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STUDY OF PHARMACOLOGICAL PROPERTIES AND SORPTION ABILITIES OF THE NEW SORBENT FERBENSORB

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ABSTRACT

The use of sorbents helps to reduce the toxic load when toxic chemicals and radionuclides enter the body. This article investigates the pharmacological properties of the new ferrocyanide-bentonite sorbent Ferbensorb. Ferbensorb is a composite sorbent containing potassium-iron(III) hexacyanoferrate(II), as well as bentonite, gelatin, and macro- and microelements. The article presents the results of experimental studies conducted on laboratory animals (rats and mice). Sorption activity with the introduction of cesium, sorption capacity with the introduction of strontium radionuclides, and sorption capacity with experimental associative mycotoxicosis were determined. In addition, a pathoanatomical autopsy was performed, and changes in body weight and some internal organs of the studied animals were determined, as well as the leukocyte formula. It was found that ferrocyanide-bentonite sorbent Ferbensorb significantly reduced the functional and morphological manifestations of the pathological process caused by mycotoxins ochratoxin A, fumonisin B, and zearalenone. It increased body weight gain, indicators of natural immunological resistance, which was expressed by an increase in the percentage of neutrophils in the leukoformula, an increase in the level of bactericidal and lysozyme activity of blood serum, and animal survival.

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Introduction

Enterosorbents are medicinal preparations that bind substances in the gastrointestinal tract by adsorption, absorption, ion exchange, and complexation [1]. The use of sorbents helps to reduce the toxic load when toxic chemicals and radionuclides enter the body. In case of violation of the barrier function of the gastrointestinal tract, sorbents inhibit the absorption of toxic chyme products, which is the basis for their use in gastrointestinal diseases [2, 3].

The mechanisms of therapeutic action of enterosorbents include direct and indirect effects. The direct effect consists in the sorption of poisons and xenobiotics coming from feed, sorption of substances involved in hepato - and hemoenteral circulation, substances formed in the intestine during hydrolysis of feed, sorption of microorganisms and their toxins, gas binding, changes in the consistency of chyme, stimulation of the functional activity of the digestive organs. The indirect effects consist of the functional unloading of detoxification organs, and the correction of metabolic processes [4-6].

However, today no effective sorbent has been identified against all or most xenobiotics, which indicates that the potential of substances promising for use as enterosorbents is quite diverse, but has not been fully studied, which necessitates further research to identify and rationalize the use of those substances that will be most suitable for the prevention of feed poisoning.

Extensive production practice has proven the ability of some substances of organic and mineral origin to bind and firmly retain a wide range of toxins of various origins [7, 8]. Clay minerals (zeolites and bentonites) have successfully proven themselves on the positive side in the practice of feeding.

Bentonite is commonly referred to as clay containing at least 70% of the mineral of the montmorillonite group [9].

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Montmorillonite is a highly dispersed layered aluminosilicate in which, due to non-stoichiometric substitutions of crystal lattice cations, an excessive negative charge appears, which is compensated by exchange cations located in the interlayer space. This is due to the high hydrophilicity of bentonite [10].

When bentonite is sealed with water, it penetrates the interlayer space of montmorillonite, hydrates its surface, and exchanges cations, which causes the mineral to swell. Upon further dilution with water, bentonite forms a stable viscous suspension with pronounced thixotropic properties [11]. Montmorillonite has high cation exchange and adsorption properties. It is known that small amounts of bentonite clay added to animal feed have a positive effect on their growth and animal health because bentonite is a valuable natural polymineral top dressing and contains several vital trace elements [12].

Bentonite has a beneficial effect on the digestive process of animals and birds. It has a high ability to absorb alcohols, microbial cells, and their toxins [13]. It is a good hepatoprotector and normalizes the activity of organs of the reproductive system [14]. The inclusion of inorganic silicon compounds in the diet in the form of bentonite has a significant effect on the correction of mineral metabolism, contributing to an increase in the concentration of trace elements vital for the body in the blood - iron, copper, and zinc [15]. Thus, the level of copper in the blood serum increases by 9.7%, iron - by 17.4%, and zinc - by 42.3%.

