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REPORT

on toxicological examination of silver spray generated by the DEW POCKET device

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ABSTRACT

The report contains: 18 pages, annotation, 1 figure and 14 tables. Bibliography: 20 sources.

A comparative evaluation of the harmfulness of Dew Pocket silver spray was performed.

The scope of the study included the study of acute subacute toxicity and local irritation.

It was found that the acute effect of the studied drug at a dose of 3 g / kg did not cause death of animals. Clinical symptoms of intoxication, changes in the studied parameters were absent.

A study of Dew Pocket silver spray toxicity on rats showed that the application of Dew Pocket spray at a dose of 3 g / kg (1/10 of the maximum test in an acute experiment) on the skin for a month did not have a toxic effect on general condition, animal behavior, body weight and peripheral blood markers and indicators that characterize the functional state of the central nervous system, heart, liver and kidneys.

The Dew Pocket silver spray did not show local irritation effect in the study with rats.

CONTENT

Sources

INTRODUCTION

The main action of silver used in the clinic is its antimicrobial action, which appears against bacteria, fungi, viruses and protozoa (about 700 species of microorganisms), including strains that cause human disease.

Silver has the highest activity against bacteria. Bacteriostatic (at lower concentrations) and bactericidal (at higher concentrations) action of silver is appears against most gram-positive and gram-negative bacteria, including those that cause cause children and adults acute respiratory disease - Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis and others. Experimental studies shows that at a concentration of 1 μg / ml, silver ions can control most bacterial and fungal pathogens [1].

An important aspect of the silver action is the fact that microorganisms do not develop resistance to it [2].

The antimicrobial activity of silver substance is determined by the presence of its ionic forms - Ag^+ ions, because they interact with cellular membrane microorganisms. The antimicrobial effect is proportional to the degree of released ions from metal silver substance. Ionization of silver increases its activity in aqueous solutions.

Currently, the main areas of application of silver substances are: water disinfection, wound healing, infection prevention, oral hygiene (prevention and control of gingivitis, periodontitis), eye hygiene, including in newborns, treatment of nasal infections [3].

It has been found that silver solutions are an effective disinfectant in direct contact with purulent and inflamed surfaces due to bacterial contamination.

Silver is effective in open wounds, burns, bone prosthesis treatment, is used for periodontitis, in reconstructive orthopedic surgery [4].

New studies have shown its efficiency against AIDS, pneumonia, herpes and herpes [4].

Silver, especially in the form of nanoparticles, is characterized by toxicity in experiments with animals during systemic use. Thus, in a 13-week study in rats for oral use of silver at doses of 30, 125 and 500 mg / kg at two higher doses, dose-dependent hepatic hyperplasia with accumulation of silver nanoparticles was observed. The dose of 30 mg / kg was quite safe [5]. At 13 weeks terms of inhalation used by rats at doses of 49, 133 and 515 μg / m3, the target organ of the negative impact of silver was the lungs. At the highest dose, there was a chronic inflammatory process in the alveoli. The adverse effects on the liver were observed. A dose of 133 μg / m3 was considered as safe [6]. At the same time, when applied to the skin and applied to the eyes of rabbits, silver did not cause erosions and was classified as a mild irritant. Acute oral and dermal application of silver did not cause death of animals and signs of intoxication in doses up to 2000 mg / kg [7].

The aim of these studies was to study ionized silver spray synthesized by the Dew Pocket device (Fig. 1) according to the manufacturer's instructions [8].

The object of the study were samples of Dew Pocket spray , synthesized by the device Dew Pocket S series DP v 10.0.3, serial number S / N just_dewpocket, the following composition: 1 ml of spray contains ionized silver 0.15 ± 0.05 μg. One spray will contain 0.3 ml, which is 0.045 ± 0.05 μg of ionized silver.

The studies were performed on laboratory animals in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" [9].

Fig. 1 Dew Pocket S DP v 10.0.3 series, that synthesizes Dew Pocket spray. Appearance.

1 STUDY OF ACUTE TOXICITY OF DEW POCKET SILVER SPRAY

The aim of these studies was to study the toxicity of Dew Pocket silver spray (test substance) in acute experiments on rats. The experiments were guided by guidelines [10].

1.1 Materials and methods of research

The study was performed on white outbred rats of both sexes with an initial body weight of 190 - 245 g. The experimental groups of animals consisted of 5 males and 5 females. A total of 20 rats were used in the experiment.

The animals were raised in the vivarium of the State Institution "Institute of Microbiology and Immunology" Mechnikov Institute of Microbiology and Immunology Kharkiv. During the experiment, they were in the vivarium at an air temperature of 18-200C, humidity of 50-60%, the mode of natural light "day and night", in normal cages, on a standard diet [11].

