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Fumaric and succinic acids derivatives efficiency in combination with silicon-containing enterosorbents

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Abstract.

The variety of harmful agents creates the danger that several factors may have simultaneous effects on the body. The agents synergistic interaction can significantly exacerbate the expected effects from ionizing radiation in this case. In this regard, the task of a comprehensive analysis of the body's reactions to the combined effect from the harmful agents of different nature has been set. This article is an attempt to analyze the effectiveness of fumaric and succinic acid salts in combination with shungite and zeolite when animals were exposed both to gamma radiation and dioxin. Six groups of experimental rabbits were formed for this purpose. The results of the research revealed that the studied medicines have a positive effect, which was manifested in the experimental groups of animals receiving salts of fumaric and succinic acids in combination with silicon-containing enterosorbents. Survival rate in these groups was 33.3%. There was 100% animals' death in the groups treated with and without the sorbents.

Key words: Dioxin; ionizing irradiation; shungite; zeolite; salts of succinic and fumaric acids

INTRODUCTION.

Human economic activity increased the number and the intensity of physical, chemical, and biological factors that have a negative impact on humans, animals, and the world. Persistent organic compounds which include dioxins and related compounds are the most dangerous environmental pollutants [1,2].

An ionizing radiation is another factor that has a negative impact on the body. The large-scale environmental contamination by the radioactive decay products, as well as the increase in the natural radiation background in the biosphere is now recognized as the most important negative changes [3,4]. In this regard, studies of the combined and mixed effects caused by various environmental factors are of great importance for risk assessment, dose normalization, as well as for treatment methods and development [5,6]. Succinic, fumaric acids, succinates, and fumarates have been proved to be adaptogens. They stimulate the process of oxygen supply to cells, relieve stress, restore energy exchange, normalize the process of new cells production, have general strengthening and restoring properties [7,8].

Sorbents are the effective medicines used for the animals poisoned with toxicants. They reduce toxicants biological availability, slow their absorption in the gastrointestinal tract, reduce the toxic effect on the body, and protect livestock products from contamination. Nutritional value of the feed is not changed [9,10].

Therefore, a complex of therapeutic measures for such lesions development is an urgent task. In this context, our study was aimed at such lesions treatment schemes development with the help of fumaric and succinic acids salts combined with silicon-containing enterosorbents.

MATERIAL AND METHODS.

Six experimental groups of rabbits were formed, three heads in each, with a live weight of 2.7–2.8 kg to conduct the experiment. The first group served as a biological control and received a normal diet; the second experimental group was subjected to ionizing irradiation of 7 Gy and primed with dioxin at a dose of 1/100 LD_{50} (0.3 mkg/kg); for the third group, the gamma radiation was at a dose of 7 Gy + dioxin at a dose of 1/100 LD_{50} (0.3 mkg/kg) + calcium succinate at a dose of 25 mg/kg + shungite (1% of dry substance) + zeolite (1% of dry substance); for the fourth group, the gamma radiation dose was 7 Gy + dioxin at the dose of 1/100 LD_{50} (0.3 mkg/kg) + ammonium fumarate at a dose of 25 mg/kg + shungite (1% of dry substance) + zeolite (1% of dry substance); for the fifth experimental group of rabbits, the gamma-radiation was at a dose of 7 Gy + dioxin at the dose of 1/100 LD_{50} + succinic acid at a dose of 25 mkg/kg + shungite (1% of dry substance) + zeolite (1% of dry substance); and for the sixth group, the gamma-radiation was at a dose of 5 Gy + dioxin in the dose of 1/100 LD_{50} + succinic acid and 25 mkg/kg of calcium succinate.

Animals' clinical conditions, feed, and water consumption were evaluated during the experiment. Body mass weight was determined and blood was taken at the beginning of the experiment and then every 10 days after irradiation, primary toxicants injections, and medicines testing. Hematological analyzer Mythic 18 was used for the hematological investigations.

The level of T-lymphocytes in the peripheral blood was detected by spontaneous rosette formation with heterogeneous erythrocytes (E-ROC). The identification of B-lymphocytes was performed by the EAS-rosette method. Cellular protection factors were studied by the neutrophils phagocytic ability in the peripheral blood.