Normalization of mineral metabolism contributes to the activation of hematopoiesis processes, primarily erythro- and hematopoiesis [16]. At the same time, the level of erythrocytes increases from the initial values by 12.3-20.2%, and the concentration of hemoglobin increases by 13.5-18.9%, respectively [17]. Inorganic complex compounds from the group of hexacyanoferrates – ferrocyanides have been known as dyes since the end of the 19th century. Experimental study of the possibility of using ferrocyanides to reduce the transition of cesium into the body and accelerate the removal from the body of animals and humans began after tests in the atmosphere of nuclear weapons in 1954-1964. By the mid-60s, experiments on laboratory animals found that the introduction of ferrocyanides simultaneously with radioactive cesium reduces the transition of radioactive cesium into organs and tissues to 95-98% [18]. Ferrocyanide-bentonite sorbent Ferbensorb is a composite sorbent containing potassium-iron(III) hexacyanoferrate(II), as well as bentonite, gelatin, and macro-trace elements.

Materials and Methods

To determine the sorption of strontium radionuclides, a study was conducted on 28 mongrel white rats weighing 280 ± 12 g, divided into 7 groups of 4 animals each, taking into account body weight. Animals were injected with a metal probe with a solution of 90 Sr nitrate in the amount of 197 kBq/animal and sorbents in the form of a suspension in 1.5% starch gel. After 3 days, the animals were killed by decapitation, and bone samples were taken for radiometry at the UMF-1500 installation.

To determine the sorption properties of the sorbent Ferbensorb in experimental associative mycotoxicosis, a study was conducted on Wistar rats weighing 160-200 g, divided into 4 groups of 8 heads each. The animals of the 1st group received clean food and served as a control. Group 2 rats were fed with a mixture of mycotoxins and 0.5% sorbent Ferbensorb (5 g per 1 kg of feed). A mixture of mycotoxins and 1% Ferbensorb (10 g per 1 kg of feed) was added to the feed of the 3rd group of rats. The animals of the 4th group received food with mycotoxins but without sorbent. A mixture of mycotoxins was added to the feed at the rate of 5 mg per 1 kg of feed.

The animals were monitored for 30 days. Before the start of the experiment, the rats were weighed, after 15 days the weighing was repeated and a controlled slaughter of 2 rats from each group was carried out. The final slaughter and analysis of the results were carried out after 30 days.

The dead rats were dissected and pathologic-anatomical changes in organs and tissues were recorded. At the end of the experiment, the surviving animals were killed and also subjected to pathologic and anatomical examination, and in the selected venous blood samples, the leukocyte formula, and bactericidal and lysozyme activity of blood serum were studied using conventional methods.

Results and Discussion

The sorption activity of ferrocyanide-bentonite sorbent Ferbensorb, depending on the dose of the drug, was studied in experiments on 20 Wistar rats weighing 180-200 g, divided according to the principle of analogs into 4 groups.

After a single administration of cesium-137 at a dose of 370 kBq/ animal, Ferbensorb was administered to rats of the first three groups in the form of a 50% aqueous suspension in amounts of 1.0; 1.5 and 2.0 ml/animal (0.5; 0.75; 1.0 g/animal), respectively. The sorbent was not used in animals of the 4th group after the introduction of the isotope, they served as a control. The results of determining the content of ^{137}Cs in some organs and tissues of rats killed on the 7th day after the introduction of the isotope and sorbents are shown in **Table 1**.

Table 1. The concentration of ^{137}Cs in rat organs and tissues after administration of various amounts of Ferbensorb sorbent (in the numerator – Bq/g, in the denominator - % of control) (M±m; n=20)

Quantity of sorbent g/head	Name of the organ or tissue					
	Liver	**Kidney**	**Lung**	**Spleen**	**Heart**	**Muscle**
0.5	210.5±24.5	378.6±34.4	155.7±18.7	205.5±11.2	248.8±30.5	492.0±24.6
	22.4	28.9	22.3	24.4	25.8	24.7
0.75	242.4±30.2	350.7±36.5	148.8±20.2	199.6±8.9	236.4±21.8	406.6±34.0
	26.9	25.8	21.4	23.7	24.5	20.4

