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STUDY OF THE TOXICITY OF THE DRUG MONIZEN® FORTE

Engasheva Ekaterina Sergeevna[1]*, Dorozhkin Vasily Ivanovich[1], Pavlenko Galina Ivanovna[1], Volkov Alexey Anatolievich[2], Nikanorova Anna Mikhailovna[3]

1. ***Laboratory of Pharmacology and Toxicology, All-Russian Scientific Research Institute Veterinary Sanitation, Hygiene, and Ecology-Branch of FGBNU FNC VIEW RAN, Moscow, Russian Federation.***
2. ***Department of Animal Diseases and Veterinary and Sanitary Expertise, Saratov State Agrarian University named after N.I. Vavilova, Saratov, Russian Federation.***
3. ***Department of Veterinary Medicine and Animal Physiology, Kaluga Branch of the Russian Agrarian University-Moscow Agricultural Academy named K.A. Timiryazeva, Kaluga, Russian Federation.***

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ABSTRACT

The article presents the results of studying the acute toxicity of the drug Monizen® forte with the intragastric and subcutaneous route of administration to white male mice. Determination of the parameters of acute toxicity of the drug MONIZEN® forte was carried out in the laboratory of pharmacology and toxicology and vivarium of VNIIVSGE-a branch of the FGBNU FNC VIEW RAN. Toxicological studies were carried out according to the «Guidelines for conducting preclinical studies of drugs. Part one» (2012).
The LD_{50} value of MONISEN® forte when administered parenterally (subcutaneously) to white nonlinear mice was 2524 ± 91.5 mg/kg and when administered orally (intragastrically) was 953.82 ± 156 mg/kg. According to the parameters of acute toxicity, established in mice, under the conditions of a single injection into the stomach, MONIZEN® forte, according to the generally accepted hygienic classification GOST 12.1.007-76, belongs to the 3rd hazard class of moderately toxic compounds. The administration of overestimated doses of the drug MONIZEN® forte white to nonlinear mice both parenterally and orally causes hepatotoxic and nephrotoxic effects.

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Introduction

In the Russian Federation and other countries, parasitosis of small cattle is quite widespread, among them nematodes, cestodes, trematodes, arachnoentomoses. The damage caused by parasitosis is due to the lack of meat and animal hair. For this purpose, LLC "AVZ S-P" (Russian Federation) has developed a complex antiparasitic drug MONIZEN® forte in the form of a solution for injection and oral administration. As active ingredients, 1 ml of the drug contains 5 mg ivermectin and 60 mg praziquantel [1, 2].
Praziquantel and ivermectin, which are part of MONISEN® forte, provide a wide range of antiparasitic action of the drug. MONISEN® forte is active against the nematodes, cestodes, trematodes, gadfly larvae, sarcoptoid, gamazoid, and ixodid ticks, bloodsuckers, and malophages (lice) bird-eaters) of mammals and birds[1].
Ivermectin is a synthetic derivative of avermectin produced by the actinomycete Streptomyces avermitilis and belongs to the group of macrocyclic lactones. The mechanism of action of ivermectin is its effect on the penetration of chloride ions through the membranes of nerve and muscle cells of ecto- and endoparasites. The main targets are glutamate-gated chloride channels and gamma-aminobutyric acid receptors. A change in the process of penetration of chloride ions disrupts the

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Corresponding Author: Engasheva Ekaterina Sergeevna; Laboratory of pharmacology and toxicology, All-Russian Scientific Research Institute veterinary sanitation, hygiene, and ecology-branch of FGBNU FNC VIEW RAN, Moscow, Russian Federation. E-mail: kengasheva@vetmag.ru.

conduction of impulses, which leads to paralysis and death of the parasite[2] [3, 4].
Ivermectin has insecticidal, acaricidal, and nematicidal activity. The toxicity of ivermectin to mammals is determined by its action on the GABA receptors of the central nervous system (CNS). At therapeutic concentrations of the drug, these receptors are inaccessible, however, an increase in the dose (>10 times) causes convulsions, tremors, and coma in animals, which is characteristic of substances that damage the CNS. The LD_{50} of ivermectin for laboratory animals ranged between 25-80 mg with different routes of administration [5-8].
Praziquantel has widely been used as an antiparasitic drug since 1980 use against various cestodes, trematodes, primarily schistosomes [9]. It rapidly causes tissue damage and paralytic muscle contraction of parasites, followed by their death, exit from the body, this is due to an increase in the permeability of the cell membrane to calcium ions, and with a secondary effect on metabolism and antigenicity[3] [9-11].
Oral LD_{50} in mice, rats, and rabbits is 2454, 2840, and 1050 mg/kg, respectively[4] [10]. In dose toxicity studies, no drug-related lesions were noted in rats receiving praziquantel up to 1000 mg/kg/day for four weeks and in beagle dogs up to 180 mg/kg/day for 13 weeks[5].