Free radical homeostasis was investigated by the content of lipid peroxidation products.

RESULTS AND DISCUSSION.

Rabbits' general condition was satisfactory for the biological control group during the period under investigation. They responded properly to the external stimuli and ate food well. The skin was smooth and shiny.

Table 1. Rabbits' survival at combined damage by gamma radiation, dioxin, and treatment.

Experimental group's number	Rabbits in the experience, heads	Fixed animals' mortality, heads					Survival, heads
		Mortality days					
		20	22	23	25	26	
The 1st (control)	3						3
The 2nd group	3	1	1	1			0
The 3rd group	3			1		1	1
The 4th group	3			1	1		1
The 5th group	3			1	1		1
The 6th group	3			2	1		0

Table 2. MDA content in rabbits' blood at combined damage by gamma radiation, dioxin, and treatment.

Groups of the animals	The terms of the research			
	Background	10	20	30
The first	2.10 ± 0.13	2.15 ± 0.23	2.20 ± 0.21	2.13
The second	2.13 ± 0.15	4.20±0.21	3.70 ± 0.14	-
The third	2.10 ± 0.13	4.00 ± 0.20	3.40 ± 0.18	3.75
The fourth	2.00 ± 0.14	3.90 ± 0.16	3.35 ± 0.23	3.70
The fifth	2.15 ± 0.32	3.95 ± 0.15	3.70 ± 0.15	3.45
The sixth	2.11 ± 0.23	4.22 ± 0.11	3.75 ± 0.16	-

Rabbits of the second group did not receive medicines, clinical signs of lesions appeared on day 12. First of all, there was a decrease in feed consumption, followed by the inhibition, a decrease in motor activity, diarrhea, and a decrease in body weight (table 2). The animals began to refuse from food on the 19th day. One animal died every 20th, 21st, and 23rd day (i.e., all rabbits of this group died). Having been treated, animals also manifested symptoms of combined lesions followed by death. One rabbit died on the 23rd day of the study from the third group receiving calcium succinate and sorbents. Another died on the 26th day.

One rabbit died in the fourth and fifth groups of rabbits on the 23rd and 25th days. All animals that were treated only by adaptogens died in the sixth group by the end of the experiment (Table 1).

There was a decrease in live weight in all experimental groups, except for the biological control group. Body weight decreased by 22% and 29% in the second and sixth groups by the 20th day. Data on the body weight by the 30th day could not have been obtained due to the total animals' death in these groups by this time.

There was also a decrease in live weight in the third, fourth, and fifth groups. According to the hematological studies, red blood cell content and hemoglobin decreased by 20.2% and 15.6%, respectively, for irradiated and toxicant treated rabbits by the 20th day of the study. A tendency of leucocytes decrease by 13% and 30% was noticed by the 10th and 20th days of the study.

Changes in the hematological parameters were also observed for the animals treated with medicines. The content of red blood cells and hemoglobin decreased by an average of 15% and 20%, the number of leucocytes also tended to decrease in the third, the fourth, and the fifth groups of animals. It was 15% by the 20th day and 14% by the 30th day.

Table 3. T- and B-lymphocytes content in rabbits' blood at combined damage by gamma radiation, dioxin, and treatment

Days	T-lymphocytes, %	B-lymphocytes, %
The first group		
Background	43.0 ± 1.50	23.50 ± 1.95
10	41.5 ± 1.40	23.00 ± 2.00
20	43.0 ± 1.00	22.50 ± 1.00
30	42.00 ± 0.90	23.50 ± 0.50
The second group		
Background	43.20 ± 2.16	21.50 ± 0.55
10	31.60 ± 1.58	14.00 ± 0.50
20	33.00 ± 1.10	15.50 ± 1.00
30	-	-
The third group		
Background	42.40 ± 1.10	22.50 ± 1.00
10	33.30 ± 1.10	15.30 ± 0.80
20	34.00 ± 0.80	17.00 ± 0.90
30	36.00	17.90
The fourth group		
Background	4250 ± 0.90	23.00 ± 0.90
10	32.10 ± 1.00	16.00 ± 1.80
20	34.50 ± 1.00	16.50 ± 1.10
30	34.20	16.70
The fifth group		
Background	43.40 ± 1.00	22.00 ± 1.00
10	33.20 ± 1.50	15.90 ± 0.60
20	33.40 ± 0.80	17.00 ± 0.90
30	34.00	16.40
The sixth group		
Background	41.00 ± 0.90	21.90 ± 0.90
10	31.90 ± 0.50	17.30 ± 0.90
20	33.55 ± 1.60	16.10 ± 1.80
30	-	-