1.0	199.6±23.4 22.2	326.4±30.7 24.9	140.0±16.7 22.1	190.2±12.4 22.6	210.8±32.3 21.8	421.3±28.7 21.1
Control	898.4±48.8 100	1306.4±72.2 100	696.4±52.0 100	840.2±54.4 100	962.8±40.3 100	1990.8±52. 100

As can be seen from the materials in **Table 1**, the administration of sorbent to rats led to a decrease in the transfer of ^{137}Cs to organs and tissues by an average of 73-80% relative to the control. Dependence on the sorbent dose was manifested by a tendency to increase sorption with an increase in the amount of sorbent injected into the body. However, no significant differences were found in these dynamics.

The sorption efficiency of ferrocyanide-bentonite sorbent Ferbensorb compared with the selective sorbent cesium–ferrocin was studied in experiments on 15 rats weighing 160-180 g, which were divided into 3 groups. Animals of all groups were injected with ^{137}Cs once using a probe in the form of an aqueous solution without a carrier at a dose of 370 kBq/animal. Group 1 rats were injected with ferrocyanide-bentonite sorbent Ferbensorb in the form of a 50% aqueous suspension at a dose of 1 ml/animal after the introduction of the isotope; group 2 rats were injected with ferrocin in an amount of 150 mg/ ml animal; sorbents were not administered to rats of the 3rd group (control group). 7 days after the introduction of the radioisotope and sorbent, the animals were killed and the content of ^{137}Cs in organs and tissues was determined **(Table 2)**.

Table 2. The concentration of ^{137}Cs in rat organs and tissues after administration of the sorbent Ferbensorb and ferrocene (in the numerator – Bq/g, in the denominator - % of control) (M±m; n=15)

Sorbent	**Name of the organ or tissue**					
	Liver	**Kidney**	**Lung**	**Spleen**	**Heart**	**Muscle**
Ferbensorb	231.4±32.6 20	348.5±35.5 23.8	185.0±21.4 23.4	205.7±14.5 21.8	258.0±37.4 22.6	124.3±16.0 19.4
Ferrocene	18.9±2.7 1.7	27.6±1.63 1.9	16.0±2.2 2.2	19.3±1.9 2.0	25.0± 1.9 2.2	10.2±12.5 1.6
Ferrocene	1110.0±43 100	1465±83.5 100	788.1±52.9 100	939.8±87.2 100	1140±52.4 100	649.3±73.9 100

Studies have shown that after administration of ferrocyanide-bentonite sorbent Ferbensorb at a dose of 0.5 g / animal, the content of ^{137}Cs in organs and tissues decreased by an average of 81.6-72.7%, and when ferrocin was administered in an amount of 0.15 g/animal - by 97.8-98.3%.

Thus, experiments on rats have established the possibility of using the ferrocyanide-bentonite sorbent Ferbensorb as a means of reducing the transition of ^{137}Cs from the gastrointestinal tract to animal organs and tissues with an efficiency similar to that of the selective sorbent cesium-ferrocin.

Studies of the sorption of strontium radionuclides were carried out on 28 mongrel white rats weighing 280 ± 12 g, divided into 7 groups of 4 animals each, taking into account body weight.

Animals were injected with a metal probe with a solution of ^{90}Sr nitrate in the amount of 197 kBq / animal and sorbents in the form of a suspension in 1.5% starch gel.

After 3 days, the animals were killed by decapitation, and bone samples were taken for radiometry at the UMF-1500 installation. The experimental conditions and the obtained data are shown in **Table 3**.