The studies were performed on laboratory animals in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" [9].

The effect of the test substance was investigated by skin application. Rats hair on their backs, sides and abdomen was cutted off , which corresponds to approximately 50% of their body surface. Applications were performed once at a dose of 3.0 ml / kg. A group of rats which were depilated and applied 3.0 ml / kg of saline was used as a control.

Clinical pictures of intoxication, animal survival, food and water consumption, body weight dynamics (baseline, 3, 7, 14 days) used as criterias for spray toxicity. All biochemical studies were performed using diagnostic kits from Philisit-Diagnostics (Ukraine). The amount of total protein in the blood was determined by the biuret method, albumin - by reaction with bromocresol green, thymol test - by the method of sediment samples [12].

Animal observations were performed during 2 weeks.

On the 14th day after spray application, rats were euthanized by painless decapitation. At Roe autopsy [13], a macroscopic assessment of the condition of the internal organs was performed and their relative mass was determined.

All obtained experimental data were processed by the method of variation statistics. This report accepts the significance level $p \leq 0.05$. Calculation of statistical significance in the case of nominal variables was performed using one-way analysis of variance and analysis of variance for experiments with repeated measurement. The hypothesis about the equation of the two means was tested using Student's t-test for related samples [14,15].

1.2 The study results

Studies have shown that after application of the test substance and saline at a dose of 3.0 ml / kg on the skin of rats, they were sedentary, but no toxic effects were observed. After 45-60 minutes, the rats were active, moved around the cage, washed, drank water, ate. In the future, the behavior of rats in the experimental group does not differ from the behavior of animals in the control group.

The deaths of animals of both groups, as well as deviations in their general condition, behavior, consumption of food and water during the 14-day study period were not observed (Table 1.1).

Table 1.1 Toxicity parameters of Dew Pocket silver spray

Substance	Animals sex	Numbers of animals	Dose, ml / kg	Mortality, %
Test substance	male	5	3.0	0
	female	5	3.0	0
Saline	male	5	3.0	0
	female	5	3.0	0

The effect of the test substance on the body weight dynamics of rats are presented in table 1.2. Analysis of the obtained data showed that after application of the test substance and saline on the skin of rats by the end of the observation period there was a statistically correct increase in body weight. At the end of the experiment, differences between the two groups on these indicators haven't been found.

Table 1.2 Dynamics of body weight changes of rats during acute action of Dew Pocket silver spray

Observation period, days	**Rat weight, g**			
	Test substance		Saline	
	male	female	male	female
0	231 ±4,64	196 ±2,92	233 ±3,54	198 ±2,55
3	235 ±6,89	203 ±3,74	239 ±3,32	200 ±1,58
7	252 ±3,74 [1]	211 ±4,30 [1]	253 ±4,36 [1]	206 ±1,87 [1]
14	258 ±3,39 [1]	215 ±4,18 [1]	260 ±2,74 [1]	214 ±1,87 [1]

Note: [1] - $p \leq 0,05$ on source data;
[2] - $p \leq 0,05$ between the data of the experimental and control groups.

The results of biochemical studies (Table 1.3) showed that the test substance in the applied dose does not affect the content of total protein, albumin and thymol test in the serum of animals of all experimental groups. There were no significant differences between the parameters of the animals exposed to the test substance and saline.

Table 1.3 Biochemical parameters of blood serum of rats with acute action of Dew Pocket silver spray

Parameters	Test substance		Saline	
	male	female	male	female
Total protein, g / l	49,52 ± 2,57	37,26 ± 2,21	38,91 ± 1,11	37,74 ± 0,80
Albumin, g / l	38,20 ± 1,79	36,90 ± 2,39	37,68 ± 2,56	35,24 ± 2,34
Thymol test, unit	0,63 ± 0,15	0,56 ± 0,11	0,49 ± 0,07	0,55 ± 0,16

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

Pathomorphological study

Pathomorphological examination, which was performed 14 days after application, included autopsy and macroscopic examination of the internal organs of rats. After euthanasia, the animals were carefully examined in terms of visible pathological features.

According to the autopsy, differences from the norm haven't been detected. The coat is shiny, neat, lymph nodes are not enlarged. Visible mucous membranes are shiny, pale pink, smooth. All macroscopically examined organs (heart, lungs, thymus, stomach, liver, kidneys, adrenal glands, pancreas, spleen, gonads) had the usual size, color and consistency. The relative mass of the internal organs of rats, which applied test substance and saline (Table 1.4), kept within the physiological norm [16].

