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ACUTE AND SUB-ACUTE TOXICITY ASSESSMENT OF 'RYBOZURIL' ON COMMON CARP

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Summary. The goal of the work was to study of acute and sub-acute toxicity parameters of 'Rybozuril' biological product (AI — diclazuril) on the model of carp. This drug is effective in the treatment of diseases caused by parasitic Eimeriidae. Carp scales of two years old were used in experiments. To determine acute toxicity, the fish were prescribed with diclazuril in doses of 1 g/kg, 5, 10, and 15 g/kg of live weight. Two experimental and control fish groups of 30 individuals each were formed to determine sub-acute toxicity of 'Rybozuril'. Experimental groups of fish were prescribed with 'Rybozuril' in a dose (by AI) of 50 and 10 mg/kg for two consecutive days. Blood samples were collected from six fish from each group for clinical and biochemical indicators after 2, 7, 14, 21, and 28 days. The hemoglobin content, number of red blood cells and leukocytes blood was determined. The intensity of peroxide oxidation of lipids (POL), catalase activity, level of total antioxidant capacity (TAC), total proteins, albumin, globulins and glucose, circulating immune complexes (CIC) and seromucoids concentration, level of enzymatic activity: aspartate transaminase (AST), alanine transaminase (ALT), α-amylase blood plasma were determined.

The acute toxicity of diclazuril for carp was estimated, LD_{50} is more than 15,000 mg/kg of live weight, the toxicity of diclazuril can be classified as undifferentiated and, in terms of toxicity, it can be classified as hazard class IV. Two administrations of the drug 'Rybozuril' in a daily dose of 50.0 mg/kg of live weight, the maximum expression of metabolic changes in fish was detected from the initial terms of the studies and up to day 21. According to the results the toxic effect of the drug in fish was estimated, which did not influence to a number of indicators. The drug in such dose was shown immunosuppression and membrane-toxic effects in fish. Two-time administration of the 'Rybozuril' drug in a daily dose of 10.0 mg/kg body weight leads to metabolic alterations in fish due to the activation of detoxification processes and lipoperoxidation maintenance in cell membranes at the physiological level. At the end of the experiment the toxic effect of 'Rybozuril' in fish characterized by stable parameters in comparing to the control group.

Keywords: diclazuril, fish, lethal dose, acute toxicity, submerged toxicity, blood, clinical and biochemical indicators

Introduction. Diclazuril ($C_{17}H_9Cl_3N_4O_2$) is a benzeneacetonitrile derivative. Up to now the mechanism of action has not been well studied. It effectively prevents early developmental stages of the Protozoa. Diclazuril is intended for use in the control of eimeriosis in poultry and mammalian livestock. According to the previous studies, diclazuril is a low toxic agent. Thus, oral or subcutaneous administration of diclazuril in a dose of 5,000 mg/kg does not cause death in mice and rats. The administration of diclazuril in rats at a dose of 80 mg/kg of weight for three months did not lead to significant changes in morphological and biochemical blood parameters (Conway et al., 2001; Verheyen et al., 1988; FEEDAP, 2014, 2015; EMEA, 1996; Tokarev et al., 2012; Selifanova and Birukova, 2012).

Diclazuril is highly effective against fish diseases caused by parasitic protozoa from the family Eimeriidae of Apicomplexa (Molnár and Ostoros, 2007). The easiest families of Eimeriidae are widespread among fish inland waters of Ukraine. In the conditions of natural hydroecosystems *Goussia cyprinorum*, *G. castravetsi*, *G. metschnikowi*, *G. alburni*, *G. carpelli*, *G. leucisci*, *G. syngnathi*, *Eimeria scardini*, *E. rutili*, and *E. radae* are registered. For industrial species of pond farms of particular importance are the species *Goussia carpelli*, *G. subepithelialis*, *G. sinensis*, *G. cheni*. Tilapia is infected by *Goussia cochlidarum* in the closed water supply systems (Maltsev, 2012; Trombitskiy and Bushuev, 2012).

The 'Rybozuril' drug (contains 10% of the active substance diclazuril), according to the results of previous studies, proved to be highly effective in controlling fish diseases caused by Eimeriidae protozoa families. Therefore, determination of the impact of this drug is an important step for further studies.

The aim of the study was to determine the parameters of acute (lethal) and sub-acute toxicity of the drug 'Rybozuril' using common carp (*Cyprinus carpio*) as a model.

Materials and methods. A study on the acute toxicity of diclazuril was carried out as described (Kotsiumbas et al., 2006) modified for carp. Two-year-old carp was used for the experiments.

