VETERINARSKI ARHIV 79 (2), 179-188, 2009

Hepatotoxic potential of malathion in the freshwater teleost, *Labeo rohita* (Hamilton)

Vineetkumar Kallappa Patil, and Muniswamy David*

Karnatak University Research Laboratory, Toxicology Division, Department of Zoology, Karnatak Science College, Dharwad, Karnataka, India

PATIL, V. K., M. DAVID: Hepatotoxic potential of malathion in the freshwater teleost, *Labeo rohita* (Hamilton). Vet. arhiv 79, 179-188, 2009.

ABSTRACT

Freshwater edible fish, *Labeo rohita,* were exposed to a sublethal concentration (0.9 µl/L) of commercial grade malathion (50% Emulsified Concentration) for 5, 15 and 25 d. After each exposure period, the liver was taken, to study biochemical alterations. An increase in free amino acid, protease activity and ACh levels, in contrast to decrement in total, structural and soluble proteins and AChE activity, were observed after 5 and l5 d exposure, but on 25 d exposure all the values came nearer to normal. Restoration of protein fractions, free amino acid, protease activity, ACh levels and AChE activity to normal implies that after 15 d of exposure there seems to exist an oscillatory phase in protein turnover towards a more synthetic phase, leading to the establishment of recuperation and adaptation phenomena.

Key words: *Labeo rohita*, liver toxicity, malathion

Introduction

For centuries pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors (PRAKASAM et al., 2001). Among these pesticides, organophosphorus compounds (OPs) are commonly used as insecticides (STORM et al., 2000). Organophosphorus (OPs) pesticides have long been of serious environmental concern. They form the largest group of chemicals used in the control of pests, including invertebrates, vertebrates and, to a lesser extent, plants. There are some 200 OP pesticides available in this class, that have been formulated into literally thousands of different products (HILL, 2003).

Malathion is a non systemic, wide spectrum organophosphate insecticide. It was one of the earliest organophosphate insecticides developed (Introduced in 1950). It was used for agricultural and non-agricultural purposes. Once malathion is introduced into the

*Corresponding author:

Dr. M. David, Karnatak College Staff Quarters, H. No. C-6, Opposite Udaya Hostel, Station road, Dharwad-580 001, Karnataka, India, Mobile: +91 98457 09815; E-mail: davidma941964@rediffmail.com

ISSN 0372-5480
Printed in Croatia

environment, it may cause serious trouble to aquatic organisms and is notorious for causing severe metabolic disturbances in non-target species, like fish and fresh-water mussels (ANONYM., 2005). Organophosphorus (OP) compounds were first discovered by German scientists in the late 1930's as a by-product of nerve gas research. The group includes many general purpose insecticides with a wide range of toxicity to fishes (malathion, parathion, diazinon, chlorpyrifos and phosmet) as well as compounds that work as fumigants and as systemics (dimethoate, disulfoton and ronnel). All the organophosphates are nerve poisons. They block the active site of the enzyme acetylcholinesterase (AChE) that breaks down and hydrolyses a neurotransmitter substance, acetylcholine (ACh), from the nerve synapse. The excessive increase of acetylcholine results in symptoms of hyperactivity, including tremors, convulsions, and eventually death. Extensive use of OP compounds has resulted in the wide spread distribution of these chemicals in the environment. They are much less persistent than the organochlorines and do not accumulate in fatty tissues. Due to their rapid biodegradability and lesser persistency in the environment, the OP compounds replaced the more persistent organochlorine compounds (ANEES, 1975). Organophosphorous pesticides (OPs) to a large extent replaced the persistent chlorinated pesticides in the 1970s and at the beginning of 1980s. The main advantage of the OPs is their low cumulative ability and short-term persistence in the environment (ÖZCAN et al., 2006).

These pesticides leave residues in the soil and water for several days after their application, and pose a constant threat to non-target organisms, especially fish (MAGARE and PATIL, 2000). Therefore, assessment of protein metabolism (KAPIL and RAGOTHAMAN, 1999; MUSHIGERI, 2003) and neural physiology may be considered to be a diagnostic tool to determine the physiological process of the cell. Hence, an attempt was made in the present study to evaluate the effect of malathion on some biochemical aspects of the liver in the freshwater fish, *Labeo rohita* exposed to sublethal concentrations.

Materials and methods

Labeo rohita fingerlings (3 ± 0.5 g, 5 cm) were collected from the state fisheries department, Dharwad, Karnataka, India and acclimated to laboratory conditions for a period of 15 d in large glass aquaria, previously washed with potassium permanganate to free the walls from microbial infection, if any. The fish were maintained in dechlorinated tap water of the quality used in the test and renewed three times a week, whose physico-chemical characteristics were analyzed following the methods mentioned in (ANONYM.,1998) and found as follows: Temperature: 24 ± 2 ^{0}C, pH: 7.2 ± 0.3 at 24 ^{0}C, Dissolved oxygen: 7.9 ± 0.7 mg/L, Total hardness: 23.4 ± 3.4 mg as $CaCo_3$/L, Salinity: nil, Specific gravity: 1.00374, Conductivity less than10 µS/cm, Calcium: 14.88 ± 0.92 mg/L, Phosphate: 0.389 ± 0.002 µg/L and Magnesium: 0.78 ± 0.3 mg/L. A photoperiod

of 12-16 L was maintained throughout. The fish were well reared and fed with rice bran and groundnut oil cake to keep the test animals in a normal metabolic state.