Table 3. The concentration of ^{90}Sr in the rat skeleton after administration of ferrocyanide-bentonite sorbent Ferbensorb and manganese dioxide (M+m; n=28)

Sorbent		**% of the introduced isotope amount**	**Effectiveness, %**
type	**Quantity, mg/animal**		
Control	-	20.3±4.2	100
Ferbensorb	40	17.8±4.2	12.9
Ferbensorb	80	4.6±0.8	40.9
Ferbensorb	120	9.7±0.8	52.6
manganese dioxide	20	17.4±2.8	14.6
manganese dioxide	40	5.2±1.2	31.8
manganese dioxide	80	6.9±0.9	68.7

As can be seen from the above data, the introduction of sorbents reduced the transition of 90Sr from the gastrointestinal tract to the rat skeleton. When the ferrocyanide-bentonite sorbent Ferbensorb and manganese dioxide are administered in minimal doses, the isotope deposition in bone tissue is approximately the same. The effectiveness of manganese dioxide after using doses of 40 mg and 80 mg, respectively, is 2.5 and 1.3 times higher than with the same doses of ferrocyanide-bentonite sorbent Ferbensorb.

When determining the sorption properties of the sorbent Ferbensorb in experimental associative mycotoxicosis, laboratory animals were Wistar rats weighing 160-200 g. The rats were divided into 4 groups of 8 heads each. The animals of the 1st group received clean food and served as a control. Group 2 rats were fed with a mixture of mycotoxins and 0.5% sorbent Ferbensorb (5 g per 1 kg of feed). A mixture of mycotoxins and 1% Ferbensorb (10 g per 1 kg of feed) was added to the feed of the 3rd group of rats. The animals of the 4th group received food with mycotoxins but without sorbent. A mixture of mycotoxins was added to the feed at the rate of 5 mg per 1 kg of feed.

The animals were monitored for 30 days. Before the start of the experiment, the rats were weighed, after 15 days the weighing was repeated and a controlled slaughter of 2 rats from each group was carried out. The final slaughter and analysis of the results were carried out after 30 days.

The animals were monitored throughout the experiment, taking into account the general condition and behavior of rats, the effect of mycotoxins, and the protective effect of the sorbent Ferbensorb on body weight gain, some parenchymal organs, and blood parameters of animals.

The dead rats were dissected and pathologic-anatomical changes in organs and tissues were recorded. At the end of the experiment, the surviving animals were killed and also subjected to pathologic and anatomical examination, and in the selected venous blood samples, the leukocyte formula, and bactericidal and lysozyme activity of blood serum were studied using conventional methods.

As a result of research, it was found that prolonged administration of mycotoxins has a pronounced toxic effect on the body of intact rats (group 4). After 6-9 days, they began to show signs of general depression, lack of mobility, impaired coordination of movements, decreased activity in eating food, appeared disheveled and loss of gloss of the coat, and increased water consumption. No external changes were observed in rats receiving pure feed (group 1) and feed with sorbent (groups 2 and 3). They willingly ate food and actively moved in the cage, the coat was not changed. They had this condition until the end of the experiment.

Starting from day 16, rapid breathing and palpitations were observed in all rats of the 4th group, and body temperature increased by 0.3-0.6 ℃. The maximum temperature increase by (1 ℃) was recorded in individual rats on the 16th-22nd day of the experiment. From the 24th day of the experiment, the body temperature of the rats gradually decreased and by the end was within the normal range. The bulk of the animals had poor feed intake, two rats refused it on the 19th day, and on the 16th, 20th, and 24th days, one rat died in this group. There were no clinical signs of toxicosis in control rats and animals of the 2nd and 3rd groups fed with mycotoxins and sorbent.

The results of the increase in live weight of rats are shown in **Table 4**. The increase in body weight of rats of the 4th group, where food contaminated with the mycotoxin association was present, was characterized by low indicators and the death of 3 animals. In the control and 2 experimental groups in which the sorbent Ferbensorb was present with the association of mycotoxins, the body weight gain was 53.4; 58; and 64 g, respectively.

The body weight gain of one animal during the experiment in group 4 was 41.4 g lower than in group 1 and 46 g and 52 g lower than in groups 2 and 3. This indicates the effect of toxins on body weight gain, impaired function of the gastrointestinal tract, and increased intoxication of the body. At the same time, there were no toxicosis phenomena in groups 2 and 3. In group 2, the average daily gain exceeded group 1 by 4.6 g, and in group 3 - by 10.6 g.