Table 1.4 Coefficients of the masses of the internal organs of rats after acute exposure f Dew Pocket silver spray

Organs	Test substance	Saline
Male		
Heart	0,45 ± 0,00 8	0,44 ± 0,026
Lungs	0,77 ± 0,045	0,80 ± 0,021
Liver	3,88 ± 0,061	3,94 ± 0,168
Spleen	0,45 ± 0,028	0,47 ± 0,031
Adrenal glands	0,029 ± 0,001	0,027 ± 0,001
Kidney left	0,45 ± 0,019	0,44 ± 0,020
Kidney right	0,46 ± 0,022	0,44 ± 0,020
left testicle	0,63 ± 0,021	0,61 ± 0,035
right testicle	0,62 ± 0,025	0,61 ± 0,029
Thymus	0,09 ± 0,006	0,10 ± 0,012
Female		
Heart	0,53 ± 0,030	0,52 ± 0,047
Lungs	0,94 ± 0,022	0,99 ± 0,099
Liver	4,36 ± 0,237	4,34 ± 0,089
Spleen	0,53 ± 0,045	0,54 ± 0,014
Adrenal glands	0,042 ± 0,003	0,043 ± 0,001
Kidney left	0,47 ± 0,011	0,46 ± 0,021
Kidney right	0,47 ± 0,012	0,46 ± 0,018
Thymus	0,13 ± 0,010	0,13 ± 0,021

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

1.3 Conclusions

Based on the results of a comparative study of the acute toxicity of Dew Pocket silver spray, it can be concluded that the when test substance applied once to rats at a dose of 3.0 ml/kg:

- does not cause death of animals;
- does not have a toxic effect on the general condition, behavior, consumption of food and water, body weight of rats;
- does not cause visible changes in internal organs;
- does not affect the absolute and relative mass of internal organs.

2 STUDY OF SUBACUTE TOXICITY OF DEW POCKET SILVER SPRAY

The aim of these studies was to compare the toxicity of Dew Pocket silver spray in a subacute experiment on rats during one month applied period.

2.1. Materials and methods of research

The study was performed on mature nonlinear white rats of both sexes, with an initial body weight of 245 - 295 g. All animals were divided into groups. Each experimental group consisted of 14 rats (7 males and 7 females). A total of 28 rats were used in the experiment.

The animals were raised in the vivarium of the State Institution "Institute of Microbiology and Immunology" Mechnikov Institute of Microbiology and Immunology Kharkiv. During the experiment, they were in the vivarium at an air temperature of 18-200C, humidity of 50-60%, the mode of natural light "day and night", in normal cages, on a standard diet [11].

The studies were performed on laboratory animals in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" [9].

The test substance was applied to the depilated part of the rat's skin. The area on which the test substance was applied was 5% of the total skin surface of the animals, which corresponds to the area of skin on the back of rats measuring 4 cm x 4 cm. The dose was chosen based on experimental data obtained in the acute toxicity study. Applications were performed once at a dose of 3.0 ml / kg. A group of rats which were depilated and applied 3.0 ml / kg of saline was used as a control. Applications were made daily, once a day for a month. The spray area was depilated once a week.

Clinical pictures of intoxication, animal survival, food and water consumption, body weight dynamics, hematological parameters of blood, biochemical parameters of blood and urine, clinical analysis of urine, electrophysiological activity of the myocardium, functional state of the central nervous system. used as criterias for spray toxicity. At the end of the experiment, the animals were euthanized for microscopy of internal organs.

Clinical observations of animals by recording changes in their general condition, behavior, food and water intake were performed daily during the experiment.

Animals body weight registration was performed in the dynamics: initial data, 1st, 2nd, 3rd week and 1 month.

Evaluation of the use of test substance at the CNS station was performed by the method of "open field" in the experiment with the end [17].

The electrocardiogram was taken a month later in the second standard lead (electrocardiograph EK1K-01). The following indicators were taken into account to interpreting electrocardiograms, : RR - duration of complete cardiac cycle, duration of PQ interval, which characterizes the time of propagation of atrial excitation, duration of ventricular QRS complex and ventricular systole - QT interval, voltage and direction of teeth R and T 18].

The content of hemoglobin, the number and morphology of erythrocytes, the content of leukocytes, platelets was determined in the peripheral blood, calculated the percentage of different forms of leukocytes (leukocyte formula). Hemoglobin was determined by cyanide method, counting of erythrocytes, leukocytes, platelets and leukocyte formula was performed by conventional methods [19]. Blood from rats was taken from the tail vein in the dynamics: initial data, two weeks and 1 month.