A preliminary study was conducted to determine the approximate average lethal dose. The drug was administrated individually through a catheter in a dose of 1, 5, 10, and 15 g/kg of body weight based on 1% starch paste.

The effect of the 'Rybozuril' drug on the biochemical and clinical parameters of fish blood was determined in the dynamics of the sub-acute toxicological experiment. The experiment was conducted using a two-year-old carp. Two experimental and one control groups of 30 fishes each were formed. Fish of each group were kept in separate aquariums with capacity of 0.2 m^3 with artificial aeration and temperatures of 18–22 °C. In the first and second experimental groups, fish for two consecutive days prescribed the 'Rybozuril' in a daily dose of 50 and 10 mg/kg body weight (for AI) respectively. The drug was prescribed to fish individually using a catheter based on 1% starch paste. In the control group, a starchy paste was added without the preparation (Demidov and Berezkina, 1986).

For the purpose of clinical and biochemical studies blood sampling was carried out from six fish from each group on 2^{nd}, 7^{th}, 14^{th}, 21^{st}, and 28^{th} days. Blood samples was collected by a paste-like pipette from the tail artery according to the generally method. Obtained blood samples were stabilized with heparin, to determine the biochemical parameters of blood serum.

Fish manipulation was carried out in accordance with existing regulations governing the organization of work using experimental animals and observance of the principles from European convention for the protection of vertebrate animals used for experimental and other scientific purposes (CEC, 1986).

The intensity of peroxide oxidation processes in blood plasma was determined by the level of production of its products: primary conjugated dienes (CD) and malonic dialdehyde (MDA) under extraction conditions in a mixture of heptane isopropanol (1:1) as previously described (Gavrilov and Mishkorudnaya, 1983); at wavelengths of 233 and 247 nm; the values of CD expressed as μmol/dm^3, and MDA at specific absorption rate in 1.0 cm^3 ($\Delta D/cm^3$).

The catalase activity in blood plasma was determined, as described (Korolyuk et al., 1988), using H_2O_2 with incubation medium (0.04412 N solution of H_2O_2, 0.01 N solution of KH_2PO_4, 0.1 M Tris-HCl buffer, pH 7.4, 4.5% solution of ammonium molybdenum acid); at a temperature of 37 ± 1 °C; at a wavelength of 410 nm; expressed as μmol H_2O_2/dm^3 in 1 min.

The measurement of antioxidant activity (TOL) in blood plasma was carried out in accordance with (Klebanov et al., 1988), by the total ability of structural antioxidants to inhibit the accumulation of TBA-active products induced in a medium of 25 mM $FeSO_4$ in 0.002 N HCl; at a wavelength of 535 nm; expressed as % of the formation inhibition of TBA-active products.

The level of hematological parameters and the content of total proteins, albumin, glucose and the level of enzymatic activity: aspartate aminotransferases (AST), alanine aminotransferases (ALT), α-amylase in blood plasma were determined by generally accepted methods and using Cormay (Poland) reagent kits as described in the manual (Vlizlo, 2012). The concentration of circulating immune complexes (CIC) of the average molecular weight was determined, as described (Kondrakhin et al., 1985), by precipitation of the protein complexes antigen-antibody PEG-6000; serum cords as described (Men'shikov, 1987); at wavelength of 260 and 280 nm; expressed as mg/cm^3. Registration of biochemical parameters was carried out using a spectrophotometer Shimadzu UV-1800 (Japan).

Statistical analysis of the obtained results was carried out in accordance with the recommendations on biometrics using the Microsoft Excel for Windows XP application package.

Results and discussion. At the determining of the acute lethal dose for pond fish based on the previous experiments for estimation of limit lethal dose of diclazuril we did not observe fish death during 21 days. The administration of diclazuril in a dose of more than 15 g/kg body weight was not feasible, considering that the maximum concentration of the drug that can be created in the starch gel is 25% which is physiologically impossible to administrate starch paste in a volume of more than 60 ml/kg of weight.

Due to the absence of fish death throughout the experiment, the median lethal dose LD_{50} could not determine. At the 21^{st} day after treatment, the fishes were euthanized with the further necropsy, which showed no special changes in the internal organs.

Consequently, due to the absence of fish death, LD_{50} was not able to estimate when the maximum dose of diclazuril, but according to the results of the study, it is greater than 15 g/kg weight. In accordance with the classification proposed by Medved', Kagan and Spynu (1968) and adopted by the WHO, the toxicity of the test drug can be classified as non-expressed, and regarding the state standard (GOST 12.1.007-76) diclazuril can be classified as toxicity class IV.

Clinical and biochemical parameters of blood after administering a therapeutic dose and five times higher therapeutic dose were analyzed to determine the effect of the drug 'Rybozuril' on fish. During the experiments behavioral changes of fishes in the experimental and control groups were not observed.

