nature, and degree of activity, coordination of movements, presence of tremors, convulsions, paresis, paralysis, change in skin color, condition of fur, visible mucous membranes, relationship to feed, time of occurrence and nature intoxication, its severity, course and time of death of animals or their recovery.

When studying the subacute skin toxicity of the drug by the classical method, four groups of animals, five each, were formed according to the principle of analogs. Animals of the control group were given water on a pre-prepared area of the body, animals of the first experimental group were given the drug in a therapeutic dose (0.25 ml/kg body weight), animals of the second experimental group were given five times the therapeutic amount (0.5 ml/kg of body weight), animals of the III research group – at ten times the therapeutic dose (2.5 ml/kg of body weight).

When studying the drug's effect on the organism of laboratory animals by the method described in the OECD No. 410, 4 groups of 5 animals each were formed according to the principle of analogs. The product under study was kept in contact with the skin for six hours using a porous gauze bandage. Animals of the control group were treated with water on a pre-prepared area of the body, animals of the first experimental group were given the drug under study at a dose of 0.25 ml/kg body weight, animals of the second experimental group were injected with a dose of 0.5 ml/kg body weight, animals III experimental group – in a dose of 2.5 ml/kg body weight. After the exposure period ended, the studied drug's remains were removed using water (OECD, 1981).

In the animals that were used in the comparative evaluation of the two methods, the day before the start of the experiment, hair was removed from the dorsal surface in an area not less than 10 % of the total surface area of the animal's body. Repeated hair removal was performed weekly. The product under study was applied to the prepared dorsal surface of the skin for 28 days.

After the end of the experiment, the laboratory animals were decapitated under light ether anesthesia, blood was collected for hematological and biochemical studies, and the weight coefficients of the internal organs were determined. Blood stabilized with EDTA was used for hematological studies, and blood serum was used for biochemical studies. In the stabilized blood, the following were determined: hemoglobin content, number of erythrocytes and leukocytes, hematocrit, and red blood indices – with the help of a Mythic-18 hematological analyzer. In blood serum, the following were determined: total protein using an IRF-22 refractometer, enzyme activity (ALT, AST), creatinine content, and urea using a semi-automatic biochemical analyzer HumaLyzer 3000 using standard sets of the Human Company. The obtained data were processed statistically by determining average values, the credible interval at the available significance level of $P < 0.05$, considering the Student's criterion.

3. Results and discussion

When determining acute skin toxicity using the classical method, it was established that using the tested agent in doses of 50, 500, 2500, and 5000 mg/kg of body weight did not cause the death of laboratory animals. During the 14-day observation period, the laboratory animals were active, mobile, willingly ate feed, and the fur was thick, shiny, and well-fitting to the body. The mucous membrane of the oral and nasal cavities is shiny and pale pink; the secretion is preserved. In addition, using the drug in the doses indicated

above did not cause redness, swelling, and discoloration of the skin. Therefore, according to SOU 85.2-37-736:2011, the studied drug belongs to the IV toxicity class (low-toxic substances).

The determination of acute skin toxicity by the OECD method was carried out in two stages. In the first stage, it was established that using the tested agent at 200 mg/kg of body weight caused slight redness, which disappeared. At the same time, it was noted that using the tested agent in doses of 1000 and 2000 mg/kg of body weight on the second day caused redness, which disappeared on the experiment's third or fourth day.

At the second stage of research, it was established that using the researched agent in a dose of 2000 mg/kg of body weight did not cause the death of animals. At the same time, a slight reddening of the skin at the place of application of the drug under study was noted, which subsequently disappeared, so according to the GHS, it belongs to category 5 (class 5).

Later, in a comparative aspect, the effect of the researched product on the body of animals during long-term dermal application was studied.

Therefore, when studying the drug's effect on the body of laboratory animals using the classical method, it was established that its use did not cause significant changes in the weight coefficients of the liver, spleen, and heart. At the same time, a slight decrease in the body weight of animals and the weight coefficient of the weight of the kidneys in animals of all experimental groups were established (Table 1).