All biochemical studies were performed using diagnostic kits from Philisit-Diagnostics (Ukraine). The amount of total protein in the blood was determined by the biuret method,

albumin - by reaction with bromocresol green, thymol test - by the method of sediment samples, alanine and aspart-ataminotransferase (ALT and AST) activity - by the Reitman-Frenkel method, blood glucose content - enzymatic method, the content of urea in the blood - diacetyl monoxime method, the amount of cholesterol - by the method of Ilko [12]. The studied indicators were determined at the end of the experiment.

To assess the effect of the test substance on the functional state of the kidneys of rats determined spontaneous diuresis, the specific gravity of urine using diagnostic strips "PHAN" (firm PLIVA-Lachema), determined the pH of urine (test based on the color change of the mixed acid-base indicator in the pH range 5-9), determined urine protein (test based on color change of the acid-base indicator under the influence of proteins), determined the content of glucose in the urine (test based on the enzymatic reaction), determined the content of urea in the urine (diacetylmonooxime method) [12]. All these indicators were determined at the end of the experiment.

All animals were weighed on the day of slaughter, then - immediately autopsied in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" [9]. Removal of rats from the experiment was carried out by painless decapitation.

During the post-mortem study individually determined mass of internal organs: heart, lungs, liver, spleen, kidneys, adrenal glands, testes and thymus. Then the absolute mass of the internal organs and their weights were determined and recalculated.

All obtained experimental data were processed by the method of variation statistics. This report accepts the significance level $p \leq 0.05$. Calculation of statistical significance in the case of nominal variables was performed using one-way analysis of variance and analysis of variance for experiments with repeated measurement. The hypothesis about the equation of the two means was tested using Student's t-test for related samples [14,15].

2.2 The study results

Observations during the experiment showed no clinical signs of toxic effects of the test substance on the general condition and behavior of experimental animals. Visual observation showed that the animals looked normal, rats with signs of exhaustion or obesity were not detected. The coat is soft, shiny, close to the body surface, the condition of the skin and mucous membranes is normal. animals of the control and experimental groups had no differences in food and water consumption. No deaths were observed during the experiment.

Analysis of the data shown in table 2.1. It is shown that animals of both groups were statistically significantly added to body weight compared to primary data. There were no statistical differences between the body weight of the rats treated with the test substance and saline.

Table 2.1 Body weight (g) of rats under subacute exposure of Dew Pocket silver spray

Observation period	Test substance	Saline
	Male	
Primary data	270,71 ± 7,67	270,71 ± 7,11
1 week	291,43 ± 6,70	292,14 ± 5,10 [1]
2 week	302,86 ± 6,80 [1]	300,00 ± 5,98 [1]
3 week	306,43 ± 6,96 [1]	304,29 ± 4,68 [1]

1 month	312,86 ± 6,35 [1]	310,71 ± 4,93 [1]
	Female	
Primary data	252,14 ± 2,40	252,86 ± 2,86
1 week	260,00 ± 3,27	260,00 ± 3,62
2 week	264,29 ± 3,35 [1]	265,71 ± 3,35 [1]
3 week	267,14 ± 3,25 [1]	269,29 ± 3,52 [1]
1 month	275,00 ± 3,09 [1]	277,14 ± 2,40 [1]

Note: [1] - $p \leq 0,05$ on source data;

[2] - $p \leq 0,05$ between the data of the experimental and control groups.

The study results of the functional state of the central nervous system of rats showed that the Dew Pocket silver spray does not affect the indicators that characterize the horizontal and vertical motor activity, and does not affect the emotional reactivity of rats in comparison with the control group (Table 2.2).

Analysis of data characterizing the electrophysiological activity of the myocardium of rats showed that the use of the test substance for a month does not cause significant changes in the duration of the intervals PQ, QRS, QT and RR, and does not affect the height of the teeth P, R and T (Table 2.3).

Table 2.2 Indicators of the CNS of rats after subacute exposure of the Dew Pocket silver spray

Indicators	**Test substance**	**Saline**
Male		
Amaunt: crossed squares	12,71 ± 2,64	12,86 ± 1,97
Racks	4,00 ± 1,11	3,71 ± 1,21
Washing	1,14 ± 0,34	1,29 ± 0,42
Defecation	3,43 ± 0,92	3,00 ± 0,93
Female		
Amaunt: crossed squares	29,57 ± 5,16	27,43 ± 4,48
Racks	9,29 ± 1,17	7,71 ± 2,12
Washing	0,86 ± 0,34	1,00 ± 0,22
Defecation	2,71 ± 0,99	3,86 ± 0,83

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

The indicators presented in tables 2.4 and 2.5 characterize the peripheral blood. The obtained results indicate that skin applications of the test substance for a month did not have a toxic effect on hematological parameters of the blood - the concentration of hemoglobin, the number of erythrocytes, leukocytes and platelets did not differ from similar indicators of the control group of animals (Tables 2.4 and 2.5). During the entire observation period in the leukocyte formula of the blood of male and female rats of the experimental group there were no changes compared with the control group.