Study Purpose
The present study aimed to determine the parameters of the acute toxicity of the drug MONIZEN® forte when administered orally and parenterally to male mice.

Materials and Methods

Determination of the parameters of acute toxicity of the drug MONIZEN® forte was carried out in the laboratory of pharmacology and toxicology and vivarium of VNIIVSGE-a branch of the FGBNU FNC VIEW RAN.
The studies were carried out under the "Rules of laboratory practice in the Russian Federation" and methodological instructions "Guidelines for conducting preclinical studies of medicinal funds. Part one" [12, 13]. Experiments on animals were carried out following the rules adopted by the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes[6] [14, 15]. The studies were performed according to an approved written protocol and in accordance with the Investigator's Standard Operating Procedures (SOP).
The values of LD_{50} and other parameters of acute toxic effect were determined by probit analysis [16].
The hazard class of the drug was determined in accordance with GOST 12.1.007-76 [17].
In experiments to determine the parameters of acute toxicity of the drug, we used 152 clinically healthy 60-75 days-old male rats. Before the start of the experiment, the animals were kept in quarantine for 15 days; they were monitored daily. Each group of mice, when administered subcutaneously and intragastrically, consisted of 10 animals. Before the experiment, the mice were kept on a starvation diet for 4 hours.
When assessing subcutaneous toxicity, the drug was administered subcutaneously to mice at doses of 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, and 2700 mg/kg in dosage form using disposable syringes.
Control mice were injected once subcutaneously with water for injection in a maximum allowable volume of 0.2 ml. When assessing oral toxicity, the test drug was administered to the stomach of mice using a gastric tube at doses of 300, 500, 800, 1000, 1500, and 2000 mg/kg in the dosage form. Doses were calculated for 100% dosage form. Control mice were injected once intragastrically with water in a maximum allowable volume of 0.2 ml.
The general condition and behavior of the animals were observed for 14 days and the death rate, manifestation of intoxication symptoms, behavioral features, food, and water intake status, as well as the condition of the hair, mucous membranes, etc., were recorded.
Experimenced and control animals were weighed before drug administration, and 1, 3, 7, and 14 days after drug administration. The relative weight gain was determined in relation to the initial body weight (%). On the 14th day after administration of the preparation, the surviving mice were subjected to necropsy. At the same time, the mass coefficients of internal organs (heart, liver, kidneys, spleen) were determined. Determination of the mass coefficient of internal organs allows identify the target organ of a toxicant, to reveal signs of endocrine-related effects[7].
When processing data on the dynamics of body weight gain and coefficients of internal organs, statistical analysis of the data was carried out by the method of variation statistics using a simple comparison of the mean by the two-sided Student's t-test. Differences were determined at the 0.05 significance level.

Results and Discussion

The results of subcutaneous administration of the test drug MONISEN® forte to white nonlinear mice are shown in **Table 1**.

[2] haf-haf.am; www. mif-ua.com Actual infectious diseases №1 (10) -2016
[3] whqlibdoc.who.int
[4] whqlibdoc.who.int
[5] whqlibdoc.who.int
[6] www.mdpi.com
[7] doclinika.ru

Table 1. Death rate of nonlinear white mice after subcutaneous injection of MONISEN® forte

Drug dose (mg/kg)	The number of mice in the experiment	The number of mice killed after a single injection of the drug in various doses every other day								Total result
		1	2	3	4	5	6	7	14	
1800	10	0	0	0	0	0	0	0	0	0/10
1900	10	0	0	0	0	0	0	0	0	0/10
2000	10	1	0	1	0	0	0	0	0	2/10
2100	10	1	1	0	0	0	0	0	0	2/10
2200	10	1	1	0	0	0	0	0	0	2/10
2300	10	1	2	0	0	0	0	0	0	3/10
2400	10	2	1	0	0	0	0	0	0	3/10
2500	10	1	3	0	0	0	0	0	0	4/10
2600	10	4	1	1	0	0	0	0	0	6/10
2700	10	5	1	0	1	0	0	0	0	7/10
The control	10	0	0	0	0	0	0	0	0	0/10

