

Effects of microbicide based on lactic acid and metal nanoparticles on laboratory animals

G.V. Ponomarenko¹, V.L. Kovalenko², O.V. Ponomarenko¹, Yu.O. Balackiy³

¹*Kharkiv State Zooveterinary Academy*

Kharkiv, Ukraine. E-mail: gpkh1966@gmail.com

²*State Scientific Control Institute of Biotechnology and Strains of Microorganisms*

Kyiv, Ukraine. E-mail: kovalenkodoktor@gmail.com

³*Bila Tserkva National Agrarian University*

Bila Tserkva, Ukraine

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Modern complex disinfectants should not only be highly effective against most pathogens, but also safe for the body of laboratory and farm animals. To determine the effect of microbicides on animal organism, there are several commonly accepted parameters that are regulated by relevant methodological documents. Taking into account the urgency of the development and implementation of modern disinfectants, the purpose of the research was to study the effects of the drug "Orgsept" on the indicators of acute toxicity and its harmful effects on laboratory animals. The determination of acute toxicity, the study of the cumulative and skin-resorptive action of the drug "Orgsept" was performed on clinically healthy white mice, and the irritating effect and sensitizing properties were studied on Guinea pigs. The studies were conducted using generally accepted techniques (Yakubchak et al., 2005). According to the results of the research, acute toxicity, cumulative, sensitizing, irritating, skin-resorptive action of the disinfectant based on lactic acid and nanoparticles of silver "Orgsept" on laboratory animals were determined. It has been determined that the investigated preparation according to sanitary-and-hygienic norms GOST 12.1.007-76 belongs to the 3 groups of toxicity in concentrations which are significantly higher than bactericidal, it does not have a pronounced cumulative, sensitizing and skin-resorptive action.

Key words: lactic acid; nanoparticles of metals; microbicide; toxicity; laboratory animals

Introduction

Creation of disinfectants is aimed at the development of modern, safe, complex disinfectants and effective methods for assessing the degree of their harmlessness to the organism of laboratory and agricultural animals (Yakubchak et al., 2010; Kovalenko et al., 2016). At the present stage, improvement of the development and introduction of disinfectant with the active substance of lactic acid and nanoparticles of metals, which is an effective fungicide and antiseptic against gram-positive and gram-negative microflora by sanitary and hygiene indices, is carried out (Kotsyumbas et al., 2006; Nikolaenko and Tsapko, 2008; Kovalenko et al., 2016).

The study of microbicidal properties of silver nanoparticles and their use to create new complex disinfectants is a promising area of research in many countries around the world (Lopes et al., 2016; Ivask et al., 2014; De Gussemme et al., 2010). At the same time, in the literature there is enough information on the potential toxicity of both silver nanoparticles and other nanoparticles in certain concentrations not only on pathogenic microorganisms, but also on the body of different species of animals (Ivask et al., 2014; Armstrong et al., 2013; Likus et al., 2013).

In addition, nanomaterials are sufficiently dynamic in biological and ecological environments. This results in various physical and chemical transformations that affect the properties of nanomaterials and their toxicity, which must be taken into account in the study of potential environmental risks (Levard et al., 2013).

The above-mentioned stipulates the necessity of conducting researches on the study of toxicity and various effects of disinfectants based on silver nanoparticles on the organism of laboratory animals.

The purpose of the research was to determine the acute toxicity and to establish the harmful effect of the drug "Orgsept" with the active ingredient of lactic acid and silver nanoparticles on laboratory animals.

Material and methods

The research was carried out in accordance with current methods (Yakubchak et al., 2005). For experiments, clinically healthy white mice weighing 18-20 g were taken, which were observed for 6 days, weak mice were removed from the group.

Prior to the determination of acute toxicity (LD_{50}), LD_{100} and LD_0 were pre-determined. For this purpose, the minimum concentration of the drug was determined, at which the death of all experimental animals occurred, and the maximum concentration, at which all the animals remained alive. Doses of the drug for the next stage were calculated so that the lower dose did not cause death of mice, the higher would cause 100% death, and between them there were at least 4 intermediate doses causing the death of more or less than 50% of mice.