Table 2.3 ECG parameters of rats after subacute exposure of Dew Pocket silver spray

Parametr	**Test substance**	**Saline**
Male		
Duration of intervals, sec: PQ	0,030 ± 0,003	0,034 ± 0,002
QRS	0,026 ± 0,002	0,024 ± 0,002
QT	0,070 ± 0,003	0,070 ± 0,003
RR	0,120 ± 0,000	0,120 ± 0,000
Height of teeth, mv:		

P	0,070 ± 0,012	0,090 ± 0,019
R	0,460 ± 0,029	0,400 ± 0,052
T	0,150 ± 0,000	0,140 ± 0,019
Female		
Duration of intervals, sec:		
PQ	0,034 ± 0,002	0,030 ± 0,000
QRS	0,028 ± 0,002	0,028 ± 0,002
QT	0,076 ± 0,004	0,072 ± 0,004
RR	0,127 ± 0,005	0,123 ± 0,005
Height of teeth, mv:		
P	0,090 ± 0,010	0,100 ± 0,000
R	0,390 ± 0,058	0,260 ± 0,040
T	0,130 ± 0,012	0,150 ± 0,000

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

Table 2.4 General analysis of blood of male rats under subacute exposure of Dew Pocket silver spray

Parametr	Test substance	Saline
Initial data		
Hemoglobin, g / l	128,30 ± 1,63	128,90 ± 0,96
Erythrocytes, ´10 12 / l	5,76 ± 0,24	5,93 ± 0,14
Leukocytes, ´10 9 / l	9,21 ± 0,42	9,50 ± 0,49
Platelets, ´10 9 / l	470,83 ± 22,59	472,75 ± 22,51
Leukogram,%		
Neutrophils sticks.	0,43 ± 0,30	0,43 ± 0,20
Neutrophils seg.	19,14 ± 1,24	16,43 ± 1,65
Eosinophils	1,57 ± 0,30	1,43 ± 0,20
Monocytes	1,57 ± 0,20	1,71 ± 0,47
Lymphocytes	77,29 ± 1,38	80,00 ± 2,09
2 weeks		
Hemoglobin, g / l	128,60 ± 0,75	128,40 ± 0,97
Erythrocytes, ´10 12 / l	5,89 ± 0,07	5,86 ± 0,14
Leukocytes, ´10 9 / l	9,94 ± 0,55	9,93 ± 0,54
Platelets, ´10 9 / l	495,11 ± 19,47	494,09 ± 19,92
Leukogram,%		
Neutrophils sticks.	0,29 ± 0,18	0,29 ± 0,18
Neutrophils seg.	18,43 ± 0,75	16,43 ± 1,04
Eosinophils	1,29 ± 0,18	1,14 ± 0,14
Monocytes	1,71 ± 0,47	1,57 ± 0,48
Lymphocytes	78,29 ± 0,92	80,57 ± 1,17
1 month		
Hemoglobin, g / l	128,10 ± 0,99	127,90 ± 1,61
Erythrocytes, ´10 12 / l	5,86 ± 0,12	5,82 ± 0,17
Leukocytes, ´10 9 / l	9,66 ± 0,56	9,96 ± 1,06
Platelets, ´10 9 / l	486,69 ± 15,84	436,79 ± 23,55
Leukogram,%		
Neutrophils sticks.	0,43 ± 0,20	0,43 ± 0,20
Neutrophils seg.	18,29 ± 0,71	17,86 ± 1,68
Eosinophils	1,57 ± 0,30	1,86 ± 0,70
Monocytes	1,86 ± 0,59	2,00 ± 0,53
Lymphocytes	77,86 ± 1,16	77,86 ± 2,09

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

The results of biochemical studies (Table 2.6) showed that the test substance did not affect the parameters that characterize protein metabolism (total protein content, albumin concentration, thymol test), carbohydrate metabolism (glucose concentration), lipid metabolism

(cholesterol concentration), as well as liver function (activity of alanine and aspartate aminotransferase enzymes).