Table 1 shows the dynamics of hematological parameters in each group.

The data present in Table 1 indicates no statistical changes in the hematological parameters for both doses.

Table 2 shows the results of determining the basic biochemical parameters of blood and markers of congenital immunity of fish during 'Rybozuril' exposure.

Table 1 — Hematological parameters in carp after two administrations of 'Rybozuril' in a daily dose of 50.0 and 10 mg/kg body weight (n = 6; M ± m)

Parameters	Groups	Period of experiment, day				
		2	7	14	21	28
Hemoglobin, g/l	I	98.3 ± 1.8	96.8 ± 4.3	96.6 ± 1.7	98.8 ± 1.8	101.3 ± 1.4
	II	98.4 ± 2.0	93.2 ± 1.0	97.5 ± 3.9	98.8 ± 1,5	98.6 ± 3.1
	Control	99.5 ± 3.7	92.2 ± 1.5	96.3 ± 5.1	100.2 ± 2.7	101.3 ± 0.9
Red blood cells, 10^{12}/l	I	7.6 ± 0.4	7.7 ± 0.3	7.9 ± 0.1	7,6 ± 0,1	7.6 ± 0.2
	II	7.8 ± 0.5	8.2 ± 0.3	8.2 ± 0.3	7.9 ± 0,3	7.8 ± 0.1
	Control	7.6 ± 0.2	7.8 ± 0.4	7.6 ± 0.1	7.8 ± 0.1	7.8 ± 0.3
White blood cells, 10^9/l	I	19.2 ± 0.2	19.2 ± 0.4	18.9 ± 0.2	19.5 ± 1.1	19.2 ± 0.7
	II	20.1 ± 0.7	19.5 ± 0.3	19.3 ± 04	19.2 ± 0.9	19.3 ± 0.7
	Control	19.6 ± 0.6	19.6 ± 0.5	19.8 ± 0.9	19.8 ± 0.6	19.9 ± 0.6

Notes: * — p ≤ 0.1, ** — p ≤ 0.05, *** — p ≤ 0.01, **** — p ≤ 0.001.

Table 2 — Biochemical parameters in carp after two administrations of 'Rybozuril' in a daily dose of 50.0 and 10 mg/kg body weight (n = 6; M ± m)

Parameters	Group	Period of experiment, day				
		2	7	14	21	28
Total protein, g/l	I	32.86 ± 1.08	32.60 ± 0.56	32.24 ± 1.60	32.42 ± 0.68	32.60 ± 1.37
	II	32.69 ± 1.02	31.96 ± 0.66	31.82 ± 0.76	31.92 ± 1.14	33.02 ± 1.03
	Control	33.42 ± 1.0	34.21 ± 1.01	33.81 ± 1.14	33.62 ± 1.05	32.62 ± 0.76
Albumins, g/l	I	19.38 ± 0.39	18.41 ± 0.59	18.96 ± 0.97	19.18 ± 0.68	19.59 ± 1.20
	II	19.66 ± 0.22	18.74 ± 0.15	19.04 ± 0.49	18.66 ± 0.56	19.74 ± 0.29
	Control	19.60 ± 0.30	20.12 ± 1.28	20.90 ± 1.03	19.83 ± 0.78	19.64 ± 1.04
Globulins, g/l	I	13.48 ± 1.04	14.20 ± 0.93	13.28 ± 1.72	13.24 ± 0.93	13.02 ± 2.14
	II	13.03 ± 1.14	13.22 ± 0.80	12.79 ± 0.71	13.26 ± 1.18	13.28 ± 1.0
	Control	13.82 ± 0.71	14.10 ± 1.88	12.91 ± 0.66	13.79 ± 0.66	12.98 ± 0.84
CIC, mg/ml	I	0.13 ± 0.005**	0.115 ± 0.012	0.110 ± 0.004	0.118 ± 0.003	0.115 ± 0.006
	II	0.105 ± 0.003	0.113 ± 0.005	0.110 ± 0.004	0.115 ± 0.003	0.118 ± 0.009
	Control	0.110 ± 0.004	0.118 ± 0.009	0.120 ± 0.004	0.120 ± 0.004	0.118 ± 0.007
Sm, mg/ml	I	0.29 ± 0.01****	0.28 ± 0.01****	0,26 ± 0.01****	0.230 ± 0.004***	0.20 ± 0.01
	II	0.18 ± 0.01	0.20 ± 0.01	0,178 ± 0,009	0,20 ± 0,004	0,20 ± 0,004
	Control	0.17 ± 0.01	0.18 ± 0.01	0.178 ± 0.005	0.19 ± 0.01	0.193 ± 0.003

Notes: * — p ≤ 0.1, ** — p ≤ 0.05, *** — p ≤ 0.01, **** — p ≤ 0.001.

