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ORIGINAL ARTICLE

Toxicants in the degradation of lipids in the orginism scaly carp

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The purpose of this work was to study the content of the products of the LPO (new conjugates, hydroperoxides and malonic dialdehyde) in tissues and organs (liver, white muscle, brain and gills) two-year-old carp scouring under the influence of toxicants of various chemical nature (heavy metal ions-Pb^{2+} and Cd^{2+}, surfactants-sodium lauryl sulfate and Tween-80) both individually and in combination in different combinations. We do not find in the available literature data on the effect of these toxicants on the activity of the antioxidant system in the flocculent carp.
Keywords: Tissue; organs; body; scaly carp; surface-active substances; heavy metals; xenobiotics; lipid peroxide oxidation; antioxidant protection system

Introduction

Biological membranes that constructed of lipids and their natural complexes ensure the functioning of cells in the multicellular organism (Vladimirov & Archakov, 1972). Oxidation of lipids constantly occurring in organs and systems of an organism and it plays an important role both in normal and in pathology. The activation of free radical oxidation of unsaturated fatty acids and lipid peroxidation (LPO) leads to the appearance of products of lipoperoxidation (alcohols, ketones, aldehyde and other compounds), a significant accumulation of which puts a threat to the body. Lipoperoxide that is transmitted by blood from places of formation to other organs and tissues and can cause their damage. There is an antioxidant defense system (AOP) in the body, which also counteracts the LPO. The equilibrium detween these components (AOP and LPO) of the oxidation-antioxidant system ensures the existence of the organism, and imbalance leads to cell death due to chain reactions of peroxidation. The functional state of cellular and subcellular membranes reflects a fairly well-defined flow of processes in the LPO system. Violation of the functional state of the lipid layer leads to damage to the cellular membranes, and to the development of this or that pathological process. We were presented earlier the influence of xenobiotics on the activity of the antioxidant system in carp tissues (Simonova & Mekhed, 2017). However, the combined effect of toxicants on the state of AOS carp in different combinations remains interesting and poorly investigated.

Materials and methods of research

In studies, we used samples of tissues of carp of two-year-old. The research was carried out in April-July 2016 on the two-year-old of carp (*Cyprinus carpio L.*) weighing 200-250 g. The fish were kept in groups of 5 animals for 14 days in aquariums of 200 liters. One group was control, and the others in the water were added toxicants in a concentration that corresponded to 2 maximum permissible concentrations (2 MPCs). The fish were not fed. Control carried out and maintained constant hydrochemical mode of water in all cases. The pH value was 7.30 ± 0.27; the oxygen content-5.6 ± 0.4 mg/l, the temperature was maintained close to the natural. According to ichthyopathological observations, the skin-causing agents of parasitic diseases in fish were not detected. Band parasites are also not recorded. The inversion voltammetry method was used to study the content of heavy metals in various fish organs. Sample preparation of samples of organs of fish was carried out by the method of "wet" mineralization and dry ozonation with additives. A weighing sample weighing 1 g was mixed with 2.5 cm^3 of concentrated nitric acid in a quartz glass, heated to a temperature of 150 °C until the gas was withdrawn and evaporated to 1/3 of the original volume. Then 2.0 ml of concentrated nitric acid and 1.0 cm^3 of a 30% solution of hydrogen peroxide were added and evaporated to dryness for 60-70 minutes at a temperature of 150-350 °C. The sample was ozonized at a temperature of 450 °C for 30 minutes. The operation of adding nitric acid, hydrogen peroxide, evaporation and ozonation was repeated two to three times until a homogeneous ash of white, yellow or gray color was obtained. Ash was dissolved in 1 cm^3

of concentrated formic acid and diluted with bistilate to 10 cm^3. In a quartz electrochemical cell, 10 cm^3 of distilled water, 0.2 cm^3 of concentrated formic acid and an aliquot of sample of 0.5 cm^3 were added. The content of heavy metals was determined on the analyzer voltamperometric TA-Lab (NPP "Tomanalit", RF) in a three-electrode electrochemical cell. An amalgam electrode was used as an indicator electrode. The chloride electrode, filled with a solution of 1 M potassium chloride, was used as a comparison electrode and an auxiliary electrode.

The determination of metals was carried out by the method of additives using standard solutions containing 1 mg/dm^3 or 10 mg/dm^3 of each of the detectable metals, prepared on the basis of state standard samples and bistylystylate. The calculation of the concentration of metals was performed using a specialized computer program TA-Lab (version 3.6.10).

The results were processed according to the known method by the method of mathematical statistics; calculated the average and interval value with a confidence probability of 95%.

In the tissues determined the content of diene conjugates (Vlizlo, 2004), hydroperoxides (Korobeynikov, 1989) and malonic dialdehyde (Yakovenko, Tretyak, Mekhed, Khaytova & Simonova, 2017). The digitized data were statistically processed using the Microsoft EXCEL program using Student's coefficient.