From the data in table 2.7, it is seen that long-term use of the test substance did not change the main indicators characterizing the functional state of the kidneys of rats. In both groups of rats after 1 month of observations, daily diuresis, relative density and pH of urine, urea content in blood and urine were within the physiological norm [16]. Glucose was absent in the urine of animals of both groups and traces of protein were indicated. Glucose in the urine of animals of both groups was absent and traces of protein were affected. Macroscopic examination of rats of the experimental and control groups showed that the autopsied animals were normally fat, their fur is neat, shiny, close to the body, without traces of scratches, ulcers, areas of alopecia and peeling. Regional lymph nodes are not enlarged to the touch. The testicles are usually located in the scrotum. From the eyes, nose and other natural openings of secretions are not detected, the hair and skin in the area of the anus and vagina are clean, without signs of irritation. The mucous membrane of the oral cavity is shiny, clean, without ulcers and plaque, the tongue is not enlarged and not coated.

Table 2.5 General analysis of blood of male rats under subacute exposure of Dew Pocket silver spray

Parametr	Test substance	Saline
Initial data		
Hemoglobin, g / l	127,10 ± 1,03	126,90 ± 1,30
Erythrocytes, ´10 12 / l	5,71 ± 0,13	5,73 ± 0,12
Leukocytes, ´10 9 / l	9,57 ± 0,64	9,43 ± 0,79
Platelets, ´10 9 / l	454,49 ± 17,43	457,87 ± 21,77
Leukogram,%		
Neutrophils sticks.	0,29 ± 0,18	0,57 ± 0,30
Neutrophils seg.	15,00 ± 2,27	17,29 ± 2,12
Eosinophils	2,00 ± 0,31	1,86 ± 0,26
Monocytes	2,71 ± 0,61	2,00 ± 0,38
Lymphocytes	80,00 ± 2,35	78,29 ± 2,42
2 weeks		
Hemoglobin, g / l	127,70 ± 0,89	128,00 ± 1,20
Erythrocytes, ´10 12 / l	5,83 ± 0,10	5,81 ± 0,15
Leukocytes, ´10 9 / l	9,76 ± 0,73	10,29 ± 0,84
Platelets, ´10 9 / l	468,04 ± 16,35	483,77 ± 18,37
Leukogram,%		
Neutrophils sticks.	0,43 ± 0,20	0,29 ± 0,18
Neutrophils seg.	17,29 ± 1,60	16,00 ± 1,45
Eosinophils	2,00 ± 0,38	2,00 ± 0,44
Monocytes	2,00 ± 0,31	2,14 ± 0,77
Lymphocytes	78,29 ± 1,85	79,57 ± 1,93
1 month		
Hemoglobin, g / l	128,90 ± 1,40	128,70 ± 1,30
Erythrocytes, ´10 12 / l	5,87 ± 0,18	5,88 ± 0,16
Leukocytes, ´10 9 / l	11,19 ± 0,50	10,66 ± 0,66
Platelets, ´10 9 / l	449,38 ± 25,48	459,92 ± 9,86
Leukogram,%		
Neutrophils sticks.	0,29 ± 0,18	0,86 ± 0,34
Neutrophils seg.	18,71 ± 1,63	20,14 ± 1,40
Eosinophils	2,00 ± 0,31	2,43 ± 0,43
Monocytes	2,43 ± 0,48	2,14 ± 0,55
Lymphocytes	76,57 ± 1,97	74,43 ± 1,66

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

Macroscopic examination of the thoracic cavity showed that the lungs are uniform to the touch, elastic, airy, without adhesions between the leaves of the pleura, occupy the entire pleural cavity, the walls of the bronchi are not thickened. The location of the mediastinal organs corresponds to normal: trachea and esophagus passable, esophageal mucosa pink. The thymus is moderate in size, conical shape with two distinct lobes, shiny, soft to the touch, gray and pink. The heart of the usual configuration is elongated-cone-shaped, the muscle walls are dense and elastic.The cavities of the left and right ventricles are narrow and slit-like, in the cavity of the cardiac fluid is not detected, epicardial surface without features, myocardium in section is slightly fibrous.

