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DETERMINATION OF ACUTE TOXICITY OF THE 'BONDARMIN' DISINFECTANT

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Summary. In the article the results of the study of toxic effect of the designed disinfectant (active ingredient potassium peroxomonosulfate) on laboratory animals (mice) are presented. For the recent years a variety of scientific works both by domestic and by foreign scientists has been devoted to the study of different disinfectants' toxicity. However today there is a number of issues that require more detailed studying and scientific justification. Among them the problem of toxic effects of disinfectants on the animal organism occupies a special place. The aim of our work was to study the toxic effect on the laboratory animals and to assess the acute toxicity (LD_{50}) of the designed 'Bondarmin' disinfectant. Tests were carried out at the Laboratory of pharmacology and toxicology of the National University of Pharmacy (Kharkiv) and on the base of Educational and scientific laboratory of genetic and molecular research methods named after P. I. Verbitskiy in the Kharkiv State Zooveterinary Academy. Acute toxicity assessment (LD_{50}) was carried out with intragastrointestinal administration of the designed drug to laboratory animals (mice). Changes in the internal organs of animals that were removed from the experiment for humane reasons and those who died after the experiment were detected by macroscopic examination. The lethality of laboratory animals after the intragastric administration of disinfectant was determined by the Prozorovskiy method The dynamic of changes in body weight of mice after the administration of disinfectant in high doses (from 1,500 to 3,500 mg/kg) was found out. The influence of the disinfectant on the mass coefficients of the internal organs of male mice after intragastric administration was evaluated. Toxic effect of the designed disinfectant 'Bondarmin', when using intragastric method of administration to laboratory animals (mice), according to the age and sexual index (LD_{50} = 2,702.40 ± 156.32 mg/kg), was established. Disinfectant 'Bondarmin' refers to IV toxicity class (low toxic substances).

Keywords: disinfectant, laboratory animals, toxic effect, lethal dose

Introduction. It is important to create scientifically grounded and comfortable animal housing conditions to ensure the high resistance of animals to diseases of different etiology (Chumachenko, Chumachenko and Pavlenko, 2004).

Timely and effective disinfection of premises and equipments has fundamental importance in the system of measures for the prevention and control of infectious diseases of farm animals (Kondratjuk, 2009; Holovko, Kochmarskyi and Tupozlieiev, 2011; Paliy, Paliy and Naumenko, 2015).

Today during disinfection when animal tuberculosis is declared, disinfectants, the active substance of which is glutaraldehyde, which interacts with the protein components of the membrane structures of bacterial cells, disrupting the processes of their synthesis and ultimately causing their death, are widely used (Chupakhin et al., 1987; Gutyj et al., 2017; Paliy et al., 2018).

At the same time, traditional means, based on chlorinated compounds, have high antimicrobial activity against mycobacteria. The bactericidal action of these means lies in the denaturation of protein and nucleic acids (Dychdala, 2001; Chapman, 2003; Zavgorodniy et al., 2013).

Taking into consideration the risk of infectious diseases, the improvement of the existing, finding new and developing more effective, environmentally safe, relatively cheap, technological, simple and accessible for use disinfectants with high bactericidal properties against mycobacteria remains the task of great importance (Paliy et al., 2015; Kozlovskaya, 2016).

Alongside, one of the main requirements for disinfectants is their low toxicity to animals and humans, which allows more extensive use of these drugs in the agriculture (Zavgorodniy et al., 2013).

It is necessary to start determination of the toxicity of the developed new veterinary medicines with an acute test, that is, with obtaining information about the dangers of the main experimental active substance for animal health after a short-term presence, as a result of which data on the lethal dose and concentration are expected (Kotsiumbas et al., 2006; Todoriuk et al., 2018).

'Bondarmin', a modern, powdered form disinfectant, well soluble in water, odorless, the active ingredient of which is potassium peroxomonosulfate, has been developed.

The aim of the study was the determination of the toxic effect of the 'Bondarmin' disinfectant on laboratory animals.

Material and methods. The toxicological properties of the 'Bondarmin' disinfectant, which is a domestic import-substituting development, have been identified. Studies were carried out using a rapid method to study moderately lethal doses of chemical compounds (Pastushenko et al., 1985).

