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EVALUATION OF TOXICOLOGICAL PARAMETERS OF DAPS-25K AFTER A SINGLE INTRAGASTIRIC ADMINISTRATION TO MICE AND GUINEA PIGS

R.O. Vasiliev [1*], E.I. Troshin [1], S.A. Brevnova[1], N. Yu. Yugatova [1], A.V. Shishkin [2], I.L. Vasilyeva [3], A. N. Kulikov [3]

[1] *Department of Veterinary Radiobiology and BZHCHS, St. Petersburg State Academy of Veterinary Medicine, St. Petersburg, RUSSIAN FEDERATION*

[2] *Department of Anatomy and Physiology, Izhevsk State Agricultural Academy, Izhevsk, RUSSIAN FEDERATION*

[3] *Department of Veterinary Sanitary Expertise and Radiobiology, Izhevsk State Agricultural Academy, Izhevsk, RUSSIAN FEDERATION.*

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ABSTRACT

Lack of selenium in food chains contributes to the development of about 40 diseases in animals. In conditions of acute selenium deficiency in agroecosystems, enrichment of plant feed for animals with selenium with the help of food additives is becoming increasingly important. However, inorganic forms of selenium are highly toxic and their use requires strict veterinary control. Therefore, feed additives containing organoselenium compounds are of the greatest interest. One of these additives is DAPS-25k, a new generation organoselenium preparation synthesized by domestic scientists. Thus, this work aimed to determine the parameters of acute toxicity of DAPS-25k after a single intragastric administration to mice and guinea pigs. It was found that high doses (25-50 mg/kg) of DAPS-25k after a single intragastric administration cause a decrease in general body temperature and live weight accompanied with depression and pulmonary edema. Guinea pigs were more sensitive to DAPS-25k, and LD_{50} in them was 15.8 ± 3.45 mg/kg, and in mice 19.6 ± 4.452 mg/kg.

Disciplinary: Veterinary Medicine.

1. INTRODUCTION

The great biological importance of selenium (Se) and its essentiality for humans and animals determine the priority of assessing selenium status of territories for many countries in the world. Mapping of territories by selenium level in the soil is becoming increasingly important and is fundamental in defining environmental risks and developing effective measures to optimize selenium status (Berdnikov & Dyachenko, 2013; Golubkina et al., 2012; Stoffaneller & Morse, 2015).

*Corresponding author (R.O. Vasiliev). Email: veterenar4ik@mail.ru

Compared with other regions of the world, Russia has not only a vast territory but a significant variety of geochemical characteristics of soil which is the main source of trace elements in the "soil-plant-animals" food chain (Ermakov & Vorontsova, 2010; Korobova et al., 2011). A significant part of the territory of Russia belongs to the biogeochemical provinces due to the insufficient content of this element in soil, water, feed, and products what makes veterinarian specialists constantly prevent hyposelenosis in animals using selenium-containing preparations and feed additives. Perm, Chelyabinsk, Novgorod, Pskov, and Leningrad Regions are among the most selenium-deficient ones (Skalny & Kiselev, 2010). Selenium content in soil is mainly determined by source and underlying rocks, climatic characteristics of the region, as well as the conditions of their cultivation (increasing the content of nutrients, humus, and improvement of environmental response).

It is known that the trace element selenium is the part of most hormones and enzymes that reveals its active role in metabolism (Stepanov, 2012). In 1980, WHO ranked selenium as an indispensable nutritional factor. In particular, it is involved in the metabolism of thyroid hormones, since it is a component of deiodinases involved in the conversion of thyroxine and triiodothyronine (Vorobyev et al., 2014; Gromova, 2010). Se deficiency results in inhibition of deiodinase activity, increasing T4 in peripheral tissues, and decreasing T3, thus exacerbating iodine deficiency if any. In this case, the isolated reverse of Se deficiency without stopping iodine deficiency leads to decreased thyroid function (Stekolnikov et al., 2015; Fordyce, 2012).

Selenium has antioxidant and adaptogenic properties, as it regulates immunogenetic through the process of lipid peroxidation and has a stimulating effect on the immune system (Combs, 2015). This trace element is included in the active center of glutathione peroxidase which is found in red blood cells and platelets and protects cells from the accumulation of peroxidation products, thereby preventing damage to its nuclear and protein-synthesizing apparatus. Besides, it takes an active part in the synthesis of such vital enzymes and cofactors as glycine reductase and coenzymes A and Q. Metabolism of selenium which is a synergist of vitamin E is closely related to its metabolism, polyunsaturated fatty acids and other components of the antioxidant system of the body (Vtorushina et al., 2009). Among other issues concerning the lack of essential elements, there is the fact that hypoelementoses often occur in a latent form, up to the development of irreversible pathological changes. We should consider the fact that blood morphological composition is sensitive to all changes in an animal's body under the influence of external and internal factors, even these in the latent form (Glotova & Vtorushina, 2010).

Lack of selenium leads to the development of an endemic disease which is based on metabolic disorders; thus leading to a decreased productivity of farm animals, impaired fetal development, and increased morbidity in the postpartum period (Aleksandrova & Sotnikov, 2014). Insufficient intake of selenium with feeding causes disorders in the metabolism of proteins, fats, carbohydrates and contributes to the development of the white-muscle disease, liver necrosis, exudative diathesis, anemia, erythrocyte hemolysis, ovarian degeneration, decreased resistance and light perception. Growing and young animals are especially affected by selenium deficiency. Many authors noted that in areas with insufficient selenium intake there is an increase in the number of oncological diseases (Shakirov et al., 2012).

Currently, there is a wide range of modern organoselenium preparations and feed additives (Davis et al., 2012). Using various drugs with selenium in their content for animal husbandry is of scientific and practical interest (Budulov et al., 2014; Tolmachev & Kilmetova, 2011). In the course

of conducting research, the emphasis is placed on finding forms of trace elements that contribute to increasing the productivity of animals while improving the quality of products (Troshina et al., 2007).