Table 2.6 Biochemical parameters of serum of rats after subacute exposure of Dew Pocket silver spray

Parameters	Test substance	Saline
Male		
Total protein, g / l	67,32 ± 4,84	69,07 ± 2,21
Albumin, g / l	36,22 ± 3,06	36,63 ± 1,49
Thymol test, unit	1,54 ± 0,16	1,66 ± 0,13
АлАТ, мккат / л	0,34 ± 0,03	0,35 ± 0,06
АсАТ, мккат / л	0,62 ± 0,04	0,62 ± 0,06
Glucose, mmol / l	4,71 ± 0,36	4,68 ± 0,19
Cholesterol, mmol / l	2,35 ± 0,18	2,32 ± 0,23
Female		
Total protein, g / l	67,32 ± 4,84	69,07 ± 2,21
Albumin, g / l	36,22 ± 3,06	36,63 ± 1,49
Thymol test, unit	1,54 ± 0,16	1,66 ± 0,13
АлАТ, мккат / л	0,34 ± 0,03	0,35 ± 0,06
АсАТ, мккат / л	0,62 ± 0,04	0,62 ± 0,06
Glucose, mmol / l	4,71 ± 0,36	4,68 ± 0,19
Cholesterol, mmol / l	2,35 ± 0,18	2,32 ± 0,23

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

Table 2.7 Indicators of the functional state of the kidneys of rats after subacute effects of Dew Pocket silver spray

Parameters	Test substance	Saline
Male		
The amount of urine, ml	3,02 ± 0,36	2,82 ± 0,62
pH	6,50 ± 0,27	6,30 ± 0,20
Specific density	1,041 ± 0,003	1,042 ± 0,004
Blood urea, mmol / l	5,70 ± 0,21	5,72 ± 0,38
Urine urea, mmol / l	663,00 ± 18,56	671,20 ± 54,59
Самиці		
The amount of urine, ml	2,66 ± 0,60	2,62 ± 0,56
pH	6,20 ± 0,12	6,30 ± 0,30
Specific density	1,040 ± 0,004	1,042 ± 0,003
Blood urea, mmol / l	6,41 ± 0,38	6,47 ± 0,21
Urine urea, mmol / l	693,40 ± 26,41	707,00 ± 58,01

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

The position of the abdominal organs is anatomically correct. In the subcutaneous tissue - moderate fat deposition, the peritoneum is transparent, smooth, without hemorrhage. No foreign contents were detected in the cavity.

In a liver all particles are well defined, its capsule is not strained, its surface is not rounded and smooth, without nodules, parenchyma in sections of equal red-brown color. The pancreas is a weakly branched loose cord, parenchyma of the gland of all animals pale, pinkish-yellow color, without signs of hemorrhage and fatty necrosis. The spleen is elastic, reddish-brown. The section shows the fine-grained fabric. Kidneys with a capsule that is easily removed, in section dark red, solid, with a preserved pattern of layers. The adrenal glands are small, rounded, yellowish-white, located in the retroperitoneal space in close proximity to the kidneys. Gastric mucosa with a characteristic relief of folds, without hemorrhages, edema, erosive lesions. Intestines and pelvic organs without visible changes. Internal genitalia are normal. The skull and spinal cord have not been studied.

The coefficients of mass of the studied organs did not differ from the control indicators and did not exceed the physiological norm for this species of animals (table.2.8).

Table 2.8 The relative mass of the internal organs of rats after subacute exposure of of Dew Pocket silver spray

Organs	Test substance	Saline
Male		
Heart	0,35 ± 0,017	0,39 ± 0,012
Lungs	0,66 ± 0,012	0,64 ± 0,050
Liver	3,37 ± 0,091	3,45 ± 0,092
Spleen	0,35 ± 0,012	0,37 ± 0,018
Adrenal glands	0,021 ± 0,0007	0,019 ± 0,0023
Kidney left	0,32 ± 0,011	0,34 ± 0,005
Kidney right	0,33 ± 0,011	0,35 ± 0,008
left testicle	0,50 ± 0,019	0,53 ± 0,021
right testicle	0,49 ± 0,021	0,54 ± 0,019
Thymus	0,07 ± 0,011	0,06 ± 0,002
Female		
Heart	0,38 ± 0,015	0,38 ± 0,018
Lungs	0,76 ± 0,053	0,78 ± 0,080
Liver	3,26 ± 0,138	3,67 ± 0,225
Spleen	0,38 ± 0,047	0,33 ± 0,021
Adrenal glands	0,030 ± 0,0017	0,029 ± 0,0018
Kidney left	0,32 ± 0,014	0,34 ± 0,013
Kidney right	0,33 ± 0,010	0,36 ± 0,014
Thymus	0,11 ± 0,007	0,06 ± 0,009 [1]

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

2.3. Conclusions

The study of subacute toxicity of Dew Pocket silver spray at a dose of 0.3 ml / kg (1/10 of the maximum dose in the acute experiment) allows us to draw the following conclusions:

- spray does not cause death of rats;
- spray does not have a toxic effect on their general condition, behavior and dynamics of body weight;
- spray does not affect the indicators of the functional state of the central nervous system and the physiological activity of the myocardium;
- spray does not have a negative effect on peripheral blood parameters;
- spray does not change the biochemical parameters that characterize the functional state of the liver and kidneys.