The least squares method was used to analyze the lethality curves (Prozorovskiy, 1962).

For experimental studies 40 CD-1 mice were used. Mice were grown in the vivarium of the Kharkiv State Zooveterinary Academy and prior to the experiment they had undergone acclimatization under the conditions of the testing room for 7–10 days. The animal keeping conditions complied with the current rules for vivarium devices, equipment and maintenance. In accordance with the code of practice, animals received standard nutrition (CEC, 2010).

Animals were treated in accordance with the requirements of the Commission on Bioethics and the General Ethical Principles of Experiments on Animals, consistent with the provisions of the 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes' (CEC, 2010).

The acute toxicity of 'Bondarmin' was studied on laboratory animals (mice) trough intragastric injection to reproduce the acute poisoning clinic and to determine the LD_{50}.

Results and discussions. The determination of the 'Bondarmin' acute toxicity (LD_{50}) trough intragastric injection to laboratory animals (mice) was the primary stage of our research. For this purpose, laboratory animals (mice) were divided into groups of three. A dose range from 1,580 to 4,470 mg/kg was chosen. The tested disinfectant was injected intragastrically to the animals in the form of an aqueous mixture through a metal probe at a rate of 0.2 ml/10 g of body weight. The results of the experiment are presented in the Table 1.

According to the study results, the death of mice was noted when they were administered a dose of 2,000 mg/kg and higher. The required dose range for calculating LD_{50} was highlighted. For this purpose a sequence of animal death 1–3–3 was used. In doses for mice it is 2,000, 2,820, and 3,160 mg/kg. With the help of the table express method by Pastushenko et al. (1985) the LD_{50} was determined. According to this method, lower doses of the selected interval in the table find the LD_{50} value and its confidence limits for mice — 2,580 (1,930–3,220) mg/kg.

In order to confirm the above mentioned results of LD_{50} and to study other toxicity parameters, the acute disinfectant toxicity studies with intragastric administration using the least squares method for probit analysis of mortality curves according to Prozorovskiy (1962) was conducted (Table 2).

Table 1 — Lethality rate of laboratory animals after intragastric administration of the 'Bondarmin' disinfectant (n = 3)

Animal species, sex	Dose, mg/kg	Received effect, animals that died/overall number
Mice (male)	1,580	0/3
	2,000	1/3
	2,500	1/3
	2,820	3/3
	3,160	3/3
	3,550	3/3
	3,980	3/3
	4,470	3/3

Table 2 — Mortality of laboratory animals after intragastric injection of the 'Bondarmin' disinfectant according to Prozorovskiy method (n = 6)

Animal species, sex	Dose, mg/kg	Received effect, animals that died/overall number
Mice (male)	1,500	0/6
	2,000	1/6
	2,500	2/6
	2,750	3/6
	3,000	5/6
	3,500	6/6

To determine the average median lethal dose, experimental groups of six animals were formed. The animals were observed for 14 days after the injection of the disinfectant, registering manifestations of violations in the physiological state of the animals and mortality. From the data presented in Table 2, it was established that the death of mice occurs in the dose range of 2,000–3,500 mg/kg.

At the next stage calculation of the disinfectants toxic effect parameters for mice using Prozorovskiy method were carried out (Table 3).

According to the data presented in the Table 3, the calculated LD_{50} parameters for disinfectant after intragastric injection to mice are: A0 = 2.19; A1 = 0.87; LD_{16} = 2,087.71 mg/kg; LD_{50} = 2,702.40 mg/kg; LD_{84} = 3,298.53 mg/kg; m = 156.32 mg/kg.

Disinfectant 'Bondarmin' trough the intragastric injection to laboratory animals (mice) belongs to the IV toxicity class (low-toxic substances) with LD_{50} = 2,702.40 ± 156.32 mg/kg or 2,702.40 (2,378.82–3,025.97) mg/kg.