However, it is known that therapeutic application, especially of inorganic forms of selenium to animals, is limited and requires constant toxicological monitoring of using existing pharmacological agents and additives (Merzlyakova, 2008; Chugay et al., 2009). At the same time, much lesser toxicity and greater bioavailability of selenium from organic preparations are proved in comparison with commonly used sodium selenite. However, not all properties of organoselenium compounds are experimentally tested by now, and physiological mechanisms of transformations of these substances in animals are far from completely revealed (Davis et al., 2017).

Over recent years, formulations for parenteral and enteral administration of DAPS-25k to animals have been developed as a pharmacological agent for the prevention and treatment of hypomelanosis (Troshina et al., 2007). This preparation affects the animal's body similar to that of vitamin E: it participates in the processes of tissue respiration and oxidative phosphorylation, acts as an inhibitor for certain enzyme systems, has antitoxic properties, and also prevents oxidation of fatty acids and accumulation of toxic substances in the body, as well as contributes to increased activity of glutathione peroxidase that normalizes metabolism.

Despite the evidence of comparatively lower toxicological properties of organic forms of selenium in comparison with inorganic ones, the issue of dosing selenium-containing preparations remains relevant for subsequent scientific research (Fordyce, 2012). Thus, this experiment aimed to define the parameters of acute toxicity of DAPS-25k after a single intragastric administration to mice and rats.

2. MATERIALS AND METHODS

Study of parameters of acute toxicity of DAPS-25k feed additive (OOO Sulfate, Saratov, Russia) was carried out on white outbred mice with a weight of 25-27 grams and guinea pigs with a weight of 378-648 grams. Animals were brought from Federal State Unitary Enterprise "Rappolovo Bank of Laboratory Animals" which is safe in the sense of infectious and invasive diseases (village of Rappolovo, Leningrad Region, Russia). The quarantine took 15 days, adaptation 7 days. Groups were formed according to the principle of pairs of analogs. Animals were kept in cages, 6 animals per cage, with watering ad libitum and feeding once a day with compound feed following GOST R 50258-92. Animals had an automatic 12-hour regime for light and darkness. Fine wood shavings were used as bedding, its thickness was 5-7 cm.

DAPS-25k is a feed additive for animals that contains diacetophenonyl selenide at least 95%, with a mass fraction of selenium in diacetophenonyl selenide of 25%, sodium sulfite and sodium chloride no more than 1%, preformed water no more than 4%. To prepare the working solution, vegetable oil heated to the temperature of 70°C was used as a solvent.

Obtained data were subjected to statistical processing using the Statistica 10 program. Results were evaluated using the Wilcoxon signed-rank test and Mann-Whitney U-test. Differences were considered significant at $p \leq 0.05$. Data are presented as median (M) and quartile range from 25% to 75% ([Q25; Q75]). LD_{50}, LD_{16}, LD_{84}, and LD_{99} values were found by the method of probit analysis according to Finney.

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2.1 WHITE MICE EXPERIMENT SIMULATION

To experiment, animals were divided into several groups, each of 6 males: intact mice, control animals, and 4 experimental groups. False intragastric administration was performed for intact mice, i.e. they were fixed by an assistant. The operator introduced a metal probe with olive at its end into the esophagus, but no solution was injected. Mice of the control group were once intragastrically injected with 0.5 mL/animal of vegetable oil on an empty stomach. Experimental animals received intragastrically, on an empty stomach, once, a total volume of 0.5 mL/animal of DAPS-25k oil solution at the dose of 5.0 mg/kg, 10.0 mg/kg, 25 mg/kg and 50 mg/kg, respectively, for the first, second, third and fourth experimental groups. To prepare 100 mL of solution with a working concentration of 2.6 mg/mL, we took 0.26 g of DAPS-25k and dissolved in vegetable oil using a magnetic stirrer with the function of maintaining a given temperature. This solution was a mother liquor that was used for preparing working oil solutions by the method of proportional dilutions.

The observation period was 14 days after the start. During the experiment, the clinical condition of animals, changes in general body temperature, and live weight were evaluated and deaths were registered. The experiment for survived animals was stopped through an overdose (80 mg/kg) of general anesthesia (Zoletil®, Virbac, France) followed by cervical dislocation.

2.2 GUINEA PIG EXPERIMENT SIMULATION

Animals were divided into 5 experimental groups, 6 male guinea pigs in each. For the control group, 3 mL/animal of vegetable oil was introduced intragastrically, once, on an empty stomach. For the first, second, third, and fourth experimental groups, a total volume of 3 mL/animal of DAPS-25k oil solution was introduced intragastrically, once, on an empty stomach, in the dose of 5.0, 10.0, 25 and 50 mg/kg, respectively. To prepare 100 mL of mother liquor with a concentration of 10 mg/mL, 1 gram of DAPS-25k was taken and dissolved in vegetable oil heated to 70°C. Working oil solutions were prepared from the mother liquor by the method of proportional dilutions for use in animals.

The observation period was 14 days after the start. During the experiment, the clinical condition of animals, changes in general body temperature, and live weight were evaluated and deaths were registered. Experiment for survived animals was stopped through an overdose (120 mg/kg) of general anesthesia (Zoletil®, Virbac, France) followed by decapitation on the guillotine.