When determining the hematological parameters (Table 2), it was established that the use of the researched product for 28 days in the animals of the I, II, and III experimental groups did not cause significant changes in the concentration of hemoglobin, the number of erythrocytes, leukocytes, the value of the hematocrit, the average concentration of hemoglobin in the erythrocyte (MCHC), average erythrocyte volume (MCV), moderate hemoglobin content in erythrocytes (MCH) and platelet count.

When determining the effect of the researched agent on the biochemical indicators of blood serum (Table 3), it was established that the use of the researched drug based on ichthyol in the animals of the experimental groups did not cause significant changes in the content of total protein, creatinine, and AST activity. However, a slight increase in the level of urea and ALT was noted compared to the indicators of the control group.

Table 1

Weight coefficients of the mass of internal organs of white rats on 29th day of the experiment (M ± m, n = 5)

Internal organs	Group of animals			
	Control	I group	II group	III group
Liver	26.5 ± 0.79	26.1 ± 0.79	25.8 ± 1.63	25.6 ± 1.29
Spleen	2.04 ± 0.08	2.02 ± 0.05	2.17 ± 0.20	2.11 ± 0.10
Heart	3.37 ± 0.12	3.55 ± 0.25	3.46 ± 0.25	3.42 ± 0.17
Lungs	6.66 ± 0.62	7.06 ± 0.16	6.2 ± 1.05	7.86 ± 1.57
Kidneys	7.12 ± 0.25	6.67 ± 0.14	6.94 ± 0.15	6.91 ± 0.48
Body weight	254.0 ± 4.0	250.0 ± 7.25	246.2 ± 6.44	245.0 ± 8.94

Table 2

Hematological indicators of the blood of white rats for the study of toxicity by the classic method (M ± m, n = 5)

Indicators	Control	I group	II group	III group
Hemoglobin, g/L	168.2 ± 1.49	172.2 ± 4.89	170.2 ± 4.22	168 ± 3.18
Erythrocytes, 10^{12}/L	7.94 ± 0.12	8.43 ± 0.18	8.19 ± 0.17	8.07 ± 0.16
Leukocytes, 10^9/L	9.5 ± 0.69	10.5 ± 1.63	10.2 ± 0.76	10.1 ± 1.51
Hematocrit, %	40.6 ± 0.85	41.1 ± 0.97	40.8 ± 1.03	40.4 ± 0.62
MCH, p/g	21.2 ± 0.18	20.5 ± 0.69	20.9 ± 0.79	20.8 ± 0.45
MCHC, g/L	41.5 ± 0.95	41.9 ± 1.14	41.7 ± 1.09	41.5 ± 0.57
MCV, μm^3	51.1 ± 1.01	48.8 ± 1.58	50.02 ± 1.99	50.1 ± 1.20
Thrombocytes	825 ± 33.2	814.4 ± 26.9	789.2 ± 28.3	797.6 ± 20.9

Table 3

Biochemical indicators of blood serum of white rats for the study of toxicity by the classic method (M ± m, n = 5)

Indicators	Control	I group	II group	III group
Total protein, g/L	76.6 ± 1.59	76.2 ± 1.82	75.8 ± 1.15	75.6 ± 1.09
Urea, mmol/L	7.86 ± 0.88	8.16 ± 0.69	8.1 ± 0.41	8.34 ± 0.53
Creatinine, μmol/L	75.5 ± 1.34	74.9 ± 1.77	76.2 ± 1.84	77.9 ± 1.93
AST, unit/L	204.8 ± 19.4	200.8 ± 11.7	206,1 ± 9.75	208.0 ± 13.5
ALT, unit/L	72.1 ± 5.5	72.9 ± 4.19	73.7 ± 7.62	74.8 ± 5.66

Therefore, the study of the skin toxicity of the product under study by the classic method showed that in the animals of the experimental groups, long-term use of the drug did not cause significant changes in the concentration of hemoglobin, the number of erythrocytes, leukocytes, the value of hematocrit, the average concentration of hemoglobin in the erythrocyte (MCHC), the average volume of the erythrocyte (MCV), the average content of hemoglobin in the erythrocyte (MCH) and the number of platelets, the content of total protein, the level of creatinine and the activity of AST. At the same time, in the animals of the I, II, and III experimental groups, a tendency to a slight increase in

the level of urea and ALT was noted compared to the indicators of the control group.