An acute toxicity (LC_{50}) test by the static renewal bioassay method was conducted to determine the toxicity of malathion (50% emulsified concentration, EC) in the freshwater fish, *L. rohita* which were exposed to various concentrations of malathion for 96 h and the pesticide was procured from the scientific fertilizer company Pvt. Ltd., Tiruchirapalli, Tamil Nadu, India. The expiry date of the test substance checked prior to initiation of the treatment was found suitable for the exposure. The required quantity of malathion was drawn directly from this emulsified concentration using a variable micropipette. After 96 h of exposure the data obtained was subjected to Finney's probit analysis method (FINNEY, 1971) and Dragstedt-Beheren's equation (CARPENTER, 1975) as mentioned by BHARGAVA and RAWAT (1999) to determine LC_{50} value. The concentration at which 50% survival/mortality occurred in malathion treated fishes was taken as the median lethal concentration (LC_{50}) for 96 h, which was 9.0 µl/L. One tenth of the LC_{50} value (0.9 µl/L) was taken for the sublethal studies according to SPRAGUE (1973).

Estimation of soluble, structural and total proteins. The soluble, structural and the total proteins in the liver were estimated using the folin-phenol reagent method as described by LOWRY et al. (1951). 1% homogenate (W/V) was prepared in an ice-cold 0.25 M sucrose solution. For soluble and structural proteins, 1.0 mL of the homogenate was taken and centrifuged at 3000 rpm for 10 min. The supernatant was separated and to both the supernatant and residue, 3 mL of 10% trichloroacetic acid (TCA) was added and again centrifuged at 3000 rpm. The supernatants were discarded and the residues were taken for experimentation. For total proteins, 1 mL of homogenate was taken; to it 3 mL of 10% TCA was added and centrifuged at 3000 rpm. The supernatant was discarded and the residue was taken for experimentation. All three residues were dissolved in 5 mL of 0.1 N sodium hydroxide and to 1 mL of each of these solutions, 4 mL of reagent -D (mixture of 2% sodium carbonate and 0.5% copper sulphate in 50:1 ratio) was added. The samples were allowed to stand for 10 min, at the end of which 0.4 mL of folin-phenol reagent (diluted with double distilled water in 1:1 ratio before use) was added. Finally, the optical density of the colour developed was measured using a spectrophotometer at a wavelength of 600 nm. A mixture of 4 mL of reagent-D and 0.4 mL of folin-phenol reagent was used as a blank. Bovine serum albumin was used for the preparation of protein standards. The protein content is expressed as mg/g wet wt. of the liver.

Estimation of free amino acids: A free amino acid level in the liver was estimated by the ninhydrin method as described by MOORE and STEIN (1954). 5% organ homogenates (W/V) were prepared in 10% TCA and centrifuged at 2000 rpm for 15 min. To 0.2 mL of supernatant, 2.0 mL of ninhydrin reagent was added and the contents were boiled for exactly 5 min. They were cooled under tap water and the volume was made to 10 mL

with distilled water. The optical density of the colour developed was measured using a spectrophotometer at a wavelength of 570 nm. A blank using distilled water and amino acid standards were also run similarly. The free amino acid levels are expressed as mg amino acid nitrogen released/g wet wt. of the liver.

Estimation of protease activity. Protease activity in the liver was estimated using the ninhydrin method as described by DAVIS and SMITH (1955). 1% homogenate (W/V) was prepared in distilled water. To 2.0 mL of homogenate 0.5 mL of 1% casein and 2.0 mL of 0.1 M phosphate buffer (pH 5.0) were added. The contents were mixed well and incubated at 30 ^{o}C for 30 min. The reaction was stopped by adding 2 mL of 2% ninhydrin reagent. Again the contents were mixed thoroughly and placed in a boiling water bath for 20 min. The solution was cooled and made to 10 mL with diluents (distilled water and n-propanol in 1:1 ratio). The optical density of the colour developed was measured using spectrophotometer at a wavelength of 570 nm. A blank taking 2.0 mL of distilled water and a control taking 2.0 mL of boiled enzyme were also run similarly. Amino acid standards were prepared alongside for comparison. The protease activity is expressed as μ moles amino acid nitrogen released/mg protein/h.