The chronic course of associated mycotoxicosis in rats with the administration of 5 mg of toxins per 1 kg of feed was manifested by the death of three out of eight experimental animals within 30 days with an average life expectancy of 20 days. The sorbent Ferbensorb in concentrations of 0.5% and 1% in the feed prevented deaths.

On the 15th day of the experiment, after weighing the animals, a controlled slaughter of 2 rats in each group was performed. No changes were found in the gastrointestinal tract and parenchymal organs in the section of slaughtered animals in the control and the first two experimental groups. In animals of the 4th group treated with toxins, redness, and spot hemorrhages were found in the stomach and small intestine, the liver was enlarged and unevenly colored, and the bile duct was moderately filled with yellow-brown bile.

Table 4. Dynamics of the course of associative mycotoxicosis in rats and the effect on their body weight gain with the introduction of the sorbent Ferbensorb, g (M+m; n=32).

Before the introduction of toxins and sorbent				**15 days**				**30 days**			
Group				**Group**				**Group**			
1	2	3	4	1	2	3	4	1	2	3	4
157	175	150	125	193	225	197	186	210	221	211	151
161	175	151	131	194	207	195	257	215	212	226	190
142	144	155	185	189	245	217	201	237	248	242	172
153	190	165	181	187	186	183	170	201	190	197	fallen
142	129	130	154	191	214	190	190	212	210	217	fallen
169	130	182	138	193	192	190	178	207	202	201	fallen
163	190	139	224	204	207	202	162	killed	killed	killed	killed

165	118	146	137	207	172	250	183	killed	killed	killed	killed
156.5 ±10.2	156.4 ±29.3	152.2 ±15.9	159.4 ±34.4	194.8 ±7.07	206 ±22.9	203 ±21.5	190.9 ±29.3	213.7 ±12.4	213,8 ±19,7	215,7 ±16,7	171 ±19,5
Body weight gain				+34	+50	+51	+32	+53.4	+58	+64	+12

During the experiment, one rat died in the 4th group on the 16th, 20th, and 24th days. An external examination of the dead animals revealed cyanosis of the mucous membranes of the oral and nasal cavities, conjunctival hyperemia, and contamination of the coat.

During the pathoanatomical autopsy, they found:

The lungs are bright red, and there is a foamy pink liquid on the incision in the lumen of the trachea and bronchi. The liver is flabby, enlarged, unevenly colored, cherry-colored, with areas of necrosis. The bile duct is filled with yellow-brown bile. The kidneys are slightly enlarged with hemorrhages, and the border of the cortical and cerebral layers is weakly expressed. The ureters are enlarged, and the mucous membrane of the bladder with striated hemorrhages.

The stomach is swollen, and there are banded hemorrhages on the mucous membrane of the fundal part.

There are spot hemorrhages on the mucous membrane of the small intestine, and the contents of the large intestine with air bubbles.

The heart is enlarged, the myocardium is flabby, and there are spot hemorrhages on the epicardium. There is poorly clotted blood in the cavities of the heart.

Liver, spleen, and heart muscle samples were taken for histological examination.

The most pronounced changes were found in the structure of liver tissue. In all cases, there was a violation of the normal girder structure of the organ, they were susceptible to karyopycnosis, karyorexis, and karyolysis.

Congestion and circulatory disorders in the lungs. The capillaries are dilated, in places they protrude into the lumen of the alveoli. There is an accumulation of homogeneous oxyphilic substances in the alveoli with the inclusion of red blood cells and epithelial cells.

Pronounced changes in immunocompetent organs. The vessels of the spleen are hyperemic, the boundaries of the white and red pulp are smoothed, and the size of the pulp and follicles are reduced. The bright centers of the vesicles are represented by sparsely located lymphocytes of medium and small size with pale-colored nuclei. Histological changes in kidney tissue consist of granular dystrophy of the epithelium of the convoluted renal tubules and its desquamation in some areas.

