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Study of the toxicity and effectiveness of an antiparasitic agent based on tinidazole and fenbendazole

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The development of new import-substituting highly effective and environmentally friendly complex antiparasitic drugs and their implementation in a scientifically grounded system of measures to combat the causative agents of the main invasive diseases of farm animals and poultry remains an urgent task of veterinary medicine. Based on the results of studies of anthelmintic and antiprotozoal action of agents on exogenous forms of parasites, an experimental form of a complex antiparasitic agent "Tinidafen" was created and investigated. An experimental sample in the form of a powder was created on the basis of two active substances – tinidazole and fenbendazole with the addition of excipients (talc, starch). It has been established that the LD_{50} of "Tinidafen" for white rats with a single dose is 2564.36±349.13 mg/kg. The therapeutic dose of "Tinidafen" for rabbits is 7.5 g per 10 kg of live body weight. The extensefectevity of "Tinidafen" is 99.5% for helminths and 98.7% for eimeria. After administered orally of the agent, helminth eggs in the feces of infested animals were not registered from day 3, and the release of eimeria oocysts was observed until day 5 of the experiment.

Key words: mixinvasions, tinidazole, fenbendazole, "Tinidafen", toxicity, helminth eggs, eimeria oocysts.

Introduction

The development of the livestock industry and the receipt of high-quality and safe products is the main task of veterinary specialists in many countries of the world. Only a scientifically grounded complex of veterinary, sanitary and technological measures ensures the conduct of animal husbandry at a high level (Tomley & Shirley, 2009; Shkromada et al., 2019a; Palii et al., 2020a). In turn, infectious (Stegniy et al., 2019; Kolchyk et al., 2020; Palii et al., 2020b) and parasitic (Majeed et al., 2015; Paliy et al., 2018b; Liu et al., 2020) animal diseases are a significant limiting factor in the development of many livestock industries. Invasive diseases cause tremendous harm to the animal's organism because parasites destroy organs and tissues, poison the whole body with their metabolic products, which cause hypovitaminosis, and weaken immunity, creating conditions for the occurrence of other diseases (Britton et al., 2011). Invasive diseases pose a great danger for young animals and birds, since they often have myxinvasions (Barber & Dingemanse, 2010; Paliy et al., 2020c; Bogach et al., 2020).

The large-scale spread of pathogens of parasitic diseases depends on a number of factors, among which the main ones are environmental, biological and anthropogenic (Kołodziej-Sobocińska, 2019). The role of insects in the spread of helminth eggs has been proven (Paliy et al., 2018d), but some of them are predators of animal ectoparasites (Paliy et al., 2020a).

Exogenous forms of helminths are found both at animal husbandry facilities (Majeed et al., 2015; Paliy et al., 2018c) and at many objects of social-epidemic control (Stojanov et al., 2018; Paliy et al., 2019). Disinfection of contaminated objects through the use of highly effective disinfectants remains the basis for the prevention of both infectious and invasive diseases (Maertens et al., 2020; Paliy et al., 2016; 2020d). The scientifically based use of disinfectants makes it possible to control the epizootic situation for many animal diseases (Howard et al., 2015; Paliy et al., 2018a; 2020b).

An important problem in veterinary medicine, as before, remains the treatment of parasitic diseases of farm animals. Science and practice have accumulated significant experience in the use of various antiparasitic agents in animal husbandry (Bustnes et al., 2006). They belong to different classes of compounds and, as a rule, are effective against a narrow range of parasites. Most of them are toxic to the organism of animals and are ecologically faulty (Katz, 1982).
The prospects for the discovery of new antiparasitic drugs for veterinary medicine are determined by economic, social and scientific factors. Consolidation in the pharmaceutical industry in general and the animal health industry in particular, changes the business conditions in which drug discovery for veterinary medicine occurs. Social pressures on traditional animal agriculture and companion animal ownership have shifted the interest of animal companies primarily to pet medicine. Antiparasitic drug discovery is more than ever targeted to the most lucrative market segments, but the excellence of available drugs, and the apparent lack of resistance in important parasites, reduces industrial motivation to invest in parasitology (Geary & Thompson, 2003).
So the creation of complex products is a promising direction in veterinary pharmacy.