3. RESULTS

3.1 CLINICAL OBSERVATION OF MICE AFTER INTRAGASTRIC ADMINISTRATION OF DAPS-25K IN THE DOSE RANGE

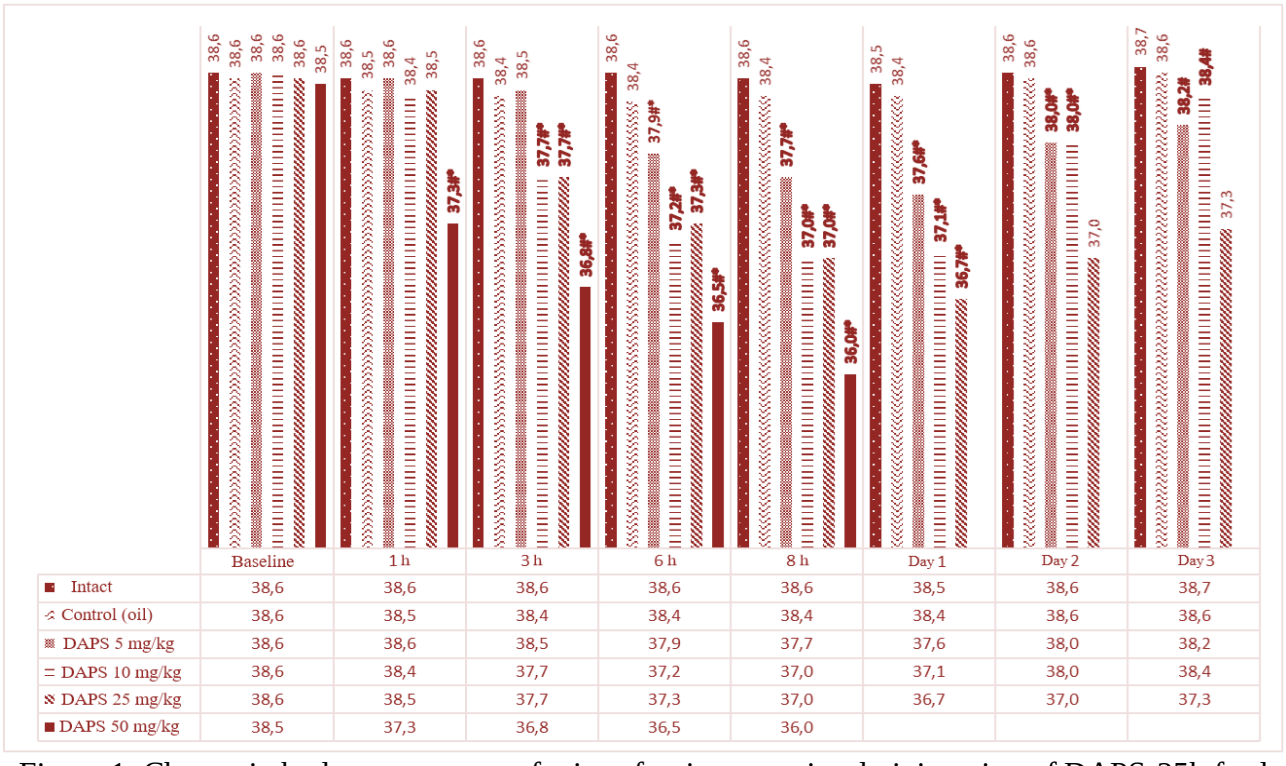
Immediately after the intragastric administration of solutions to control and experimental animals, we noted increased motor activity, ruffled fur, frequent rubbing of the face with forepaws, frequent and shallow thoracic breathing due to stress reaction to manipulation performed. These signs disappeared in all animals in 10-15 minutes.

In mice that received DAPS-25k at the dose of 50 mg/kg, after 3 h, signs of depression were observed, i.e. decreased motor activity, decreased response to external stimuli, and lack of appetite and thirst. After 6 hours, animals developed signs of shortness of breath of a mixed type, cyanosis of the nose. All animals died in the range of 10-12 hours after the start. Before death, animals were in a state of significant depression, reaction even to very strong external stimuli was sharply reduced, mice were on their stomach, respiratory movements were irregular.

The general condition of the mice of the first, second, and third experimental groups 6 hours after the start was characterized as satisfactory, and food activity remained. However, by day 1, all mice of the second and third experimental groups received 10.0 and 25.0 mg/kg of DAPS-25k, respectively, showed signs of depression, decreased appetite, ruffled fur, reduced motor activity compared to control and intact animals. 36 h after drug instillation, 4 mice taken 25 mg/kg of DAPS-25k showed signs of dyspnea of a mixed type, nasal skin cyanosis, physical inactivity, decreased response to external stimuli, lack of appetite and thirst, and ruffled fur; after 48 h, 4 of 6 mice in the third experimental group died.

By day 3, the general condition of all survived animals differs not much from that of intact and control mice, food activity remained, response to external stimuli was adequate, fur was smooth and shiny.

Figure 1 shows changes in the general body temperature of studied animals after the intragastric administration of DAPS-25k in the dose range, from the start to Day 3. Figure 1 shows a progressive decrease in the general temperature of mice of the fourth experimental group during 1-8 hrs by 1.2-2.4°C ($p \leq 0.01$) in comparison with control values. When using DAPS-25k at the dose of 10 mg/kg and 25 mg/kg, general body temperature was significantly reduced by 0.7°C ($p \leq 0.01$) in comparison with control values. By 6 hrs, body temperature values in mice of the first, second and third experimental groups were 37.9 ($p \leq 0.01$), 37.2 ($p \leq 0.01$) and 37.3 ($p \leq 0.01$) °C versus 38.4°C in control animals and 38.6°C in healthy mice. A similar trend persisted at 8 hours and 1 day after drug administration. By day 2 and 3, total body temperature in mice while using DAPS-25k at the dose of 5 and 10 mg/kg rose to 38.0 and 38.3°C but remained significantly lower than control values.



	Baseline	1 h	3 h	6 h	8 h	Day 1	Day 2	Day 3
Intact	38,6	38,6	38,6	38,6	38,6	38,5	38,6	38,7
Control (oil)	38,6	38,5	38,4	38,4	38,4	38,4	38,6	38,6
DAPS 5 mg/kg	38,6	38,6	38,5	37,9	37,7	37,6	38,0	38,2
DAPS 10 mg/kg	38,6	38,4	37,7	37,2	37,0	37,1	38,0	38,4
DAPS 25 mg/kg	38,6	38,5	37,7	37,3	37,0	36,7	37,0	37,3
DAPS 50 mg/kg	38,5	37,3	36,8	36,5	36,0			

Figure 1. Change in body temperature of mice after intragastric administration of DAPS-25k feed additive between the start of experiment and day 3 (n = 6).

– $p \leq 0,01$ – significance of differences between control and experimental groups in comparison with the values in intact animals (Mann-Whitney U-test);

* – $p \leq 0,01$ – the significance of differences between experimental groups in comparison with the values in control animals (Mann-Whitney U-test).