In the study of subacute toxicity by the OECD method, it was established that the 28-day dermal application of the drug under study did not cause illness or death in laboratory rats. The animals were active, mobile, and willingly ate fodder; the fur was thick, shiny, and well fitting to the body. The mucous membrane of the oral and nasal cavities is shiny and pale pink; the secretion is preserved.

Skin application of the researched agent for 28 days (Table 4) in animals of the I, II, and III experimental groups did not cause probable changes in the weight coefficients of the mass of the spleen, lungs, and heart. At the same time, a tendency towards an increase in the weight coefficient of the kidney mass against the background of a decrease in the weight coefficients of the liver mass and body weight was noted.

Table 4

Weight coefficients of the mass of internal organs of white rats on 29th day of the experiment (M ± m, n = 5)

Internal organs	Groups of animals			
	Control	I group	II group	III group
Liver	26.1 ± 0.92	25.2 ± 0.65	24.9 ± 0.67	24.5 ± 0.85
Spleen	2.08 ± 0.09	1.88 ± 0.06	2.48 ± 0.39	1.97 ± 0.16
Heart	3.26 ± 0.16	3.62 ± 0.11	3.40 ± 0.21	3.13 ± 0.09
Lungs	7.44 ± 0.87	6.62 ± 0.34	7.64 ± 0.98	6.46 ± 0.68
Kidneys	6.54 ± 0.20	7.1 ± 0.29	6.92 ± 0.35	6.68 ± 0.17
Body weight	264.0 ± 4.85	253.8 ± 4.68	250.2 ± 3.43*	247.6 ± 3.50*

Note: * – P < 0.05

Table 5

Hematological indicators of the blood of white rats on the 29th day of the experiment (M ± m, n = 5)

Indicators	Group of animals			
	Control	I group	II group	III group
Hemoglobin, g/L	163.8 ± 1.93	171.8 ± 3.67	165.2 ± 2.39	163.2 ± 4.12
Erythrocytes, 10^{12}/L	7.72 ± 0.12	8.55 ± 0.16**	8.19 ± 0.12*	8.3 ± 0.16*
Leukocytes, 10^{9}/L	8.06 ± 0.81	9.58 ± 1.3	7.38 ± 0.74	9.52 ± 0.75
Hematocrit, %	41.9 ± 0.58	43.1 ± 0.76	42.02 ± 0.70	41.9 ± 1.09
MCH, p/g	21.2 ± 0.19	20.1 ± 0.29*	20.1 ± 0.24**	19.7 ± 0.19***
MCHC, g/L	39.1 ± 0.10	39.9 ± 0.16*	39.3 ± 0.17	38.9 ± 0.30
MCV, μm^3	54.3 ± 0.42	50.5 ± 0.67**	51.3 ± 0.73**	50.4 ± 0.48***
Thrombocytes	884.0 ± 26.8	879.0 ± 19.95	869.8 ± 20.8	780.4 ± 36.0

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001

Long-term dermal application of the product under study (Table 5) in animals of the I, II, and III experimental groups caused an increase in the number of erythrocytes, respectively, by – 10.7 (P < 0.01), 6.0 (P < 0.05) and 7.5 % (P < 0.05). At the same time, the concentration of hemoglobin did not change. In addition, in the animals of the I and II experimental groups, a tendency to increase the value of hematocrit was established. Under these conditions, changes in the erythrocyte index were noted in the animals of the I, II, and III research groups; in particular, a probable decrease in the average content of hemoglobin in the erythrocyte (MCH) was established, respectively by – 5.2 (P < 0.05), 5.2 (P < 0.01) and 7.1 % (P < 0.001) of the average erythrocyte volume (MCV), respectively, by – 6.9 (p<0.01), 5.5 % (P < 0.01) and 7.2 % (P < 0.001). Under these conditions, an increase in the average concentration of hemoglobin in erythrocytes (MCH) was noted in the animals of the I and II research groups. Whereas, in the animals of the III experimental group, a slight decrease in this indicator was noted compared to the values of the control group.

When determining the biochemical indicators of blood serum, the data shown in Table 6 were obtained.