It was found that in blood plasma of fish from experimental groups I and II, there was no statistical changes in the content of total proteins during the experiment. An exception was the tendency to decrease its level on 21st day after the drug administration. However, during the introduction of the drug in both doses on the 7th and 14th day of the experiment, a tendency to decrease their albumin fraction was determined, which for the fish from group I and II averaged at 8.9 and 7.2% respectively. At the end of the experiment, the value of the indicator did not acquire statistical changes and close to their control level.

In addition, the toxic manifestation of the action of the drug at a higher dose (experiment I) indicates an increase of the level of the formation of osmotic phase proteins — seromucoids, the maximum content of which in the blood plasma of fish was set on the 2nd day after the introduction of 'Rybozuril' (70.6%, $p < 0.05$). On the 7th, 14th, and 21st day of the experiment, an increase in serum levels in the blood plasma of fish and experimental group was 55.6, 44.4, and 21.1% ($p < 0.05$) respectively, relative to the control values of the indicator. The obtained results are consistent with the increase of the level of circulating immune complexes (CIC) of the average molecular weight in blood plasma of fish in this group on the 2nd day after administration of the drug, which is an average of 16.3% ($p < 0.05$) relative to control.

The CIC are constantly circulating in the blood, which are important immune homeostasis components, and their elimination occurs by the cells of the reticuloendothelial system (Levinsky, 1981). It should be noted that the probable CIC increasing the physiological

products of the reaction 'antigen'-'antibody', which was determined by the effect of the drug in a toxic dose (experiment I), might be explained either by the antibodies development due to dysfunction of mechanisms of nonspecific protection in the forced mode, or by a shift in the elimination of CIC due to the functioning of the reticuloendothelial system (Vlizlo, 2012).

This fact, along with the increasing in the formation of serum counts in fish from this experimental group can reflect the phases of the development of immunotoxic reactions in their organisms.

The protein metabolism activity, as well as the enhancement of the formation of lipoproteins, which are necessary for the restoration of affected cell membranes, is shown by the action of a number of xenobiotics (Al-Akel et al., 2010).

The intensity of the processes of lipoperoxidation and the activity of the antioxidant system in carp by using the 'Rybozuril' drug is shown in Table 3.

Table 3 — The intensity of lipoperoxidation processes and the activity of the antioxidant system in carp after two administrations of 'Rybozuril' in a daily dose of 50.0 and 10 mg/kg body weight (n = 6; M ± m)

Parameters	Group	Period of experiment, day				
		2	7	14	21	28
Peroxide oxidation of lipids (POL)						
Conjugated dienes, μmol/l	I	15.60 ± 0.87****	22.41 ± 0.65*	27.86 ± 1.63	27.22 ± 1.66	25.04 ± 0.47
	II	25.33 ± 0.95	24.98 ± 1.15	25.42 ± 1.57	28.97 ± 1.48	25.50 ± 2.08
	Control	26.02 ± 1.51	25.85 ± 1.67	26.15 ± 0.42	26.31 ±0.28	25.96 ± 2.08
Malonic dialdehyde, $\Delta D/cm^3$	I	3.38 ± 0.37**	4.08 ± 0.47**	4.40 ± 0.28**	4.44 ± 0.37**	4.54 ± 0.28**
	II	5.44 ± 0.50	5.75 ± 0.11	5.85 ± 0.70	5.40 ± 0.50	6.10 ± 0.17
	Control	5.64 ± 0.28	5.74 ± 0.27	5.74 ± 0.38	5.80 ± 0.16	5.81 ± 0.41
Level of total antioxidant capacity (TAC)						
Catalase activity, μmol H_2O_2/dm^3 in 1 min	I	21.6 ± 0.3*	20.9 ± 0.5	20.8 ± 1.0	20.1 ± 0.8	19.3 ± 0.3
	II	26.9 ± 1.5***	28.0 ± 0.6****	30.4 ± 3.0**	26.2 ± 1.4***	22.3 ± 0.6*
	Control	19.6 ± 0.7	19.6 ± 0.6	20.1 ± 0.3	19.9 ± 0.6	20.2 ± 0.7
Inhibition, %	I	58.0 ± 4.9	59.2 ± 2.7	58.4 ± 1.8	58.9 ± 2.6	58.6 ± 2.3
	II	52.2 ± 9.8	53.1 ± 4.4	47.3 ± 3.4**	43.0 ± 5.8**	35.5 ± 2.6****
	Control	57.7 ± 4.6	58.6 ± 6.3	58.7 ± 1.1	57.3 ± 0.7	59.1 ± 1.6

Notes: * — $p \le 0.1$, ** — $p \le 0.05$, *** — $p \le 0.01$, **** — $p \le 0.001$.