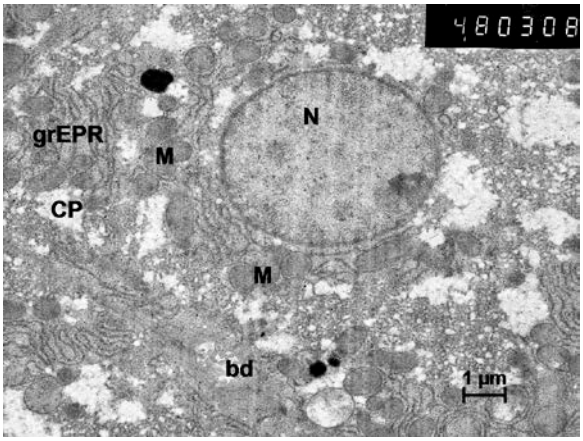

Figure 1. Hepatocytes' fragments with the rabbit's bile duct of the control group.M = mitochondria, N = nucleolus, CP = cytoplasm, grERP = granular endoplasmic reticulum, BD = bile duct.

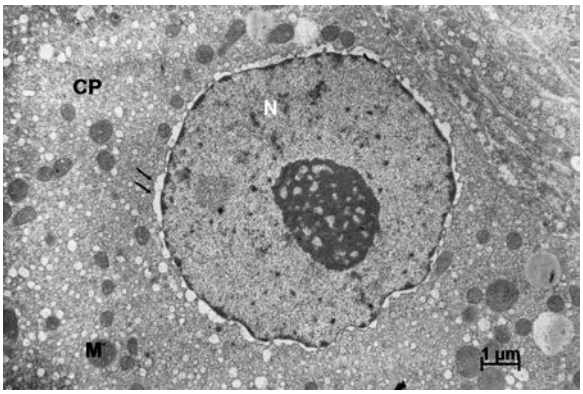

Figure 2. Rabbit's hepatocyte fragment after ionizing radiation exposure at a dose of 7 Gy and dioxin at a dose of 1/100 LD_{50}. M = mitochondria, N = nucleolus, CP = cytoplasm, and arrows indicate the swelling of the nuclear envelope perinuclear space.

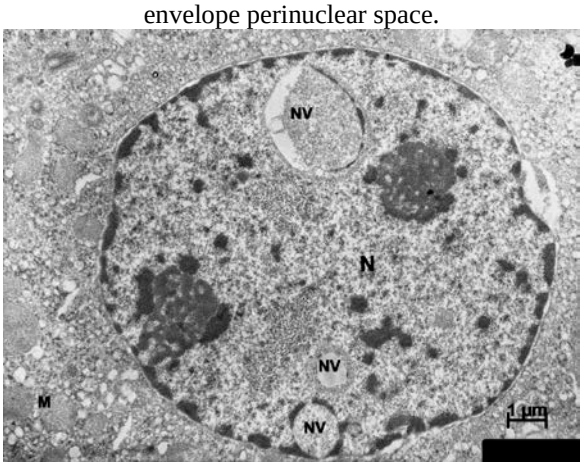

Figure 3. Rabbit's hepatocyte fragment after combined exposure to ionizing radiation at a dose of 7 Gy, dioxin at a dose of 1/100 of LD_{50}, ammonium fumarate at a dose of 25 mg/kg bwt, shungite, and zeolite at doses of 1% dry substances, respectively. M = mitochondria, N = nucleolus, NV = intranuclear vacuoles, CP = cytoplasm