Table 6

Biochemical indicators of blood serum of white rats on the 29th day of the experiment (M ± m, n = 5)

Indicator	Group of animals			
	Control	I group	II group	III group
Total protein, g/L	71.1 ± 1.11	67.9 ± 3.62	67.1 ± 1.91	65.8 ± 1.45*
Urea, mmol/L	8.5 ± 0.53	8.46 ± 0.44	8.78 ± 0.89	9.22 ± 0.55
Creatinine, µmol/L	64.7 ± 1.09	64.5 ± 2.42	65.9 ± 2.47	69.9 ± 1.95*
AST, unit/L	201.2 ± 11.3	204.6 ± 4.55	205.8 ± 7.31	207.6 ± 12.7
ALT, unit/L	69.2 ± 2.47	73.8 ± 4.73	76.4 ± 1.60*	78.5 ± 2.88*

Note: * – P < 0.05

The use of the drug in a therapeutic dose caused a slight decrease in the level of total protein, urea, and creatinine, respectively, by – 4.5, 0.5, and 0.3 %. Under these conditions, a slight increase in the activity of AST and ALT was noted, respectively, by – 1.7 and 6.6 %, compared to the values of the control group.

At the same time, the animals of the II and III research groups, which received the drug under investigation at five

times and ten times the therapeutic dose, noted an increase in the level of urea, respectively, by – 3.3 and 8.5 %, and creatinine, respectively, by – 1.9 and 8.0 % ($P < 0.05$), the activity of AST, respectively, by – 2.3 and 3.2 %, and ALT, respectively, by – 10.4 ($P < 0.05$) and 13.4 % ($P < 0.05$) compared to the values of the control group.

4. Conclusions

A comparative assessment of the study of the skin toxicity of an antiseptic preparation based on ichthyol by the classic method and the method according to the OECD No. 410 indicates that the latter is more informative. In particular, the study of skin toxicity of the researched product by the classic method showed that long-term use of the drug in experimental animals did not cause significant changes in hematological and biochemical parameters. While the study of skin toxicity by the method according to OECD No. 410 showed that with long-term use of an antiseptic drug in animals of experimental groups, the number of erythrocytes increases against the background of a decrease in the content of hemoglobin in the erythrocyte (MCH), the average volume of the erythrocyte (MCV), as well as a decrease in the level total protein, urea, and creatinine.

In further studies, it is advisable to study the toxicity of different pharmacological groups of veterinary medicines in a comparative aspect.

Conflict of interest

The author declare that there is no conflict of interest.

References

Blatun, L. A. (2002). Flegmony i abscessy – sovremennye vozmozhnosti lechenija. *Lechashhij vrach*, 1-2, 30–40 (in Russian).
[Google Scholar]

Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (Text with EEA relevance).
[Article] [Google Scholar]

Di Lodovico, S., Fasciana, T., Di Giulio, M., Cellini, L., Giammanco, A., Rossolini, G.M., & Antonelli, A. (2022). Spread of Multidrug-Resistant Microorganisms. *Antibiotics (Basel),* 11(7), 832.
[Crossref] [Google Scholar]

Eltwisy, H. O., Twisy, H. O., Hafez, M. H., Sayed, I. M., & El-Mokhtar, M. A. (2022). Clinical Infections, Antibiotic Resistance, and Pathogenesis of Staphylococcus haemolyticus. *Microorganisms*, 10(6), 1130.
[Crossref] [Google Scholar]

Gutyj, B., Grymak, Y., Drach, M., Bilyk, O., Matsjuk, O., Magrelo, N., Zmiya, M., & Katsaraba, O. (2017). The impact of endogenous intoxication on biochemical indicators of blood of pregnant cows. *Regulatory Mechanisms in Biosystems*, 8(3), 438–443.
[Crossref] [Google Scholar]

Kang, S. Y., Um, J. Y., Chung, B. Y., Lee, S. Y., Park, J. S., Kim, J. C., Park, C. W., & Kim, H. O. (2022). Moisturizer in Patients with Inflammatory Skin Diseases. *Medicina (Kaunas),* 58(7), 888.
[Crossref] [Google Scholar]