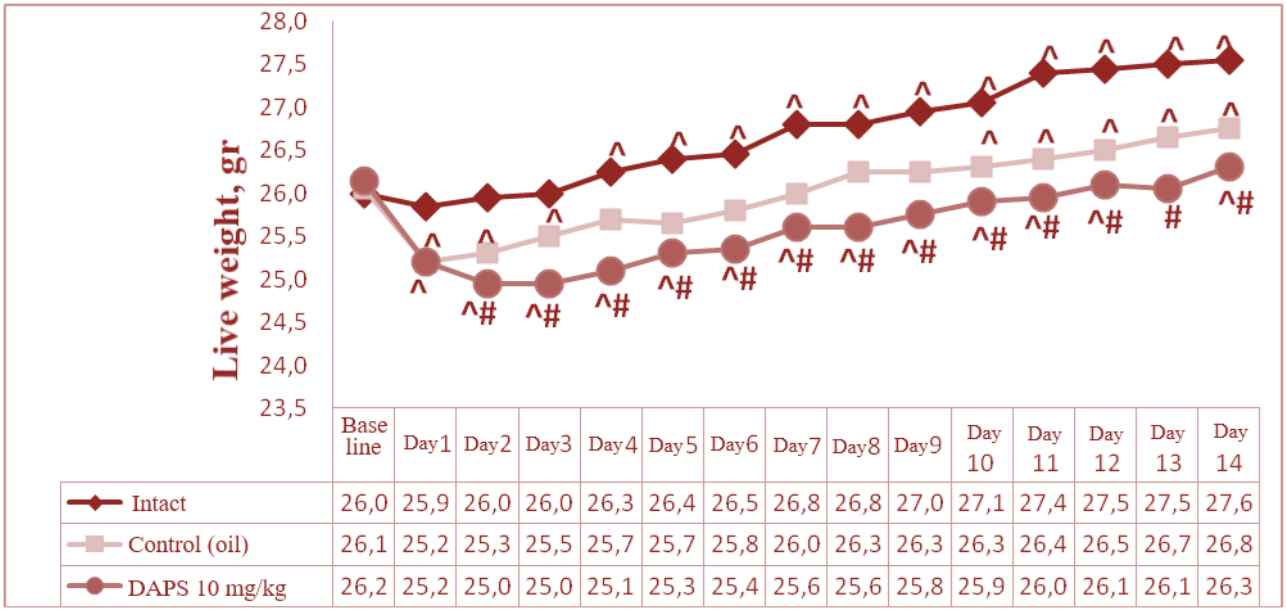
Changes in the values of general body temperature in studied animals with the intragastric administration of DAPS-25k in the dose range are shown in Table 1, from Day 4-14.

Table 1: Changes in general body temperature in °C in mice after intragastric administration of DAPS-25k, from day 4-14, M [Q25; Q75], (n = 6).

	Intact	Control (oil)	DAPS 5 mg/kg	DAPS 10 mg/kg	DAPS 25 mg/kg
Baseline	38.6 [38.5;38.8]	38.6 [38.4;38.8]	38.6 [38.4;38.7]	38.6 [38.4;38.6]	38.6 [38.4;38.7]
Day 4	38.6 [38.5;38.6]	38.6 [38.4;38.6]	38.3 [38.3;38.4]	38.5 [38.3;38.6]	37.5 [37.4;37.6]
Day 5	38.7 [38.6;38.7]	38.6 [38.5;38.6]	38.6 [38.5;38.8]	38.6 [38.4;38.6]	38.0 [37.8;38.2]
Day 6	38.7 [38.5;38.9]	38.6 [38.4;38.7]	38.6 [38.4;38.6]	38.5 [38.2;38.7]	38.3 [38.1;38.4]
Day 7	38.6 [38.5;38.8]	38.4 [38.4;38.7]	38.4 [38.3;38.6]	38.7 [38.5;38.8]	38.3 [38.2;38.4]
Day 14	38.7 [38.4;38.8]	38.6 [38.4;38.7]	38.5 [38.3;38.6]	38.6 [38.5;38.8]	38.4 [38.2;38.5]

According to Table 1, values of general body temperature in all survived animals of the first, second, third experimental groups and control animals from Day 4-14 were at the level of healthy animals at the same time of observation.

Changes in body weight in mice after intragastric administration of DAPS-25k for the period from the start of the experiment to Day 14 is shown in Figures 2 and 3.



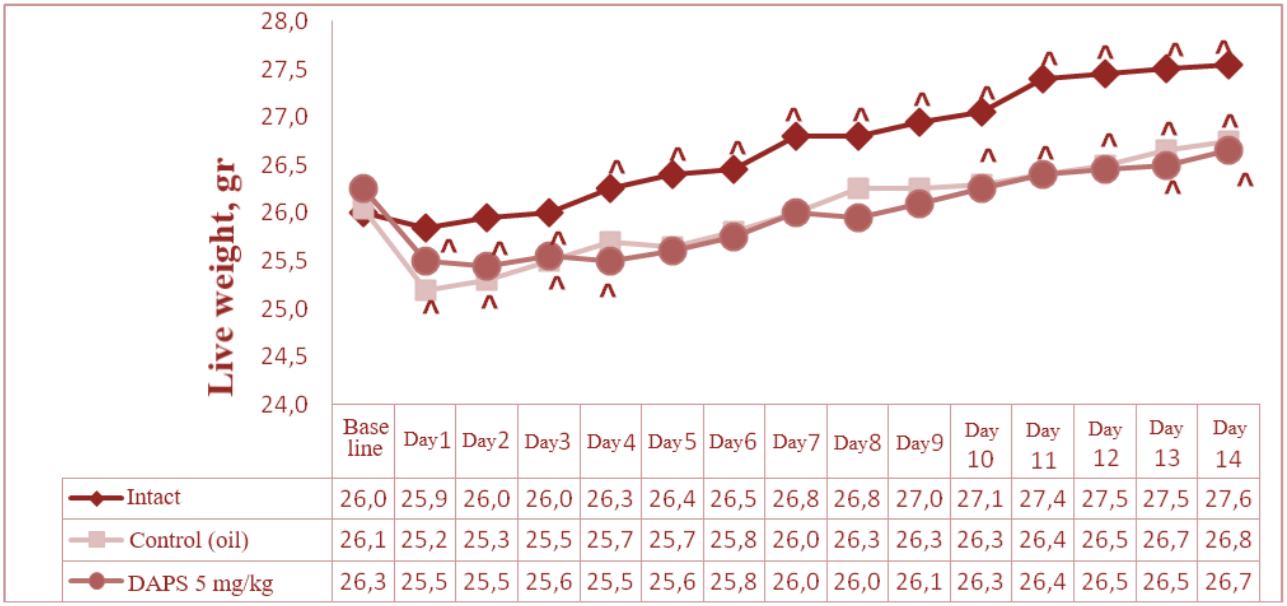
	Base line	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day 10	Day 11	Day 12	Day 13	Day 14
Intact	26,0	25,9	26,0	26,0	26,3	26,4	26,5	26,8	26,8	27,0	27,1	27,4	27,5	27,5	27,6
Control (oil)	26,1	25,2	25,3	25,5	25,7	25,7	25,8	26,0	26,3	26,3	26,3	26,4	26,5	26,7	26,8
DAPS 10 mg/kg	26,2	25,2	25,0	25,0	25,1	25,3	25,4	25,6	25,6	25,8	25,9	26,0	26,1	26,1	26,3