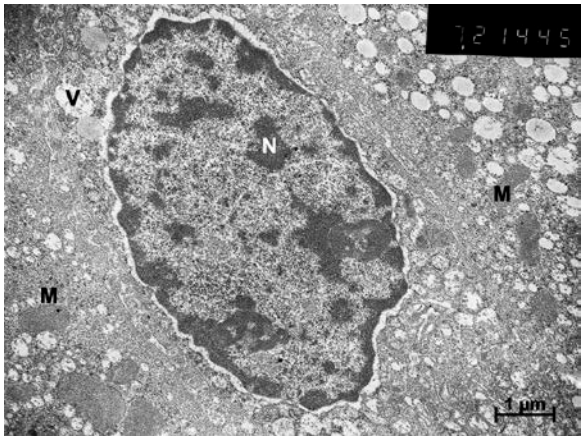

Figure 4. Rabbit's hepatocyte fragment after combined exposure to ionizing radiation at a dose of 7 Gy, dioxin at a dose of 1/100 of LD_{50}, succinic acid at a dose of 25 mg/kg bwt, shungite, and zeolite at doses of 1% dry substances, respectively. M = mitochondria, N = nucleolus, V = vacuoles.

The content of the shaped elements was an average of 18%–20% lower in the sixth group of animals.

According to the studies on the intensity of lipid peroxidation, changes in the malondialdehyde (MDA) concentration have been revealed in all experimental groups. The results are presented in Table 2.

According to Table 2, the medicine under discussion has not been changed in the biological control. The maximum increase in the content of MDA was by the 10th day and its number increased in all experimental groups. The indicator decreased by the 20th and 30th days. It was of multidirectional character depending on the period.

Exposure to radiation and dioxin also had an impact on natural resistance. Phagocytic activity, number, index, and capacity decreased by 28%, 14%, 38%, and 56% by the 20th day of study in the second group and by 29%, 10%, 28%, and 41%, respectively, in the sixth group. These indicators also decreased for the rabbits of the third, the fourth, and the fifth groups. They were an average of 15%–25% lower by the end of the experiment.

Changes in the number of T- and B-lymphocytes in the peripheral blood are one of the indicators characterizing the state of animals' immunity. The obtained results are presented in Table 3.

According to Table 3, the maximum reduction of T-lymphocytes was on the 10th day in all experimental groups. The cell content increased but did not reach the background value by the end of the experiment. A similar situation happened to the B-lymphocytes.

Our ultrastructural studies have also revealed changes in cells. The light-optical study revealed the areas of the rabbit's liver with a typical lobular structure. Hepatic lobules form the long strands of tissue (hepatic beams or trabeculae) consisting of hepatocytes radiating to the periphery of the central vein (Fig. 1).

The electron microscopic study of the rabbit's liver cells ultrastructure of the second group revealed most organelles structural abnormalities: changes in the shape of nuclei, karyoplasm consolidation, and chromatin redistribution to

the marginal state, which is an irreversible state showing the cells death. The number of nuclear pores is reduced. They are hardly visible. The perinuclear space has a different thickness throughout its length, forming a bulge, facing either inside the nucleus or to the cytoplasm (Fig. 2). The mitochondrial matrix is compacted, the crystals either disappear or become extremely small, and the intermembrane space along their entire length is of uneven thickness. This indicates a violation in the mitochondria functioning. There is probably a disconnection in the processes of redox phosphorylation. Endoplasmic reticulum fragmentation occurs, its tanks thicken, cytoplasm is vacuolized. The size and number of peroxisome increase. It is probably associated with hydrogen peroxide H_2O_2 accumulation formed during the radiolysis. Catalase peroxisome is involved in its detoxification.