Materials and methods

Research in this direction was carried out in the laboratory of veterinary sanitation and parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv). At the first stage of the research, the effectiveness of modern veterinary antiparasitic drugs used in animal husbandry and poultry farming in Ukraine was determined. For the experiments, agents with the following active substances (AS) were selected, which, according to the instructions, had an anthelmintic and eimericidal effect: niclosamide, oxybendazole, fenbendazole, albendazole, levamisole, metronidazole, tinidazole. The study of the antiparasitic effect on exogenous forms of parasites was carried out on experimental biological models: *Passalurus ambiguous* eggs and cysts of *Eimeria perforans*, *E. magna* and *E. stiedae*. Eggs of helminths and eimeria oocysts were isolated by flotation from the feces of spontaneously affected animals (Collender et al., 2015; Pouillevet et al., 2017).
One part (50%) of eggs and oocysts was infested with clinically healthy rabbits of 30 days of age (n=21). After the appearance of the first clinical signs of the disease and the detection of exogenous forms of parasites in animal feces, rabbits were divided into 7 research groups, 3 animals each. Each research group of rabbits received according to the instructions one of the indicated antiparasitic drugs. The drugs were used in the form of powders (Kotsiumbas et al., 2006). The criterion for evaluating the action of the drugs was the disappearance of clinical signs of the disease and the absence of exogenous forms of parasites in the feces of rabbits within 28 days after the administration of the drugs (Tucker et al., 2007; Seyoum et al., 2017).
At the second stage, the studies were carried out *in vitro*. At the same time, fenbendazole and tinidazole solutions were added to the culture of eggs of helminths and oocysts of eimeria, which are in the stage of cleavage, which gave the best results in the first experiment. The criterion for evaluating the effect of the drugs was the death of eggs and oocysts within 28 days after the addition of drugs (Lin et al., 2020).
At the third stage of research, an innovative antiparasitic agent was created and its effectiveness was determined on spontaneously infested animals (Partridge et al., 2020). After obtaining positive results, the formulation of the innovative antiparasitic agent "Tinidafen" was designed and its properties and effectiveness were studied. The criterion for assessing the effectiveness of the agent was the presence and number of eimeria oocysts and helminth eggs in fecal samples from research animals. Acute toxicity of the Tinidafen formulation after oral administration was determined on 48 sexually mature nonlinear male rats weighing 180±25 g (Varkholiak & Gutyj, 2018). To conduct the experiment on the principle of analogs, seven research and one control groups were formed, 6 rats each. The dose of "Tinidafen" was calculated individually, based on body weight values, while the total volume of "Tinidafen" administered orally did not exceed 2.5 cm^3. The range of doses studied ranged from 500 mg/kg to 3500 mg/kg of the body weight of a laboratory animal. The drug was administered to rats of the experimental groups using a metal probe in doses (according to AS):
Group I – 500 mg/kg, II – 1000 mg/kg, III – 1500 mg/kg, IV – 2000 mg/kg, V – 2500 mg/kg, VI – 3000 mg/kg, VII – 3500 mg/kg mass body respectively. Animals of the control group were injected with purified water in the amount of 2.5 cm^3 under similar conditions. The experiment lasted 14 days. The degree of acute toxicity of Tinidafen was assessed by changes in the general condition of the animals, taking into account the characteristics of behavior, reactions to external stimuli, the condition of the skin and mucous membranes, the consumption of food and water, the functioning of organs and systems, the timing of the development of intoxication and the death of animals was recorded (Saganuwan, 2017).
Laboratory animals, which were used for toxicological experiments, were kept under optimal vivarium conditions: room temperature was 18±5°C, relative air humidity was 65-75%; during the experiment, a “day-night” illumination cycle was provided, as well as uninterrupted air circulation. For feeding the animals, we used complete feed, which, in terms of quality and safety, corresponded to the current regulatory documents (Carter & Lipman, 2018).
In order to determine the effectiveness of the developed tool, experiments were carried out on clinically healthy male rabbits at the age of 3.5 months, which were experimentally infected with *Passalurus ambiguous* eggs and *Eimeria stiedae* cysts. To conduct research on the principle of analogs, three experimental and one control groups of animals were formed. The animals of the first experimental group (n=3) were given a single dose of "Tinidafen" at a dose of 10 g per 10 kg of body weight, the second group (n=3) – 7.5 g per 10 kg of body weight, and the third group of rabbits (n=3) received – 5 g per 10 kg of body weight. Animals of all research groups received the drug with food by the group method. Rabbits of the control group (n=3) were fed complete feed without the drug. Starting from the third day after the start of the experiment, as well as on the 7^{th}, 14^{th}, 21^{st} and 30^{th} days, fecal samples were taken for studies using standardized methods (Taglioretti et al., 2014; Abu-Madi et al., 2017; Stojanov et al., 2018) for the presence of helminth eggs and simple cystic forms. The effectiveness against parasitic treatment was calculated by the formula (1):