Figure 2: Changes in body temperature in mice after intragastric administration of a DAPS-25k feed supplement at the dose of 10 mg/kg between the start of the experiment and day 14 (n = 6).
^ – p≤0,05 – the significance of differences according to the criterion of signed ranks according to Wilcoxon within the group in comparison with baseline values;
# – p≤0,01 – the significance of differences according to the Mann-Whitney U-test between the experimental and control groups in comparison with intact animals.

According to the plot (Figure 2.), a significant increase in live weight in healthy mice was obvious, from Day 4 to 14 days to 27.6 g (p≤0.05) in comparison with baseline values. A similar trend was observed in control animals from Day 10-14. However, during Day 1-3, the live weight of mice that received vegetable oil significantly decreased in comparison with the baseline value. Live weight of mice treated with DAPS-25k at the dose of 10 mg/kg up to 11 days was in the range of 25.2-26.0 (p≤0.05) grams what is significantly lower than the baseline value and the same value for intact animals, however by 13-14 days, it was restored to the level of control values.

Changes in the live weight in mice with using DAPS-25k at the dose of 5 mg/kg (Figure 3.)

correlate with control values but are characterized by a tendency to a more progressive decrease in Days 1-2 and a slower recovery in comparison with control animals.



	Base line	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day 10	Day 11	Day 12	Day 13	Day 14
Intact	26,0	25,9	26,0	26,0	26,3	26,4	26,5	26,8	26,8	27,0	27,1	27,4	27,5	27,5	27,6
Control (oil)	26,1	25,2	25,3	25,5	25,7	25,7	25,8	26,0	26,3	26,3	26,3	26,4	26,5	26,7	26,8
DAPS 5 mg/kg	26,3	25,5	25,5	25,6	25,5	25,6	25,8	26,0	26,0	26,1	26,3	26,4	26,5	26,5	26,7

Figure 3: Changes in body temperature of mice after intragastric administration of a DAPS-25k feed additive at the dose of 5 mg/kg between the start of the experiment and day 14 (n = 6).
^ - p≤0.05 - the significance of differences according to the criterion of signed ranks according to Wilcoxon within the group in comparison with baseline;

Animal mortality rates and acute toxicity parameters of DAPS-25k feed supplement in mice, after a single intragastric administration, are shown in Table 2.

Table 2: Mortality and acute toxicity parameters of DAPS-25k feed supplement in six mice, after a single intragastric administration (n = 6)

Group of animals	Observation					
	12 hrs	Day 1	Day 2	Day 3	Day 7	Day 14
Intact animals	0/6	0/6	0/6	0/6	0/6	0/6
Control animals (vegetable oil)	0/6	0/6	0/6	0/6	0/6	0/6
Experimental group 1 (5.0 mg/kg DAPS-25к)	0/6	0/6	0/6	0/6	0/6	0/6
Experimental group 2 (10.0 mg/kg DAPS-25к)	0/6	0/6	0/6	0/6	0/6	0/6
Experimental group 3 (25.0 mg/kg DAPS	0/6	0/6	4/6	4/6	4/6	4/6
Experimental group 4 (50.0 mg/kg DAPS	6/6	6/6	6/6	6/6	6/6	6/6
LD_{50}, mg/kg (Mean±SD)	19.6±4.452					
LD_{16}, mg/kg	13.4					
LD_{84}, mg/kg	33.6					
LD_{99}, mg/kg	40.7					

According to Table 2, 100% of animals that intragastrically received DAPS-25k at the dose of 50.0 mg/kg died in 12 hr; when the drug was used at the dose of 25.0 mg/kg, mortality by day 2 was 67%. The semi-lethal dose of DAPS-25k feed additive was 19.6 ± 4.452 mg/kg, and the absolute lethal dose was 40.7 mg/kg.

Pathological changes in the internal organs of dead mice were characterized by blood congestion in pulmonary and systemic circulation and liver congestion. Lungs were filled with blood and red color; blood ran out of incisions, a white foamy fluid ran out from bronchi. Edges of the lower lobes of lungs were with the foci of emphysema. The heart was a triangular shape, of dense consistency; the

*Corresponding author (R.O. Vasiliev). Email: veterenar4ik@mail.ru Volume 11 No.10 ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8
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myocardium was pale pink, injected with coronary vessels. Heart cavities were filled with blood. The liver was of a pasty consistency, of dark cherry color, its boundaries were smooth, scraping from incision surface was large. The gallbladder was filled with light green bile.