The membrane-toxic effect of the drug at a higher dose indicates inhibition of the intensity of the processes of peroxide oxidation of lipids by the level of formation of lipoperoxidation products in fishes and experimental group (Table 3). Thus, on the 2nd and 7th day of the experiment, blood plasma of fish in this group recorded decrease in the level of primary lipid oxidation products (LOPs) — conjugated dienes an average of 40.0 and 13.3% ($p < 0.05$) relative to control. The value of the indicator, from the 14th day and at the end of the experiment (after 28 days), reached its control level.

However, from the 2nd to 28th day of the experiment, in the blood plasma of fish from group I, a decreasing of terminal membrane-toxic products of lipoperoxidation — MDA formation was determined. Thus, on 2nd, 7th, 14th, 21st, and 28th day after drug administration at a higher dose, the reduction of the MDA content in the plasma of fish was 40.0, 29.0, 23.3, 23.4, and 21.9% ($p < 0.05$) respectively, in relation to the control values.

The total antioxidant capacity (TAC) in blood plasma of fish at the effect of the drug in a lower dose had other changes. Thus, fishes of the group II during the experiment, an increase in the enzymatic activity of catalase was detected on average by 34.7% ($p < 0.05$) in comparison to the control. In the same time in the blood samples from fishes in this group, a gradual consumption of non-enzymatic levels of TAC was found on the dynamics of the overall TAC index. Thus, on 2nd, 7th, 14th, 21st, and 28th day after the introduction of the drug, the level of this indicator decreased in blood plasma of fish in comparison to its control values by 9.5, 9.4, 19.4, 25.0, and 40.0% ($p < 0.05$) respectively.

Therefore, it should be noted that during the experiment in the fish which obtained drug at a lower dose (group II), the intensity of the processes of the POL (according to the level of formation of its primary and end products) was at the level of physiological control. It could be explained by occurring of compensatory expenditure of a pool of endogenous structural antioxidants (at TAC decreasing) and adaptogenic enhancement of catalase activity: the maximum expressed changes in the values of the parameters were found on the 28th and 14th day of the experiment. It is known that catalase plays a major role in the adaptation of cells to the increased intensity of catabolic and destruction (Marques et al., 2015), and the determined dynamics of this enzyme as a result of the

introduction of the drug is cells adaptation to maintain the processes of lipoperoxidation in their membranes at the physiological level.

The dynamics of enzymes and glucose in the blood plasma of carp for the use of drug 'Rybozuril' is shown in Table 4.

Table 4 — Enzymes and glucose dynamics in blood plasma of carp after two administrations of 'Rybozuril' in a daily dose of 50.0 and 10 mg/kg body weight (n = 6; M ± m)

Parameters	Groups	Period of experiment, day				
		2	7	14	21	28
ALT, mmol/(h×l)	I	0.37 ± 0.01	0.37 ± 0.02	0.4 ±0.01	0.38 ±0.01	0.4 ±0.01
	II	0.32 ± 0.01***	0.3 ± 0.01**	0.38 ±0.02	0.44 ±0.03	0.4 ±0.03
	Control	0.36 ± 0.003	0.37 ±0.02	0.38 ±0.01	0.40 ±0.04	0.41 ±0.01
AST, mmol/(h×l)	I	1.98 ± 0.03	2.12 ±0.03	1.59 ± 0.04**	1.77 ± 0.05**	1.58 ± 0.05***
	II	2.61 ± 0.02**	1.8 ± 0.03***	1.59 ± 0.05**	2.15 ±0.04	2.17 ±0.06
	Control	2.13 ± 0.12	2.1 ±0.06	2.07 ±0.11	2.11 ±0.10	2.09 ± 0.09
α-amylase, mg/(sec×l)	I	5.10 ± 0.13	4.61 ± 0.19	4.44 ± 0.03****	4.48 ± 0.05****	5.07 ± 0.05
	II	5.07 ± 0.08	5.11 ± 0.09	5.25 ± 0.14	5.29 ± 0.13	5.10 ± 0.04
	Control	4.98 ± 0.03	4.97 ± 0.03	4.97 ± 0.03	5.01 ± 0.02	5.08 ± 0.07
Glucose, mmol/l	I	3.52 ± 0.04	3.17 ± 0.05***	3.04 ± 0.09**	3.51 ± 0.16	3.53 ± 0.13
	II	3.48 ± 0.09	3.38 ± 0.06	3.35 ± 0.07	3.45 ± 0.03	3.6 ±0.05
	Control	3.56 ± 0.06	3.51 ±0.03	3.52 ±0.14	3.57 ±0.14	3.56 ±0.13

Notes: * — $p \le 0.1$, ** — $p \le 0.05$, *** — $p \le 0.01$, **** — $p \le 0.001$.