Animals' cells exposed to the combined lesions and treated with ammonium fumarate and sorbents preserved better but their structure still has signs of different alterations. Cells acquire features that are characteristic neither for the norm nor for the pathology. Cells try to protect themselves from the effects of radiation and toxic substances. They form membrane structures inside the nucleus filled with a variety of contents: areas of cytoplasm (possible invagination of the nuclear envelope), transparent flake matrix, or dense detritus. Chromatin is evenly distributed over the nucleus, there are nucleoli there. The perinuclear space extends as a smooth ribbon. Swelling is detected only in some areas. Hyaloplasm is of the dense structure. There are many small electron-transparent vacuoles with separate fragments of endoplasmatic reticulum and mitochondria in the cytoplasm. They are with a dense flake matrix and single crystals, which are hardly observable due to the intermembrane space adhesion (Fig. 3).

There are hepatocytes with small-droplet fat dystrophy. Necrotic cells may appear. There are many red blood cells in the sinusoids of the liver.

A weak preservation of cellular organelles was noted for a group of animals, which received succinic acid and sorbents simultaneously with the combined exposure. Vacuolization (large and small vacuoles) in the cytoplasm and the swollen periplasmic space, i.e., atomized hydropic as well as hepatocytes and fat dystrophies are among the disturbed patterns (Fig. 4). Hyaloplasm is of medium electron density. Endoplasmic reticulum is very rare. It has an electron-transparent content. Mitochondria are with flake matrix and single crystals. Intra-nuclear inclusions are less often than in the previous group.

CONCLUSION.

Thus, according to the results of the studies, the medicines under the investigation have a positive effect in the form of corrective influence on the free radical and immune homeostasis observed in the third (calcium succinate + shungite + zeolite), the fourth (ammonium fumarate + shungite + zeolite), and the fifth (succinic acid + shungite + zeolite) groups. The survival rate was 33.3%, while there was 100% animals' death in the second (combined lesion) and the sixth (calcium succinate + succinic acid) by the 30th day.

REFERENCES

[1] Biktashev R.U., Bulankov S.R., Ermakova Y.I., Modified bentonite sorption properties in the experiments in vitro and in vivo. Vetkorm. 2014, 1, 2-3.

[2] Ershov A.G., Shubnikov V.L., Dioxinophobia: facts and speculation. Ecoprogress. 2012, 8, 26-31.

[3] Ivanov A.V., Papunidi K.Kh., Tremasov M.Ya., Papunidi K.Kh., The use of succinic acid and products based on it. Kolos, Kazan, 2014.

[4] Ivanov A.V., Smirnov A.M., Papunidi K.Kh., Ivanov A.A., Kadikov I.R., Dioxins (biological and veterinary aspects), Kolos, Kazan, 2015.

[5] Kadikov I.R., Papunidi K.Kh., Korchemkin A.A., Vafin I.F., Semenov E.I., Idiatov I.I, Comparative evaluation of agents for preventive therapy at combined intoxications with xenobiotics in poultry. Indian Vet. J. 2018, 95(07), 29-32.

[6] Konyukhov G.V., Papunidi K.Kh., Vasilevskiy N.M., Tarasova N.B., Nizamov R.N., Kurbangaleev Ya.M., The effectiveness of sulfotozifan in the case of external gamma. Veterinary science. 2018, 1, 10-14.

[7] Mouret A., Agnius-Delort C., Rinaldi R., Etude de lefficifeit de trios heterocycles radioprotectors sur des soures C3H irradies an cobalt-60. Acad Sci. 1972, 275(14), 5-8.

[8] Papunidi K.Kh., Kadikov I.R., Saitov V.R., Semenov E.I., Gataullin D.Kh., Korchemkin A.A., Homeostatic system of sheep against the background of combined effects of pollutants and the use of therapeutic and preventive agents. Bali Medical Journal. 2017, 6(2), 83-87.

[9] Papunidi K.Kh., Semenov E.I., Kadikov I.R., Biktashev R.U., Gataullin D.Kh., Sorbents application to prevent animals' metabolic disorders and toxicosis. Kolos, Kazan, 2018.

[10] Yoshizawa K., Marsh T., Cai B., Peddada Sh., Walker N., Mehanisms of exocrine and endocrine pancreatic toxicity induced by oral treatment with 2,3,7,8-TCDD in female rats. Toxicol. Sci. 2005, 85(1), 85-93.