3.2 CLINICAL OBSERVATION OF GUINEA PIGS AFTER INTRAGASTRIC ADMINISTRATION OF DAPS-25K IN A DOSE RANGE

During the first 15-20 minutes after the introduction of oil and DAPS-25k oil solution, anxiety, tachycardia, and tachypnea were observed in animals of all groups. After this time, the state of animals in all groups, except group 1, returned to the baseline.

In animals of the first group treated with DAPS-25k at the dose of 50 mg/kg, anxiety was replaced by depression in the first hour after administration. There was also a decrease in appetite, then a complete rejection of food and water, severe depression, and decreased reaction to external stimuli. It was noted that 67% of the animals in this group died in 2-3 hrs, other 33% in 8-12 hrs after the administration of the solution.

In pigs of the second group treated with DAPS-25k at the dose of 25 mg/kg, a decrease in appetite and depression were observed after 2 hours from the time of administration. Animal fur was ruffled, mucous membranes and skin without fur were cyanotic. 50% of animals died in 8-14 hrs; shortly before the death, deep depression was noted. One out of 6 survived; her appetite recovered in 3 days after drug administration.

In animals of the third group treated with DAPS-25k at the dose of 10 mg/kg, appetite was slightly reduced, general condition was assessed as satisfactory. The death of one animal was registered on the fifth day. The animals 50% in this group survived. In animals of 4th group treated with DAPS-25k at the dose of 5 mg/kg, mild depression was observed in 6 hrs. after drug administration, but the state of animals returned to baseline in one day after administration. Their appetite remained, mucous membranes and skin were light pinks in color. The state of the animals of the control group immediately after drug administration and throughout the experiment can be described as satisfactory.

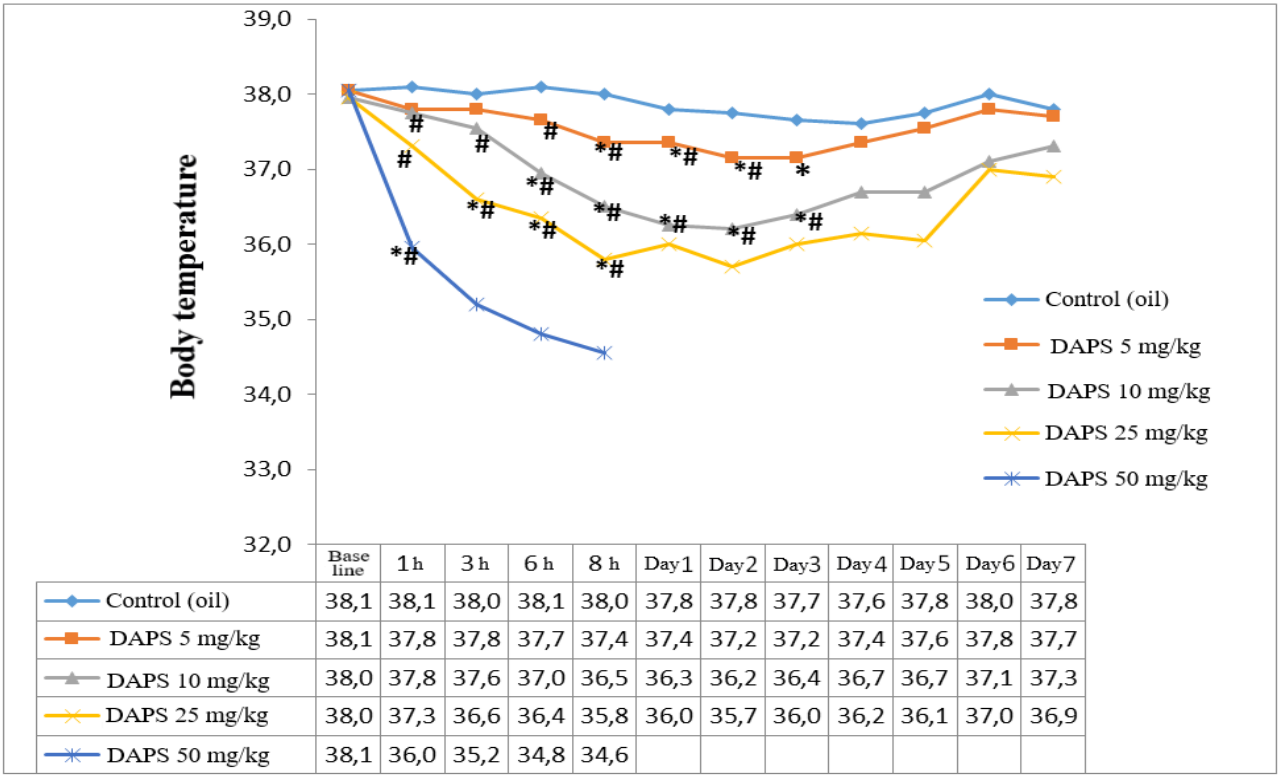
Table 3 shows data on changes in body weight in guinea pigs of experimental groups on the baseline of the intragastric administration of DAPS-25k in the dose range.

Table 3: Changes in body weight of guinea pigs in grams, after intragastric administration of DAPS-25k feed additive, from 1 hour to 7 days, M [Q25; Q75], (n = 6).