It was noticed that there is decrease the level of glucose in the blood of fish from group I on 7th and 14th day of the experiment, having valued 9.7 and 13.6% ($p < 0.05$) respectively in comparison to the control. The results suggested compensatory character and aimed at activation of detoxification processes due to administration of the drug in a toxic dose. At the end of the experiment (on the 21st and 28th day), the value of the indicator was not statistically different from the control.

It is known that the magnitude of the induction of activity of hepatic enzymes in the blood is proportional to the degree of destruction of hepatocytes and the activity of the pathological process (Khazanov, 1988, De Bono, 1994).

The drug administration at a lower dose at the beginning of the experiment affected the intensity of the transamination processes in the liver and myocardium of fish (Table 2). Thus, on the 2nd and 7th day of the experiment in blood plasma of fish from group II, a significant decrease in the enzymatic activity of ALT was recorded on average by 11.1 and 18.9% ($p < 0.05$) relative to the control.

Whereas, on the 2nd day, the AST level, which participates in providing cytoplasm to substrates for gluconeogenesis in the conversion of pyruvate to glucose (Dirksen, 1990), in the blood of fish from this group significantly increased by 22.5%, and on the 7th day decreased by 14.3% relative to its control values. Thus, the obtained results indicate, on the one hand, the temporal inhibition of the processes of transamination, and on the other hand there is intensity of detoxification systems in the liver of experimental fish.

Glucose decreasing in blood plasma of fish was observed on the 7th and 14th day as a result of drug administration in a toxic dose. It suggests the participation of α-amylase, the activity of which at this time was reduced by 7.2 and 10.7% respectively relative to the control level of the enzyme. The established changes in the indicators at the end of the experiment had a restoration to the physiological values.

Thus, the maximum severity of metabolic changes in fish after two-time administration of the 'Rybozuril' drug in a daily dose of 50.0 mg/kg body weight was found at the initial stages of the study and until 21st day. According to the obtained results, manifestations of the toxic effect of the drug in fish, that had no reciprocal character by a number of indicators. The drug in such dose was shown immunosuppression and membrane-toxic effects in fish.

The metabolic alterations in fish due to the drug effect at a lower dose (group II) influence on the activation of detoxification processes and maintenance of processes of lipoperoxidation in cell membranes at the physiological level. It was stated that at the end of the experiment dynamics of most of the investigated parameters had a reversible character and by their value approached the physiological level (control).

Conclusions. 1. Due to the absence of fish death after determining the maximum dose of diclazuril LD_{50} it was not possible to determine. However, it can be classified as hazard class IV toxicity.

2. Two-time administration of the 'Rybozuril' drug in a daily dose of 50.0 mg/kg body weight, the maximum exposure to metabolic changes in fish was observed in the initial terms of the study until 21st day. Based on the

obtained results, manifestations of the toxic effect of the drug in fish at the end of the experiment dynamics of most of the investigated parameters had not a reversible character. The drug in such dose was shown immunosuppression and membrane-toxic effects in fish.

3. Two-time administration of the 'Rybozuril' drug in a daily dose of 10.0 mg/kg body weight leads to metabolic alterations in fish due to the activation of detoxification processes and lipoperoxidation maintenance in cell membranes at the physiological level. At the end of the experiment the toxic effect of 'Rybozuril' in fish characterized by stable parameters in comparing to the control group.

Outlooks. Taking into account the obtained results and data of previous studies, it is planned to introduce the 'Rybozuril' drug into the practice of veterinary medicine and fish farming in order to control fish diseases caused by the parasitic protozoa from family Eimeriidae.

References

Al-Akel, A. S., Al-Balawi, H. F. A., Al-Misned, F., Mahboob, S., Ahmad, Z. and Suliman, E. M. (2010) 'Effects of dietary copper exposure on accumulation, growth, and hematological parameters in *Cyprinus carpio*', *Toxicological and Environmental Chemistry*, 92(10), pp. 1865–1878. doi: 10.1080/02772248.2010.486230.

CEC (The Council of the European Communities). (1986) 'Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes', *The Official Journal of the European Communities*, L 358, pp. 1–28. Available at: http://data.europa.eu/eli/dir/1986/609/oj.