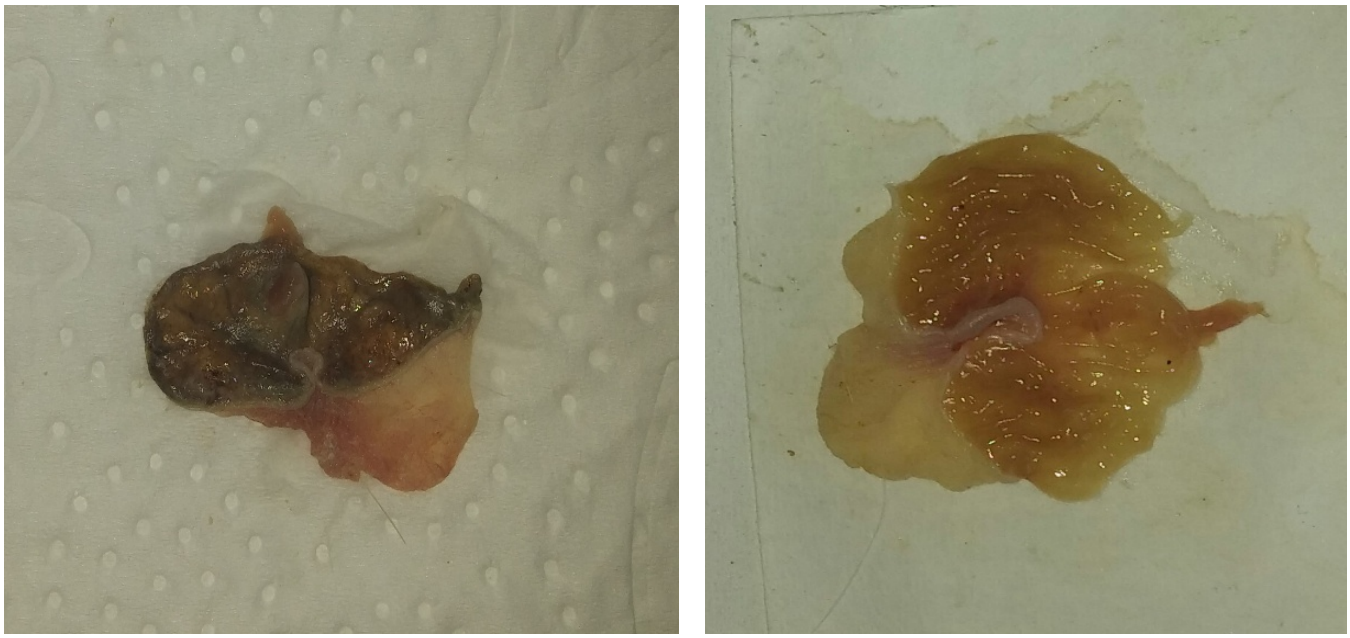
Table 3 — Calculation data for the determination of the LD_{50} of disinfectant in mice after intragastric injection by Prozorovskiy method

Dose, mg/kg	Lethality, %	Dose place (X)	Probit (Y)	Weighting coefficient (B)	xB	x^2в	yB	xyB
1,500	0	1	3.27	1.6	1.60	1.60	5.23	5.23
2,000	16.67	2	4.05	3.7	7.40	14.80	14.99	29.97
2,500	33.33	3	4.56	4.6	13.80	41.40	20.98	62.93
2,750	50.00	3.5	5.00	5	17.50	61.25	25.00	87.50
3,000	83.33	4	5.95	3.5	14.00	56.00	20.83	83.30
3,500	100	5	6.73	1.6	8.00	40.00	10.77	53.84
Σ				20	62.30	215.05	97.79	322.77

After administration of toxic doses of disinfectant to animals such signs of intoxication were observed: reduced motor activity, heavy breathing, cold limbs and tail, lack of appetite, developed stupor, and then death occurred. The death of animals was observed 2–3 hours after administration of doses 2,750–3,500 mg/kg and the death was delayed from 1 to 8 days after the injection of smaller doses of the disinfectant 'Bondarmin'.

A macroscopic examination of the internal organs of animals, which were removed from the experiment according to humane motives and those who died, showed that the gastric mucosa had a broken relief of folds and was full of blood, as well as numerous point and linear ulcers were observed (Fig. 1).

As was shown by the results of survived animals' observation, they were active, had a satisfactory appetite, responded to sound and light irritators, urination and defecation were normal, respiratory failure and convulsions were not observed. All animals maintained the reflex excitability. The water and food consumption in all experimental animals did not differ from the animals of the intact control group.