As follows from the results in the table, the introduction of the test drug at a dose of 1800 and 1900 mg/kg did not lead to the death of the animals. All subsequent doses caused the death of the animals. The highest dose of 2700 mg / kg killed 70% of the mice in this group. In the control group of animals, mortality and signs of intoxication were not observed. Autopsy of the dead mice showed that: the liver was enlarged; the blood vessels of the liver were filled with blood; the spleen was enlarged and flabby; the lungs of mice were dark red, with a bluish tinge, light areas, and doughy consistency. The blood vessels were full of blood. The kidneys were enlarged, hyperemic, with punctate hemorrhages. The picture of poisoning and the dynamics of the development of intoxication symptoms depended on the administered dosage. Subcutaneous administration of MONISEN® forte to white nonlinear mice in the dose of 1800-2700 mg/kg led to a decrease in the dynamics of weight gain [18, 19].

At the same time, when the drug was administered in the dose of 1800-2100 mg/kg of body weight, although it led to a decrease in the dynamics of weight gain, nevertheless, on the 14th day after administration of the drug, these animals showed a positive average daily weight gain for 14 days and amounted to 108.19 ± 2.3; 108.15 ± 3.23; 97.39 ± 3.07, and 101.54 ± 3.08% versus the control value of 129.10 ± 4.55%. While a single subcutaneous injection of the drug MONISEN® forte, white nonlinear mice at doses of 2200, 2300, 2400, 2500, 2600, and 2700 mg/kg in the dosage form led to a decrease in the body weight of mice, both relative to the control and the initial values and amounted to 99.26 ± 0.89, 98.12 ± 1.35, 97.37 ± 2.67, 97.43 ± 2.5, 98.5 ± 2.12, and 94.54 ± 1.01%, respectively; versus the control value of 129.10 ± 4.55%[8]. This fact indicates that all administered doses had a general toxic effect on the animal organism. At the same time, a dose-dependent general toxic effect of MONISEN® forte on white nonlinear mice was noted, which is manifested in the intensity of the decrease in the dynamics of average daily weight gain, depending on the dose of the drug. The higher the dose, the more intense the reduction in weight gain. Along with this, should pay attention to the indices of the coefficients of the internal organs of white nonlinear mice on the 14th day after a single subcutaneous injection of MONISEN® forte[9].

So, a subcutaneous, single administration of the drug MONISEN® forte to white nonlinear mice in the dose range from 1800 to 2600 mg/kg body weight d not cause statistically significant changes in the coefficients of internal organs. Whereas a dose of 2700 mg/kg leads to a increase in the liver mass coefficient to 0.07 ± 0.002 compared to control animals (0.05 ± 0.0049)[10]. This fact indicates the hepatotoxic effect of lethal doses of MONISEN® forte. The results of intragastric introduction of the test drug MONISEN® forte to white nonlinear mice are shown in **Table 2**[11].

Table 2. Death rate of nonlinear white mice after intragastric introduction of MONISEN® forte

Drug dose (mg/kg)	The number of mice in the experiment	The number of mice killed after a single injection of the drug in various doses every other da								Total result
		1	2	3	4	5	6	7	14	
300	6	0	0	0	0	0	0	0	0	0/6
500	6	0	1	0	0	0	0	0	0	1/6
800	6	0	1	1	0	0	0	0	0	2/6
1000	6	0	4	0	0	0	0	0	0	4/6

[8] www.mdpi.com
[9] eur-lex. europa.eu
[10] eur-lex. europa.eu
[11] eur-lex. europa.eu

1500	6	0	4	1	0	0	0	0	0	5/6
2000	6	0	5	1	0	0	0	0	0	6/6
The control	6	0	0	0	0	0	0	0	0	0/6