Results and discussion

Our research has shown that the activity of the antioxidant defense system in the tissues of the carp (*Cyprinus carpio L.*) is changing with the action of the toxicants.

In particular, from the data presented in Tables 1-3 it is evident that the content of diene conjugates increases significantly in investigated tissues of fish that were in a toxic effect.

Table 1. The content of LPO products in the white muscle of the scaly carp (M ± m, n=10).

Conditions of detention	Diene conjugates, Kmol/kg tissues	Hydroperoxide Optical density unit/g tissues	Malonic dialdehyde, nmol/g tissues
Control group	89.24 ± 3.12	7.80 ± 0.15	20.04 ± 1.45
Pb^{2+}	94.34 ± 4.14	9.18 ± 1.12	18.99 ± 2.18
Cd^{2+}	92.88 ± 1.80	8.12 ± 2.14	21.21 ± 2.12
SLS	102.14 ± 5.15	13.15 ± 0.98	26.14 ± 4.15
Tween-80	95.64 ± 8.11	14.92 ± 0.55	24.18 ± 3.11
Pb^{2+}+ SLS	120.54 ± 6.15	19.98 ± 1.15	28.14 ± 5.12
Pb^{2+}+Tween-80	103.56 ± 12.10	11.6 ± 2.11	26.24 ± 4.40
Cd^{2+}+SLS	98.45 ± 6.16	7.18 ± 0.96	24.95 ± 2.21
Cd^{2+}+Tween-80	98.35 ± 3.14	9.19 ± 1.96	28.12 ± 4.04

It was found that when fish were kept under the influence of Pb^{2+}, the changes in the content of diene conjugates and hydroperoxides increased-105.7 and 117.7%, respectively, but considering the effect of Pb^{2+} on quantitative indicators of malondialdehyde, reaching 94.8% compared with control, we conclude that there is no noticeable change in this group. Cadmium is a heavy metal, which is now considered one of the most widely spread pollutants. Violations of activity of all systems of an organism are detected by the long action of Cd^{2+}, during the determination of the influence of Cd^{2+} there is an increase in the amount of the final product of peroxidation of lipids in all the indicators-DC and hydroperoxides-104% in the group of malon dialdehyde-105.8%.

Investigation of the influence of surfactants-sodium lauryl sulfate and Tween-80, reflects the change of indicators in all groups of markers. The largest change in the action of surfactant occurs in the group of hydroperoxides, where the effect of SLS-168.6% and Tween-80-191.3%. Malonium dialdehyde by the action of surfactant, has an average change in this group, 130.4% and 120.7%. The least effect is observed in the quantitative indices of diene conjugates-114.5% and 107%. Regular admission to the body of xenobiotics leads to the development of free radical pathology, which is based on the intensification of free radical oxidation by detergents. It is known that the adverse effect of surfactant leads to the development of hypoxia, changes in metabolic processes, and violations of normal functioning of the body.

Based on the combined effect of surfactants and heavy metals, the following conditions were taken: Pb^{2+}+SLS, $Pb2^{+}$+Tween-80, Cd^{2+}+SLS and Cd^{2+}+Tween-80. Analyzing data on the influence of toxicants on white muscle, the greatest changes occur in the group Pb^{2+}+LSN-256%. The least noticeable changes for white muscle was the combined effect of Cd^{2+}+LSN-92%.

Analyzing the results of the accumulation of LPO products in the liver of scaly carp, it can be concluded that significant changes occur in the group of combined effects of Pb^{2+}+LSN; the change in the indicator is characterized by the action of Cd^{2+} in the group of heavy metals (Table 2).

Table 2. The content of LPO in the liver of scaly carp (M ± m, n=10).

Conditions of detention	Diene conjugates, Kmol/kg tissues	Hydroperoxide Optical density unit/g tissues	Malonic dialdehyde, nmol/g tissues
Control group	147.26 ± 3.12	6.40 ± 0.25	27.44 ± 3.48
Pb^{2+}	202.45 ± 10.24	7.12 ± 0.65	31.25 ± 3.35
Cd^{2+}	167.64 ± 12.11	6.80 ± 1.13	30.21 ± 4.20
SLS	187.85 ± 18.12	10.24 ± 2.20	39.27 ± 2.20
Tween-80	197.12 ± 12.14	9.26 ± 1.80	31.31 ± 3.50
Pb^{2+}+SLS	212.24 ± 15.42	11.25 ± 2.14	39.11 ± 4.16
Pb^{2+}+Tween-80	202.12 ± 10.12	9.12 ± 1.80	42.12 ± 2.26
Cd^{2+}+SLS	187.11 ± 8.11	10.11 ± 2.02	28.28 ± 3.36
Cd^{2+}+Tween-80	199.14 ± 16.12	9.14 ± 1.85	29.29 ± 2.24

Chemical compounds and surfactants accumulate in the body of fish in different tissues of varying concentrations. Salts of heavy metals such as lead, copper and cadmium accumulate mainly in the liver. When poisoning fish with toxicants, the liver becomes flabby consistency, pale appearance with local hemorrhages. Analyzing the data of the conducted experimental study, in the carpal liver, when heavy metals are contaminated by Cd^{2+}, Pb^{2+}, an increase in the content of malondialdehyde in the action of Pb^{2+} is observed-the change is 137.5% compared with the control group.