A **B**

Figure 1. Mice stomach: A — after injection of a disinfectant in a dose of 2,750 mg/kg, B — intact control

The study of mice body weight showed (Table 4) that the mass of survived animals was not statistically different from the values of the control group during the study period.

As to the other indicators of the internal organs of the survived animals, the use of disinfectant did not lead to their change compared with animals of the intact control group. Indicators were within the physiological range (Table 5).

Calculation and statistical analysis of the mass coefficient indicators of the mice internals showed that intragastric injection of the 'Bondarmin' disinfectant in high doses resulted in decrease of the thymus mass coefficient.

Table 4 — Dynamics of the mice body weight after the injection of the 'Bondarmin' disinfectant in high doses (n = 6)

Group	Dose, mg/kg	Animal body weight, g			
		Data line	For the 3rd day	For the 7th day	For the 14th day
Intact control	—	26.7 ± 1.4	27.1 ± 1.3	26.9 ± 1.2	27.1 ± 1.1
Experimental	1,500	27.6 ± 1.1	26.2 ± 1.3	26.4 ± 1.1	27.7 ± 1.1
	2,000	25.0 ± 0.8	23.9 ± 0.8	24.9 ± 0.9	25.1 ± 0.9
	2,500	25.9 ± 1.0	24.5 ± 0.2	24.9 ± 0.3	25.5 ± 0.2
	2,750	25.5 ± 1.5	24.5 ± 0.2	24.9 ± 0.4	25.1 ± 0.4
	3,000	26.3 ± 1.7	22.2	22.0	23.2
	3,500	25.1 ± 0.9	—	—	—

Table 5 — Mass coefficient of the male mice internals after intragastric injection of a disinfectant (n = 6)

Internals	Intact control (n = 6)	Disinfectant, mg/kg				
		1,500 (n = 6)	2,000 (n = 5)	2,500 (n = 4)	2,750 (n = 3)	3,000 (n = 1)
Liver	5.20 ± 0.21	5.59 ± 0.30	5.43 ± 0.37	5.16 ± 0.36	5.05 ± 0.33	6.03
Right kidney	0.62 ± 0.05	0.69 ± 0.05	0.79 ± 0.07	0.67 ± 0.08	0.66 ± 0.07	0.95
Left kidney	0.61 ± 0.04	0.66 ± 0.04	0.75 ± 0.07	0.69 ± 0.09	0.67 ± 0.08	0.82
Heart	0.57 ± 0.03	0.54 ± 0.06	0.66 ± 0.04	0.54 ± 0.04	0.53 ± 0.03	0.66
Lungs	0.70 ± 0.03	0.81 ± 0.06	0.88 ± 0.06	0.72 ± 0.04	0.71 ± 0.04	0.76
Spleen	0.79 ± 0.06	0.98 ± 0.31	1.00 ± 0.04	0.90 ± 0.04	0.88 ± 0.03	0.56
Adrenal	0.061 ± 0.005	0.052 ± 0.006	0.061 ± 0.008	0.052 ± 0.002	0.051 ± 0.002	0.056
Thymus	0.298 ± 0.009	0.203 ± 0.022*	0.205 ± 0.017*	0.200 ± 0.004*	0.196 ± 0.035*	0.320
Right spermary	0.38 ± 0.04	0.34 ± 0.03	0.48 ± 0.05	0.40 ± 0.03	0.39 ± 0.03	0.42
Left spermary	0.37 ± 0.03	0.34 ± 0.03	0.44 ± 0.03	0.38 ± 0.02	0.38 ± 0.03	0.37

Note: * — the deviation of the indicator is statistically significant relative to the values of the intact control group (Man-Whitney test with Bonferoni correction), $p < 0.01$.

Conclusions. The toxic effect of the newly designed disinfectant 'Bondarmin' was established during the intragastric injection to laboratory animals (mice).

The median lethal dose of the 'Bondarmin' disinfectant by Prozorovskiy method is LD_{50} = 2,702.40 ± 156.32 mg/kg. The disinfectant belongs to the IV toxicity class (low toxic substances).

The results of toxicological studies allow us to recommend the use of 'Bondarmin' for disinfecting livestock facilities.

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