Conway, D. P., Mathis, G. F., Johnson, J., Schwartz, M. and Baldwin, C. (2001) 'Efficacy of diclazuril in comparison with chemical and ionophorous anticoccidials against *Eimeria* spp. in broiler chickens in floor pens', *Poultry Science*, 80(4), pp. 426–430. doi: 10.1093/ps/80.4.426.

De Bono, D. (1994) 'Free radicals and antioxidants in vascular biology: the roles of reaction kinetics, environment and substrate turnover', *QJM: Monthly Journal of the Association of Physicians*, 89(8), pp. 445–453. doi: 10.1093/oxfordjournals.qjmed.a068954.

Demidov, N. V. and Berezkina, S. V. (1986) *Guidelines for assessment of anthelmintics in veterinary medicine [Metodicheskie rekomendatsii po otsenke angel'mintikov v veterinarii]*. Moscow: VASKhNIL. [in Russian].

Dirksen, G. (1990) 'Liver [Leber]', in Dirksen, G., Gründer, H. D. and Stöber, M. (eds.) Rosenberger, G. *Clinical examination of cattle [Die klinische Untersuchung des Rindes]*. 3rd ed. Berlin, Hamburg: Paul Parey, pp. 367–384. ISBN 9783830440383. [in German].

EMEA (European Medicines Agency) (1996) *Diclazuril — summary report (1)*. Report of the Committee for Veterinary Medicinal Products. EMEA/MRL/086/96-FINAL. Available at: https://www.ema.europa.eu/en/documents/mrl-report/diclazuril-summary-report-1-committee-veterinary-medicinal-products_en.pdf.

FEEDAP (EFSA Panel on Additives and Products or Substances used in Animal Feed) (2014) 'Scientific Opinion on the safety and efficacy of Coxiril® (diclazuril) as a feed additive for turkeys for fattening: Coxiril® (diclazuril) for turkeys for fattening', *EFSA Journal*, 12(6), p. 3729. doi: 10.2903/j.efsa.2014.3729.

FEEDAP (EFSA Panel on Additives and Products or Substances used in Animal Feed) (2015) 'Scientific Opinion on the safety and efficacy of Coxiril® (diclazuril) for rabbits for fattening and breeding: Coxiril® (diclazuril) for rabbits for fattening and breeding', *EFSA Journal*, 13(1), p. 3968. doi: 10.2903/j.efsa.2015.3968.

Gavrilov, V. B. and Mishkorudnaya, M. I. (1983) 'Spectrophotometric determination of the content of lipid hydroperoxides in blood plasma' [Spektrofotometricheskoe opredelenie soderzhaniya gidroperekisey lipidov v plazme krovi], *Laboratory Science [Laboratornoe delo]*, 3, pp. 33–36. [in Russian].

GOST 12.1.007-76. *Occupational safety standards system. Noxious substances. Classification and general safety requirements [Sistema standartov bezopasnosti truda. Vrednye veshchestva. Klassifikatsiya i obshchie trebovaniya bezopasnosti]*. [in Russian].

Khazanov, A. I. (1988) *Functional diagnosis of liver diseases [Funktsional'naya diagnostika bolezney pecheni]*. 2nd ed. Moscow: Meditsina. ISBN 522500069X. [in Russian].

Klebanov, G. I., Babenkova, I. V., Teselkin, Yu. O., Komarov, O. S. and Vladimirov, Yu. A. (1988) 'Evaluation of the antioxidative activity of blood plasma using yolk lipoproteins' [Otsenka antiokislitel'noy aktivnosti plazmy krovi s primeneniem zheltochnykh lipoproteidov], *Laboratory Science [Laboratornoe delo]*, 5, pp. 59–62. PMID: 2459459. [in Russian].

Kondrakhin, I. P., Kurilov, N. V., Malakhov, A. G., Arkhipov, A. V., Belov, A. D., Belyakov, I. M., Blinov, N. I., Korobov, A. V., Frolova, L. A. and Sevast'yanova, N. A. (1985) *Clinical laboratory diagnostics in veterinary medicine [Klinicheskaya laboratornaya diagnostika v veterinarii]*. Moscow: Agropromizdat. [in Russian].

Korolyuk, M. A., Ivanova, L. I., Mayorova, I. G. and Tokarev, V. E. (1988) 'Determination of catalase activity' [Opredelenie aktivnosti katalaz], *Laboratory Science [Laboratornoe delo]*, 1, pp. 16–18. PMID: 2451064. [in Russian].

Kotsiumbas, I. Ya., Malyk, O. H., Patereha, I. P., Tishyn, O. L. and Kosenko, Yu. M. (2006) *Preclinical studies of veterinary drugs [Doklinichni doslidzhennia veterynarnykh likarskykh zasobiv]*. Lviv: Triada plus. ISBN 9667596648. [in Ukrainian].