The smallest change in the behavior of heavy metals is observed in the Cd^{2+}group on hydrogen peroxide-106.3%.

Influence of surfactant on peroxidation processes mark an important role of oxidative stress, in mechanisms of biological action for detergent interference. Considering the influence of SLS and Tween 80 on the liver, the greatest changes are observed in the group of hydroperoxides. The SLS score is 160%, and the Tween 80 effect is more than 155% compared with the control group. According to the action of heavy metals and surfactants, we observe the largest changes in the group of hydroperoxides, under the conditions of Pb^{2+}+LSH-make up the indicator-175.8%, which is the largest among the group of combined effects. The slightest changes occur in the group with malonic dialdehyde, with the effect of Cd^{2+} with LSH percentage is an increase of only 3%. With the use of Tween-80, the growth of rate was only 6%.

Summing up in the study of the liver, heavy metals, surfactants and their combined action, it can be noted that the effect of Pb^{2+}+ LSH has the most harmful effects on the organism of fish. The smallest percentage gain for all groups is the influence of heavy metal Cd^{2+}-compared with the control group, the data do not exceed the change by more than 13% for all indicators, which is to the liver. Results of the study of the content of LPO products in the brain of fish are presented in Table 3.

Table 3. The content of LPO in the brain of the scaly carp (M ± m, n=10).

Conditions of detention	Diene conjugates, Kmol/kg tissues	Hydroperoxide Optical density unit/g tissues	Malonic dialdehyde, nmol/g tissues
Control group	23.23 ± 3.12	3.44 ± 0.25	12.44 ± 2.48
Pb^{2+}	25.28 ± 2.24	4.12 ± 0.80	14.14 ± 4.20
Cd^{2+}	26.26 ± 4.12	4.12 ± 0.44	13.12 ± 2.11
SLS	24.12 ± 2.28	3.88 ± 0.65	14.45 ± 0.56
Tween-80	26.67 ± 3.28	5.12 ± 0.28	14.46 ± 0.96
Pb^{2+} + SLS	28.14 ± 4.13	4.80 ± 0.34	15.54 ± 3.12
Pb^{2+}+Tween-80	26.11 ± 5.11	3.98 ± 0.56	14.96 ± 4.05
Cd^{2+}+SLS	28.44 ± 4.45	4.56 ± 0.65	15.56 ± 3.35
Cd^{2+}+Tween-80	28.28 ± 6.02	4.46 ± 0.80	14.96 ± 3.12

Investigating the influence of heavy metals on the tissues of the brain, it can be noted that the slightest effect has Cd^{2+} on malonic dialdehyde-the change was 105%. Considering the group of hydroperoxides, changes in the action of Pb^{2+} and Cd^{2+} do not exceed 120% compared with the control. The content of new conjugates is characterized by slight changes in the indicators, Pb^{2+} and Cd^{2+}-108% and 113%. The accumulation of hydroperoxides is most favored by Tween- 80-compared with the control, the change in the indicator is 148.8%. Malone dialdehyde is a marker of intensification and an intermediate product of LPO processes. Similar results-116% were observed in the study of the action of SLS and Tween-80, on the content of malonic dialdehyde. The combined action of metals and surfactants in the brain reflects the tendency for a maximum increase in the group of hydroperoxides, SLS with the addition of heavy metal. The major changes were-Pb^{2++} of SLS-139.5% and $Cd^{2++}$of SLS-132.6. The minor indicator was observed when heavy metal is combined and the surfactant is monitored for Pb^{2++} Twin 80, where the change is only 12%. The content of hydroperoxides is characterized by certain tissue features. The content of hydroperoxides serves as a marker for the study of toxic effects on the body. When analyzing data, we observe an

increase in the content of hydroperoxides. The content of LPO products in the gills of carp for the effects of toxicants of various chemical structures is presented in Table 4.

Table 4. The content of LPO products in the grill of scaly carp (M ± m, n=10).