To determine the acute toxicity of the drug, 7 experimental and one control groups of white mice weighing 18-20 g, 7 heads in each group were formed. A solution of disinfectant was injected directly into the stomach in a volume of 0.5 ml. The amount of active substance administered per 1 kg of living weight was calculated by the concentration of the study drug and the administered volume. Calculations were carried out using the Kerber method. The trial lasted 15 days. During the experiment, we observed the behavior of animals, recorded the number of deaths in each group. Pathologically-anatomical section of dead mice was carried out.

In order to study the cumulative effect of the drug "Orgasept", the experimental and control groups of mice were formed, 7 heads in each group, weighing 18-20 g. The control group of animals daily was fed the studied drug with milk at the rate of 1/5 of LD_{50} , the experimental group - the studied drug in the same dose. The research lasted for 60 days.

The irritating effect of the drug "Orgasept" was studied on the skin of Guinea pigs, the fur of these Guinea pigs was cut off on the day before the experiment in the area of the back on both sides. Three experimental groups and one control group of Guinea pigs were formed, 5 heads in each group. Twice a day, on a bald surface, solutions in concentrations of 1% and 3% were evenly applied. Animals of the control group were applied water on the surface with cut fur. The research lasted for 30 days. The study of sensitizing properties was carried out on Guinea pigs weighing 340-380 g. In experimental and control groups, there were 5 animals in each. Previously, a selection of sensitizing and testing concentrations of the drug was performed on 3 Guinea pigs. A drug in a native form, 25% and 10% concentrations of aqueous solutions, was applied on the skin of animals, 0.2 ml per animal, for 10 days. To sensitize the organism, the substance was injected intradermally into the outer ear to the animals of experimental group in a volume of 0.02 ml once a day, control animals were injected with 0.02 ml of distilled water.

Starting from the 12th day, the experimental animals were treated with 0.02 ml of 25% aqueous solution for 7 days, the substance was applied on the bald areas, and the same amount of distilled water was applied to the experimental animals. Animal testing was performed on the 10th and 20th day of the experiment when 0.2 ml of the drug was applied natively to the intact areas of the skin of the experimental and control animals. After applying the test concentration of the drug to the skin, the animals were monitored 24 and 48 hours later. The skin reaction was evaluated visually by a five-point unified scale.

The skin-resorptive effect of the test substance was studied on white mice weighing 18-20 g, the skin of which had no available signs of pathology. Within 15 days, two hours a day, experimental mice tail (five heads) were immersed in test tubes with 1% and 3% solution of the drug for 2 hours. Tails of control animals were placed in test tubes with water.

After administration of 0.5 ml of the test drug solutions in concentrations of 0.05% to 0.2%, which is 10 times higher than bactericidal dilution, in the stomach of mice, slaughter of mice (10 heads) was performed for the purpose of pathoanatomical separation, sampling and testing blood samples.

The results and discussion

After calculating the doses of the drug for 1 kg of live weight of experimental animals, the drug was injected into the gastrointestinal tract of the mice of the first group. Mice of the control group were injected 0.5 cm³ of water. During the study period, all animals remained alive in the first experimental and control groups. The death of animals of other groups was mostly observed from the 1st to the 10th day of observations. According to the results $LD_{50} = 3730$ mg / kg of live weight.

During the autopsy of dead mice (LD_{100} dose), changes in the gastrointestinal tract were observed, namely, redness and debris of the mucous membrane of the stomach and small intestine, liver enlargement, and the presence of foam fluid in the abdominal and thoracic cavities.