$$E_o = \frac{O_1 - O_2}{O_1} \times 100$$

where, E_o – processing efficiency in %;
O_1 – number (eggs or cysts) before processing;
O_2 – the number (eggs or cysts) after processing;
100 – coefficient of expression of the result in percent.
Before the start of the experiment, on the 3rd, 7th, 14th and 30th days of the experiment, blood samples were taken from rabbits. In order to study the effect of the antiparasitic agent on the hematological parameters of the blood of animals, the number of erythrocytes and the concentration of total hemoglobin, the total number of leukocytes, were determined, and the leukogram was calculated (Özkan et al., 2012; Ochiai et al., 2018). The number of leukocytes was counted in a counting chamber on a Goryaev grid. Differential counting of leukocytes was carried out by microscopic evaluation of dry blood smears fixed with methyl alcohol and stained with Romanovsky-Giemsa dye. In blood smears, the percentage of erythrocytes with signs of toxic granularity was determined. The leukocyte intoxication index (LII) was determined (Radsikhovskii et al., 2018) using the formula (2):

$$LII = (4M+3Yu+2P+S) \times (Pl.+1) / (L+Mo) \times (E+1)$$

where, LII – is the leukocyte index of intoxication; M – Myelocytes; Yu – young leukocytes; P – stab leukocytes; S – Segmented leukocytes; Pl. – plasma cells; Mo – monocytes; L – Lymphocytes; E – Eosinophils.

The biochemical parameters of blood, reflecting the functional state of the liver, were studied. Namely: the dynamics of the content of total protein and its fractions (albumin and globulins) and the activity of enzymes (AST and ALT), were investigated according to generally accepted methods (Ahamefule et al., 2006; Melillo, 2007; Nakyinsige et al., 2013).
Experiments on animals were performed in compliance with modern bioethical requirements (Festing & Wilkinson, 2007; Kabene & Baadel, 2019). Statistical processing of the results obtained was carried out in accordance with the recommendations for biometrics using the Microsoft Excel for Windows XP computer program.

Results and discussion

When screening modern veterinary commercial anthelmintic (powder and tableted) drugs, it was found that in 27% of drugs the active substances (AS) is albendazole and its derivatives, in 12.3% – fenbendazole, in 41.5% – praziquantel, and 19.2% contains several active substances. The criterion for evaluating the effectiveness of the antiparasitic action was the presence of anthelmintic and eimericidal action on eggs of *Passalurus ambiguous* helminths and oocysts of *Eimeria perforans*, *E. magna*, *E. stiedae*. Anhelminthic and eimericidal properties in antiprotozoal agents have also been tested (Table 1).

Table 1. Antiparasitic action of substances on exogenous forms of rabbit parasites (n=3)

Active substances	Eggs of helminths and oocysts of *Eimeria*			
	Passalurus ambiguous	*Eimeria perforans*	*Eimeria magna*	*Eimeria stiedae*
niclosamide	+	–	–	–
oxybendazole	+	–	–	–
fenbendazole	+	+	+	–
albendazole	+	+	–	–
levamisole	+	–	–	–
tinidazole	–	+	+	+
metronidazole	–	–	–	–

Note: "+" – there is an antiparasitic effect; "–" – there is no antiparasitic effect.

According to the results obtained (Table 1), it was found that albendazole and fenbendazole have the same anthelmintic effect, but the eimericidal effect is stronger for fenbendazole. The culture of *Eimeria stiedae* proved to be resistant to the action of both of these anthelmintics. Along with this, anthelmintics niclosamide, oxybendazole, levomizole did not show eimericidal action. So in our experiments, fenbendazole, albendazole and tinidazole turned out to be the most effective.
The extensibility (EE) of fenbendazole, albendazole and tinidazole was tested on *Passalurus ambiguous* and *Eimeria intestisnalis* egg cultures (Table 2).