Conditions of detention	Diene conjugates, Kmol/kg tissues	Hydroperoxide Optical density unit/g tissues	Malonic dialdehyde, nmol/g tissues
Control group	73.63 ± 3.82	3.84 ± 0.25	13.64 ± 3.44
Pb^{2+}	75.48 ± 5.25	4.31 ± 0.62	15.24 ± 2.28
Cd^{2+}	88.16 ± 8.12	4.17 ± 0.43	16.12 ± 3.12
SLS	89.66 ± 6.03	5.23 ± 0.32	14.44 ± 2.14
Tween-80	92.18 ± 12.05	5.28 ± 0.65	15.67 ± 4.12
Pb^{2+} + SLS	104.30 ± 6.12	5.89 ± 0.12	18.19 ± 2.14
Pb^{2+}+Tween-80	98.20 ± 5.14	5.44 ± 0.54	19.29 ± 4.13
Cd^{2+}+SLS	100.18 ± 8.80	4.16 ± 0.42	16.16 ± 3.15
Cd^{2+}+Tween-80	98.35 ± 4.12	5.05 ± 0.90	17.22 ± 2.15

The gills are more protected from LPO under conditions of toxicity. Significant increase in the content of LPO, in the group of heavy metals, registered in them only under the influence of Cd^{2+}. For the action of new conjugates, the change is more than 120%. An adequate degree of correlation was noted in the group of exposure to surfactants, where the highest change rate was more than 138% for the Tween-80 activity in hydroperoxides, and the smallest in the group of malonic dialdehyde in the SLS-105.9%. We observe similar changes in 141% for hydroperoxides and malonic dialdehyde when combined Pb^{2+}+Tween-80 with respect to the combined effect of surfactant and heavy metal ions. These figures are also the largest in this group. The combined effect of xenobiotics, when exposed to hydroperoxide, is the smallest in this group-its index is 108, 3%. The transition of the values of the correlation coefficient detween the activity of toxicants and the content of LPO products in gills from positive in the control group to negative in experimental group of fishes indicates the detrimental effect of toxicants. The ambiguous effect of the under study substances, both individually and in various combinations, on the content of LPO products in fish tissues interested us with the question of the accumulation of heavy metals in the organs and tissues of fish under different conditions of detention. The content of the metal in the samples calculated from the voltamper curves is presented in Table 5.

Table 5. The content of heavy metals in the organs of fish.

Organ	Surfactant	Cd^{2+}	Pb^{2+}
Muscles	SLS	0.000070 ± 0.000022	0.068 ± 0.024
	-	0.05 ± 0.001	0.81 ± 0.05
	Tween-80	0.0024 ± 0.0005	0.19 ± 0.07
Liver	SLS	0.50 ± 0.16	0.68 ± 0.20
	-	0.87 ± 0.23	1.4 ± 0.4
	Tween-80	0.33 ± 0.09	1.1 ± 0.3
Gills	SLS	0.030 ± 0.008	1.1 ± 0.3
	-	1.5 ± 0.05	2.3 ± 0.7
	Tween-80	0.21 ± 0.06	1.2 ± 0.4
Brain	SLS	0.032 ± 0.010	0.48 ± 0.04
	-	0.48 ± 0.013	1.1 ± 0.3
	Tween-80	0.36 ± 0.18	0.21 ± 0.06

The data given in the table indicates the maximum accumulation of heavy metals in the conditions of their exclusive presence in water of aquariums, the presence of surface-active substances in water and the accumulation of metals. If we compare the ability to accumulate metals in different tissues, we can observe that the highest content of the plum is in the tissues of the gills, and the smallest is, respectively, in the white muscle of animals. A similar trend towards accumulation was also observed for cadmium.

Conclusions

Therefore, in the study of tissues, which are affected by extreme environmental factors, found that in fish organism initiation

of lipid peroxidation products.

In the group of diene conjugates, the largest change indicator is the combined effect of Pb^{2+}+LSH in the determination of SLS products in the liver. As is known in large quantities, Pb^{2+} can have a toxic effect on the growth and development of organisms. The liver is a definite filter, a barrier to the development of LPO in the organisms of fish, it takes an important part in the elimination of toxic substances in the body. Determining the content of malon dialdehyde, we can conclude that this indicator has the greatest increase in the tissues of the liver. The combined action of Pb^{2+}+Tween-80 causes changes in the MD content to reach over 153.5%.

The group of hydroperoxides has the highest rates in all tissues. The change in white muscle for the combined action of Pb^{2+}+LSW is over 256%. A similar trend is observed in the liver-an indicator reaching 175.8%. Investigation of the gill apparatus of the carp by the action of toxicants of different chemical nature makes it possible to see that the simultaneous influence of Pb^{2+}+LSN causes changes to 153.3%. The effect of toxicants on the brain is 139.5%, but in this group also the high rate has the effect of Twiw-80-more than 148%. Investigated tissues in order of increasing toxicity for the use of the most active toxicant Pb^{2+}+LSN: white muscle-liver-gills-brain.

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