It has been established, that this preparation at introduction in a stomach, in accordance with sanitary-hygienic norms GOST 12.1.007-76, belongs to the 4th class of danger. When studying the cumulative effect of the drug during the observation of experimental and control animals, no deviations in the behavior and physiological functions of the experimental animals were detected. No animal died. After the completion of the experiment and the autopsy of the slaughtered mice, macroscopic changes in the internal organs were not established. When studying the irritating effect during the trial period (30 days), applying 1% and 3% disinfectant solutions we did not reveal any visible changes on both the skin surface and the physiological functions of the experimental animals. When studying the sensitizing properties, it was found that when the drug is applied to the skin of the animals in all investigated concentrations, the irritating action was not detected. After intradermal administration in the ear, there was not observed any changes on the skin of Guinea pigs. Application of the drug in sensitizing concentrations for 7 days did not have an irritant effect on the skin of Guinea pigs. Throughout the experiment, the skin was clean, of normal color. After 24-48 hours after the first and the second testing in experimental and control animals, the skin reaction to the action of antigen was 0 points.

During the research on the determination of skin-resorptive action of the drug "Orgasept" and after their completion, no signs of toxic 1% and 3% solutions in mice were detected.

It should be noted that in the recommended working concentration – 0.05-0.2% the preparation administered orally in a dose of 0.5 cm³, did not cause visible pathoanatomical changes during the autopsy of slaughtered mice. The results of blood testing of slaughtered mice are presented in Table 1 that indicate changes in blood characteristics of mice in 3 hours after disinfection. Thus, in comparison with the initial data, the indicators of leukocytes, basophils, eosinophils, lymphocytes were significantly increased. There was also an increase in the number of monocytes, a slight increase in hemoglobin and a slight decrease in red blood cells. The number of stab and segmented neutrophils was within the normal range.

Table 1. Morphological parameters of blood of mice after treatment with working solutions of the drug "Orgasept", M ± m, (n = 10)

Characteristics	Control	Before treatment	3 hours after treatment	7 days after treatment	14 days after treatment
Hemoglobin, g/L	103.0±1.0	97.0±3.1	107.0±1.7**	95.2±2.2	103.1±2.1**
Erythrocytes, T/L	9.5±0.1	9.3±0.21	8.9±0.3**	9.2±0.1	9.0±0.1**
Leucocytes, g/L	10.5±0.3	11.3±0.1	13.4±0.1**	12.5±0.2	10.3±0.4**
Leukogram, %:					
basophils	0	1.0±0.1	2.0±0.3	0	0
eosinophils	1.0±0.1	1.0±0.1	2.0±0.1	1.0±0.1	1.0±0.1
neutrophils	–	–	–	–	–
myelocytes	–	–	–	–	–
young	–	–	–	–	–
stab	3.0±0.1	2.0±0.2	3.0±0.1	2.0±0.1	2.0±0.2
segmented	22.0±1.1	27.0±1.5	31.0±1.3	29.0±1.6	27.0±1.1
lymphocytes	62.0±1.2	73.0±3.3	75.0±5.3	68.0±3.1	69.0±3.7
monocytes	1.0±0.1	2.0±0.1	2.0±0.1	2.0±0.1	1.0±0.1

Note: * - P < 0.05, ** - P < 0.01 in comparison with the control

In control mice, during the experiment, no significant changes in the leukocyte blood formula of animals were detected. Short-term medication leukocytosis, which arose after the external treatment of animals, was manifested. We also observed eosinophilia at a disease that runs through the phenomena of allergy and stress. However, the prognostic value of eosinophilia should be evaluated in combination with other clinical signs and changes in blood. Lymphocytosis was short-lived, accompanied by an increase in the number of eosinophils, monocytes, a slight decrease in neutrophils without significant changes in the level of hemoglobin and erythrocytes, indicating a rapid recovery of the body.

Conclusions

The "Orgasept" drug exhibits low toxicity to the organism of mice (LD₅₀ = 3730 mg / kg live weight). The drug solutions in the recommended working concentrations, which are 10 times higher than bactericidal, are not toxic to animals, at the slaughter and pathoanatomical section, there have not been detected visible changes, at the study of blood morphological indices were not changed credibly. The preparation does not have cumulative, sensitizing, irritating or skin-resorptive action at concentrations from 1% to 3%.

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