Table 2. Extensibility of antiparasitic agents for the culture of eggs of helminths and oocysts of eimeria (n=3)

Active substances	Death of eggs of helminths and oocysts of eimeria, %	
	eggs of helminths *P. ambiguous*	oocysts of *E. intestisnalis*
fenbendazole	98.7	78.2
albendazole	83.3	–
tinidazole	–	96.8

Note: "–" – there is no antiparasitic effect.

As can be seen from the results of Table 2, the eimericidal effect of tinidazole on the oocysts of eimeria of rabbits (*E. intestinalis*) is 96.8%; eimericidal action of fenbendazole – 78.2%, albendazole has no eimericidal action on *E. intestinalis*. The anthelmintic

effect of fenbendazole on rabbit nematodes (*Passalurus ambiquus*) was 98.7%, and that of albendazole was 83.3%; tinidazole did not have it. The experimental agent included two active substances – tinidazole and fenbendazole; starch and talc were chosen as auxiliary substances (excipient).

Fenbendazole (chemical name methy l-5 (phenyl-thio)-benzimidazole-2-carbamate) is a benzimidazole compound that has a wide range of nematicidal action. The mechanism of action is associated with a violation of energy metabolism, destruction of microtubules and the development of a neurotoxic effect in helminths. Spectrum of action of fenbendazole for nematodes of the gastrointestinal tract: rabbits (*Passalurus ambiguus, Trichocephalus leporis, Trichostrongylus* spp.), poultry *(Ascaridia galli, Heterakis gallinarum, Capillaria anseris*), waterfowl tissostomis (*Amidostomum anseris, Tetrameres fissispina, Streptocara crassicauda, Hystrichis tricolor, Echinuria uncinata, Ganguleterakis dispar, Hymenolepis gracilis*). When administered orally, fenbendazole is readily absorbed in the intestines and distributed in the organs and tissues of the animal; excreted from the body unchanged and in the form of metabolites, mainly with bile and partly with urine, in lactating animals also with milk. According to the degree of exposure to the body, it belongs to moderately hazardous substances (hazard class 3 according to GOST 12.1.007-76) in recommended doses it is well tolerated by animals (Ssenyonga, 1982; Gupta et al., 2020).

Tinidazole (chemical name 1H-imidazole, 1-(2-(methylsulfonyl)ethyl)-2-methyl-5-nitro) is a synthetic drug of the imidazole group that exhibits antiprotozoal activity. It causes an intracellular reduction of the specific metabolism of anaerobic microorganisms. Influences the spiral structure of DNA, interrupts the synthesis of nucleic acids, and causes cell death. The spectrum of action of tinidazole extends to protozoa (*Balantidium coli, Entamoeba hyslolytica, Treponema hyodysenterie, Histomonas meleagridis, Lamblia intestinalis, Eimeria* spp*., Trichomonas columbae, T. gallinae*), anaerobes (*Bacteroides* spp*., Fusobacterium* spp*., Clostridium* spp.) and most gram-positive gram-negative cocci (Sawyer et al., 1976; Livengood et al., 2007).

The innovative product "Tinidafen" was prepared by simple mixing of the constituent components: fenbendazole – 240-260 mg, tinidazole – 190-210 mg, talc – 240-260 mg, starch – 190-210 mg.

The results of the action of the "Tinidafen" agent on exogenous forms of rabbit parasites are shown in Table 3.

Table 3. Anthelmintic and eimericidal efficacy of the "Tinidafen" agent on exogenous forms of rabbit parasites (n=3)

Pathogens	Efficiency, % Day				
	1	2	3	4	5
Passalurus ambiguous	96.3	98.7	99.5	–	–
Eimeria perforans	63.5	86.6	98.6	–	–
Eimeria magna	66.5	91.3	94.8	98.8	–
Eimeria stiedae	88.9	98.7	–	–	–

Note: "–" – no eggs (oocysts) in feces.

From the materials presented in Table 3, it can be seen that the EE of "Tinidafen" for helminths in rabbits was 99.5%, and for eimeria – 98.7%. Helminth eggs in feces were not detected from the 3rd day of the experiment, and eimeria oocysts were secreted up to 5 days. Eggs of helminths and oocysts of eimeria in feces were absent in all experimental animals until 28 days of observation.