In control slaughters of laboratory animals, the effect of the drug Ferbensorb on the leukocyte formula and immunological parameters of rats against the background of mycotoxicosis was studied. Under the influence of toxins in the blood of rats of groups 2, 3, and 4, compared with the control level (group 1), there was a significant decrease in the percentage of rod-shaped neutrophils **(Table 5)**. A significant decrease in segmented neutrophils was found only in group 4. The content of lymphocytes in the blood of rats under the influence of toxins in group 4 compared with group 1 significantly increased. In rats of the 2nd and 3rd groups, this increase was insignificant.

Table 5. The effect of the sorbent Ferbensorb on the leukocyte formula of rat blood (M+m; n=32)

Group	Neutrophils			Eosinophils	Monocytes	Lymphocytes
	young	rod-shaped	segmented			
1 Control	0.0	1.5±0.22*	30.6±2.48*	4.2±0.49	3.4±0.5	58.3±2.03
2 Toxins +0.5%Ferbensorb	0.0	0.2±0.17x	27.7±1.61*	3.6±0.87	3.5±0.56	65.0±2.08
3 Toxins+1% Ferbensorb	0.0	0.5±0.22x	34.8±1.68*	2.7±0.42	4.5±0.4	57.52±2.14
4 Toxins	0.0	0.5±0.22x	13.7±1.26x	3.2±0.54	2.9±0.65	79.7±1.08x
The physiological norm	0	2.0 (1-4)	26.5(20-35)	1-5	1-5	55-75

Note: x 2,3,4 groups compared to the 1st group, p <0.001; *1,2,3 groups compared to the 4th group, p <0.001

The bactericidal activity of the blood serum of rats treated with toxins (group 4) compared with control animals (group 1) significantly decreased **(Table 6)**. The addition of a sorbent in an amount of 0.5% to the feed (group 2) did not lead to an increase in the level of bactericidal activity of blood serum, while an increase in the dose of the sorbent to 1% (group 3) activated bactericidal activity (p<0.001).

The lysozyme activity of rat blood serum under the action of toxins about the control decreased (group 4) and remained at the initial level in rats treated with sorbent (groups 2 and 3). Compared with group 4, there was a significant increase in this indicator in rats treated with sorbent in the amount of 1% to feed (group 3).

Table 6. Effect of the drug Ferbensorb on bactericidal activity and lysozyme activity of rat blood serum against the background of mycotoxicosis (M+m; n=21)

Indicator	Group			
	1	2	3	4
Bactericidal activity, %	57.03±0.38	47.63±1.76 x	62.58±0.96x*	49.36±1.61 x

Lysozyme activity, %	65.26±0.74*	61.28±0.43	64.25±0.63*	59.27±0.86 [x]

Note: [x] 2,3,4 groups compared to group 1, p<0.001, * 1,.3 groups compared to group 4, p<0.001

Thus, feeding rats with food contaminated with mushroom toxins leads to a deterioration in the general condition of animals, expressed by a decrease in appetite, increased water consumption, digestive disorders, an increase in body temperature by 0.3-0.6 ℃, redness of the conjunctiva, disheveled, stunted growth.

Morphological changes in fallen animals indicate damage to detoxification organs (liver), secretions (kidneys), digestion (intestines), immunity, lungs, and heart.

Ferrocyanide-bentonite sorbent Ferbensorb significantly reduced the functional and morphological manifestations of the pathological process caused by mycotoxins ochratoxin A, fumonisin B, and zearalenone. It increased body weight gain, indicators of natural immunological resistance, which was expressed by an increase in the percentage of neutrophils in the leukoformula, an increase in the level of bactericidal and lysozyme activity of blood serum, and animal survival.

Conclusion

Experiments on rats have established the possibility of using ferrocyanide-bentonite sorbent Ferbensorb as a means of reducing the transition of ^{137}Cs from the gastrointestinal tract to animal organs and tissues with an efficiency similar to that of the selective sorbent caesium-ferrocin.

When the ferrocyanide-bentonite sorbent Ferbensorb and manganese dioxide are administered in minimal doses, the isotope deposition in bone tissue is approximately the same. The effectiveness of manganese dioxide after using doses of 40 mg and 80 mg, respectively, is 2.5 and 1.3 times higher than with the same doses of ferrocyanide-bentonite sorbent FERBENSORB.

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