As follows from the results in the table, the introduction of the test drug at a dose of 300 mg/kg did not lead to the death of the animals [19]. All subsequent doses caused the death of the animals. The highest dose of 2000 mg/kg resulted in the death of 100% of the mice in this group **(Table 2)** [19]. In the control group, mortality and signs of intoxication were not observed. Autopsy of the dead mice showed that: the liver was enlarged; the blood vessels of the liver were filled with blood; the spleen was enlarged and flabby; the lungs of mice were dark red, with a bluish tinge, light areas, and doughy consistency. The blood vessels were full of blood. The kidneys were enlarged, hyperemic, and punctate hemorrhages were noted. Intragastric administration of MONISEN® forte to white nonlinear mice at the dose range of 300-2000 mg/kg led to a statistically significant decrease in the dynamics of weight gain [19]. At the same time, when the drug was administered at the dose of 300-800 mg/kg, although it led to a significant decrease in the dynamics of weight gain, nevertheless, on the 14th day after the administration of the drug, these animals showed a positive average daily weight gain for 14 days and amounted to 107.8 ± 3.26%, 106.3 ± 2.19%, and 100.4 ± 8.06% versus the control value of 131.4 ± 10.05%. While a single intragastric administration of the drug MONIZEN forte, white nonlinear mice at a dose of 1500 mg/kg in the dosage form led to a decrease in the body weight of mice both relative to the control and initial values and amounted to 92.7 ± 9.03% versus control value 131.4 ± 10.05%[12] [19].

This fact indicates that all administered doses have a general toxic effect on the animal organism. There is a dose-dependent effect of the general toxic effect of MONISEN® forte on the body of nonlinear white mice, which is manifested in the intensity of the decrease in the dynamics of average daily weight gain depending on the dose of the drug. The higher the dose, the more intense the reduction in weight gain. An intragastric, single administration of MONISEN® forte to white nonlinear mice at a dose of 300 mg/kg does not cause statistically significant changes in the coefficients of internal organs. Whereas doses are 500, 800, and 1000 mg/kg leads to a significant increase in the liver mass coefficient up to 5.91 ± 0.28%, 6.03 ± 0.17%, and 6.32 ± 0.23%, respectively, relative to control animals 5.5 ± 0.25%[13]. This fact testifies to the hepatotoxic effect of lethal doses of MONISEN® forte, which must be taken into account when conducting clinical trials. At the same time, with a single intragastric administration of MONISEN® forte to white nonlinear mice, it leads to an increase in kidney mass coefficients at doses of 800 (0.71 ± 0.02%) and 1000 (0.72 ± 0.05%) mg/kg body by dosage form. Whereas, in control animals, this indicator was 0.68 ± 0.03. These changes indicated the nephrotoxic effect of lethal doses of MONISEN® forte. Analyzing the data obtained, with intragastric administration of MONISEN® forte to white nonlinear mice, the following can be stated-a dose of 300 mg/kg should be considered as a tolerable dose, a dose of 500-1500 mg/kg-as lethal, a dose of 2000 mg/kg according to the dosage form is absolutely lethal (leads to the death of 100% of animals). The calculated toxicological parameters of the drug MONISEN® forte for non-linear white mice are given in **Table 3**.

Table 3. Parameters of the acute toxic effect of the drug MONISEN® forte for nonlinear white mice

A drug	LD_{10} (мг/кг)	LD_{16} (мг/кг)	LD_{50} (мг/кг)	LD_{84} (мг/кг)	LD_{90} (мг/кг)
Subcutaneous administration					
MONISEN® forte male mice	2021± 101.7	2124± 83.5	2524± 91.5	2999± 232.74	3152± 287
Intragastric administration					
MONISEN® forte male mice	258±195	411±186	953,82±156	1498±364	1650±512

Conclusion

The LD_{50} value of MONISEN® forte, when administered parenterally (subcutaneously) to white nonlinear mice, was 2524 ± 91.5 mg/kg when administered orally (intragastrically) was 953.82 ± 156 mg/kg. According to the parameters of acute toxicity, established in mice, under the conditions of a single injection into the stomach, MONIZEN® forte, according to the generally accepted hygienic classification GOST 12.1.007-76, belongs to the 3rd hazard class of moderately toxic compounds. The administration of overestimated doses of the drug MONIZEN® forte whight o nonlinear mice both parenterally and orally causes hepatotoxic and nephrotoxic effects.

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Conflict of interest: None

[12] www.mdpi.com
[13]eur-lex. europa.eu

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Ethics statement: The study was carried out following the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

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