	Control (oil)	DAPS			
		5 mg/kg	10 mg/kg	25 mg/kg	50 mg/kg
Baseline	610.0 [604.6;622.9]	600.4 [581.5;601.6]	560.8 [545.0;565.5]	611.3 [601.6;625.2]	618.6 [580.6;641.7]
1 h	606.8 [603.8;620.3]*	596.1 [580.9;597.7]*	556.3 [540.3;561.5]*	594.2 [585.2;603.0]*#	598.5 [566.5;622.1]*
3 h	599.6 [597.6;619.6]*	590.2 [579.4;593.1]*	550.2 [531.2;556.0]*	578.4 [567.2;591.6]*#	579.3 [418.1;450.9]
6 h	599.6 [587.3;617;5]*	584.3 [578.9;588.8]*	542.5 [520.6;549.3]*	561.9 [555.3;582.0]*#	560.8 [399.2;442.0]
8 h	599.3 [577.6;615.3]*	578.9 [575.3;584.2]*	535.3 [512.9;543.1]*	555.6 [547.5;575.8]*#	556.8 [398.6;436.6]
Day 1	609.0 [602.9;622.6]	575.5 [572.3;580.1]*	529.5 [506.2;542.4]*#	545.6 [536.2;554.8]*#	–
Day 2	612.2 [606.2;623.7]	570.4 [567.2;580.7]*	524.7 [497.1;534.2]*#	545.9 [534.2;575.4]	–
Day 3	611.9 [604.8;626.0]	566.6 [563.1;573;9]*	523.0 [489.3;532.1]*#	552.4 [399.4;573.1]	–
Day 4	612.7 [604.7;627.8]	563.0 [557.7;573;7]*	522.1 [471.1;543.2]	556.3 [399.7;578.6]	–
Day 5	613.7 [604.1;628.0]	564.5 [553.4;573.3]	504.4 [479.5;549.8]	555.7 [397.7;579;8]	–
Day 6	613.2 [605.3;629.1]	567.6 [549.3;572.9]	502.6 [472.5;547.9]	582.4 [291.2;581.8]	–
Day 7	615.9 [605.8;629.0]*	570.9 [549.9;577.7]	502.4 [463.2;550.0]	584.5 [292.3;582.9]	–

- p≤0.05 – the significance of differences between the values in experimental groups in comparison with these of control animals (Mann-Whitney U-test);
* - p≤0.05 – the significance of differences according to the criterion of signed ranks according to Wilcoxon within the group in comparison with baseline values.

According to the data in Table 3, a significant decrease in body weight was observed in animals of all groups already in the first hour after the administration of DAPS-25k. In the group of animals treated with DAPS-25k at the dose of 25 mg/kg, a significant decrease in body weight by 2-10% ($p \leq 0.05$) was observed in comparison with the control group, from the moment of administration and up to Day 1. Guinea pigs treated with DAPS-25k at the dose of 10 mg/kg demonstrated a decrease in live weight in comparison to the control group by 13-14.5% ($p \leq 0.05$) in Day 1-3. A decrease in body weight in comparison to baseline values in control animals was registered in the period from 1 to 8 hours; on Day 7, an increase in body weight was noted ($p \leq 0.05$). In animals of the fourth group, parameters of live weight were significantly lower than in control ones up to Day 4. Animals of the third group showed a progressive decrease in body weight in comparison with baseline values ($p \leq 0.05$) and parameters of control animals ($p \leq 0.05$) from the moment of drug administration and up to Day 3. In the second group of animals, bodyweight parameters were significantly lower than baseline values and data of the control group from the moment of administration and up to Day 1.



	Base line	1 h	3 h	6 h	8 h	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Control (oil)	38,1	38,1	38,0	38,1	38,0	37,8	37,8	37,7	37,6	37,8	38,0	37,8
DAPS 5 mg/kg	38,1	37,8	37,8	37,7	37,4	37,4	37,2	37,2	37,4	37,6	37,8	37,7
DAPS 10 mg/kg	38,0	37,8	37,6	37,0	36,5	36,3	36,2	36,4	36,7	36,7	37,1	37,3
DAPS 25 mg/kg	38,0	37,3	36,6	36,4	35,8	36,0	35,7	36,0	36,2	36,1	37,0	36,9
DAPS 50 mg/kg	38,1	36,0	35,2	34,8	34,6							

Figure 4: Changes in body temperature in guinea pigs after intragastric administration of DAPS-25k feed additive between the start of experiment and day 7 (n = 6).

* - $p \leq 0.05$ – the significance of differences according to the criterion of signed ranks according to Wilcoxon within the group in comparison to baseline values;

- $p \leq 0.05$ – the significance of differences according to the Mann-Whitney U-test between experimental and control groups.

According to the data in Figure 4, animals treated with DAPS-25k at the dose of 50 mg/kg demonstrated a significant decrease in temperature by 5% in comparison to the control group, already in the first hour after drug administration. In animals treated with DAPS-25k at the dose of 25 mg/kg, body temperature decrease by 2-5% ($p \leq 0.01$) in comparison to the control group was observed from 1 to 8 hours. The Group of animals received DAPS-25k at the dose of 10 mg/kg showed a significant

decrease in body temperature by 1-4% in 1 h to day 3, in comparison to the control group ($p \leq 0.05$). A similar trend was observed among guinea pigs treated with DAPS-25k at the dose of 5 mg/kg: their body temperature decreased by 1-2% in comparison to the control group in the period from 6 hours to Day 2 ($p \leq 0.05$). Body temperature in animals of experimental groups 4 and 3 was significantly lower than baseline values in the period from 8 and 6 hrs up to day 3 after drug administration. Animals of experimental groups 3 and 2 demonstrated a progressive decrease in body temperature from the moment of drug solution administration to days 3 and 8 hours, respectively.

Parameters of animal mortality and of the acute toxicity of DAPS-25k feed additive in guinea pigs after a single intragastric administration are shown in Table 4.

Table 4: Parameters of mortality and acute toxicity of DAPS-25k feed additive in guinea pigs, after a single intragastric administration (n = 6).

Group of animals	Observation					
	12 hours	Day 1	Day 2	Day 3	Day 7	Day 14
Control animals (vegetable oil)	0/6	0/6	0/6	0/6	0/6	0/6
Experimental group 4 (5.0 mg/kg DAPS-25)	0/6	0/6	0/6	0/6	0/6	0/6
Experimental group 3 (10.0 mg/kg DAPS-25)	0/6	0/6	0/6	0/6	1/6	1/6
Experimental group 2 (25.0 mg/kg DAPS-25)	0/6	0/6	3/6	4/6	5/6	5/6
Experimental group 1 (50.0 mg/kg DAPS-25)	6/6	6/6	6/6	6/6	6/6	6/6
LD_{50}, mg/kg (M±m)	15.8±3.45					
LD_{16}, mg/kg	8.7					
LD_{84}, mg/kg	28.8					
LD_{99}, mg/kg	35.3					

Autopsy of dead animals revealed cyanosis of the mucous membranes of mouth and eyes. Lungs were unevenly colored, with areas of deep dark red color, with foamy light pink liquid flowing out of incision surface on pressure; when immersed in water, most of the lung is beneath the water surface. The heart was cone-shaped, of dense consistency, heart cavities were equally filled with uncoagulated blood of dark cherry color, and heart vessels were injected. The myocardium of pale pink color, of dense consistency. The liver is hyperemic, dark red with a touch of violet, of floppy consistency, edges are sharp, no approximation of incision edges. The gall bladder is expanded, filled with a greenish, opalescent, clear fluid.