At the beginning of the experiment, the animals showed a lack of appetite, depression, bloating, and liquid fecal matter. After receiving the drug "Tinidafen" for two days, the rabbits completely ate the food, had a lively appearance, fecal masses were formed. In the control group, one animal perished on the fourth and sixth days.

One of the obligatory stages of approbation and introduction into production of new drugs is the study of their toxicity in laboratory animals (Bondarchuk et al., 2019; Kovalenko et al., 2020; Orobchenko et al., 2020).

The results of calculating the median lethal dose of "Tinidafen" for white rats under the conditions of oral administration are shown in Table 4.

Table 4. The results of calculating the lethal doses of the "Tinidafen" agent under the conditions of a single oral administration to white rats

№ experiment (group)	Drug dose, mg/kg (X)	Esearch results (Number of animals)		The size of the effect in probit (Y)	Probit weighting factor (Z)
		with the revealed effect	total in the group		
control	0	0	6	3.27	1.6
1	500	0	6	3.27	1.6
2	1000	0	6	3.27	1.6
3	1500	1	6	4.03	3.5
4	2000	3	6	5	5
5	2500	3	6	5	5
6	3000	4	6	5.43	4.6
7	3500	4	6	5.43	4.6

When calculating according to the data given in Table 4, it was found that LD_{16} = 1212.16; LD_{50} = 2564.36; LD_{84} = 3916.56; LD_{100} = 4592.66; S_LD_{50} = 349.13. So, according to GOST 12.1.007-76 "Harmful substances. Classification and General Safety

Requirements"according to the degree of toxicity, the preparative form of "Tinidafen" for white rats can be classified as moderately toxic substances (hazard class 3).

The next stage of our research was to determine the therapeutic efficacy of the anthelmintic and eimericidal action of "Tinidafen" in various doses (Table 5).

Table 5. The effectiveness of the action of various doses of "Tinidafen" in case of parasitic infection of rabbits (n=3, M±m)

Dose, per 10 kg of body weight	Pathogens	The number of helminth eggs (eimerium oocysts, thousand) in 1 g of feces		
		3 day	7 day	14 day
5.0 g	*P. ambiguous*	27.0±5.4	2.0±0.4	–
	E. stiedae	1.04±0.145	1.01±0.125	1.01±0.125
7.5 g	*P. ambiguous*	3.0±0.6	–	–
	E. stiedae	0.04±0.145	–	–
10.0 g	*P. ambiguous*	3.0±0.6	–	–
	E. stiedae	0.04±0.145	–	–
control	*P. ambiguous*	31.0±5.4	33.0±5.4	46.0±5.4
	E. stiedae	1.04±0.145	1.14±0.125	1.24±0.145

Note: "–" – no eggs (oocysts) in feces.

As can be seen from Table 5, after application of "Tinidafen" at a dose of 5.0 g per 10 kg of body weight, *Passalurus ambiguous* eggs in feces were detected up to 7 days, and eimerian oocysts of *E. stiedae* up to 14 days. When applying a dose of 7.5 g and 10.0 g, eggs of helminths and oocysts of Eimeria were detected on day 3 in the same amount.

The rabbits of the control group were fed complete feed without any means. *Passalurus ambiguous* eggs and eimerian oocysts of *E. stiedae* in feces were detected throughout the study, their number increased. The animals showed a lack of appetite, depression, bloating, and liquid fecal matter. During the experiment, three animals died, which is characteristic of the associated invasion. Hematological parameters of blood in animals after the use of "Tinidafen" in various doses are presented in Table 6.

Table 6. Hematological blood parameters in rabbits under the influence of the drug "Tinidafen"

Blood parameters	Dose drug			
	infested rabbits	5.0 g on 10 kg of body weight	7.5 g on 10 kg of body weight	10.0 g on 10 kg of body weight
Hemoglobin, g/l	70.2±1.3	100.0±0.2	109.0±0.2	109.0±0.4
Erythrocytes	3.2±0.5	3.9±0.8	5.2±0.8	5.5±0.8
Leukocytes	12.5±0.7	9.6±0.7	8.7±0.4	9.7±0.4
Young leukocytes	3.0	3.0	3.0	3.0
Stab leukocytes	3.2	3.2	3.2	3.2
Segmented leukocytes	3.0	3.0	3.0	3.0
Plasmacytes	1	0	0	0
Myelocytes	3.0	3.0	3.0	3.0
Eosinophils	6	2.0	2.0	1
Monocytes	12	10	10	10
Lymphocytes	18	40	45	65

The percentage of erythrocytes with signs of toxic granularity in animals receiving the drug was 6-12%, and in the control group it was 27%. Leukocyte intoxication index (LII) was: in the control group – 0.38, which indicates an average severity of the disease; in experimental groups – 0.2 and 0.18, and it decreased, which indicates the therapeutic efficacy of the drug, but when the dose was increased to 10 g, it began to grow.