3 STUDYING THE LOCAL IRRITANT EFFECT OF DEW POCKET SPRAY

The study of the local irritant effect was carried out as part of an experiment to study the subacute toxicity of Dew Pocket silver spray. The experiment was guided by guidelines [20].

3.1 Materials and methods of research

Studies were performed on nonlinear white rats with a body weight of 245 - 295 g.

The local irritant effect was evaluated by daily skin application of test substance at a dose of 0.3 ml / kg for 1 month. A group of animals treated with saline was used as a control.

Assessment of local irritation included: daily macroscopic control of the spray application area (depilated areas), as well as macroscopic examination of the treated skin areas after the experiment.

During the collection of biological material, a visual assessment of the treated areas was performed.

The skin reaction was evaluated in according to the points of modified Draize scale [20] for the presence and indicators of erythema, scab, edema and necrosis:

Erythema and scab formation

no erythema - 0 points

minor erythema - 1 point

clear erythema - 2 points

moderate erythema - 3 points

severe erythema, mild scab formation - 4 points

necrosis - + H

scab - + C

Edema

no edema - 0 points

minor edema - 1 point

slight edema - 2 point

moderate edema - 3 point

severe edema - 4 point

The total degree of irritation was calculated for each animal by the individual sum of points. The index of direct skin irritation was calculated by dividing the total by the number of observations. The degree (index) of irritation was evaluated by follows:

0.0 - no irritant effect;

0.0 - 0.5 - irritation that can be neglected;

> 0.5-2.0 - moderate irritation;

> 2.0-5.0 - average irritation;

> 5.0-8.0 - severe irritation.

The thickness of the skin folds of the treated by test substance areas was determined using a micrometer at the beginning of the experiment and at the end of the experiment.

3 .2 Research results

Postmortem macroscopic examination. After autopsy of rats, it was found that the integrity of the skin at the treated by test substance areas was preserved. In the depilated area there were no signs of peeling, hyperkeratosis, erythematous rash, hemorrhage, swelling, depigmentation and other visible signs of damage, hair was grown thick and uniform (Table 3 .1).

Table 3.1 Evaluation of irritation by the results of a macroscopic study of the subacute effects of Dew Pocket silver spray

A group of animals	Average score	Irritation
Test substance	0	are absent
Saline	0	are absent

The results of the assessment of the thickness of the skin folds of rats are shown in table 3.2. Silver spray did not affect this indicator, which indicates the absence of adverse effects on the skin and subcutaneous fat.

Table 3.2 The thickness of the skin folds of rats after subacute exposure to silver spray Dew Pocket

Observation period	**The thickness of the skin folds, mm**	
	Test substance	**Saline**
Male		
Initially	2,95 ± 0,09	3,1 9 ± 0,60
After 1 month	3,07 ± 0,36	3,24 ± 0,44
Female		
Initially	2,67 ± 0,25	2,87 ± 0,52
After 1 month	2,85 ± 0,53	2,76 ± 0,38

Note: [1] - $p \leq 0,05$ between the data of the experimental and control groups.

3.3 Conclusions

Thus, as a result of the conducted research it is possible to draw a conclusion that Dew Pocket silver spray at subacute skin influence in dermal apply does not have local irritating action.

CONCLUSIONS

The report contains materials of the safety studying (acute, subacute toxicity and local irritation) of Dew Pocket silver spray in experiments on rats.

It was found that the acute effect of the test substance at a dose of 3.0 ml / kg does not cause death of rats. According to the clinical symptoms of animal intoxication, the studied parameters and the level of acute toxicity, there are no differences from the effect of placebo - saline.

Dermal application of Dew Pocket silver spray to rats at a dose of 0.3 g / kg (1/10 of the maximum tested in the acute experiment) for 1 month does not have a toxic effect on the general condition of animals, behavior, food and water consumption, CNS function myocardial activity, peripheral blood parameters, does not cause pathological changes in the main biochemical parameters of blood and urine of animals that characterize metabolic processes in the liver and kidneys.

Based on the results of pathomorphological research it is established that the test substance does not cause visual changes in the appearance of internal organs. The weight of the organs do not differ from the control indicators - saline.

Dew Pocket silver spray has no local irritant effect.

Thus, the results of the safety study allow us to conclude that the Dew Pocket silver spray in terms of acute and subacute toxicity, as well as local irritation, is quite safe.

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