4. DISCUSSION

Despite the pronounced antioxidant properties of the element when using in therapeutic doses, selenium is metabolized to free radicals, such as superoxide and hydrogen peroxide, which can cause oxidative damage, especially in energy-intensive tissues such as the myocardium. In macro- and microscopic studies of organs and tissues of animals that died as a result of selenium poisoning, there were multifocal foci of pale color in myocardium what is the sign of cardiac muscle necrosis; also there was dilatation of ventricles, pulmonary edema, fatty liver. Histologically, multifocal pale foci of myocytes with different degrees of swelling, sarcoplasma fragmentation, nuclear pycnosis, and karyorrhexis were found in the myocardium. Similar changes were revealed in skeletal muscles. The heart is a critical organ in comparison with selenium toxicosis; this is clinically manifested by the development of signs of acute and subacute heart failure and causes secondary lesions, including

pulmonary edema, hydrothorax, and hydropericardium. Clinical signs of selenium poisoning described in mice, such as significant general depression, shortness of breath, nasal skin cyanosis, correspond to scientific literature data (Merzlyakova, 2008; Davis et al., 2017; McKenzie, 2017). The decrease in general body temperature in animals is associated with the development of general intoxication due to impaired cardiac and respiratory failure with underlying selenium poisoning.

5. CONCLUSION

From the experimental results, we could establish that high doses (25-50 mg/kg) of DAPS-25k in a single intragastric administration to mice in the form of oil solution have a pronounced toxic effect on mice with the following signs: decrease in general body temperature, severe depression, pulmonary edema. Based on the data obtained, probit analysis according to Finney revealed a semi-lethal dose of DAPS-25k 19.6 ± 4.452 mg/kg and found LD_{16}, $LD_{84,}$ and LD_{99} which amounted to 13.4 mg/kg, 33.6 mg/kg, and 40.7 mg/kg, respectively.

Guinea pigs appeared to be more sensitive to DAPS-25k with single intragastric use in the dose range. Thus, according to Finney probit analysis, LD_{50} was 15.8 ± 3.45 mg/kg, LD_{16} was 8.7 mg/kg, LD_{84} was 28.8 mg/kg, and LD_{99} was 35.3 mg/kg.

Obtained results allow conducting further pharmacodynamic and pharmacokinetic studies of the components of DAPS-25k with the subsequent addition and clarification of the physiological mechanisms of diacetophenonyl selenide conversion in animals.

6. AVAILABILITY OF DATA AND MATERIAL

Information about this study can be made available by contacting the corresponding author.

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Roman Olegovich Vasiliev an Associate Professor of the Department of veterinary radiobiology and BZHCHS, the St. Petersburg State Academy of Veterinary Medicine (St. Petersburg). He is a Candidate of Biological Sciences. He graduated with Honors from the Izhevsk State Agricultural Academy in veterinary medicine. His research interests are Radiobiology, Anti-Radiation Protection of Animals, Pharmacology and Toxicology of Iodine and Selenium Compounds, Radioecology, Biochemistry, Hematology, Histology, Internal Non-Infectious Diseases.

Professor Dr.Troshin Yevgeny Ivanovich is Professor and Head of the Department of Veterinary Radiobiology and BZHCHS, the St. Petersburg State Academy of Veterinary Medicine. He holds a Doctor of Biological Sciences. His research interests include Radiobiology, Anti-Radiation Protection Of Animals, Pharmacology and Toxicology of Iodine and Selenium Compounds, Radioecology, Hematology, Histology, Reproduction.

Sofia Andreyevna Brevnova is a Postgraduate Student at the Department of veterinary radiobiology and BICS. She graduated from the St. Petersburg State Academy Of Veterinary Medicine Research interests: radiobiology, veterinary laboratory diagnostics, Microbiology, Hematology.

Yugatova Natalya Yuryevna is an Assistant Professor at the Department of Veterinary Radiobiology and BZhChS.. she graduated in Veterinary Medicine from the Belgorod Agricultural Academy and Voronezh State Agrarian University. Her scientific interests are Influences of Anthropogenic Factors on Livestock Production, in particular, the Effect of Ultraviolet and Ionizing Radiation. She involves in Toxicology of Iodine and Selenium Preparations.

Dr.Shishkin Alexander Valentinovich is Professor at Department of Anatomy and Physiology, Izhevsk State Agricultural Academy (Izhevsk). He holds a Doctor of Medical Sciences. His research interests are Development of Systems for Immune Analysis, Development of Medical and Veterinary Equipment, Biochemistry, Cytology, Pathological Physiology, Hematology.

Vasilyeva Irina Lvovna is an assistant at the Department of Veterinary Sanitary Expertise and Radiobiology of the Izhevsk State Agricultural Academy (Izhevsk). She graduated from the Izhevsk State Agricultural Academy (Izhevsk). Her research interests are Pharmacology and Toxicology of Selenium Preparations, Radiobiology, Veterinary and Sanitary Examination.

Kulikov Andrey Nikolaevich is an Assistant Professor at the Department of Veterinary Sanitary Expertise and Radiobiology of the Izhevsk State Agricultural Academy (Izhevsk). He studied at the Graduate School of the Izhevsk State Agricultural Academy (Izhevsk) and St. Petersburg State Academy of Veterinary Medicine (St. Petersburg). He is a Candidate of Veterinary Sciences. His research interests encompass Veterinary Pharmacology with Toxicology, Biochemistry.

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