Karbovskyi, V. L., Shevchuk, I. A., Kurkina, O. V., & Makovska, T. Ye. (2016). Eksperymentalne doslidzhennia pidhostroi toksychnosti preparatu «Dermabin». *Farmatsevtychnyi chasopys*, 3, 45–51 (in Ukrainian).
[Crossref] [Google Scholar]

Karpenko, Y., Hunchak, Y., Gutyj, B., Hunchak, A., Parchenko, M., & Parchenko, V. (2022). Advanced research for physico-chemical properties and parameters of toxicity piperazinium 2-((5-(furan-2-YL)-4-phenyl-4H-1,2,4-triazol-3-YL)THIO)acetate. *ScienceRise: Pharmaceutical Science*, 2(36), 18–25.
[Crossref] [Google Scholar]

Koller, U., & Bauer, J. W. (2022). Molecular Research and Treatment of Skin Diseases. *International Journal of Molecular Sciences*, 23(10), 5435.
[Crossref] [Google Scholar]

Kravchenko, V. H., Khadzhai, Ya. I., Lukashov, S. V., & Levchenko, V. V. (2016). Eksperymentalna otsinka hostroi toksychnosti Tsydipolu – antyseptychnoho likarskoho zasobu. *Dermatovenerologija. Kosmetologija. Seksopatologija*, 1-4, 74–77 (in Ukrainian).
[Article] [Google Scholar]

Kukhtyn, M., Malimon, Z., Salata, V., Rogalskyy, I., Gutyj, B., Kladnytska, L., Kravcheniuk, Kh., & Horiuk, Y. (2022). The Effects of Antimicrobial Residues on Microbiological Content and the Antibiotic Resistance in Frozen Fish. *World's Veterinary Journal*, 12(4), 374–381.
[Crossref] [Google Scholar]

Kushnir, V. I., Kushnir, I. M., Patereha, I. P., Kutsan, O. T., Zhovnir, O. M., & Gutyj, B. V. (2022). Comparative assessment of various methods of studying the skin toxicity of a wound-healing drug. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 5(2), 3–7.
[Crossref] [Google Scholar]

Maksimovskij, Ju. M., Chirkova, T. D., & Ul'janova, N. A. (2010) Preparat «Stomatofit» v kompleksnom lechenii vospalitel'nyh zabolevanij parodonta. *Sovremennaja stomatologija*, 3, 45–47 (in Russian).
[Google Scholar]

Martyshuk, T, Gutyj, B, Vyshchur, O., Paterega, I., Kushnir, V., Bigdan, O., Bushueva I., Parchenko V., Mykhailiuk E., Aleksieiev O., Tkachenko N. (2022). Study of Acute and Chronic Toxicity of "Butaselmevit" on Laboratory Animals. *Archives of Pharmacy Practice,* 13(3), 70–75.
[Crossref] [Google Scholar]

OECD (1981). Test No. 410 "Repeated Dose Dermal Toxicity: 21/28-day Study".
[OECD] [Google Scholar]

OECD (2017). Test No. 402: Acute Dermal Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.
[OECD] [Google Scholar]

Petronic-Rosic, V. (2022). Treatment of Skin Disease: Comprehensive Therapeutic Strategies. *Skinmed*, 20(5), 400.
[Article] [Google Scholar]

Todoriuk, V., Hunchak, V., Gutyj, B., Gufriy, D., Hariv, I., Khomyk, R., & Vasiv, R. (2018). Preclinical research of the experimental preparation "Ferosel T". *Ukrainian Journal of Veterinary and Agricultural Sciences*, 1(1), 3–9.
[Crossref] [Google Scholar]

Tsulun, O. V., Derymedvid, L. V., & Zelenin, Yu. V. (2014). Doslidzhennia hostroi toksychnosti kombinovanykh mazei pryrodnoho pokhodzhennia. *Problemy ekolohichnoi ta medychnoi henetyky i klinichnoi imunolohii,* 1, 264–272 (in Ukrainian).
[Article] [Google Scholar]

Varcholyak, I. S., & Gutyi, B. V. (2019). Determination of the chronic toxicity of preparation "Bendamin" on laboratory animals. *Theoretical and Applied Veterinary Medicine*, 7(2), 63–68.
[Crossref] [Google Scholar]