Levinsky, R. J. (1981) 'Role of circulating immune complexes in renal diseases', *Journal of Clinical Pathology*, 34(11), pp. 1214–1222. doi: 10.1136/jcp.34.11.1214.

Maltsev, V. N. (2012) 'About parasitological situation in carp hatcheries of the south regions of Ukraine' [O parazitologicheskoy situatsii v karpovykh rybopitomnikakh yuzhnykh oblastey Ukrainy], *Modern Problems of Theoretical and Practical Ichthyology: Proceedings of the 5th International Ichthyologic Scientific and Practical Conference (Chernivtsi, 13–16 September 2012) [Suchasni problemy teoretychnoi i praktychnoi ikhtiolohii: materialy V mizhnarodnoi ikhtiolohichnoi naukovo-praktychnoi konferentsii (Chernivtsi, 13–16 veresnia 2012 r.)]*. Chernivtsi: Knyhy-XXI, pp. 142–148.

Available at: https://www.eco-tiras.org/docs/berg/Cernauti-kof_ichthyo_chernivtsi_2012.pdf. [in Russian].

Marques, G. L., Neto, F. F., Ribeiro, C. A. de O., Liebel, S., Fraga, R. de and Bueno, R. da R. L. (2015) 'Oxidative damage in the aging heart: an experimental rat model', *The Open Cardiovascular Medicine Journal*, 9(1), pp. 78–82. doi: 10.2174/1874192401509010078.

Medved', L. I., Kagan, Yu. S. and Spynu, Ye. I. (1968) 'Pesticides and health problems' [Pestitsidy i problemy zdravookhraneniya], *Journal of the D. I. Mendeleev All-Union Chemical Society [Zhurnal Vsesoyuznogo khimicheskogo obshchestva imeni D. I. Mendeleeva]*, 13(3), pp. 263–271.

Men'shikov, V. V. (ed.) (1987) *Laboratory research methods in the clinic [Laboratornye metody issledovaniya v klinike]*. Moscow: Meditsina. [in Russian].

Molnár, K. and Ostoros, G. (2007) 'Efficacy of some anticoccidial drugs for treating coccidial enteritis of the common carp caused by *Goussia carpelli* (Apicomplexa: Eimeriidae)', *Acta Veterinaria Hungarica*, 55(1), pp. 67–76. doi: 10.1556/AVet.55.2007.1.7.

Selifanova, E. A. and Birukova, N. P. (2012) 'Subchronic toxicity of Eimeterm diclazuril' [Subkhronicheskaya toksichnost' preparata Eymeterm diclazuril], *Theory and Practice of Struggle Against Parasitic Diseases [Teoriya i praktika bor'by s parazitarnymi boleznyami]*, 13, pp. 385–387. Available at: https://elibrary.ru/item.asp?id=30311107. [in Russian].

Tokarev, A. N., Selifanova, E. A., Napalkova, V. V. and Zhuravleva, A. Z. (2012) 'Comparative evaluation of acute toxicities of Eimeterm diklazuril and Diacox' [Sravnitel'naya otsenka ostroy toksichnosti preparatov Eymeterm diklazurila i Diakoksa], *Theory and Practice of Struggle Against Parasitic Diseases [Teoriya i praktika bor'by s parazitarnymi boleznyami]*, 13, pp. 418–421. Available at: https://elibrary.ru/item.asp?id=30311119. [in Russian].

Trombitskiy, I. D. and Bushuev, S. G. (eds.) (2011) *Comprehensive Moldovan-Ukrainian studies of the ichthyofauna of the Lower Dniester basin —2011 [Kompleksnye moldo-ukrainskie issledovaniya ikhtiofauny vodoemov basseyna Nizhnego Dnestra — 2011]*. Project report. Available at: http://www.eco-tiras.org/docs/DniesterFishReport2011.pdf. [in Russian].

Verheyen, A., Maes, L., Coussement, W., Vanparijs, O., Lauwers, F., Vlaminckx, E., Borgers, M. and Marsboom, R. (1988) 'In vivo action of the anticoccidial diclazuril (Clinacox®) on the developmental stages of *Eimeria tenella*: an ultrastructural evaluation', *The Journal of Parasitology*, 74(6), pp. 939–949. doi: 10.2307/3282213.

Vlizlo, V. V. (ed.) (2012) *Laboratory methods of research in biology, animal husbandry and veterinary medicine [Laboratorni metody doslidzhen u biolohii, tvarynnytstvi ta veterynarnii medytsyni]*. Lviv: Spolom. ISBN 9769666656776. [in Ukrainian].