Biochemical blood parameters reflecting the functional state of the liver, namely: the dynamics of the total protein and its fractions (albumin and globulins), the level of enzyme activity (AST and ALT) after the use of "Tinidafen" at a dose of 7.5 g per 10 kg of body weight are given in Table 7.

From 3 days of observation after the administration of the drug "Tinidafen" in the proteinogram, a slight increase in total protein was observed, which reached the physiological norm only on the 30 day. ALT values decreased to 0.3 mmol/l. hour, but were slightly higher than the physiological norm, which suggests that the changes found in the blood are the result of the irritating effect of metabolites of dying parasites on liver tissue.

Based on the results of the research, an experimental formula of the antiparasitic agent "Tinidafen" was created to destroy the association of helminths and protozoa in animals.

The strategy for combating parasitic diseases of animals provides for a set of measures implemented through the use of disinfecting, antiparasitic and immunostimulating drugs (Schmid-Hempel, 2009; Shkromada et al., 2019b).

Table 7. Biochemical parameters of blood when exposed to "Tinidafen" at a dose of 7.5 g per 10 kg of weight (M±m)

Total protein, g/l	Albumin, g/l	Globulins, g/l	A/G	AST, mmol/l. hour	ALT, mmol/l. hour
Indicators before the start of the experiment					
47.4±1.1	27.4±2.99	20.0±3.0	0.74±0.18	0.42±0.08	1.86±0.13
3 day of the experiment					
48.0±1.1	27.3±2.1	20.7±3.4	0.76±0.11	0.33±0.47	1.53±0.03
5 day of the experiment					
49.0±1.05	34.0±3.0	15.0±2.1	0.44±0.08	0.35±0.03	1.53±0.19
7 day of the experiment					
50.4±3.36	38.4±2.99	12.0±3.0	0.31±0.18	0.42±0.08	1.86±0.13
14 day of the experiment					
52.8±1.1	36.7±3.08	16.10±2.1	0.43±0.08	0.6±0.8	0.3±0.8
30 day of the experiment					
54.8±1.1	36.2±2.1	18.60±2.1	0.51±0.08	0.6±0.8	0.3±0.8
physiological norm indicators					
54-75	27-46	27-29	–	0.5-0.31	0.25-0.60

Conclusions

In the course of laboratory studies, it was found that not all antiparasitic drugs have anthelmintic and eimericidal effects. In *in vitro* experiments, the eimericidal effect of tinidazole on coccidia of rabbits (*E. Intestinalis*) was 96.8%. The eimericidal effect of fenbendazole was 78.2%, and the anthelmintic effect of fenbendazole on rabbit nematodes (*Passalurus ambiquus*) was 98.7%. The most effective for the destruction of the association of helminths and protozoa in rabbits is a complex remedy consisting of fenbendazole and tinidazole.

An innovative antiparasitic agent "Tinidafen" has been developed, which includes: fenbendazole – 240-260 mg, tinidazole – 190-210 mg, talc – 240-260 mg, starch – 190-210 mg.

The extensefectity of "Tinidafen" is 99.5% for helminths, and 98.7% for eimeria. After per os of the agent to spontaneously infested laboratory animals, helminth eggs in feces were not detected from 3 days, and the release of eimeria oocysts was observed until 5 days of the experiment. The therapeutic dose of "Tinidafen" for rabbits was determined, which is 7.5 g per 10 kg of live body weight.

It was found that the LD_{50} of the "Tinidafen" agent for single administration of white rats is 2564.36±349.13 mg/kg, LD_{16} – 1212.16 mg/kg, LD_{84} – 3916.56 mg/kg, LD_{100} – 4592.66 mg/kg of body weight, respectively. The "Tinidafen" agent can be classified as a moderately toxic substance – the 3rd hazard class. The results obtained make it possible to expand the range of fatherland antiparasitic agents.

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