Estimation of acetylcholine (ACh) content. The tissue ACh content was estimated by the method of Hestrin, as described by AUGUSTINSON (1957). After isolating and weighing, the liver was teased and transferred into tubes, already kept in the boiling water bath for 10 minutes, to inactivate the enzyme acetylcholinesterase and to release the bound ACh. The tubes were cooled and the contents were homogenized in 2.0 mL of distilled water, 2.0 mL of alkaline hydroxylamine hydrochloride and 1.0 mL of 1:1 diluted HCl with water was added to the supernatant. The optical density of the sample was measured at 540nm in a spectrophotometer against the blank. The blank consisted of 2.0 mL of distilled water, 2.0 mL of alkaline hydroxylamine hydrochloride, 1.0 mL of diluted HCl and 10 mL of ferric chloride solution. A standard graph was prepared with ACh and the values were expressed as μM of ACh/g wet wt. of liver.

Estimation of acetylcholinesterase (AChE, EC 3.1.1.7) activity. Acetylcholinesterase activity was estimated by the method of METCALF (1951). 3% homogenate of liver was prepared in cold 0.25 M sucrose solution and the homogenate itself was used for the enzyme assay. The reaction mixture of 3.0 mL contained 12 μM of acetylcholine chloride, 100 μM of sodium phosphate buffer (pH 7.4) and 1.0 mL of homogenate. After incubating at 37 ^{0}C for 30 min and the reaction was stopped by adding 2.0 mL of alkaline hydroxylamine hydrochloride solution followed by 1.0 mL of HCl (1:1, HCl:H_2O). The unincubated samples were treated with 2.0 mL of alkaline hydroxylamine hydrochloride followed by 1.0 mL of HCl prior to the addition of the homogenate. The contents were thoroughly mixed and filtered. To the clear filter 1.0 mL of 0.37 M ferric chloride solution was added and the colour was read at 540 nm in a spectrophotometer using the blank. The

blank preparation is the same as the homogenate. The experimental except distilled water substitutes as μM of acetylcholine hydrolyzed/mg protein/h.

Statistical analysis. The data were subjected to analysis of variance and the means were compared by Duncan's new multiple range to test at 0.05% confidence level (DUNCAN, 1955) to find the mean comparison among the results.

Results and discussion

Biochemical alterations in the liver of the fish exposed to malathion were present (Table 1, 2 and 3). The total, structural and soluble proteins and AChE activity were decreased, whereas free amino acids, protease activity and ACh were found to increase in fish exposed for 5 and 15 d. But in fish treated with malathion for 25 d all the values were nearer to the control and recovering to normalcy.

The decrement in the total, structural and soluble proteins suggests the existence of high proteolytic activity, and impairment in the protein biosynthesis. The decrease in protein levels may also be due to their degradation. The degradation is due to oxidative stress (HAI et al., 1995) which is a characteristic of OP compounds, besides their inhibitory effect on AChE. Oxidative stress also induces changes in free radical production. When the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage and death. All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals (CHEESEMAN and SLATER, 1992).

The degradation products may in turn be fed into a tricarboxylic acid cycle, through the aminotransferase system, to cope with the high energy demands augmented during malathion stress (MALLA REDDY, 1987; BASHAMOHIDEEN, 1988). Total, Structural and soluble protein contents were depleted in the liver tissue exposed to the lethal concentration of fenvalerate, indicating the breakdown of these proteins due to the acute pesticide toxic stress, and generally the breakdown of proteins dominates over synthesis under enhanced proteolytic activity (DAVID et al., 2004). To have a more precise understanding of the variation among structural, soluble and total proteins, the ratios, namely Sop/Tp, Stp/Tp and Sop/Stp, were calculated. The Stp/Tp values were correspondingly high over Sop/Tp and Sop/Stp values. These ratios clearly indicate that recycling of soluble proteins was considerably higher compared with structural proteins. However, the net protein budget was balanced at the total proteins level. Hypoprotenemia was observed in the liver of fish exposed to OP pesticides by various investigators, thus supporting the findings of the present study (RAMALINGAM, 1982; DEVA PRAKASH RAJU, 2000).

The elevation of free amino acids has a functional relevance so as to meet energy demands and is also involved in osmoregulation (MURTHY, 1984). The increase in protease activity under stress conditions clearly suggests that malathion induces high protease activity, which leads to the formation of higher free amino acid content,

causing hepatotoxicity. The liver is found to be affected more than any other tissues (MALLA REDDY, 1987; BASHAMOHIDEEN, 1988) because it is the metabolic centre for detoxification and also the host in absorbing greater OP pesticide residues. The restoration of the protein level to normalcy indicates that after 15 d of exposure an oscillatory phase on protein turnover seems to exist towards a more synthetic phase or a less degradative phase, leading to the establishment of recuperation and adaptive phenomena. Table 2. Protease activity (μM of amino acids/mg protein/h) and free amino acid (mg/g wet wt.)in the liver of the fish, *Labeo rohita,* following exposure to sublethal concentrations of malathion.

Table 1. Total, structural and soluble proteins (mg/g wet wt.) in the liver of the fish, *Labeo rohita* following exposure to sublethal concentration of malathion

Parameters	Control	Sublethal exposure period in days		
		5	15	25
Totalproteins (Tp)	141.51[a]	108.61[c]	79.48[d]	109.65[b]
± SD	2.76	1.94	1.98	2.33
% change	-----	-23.2492	-43.8344	-22.5143
Structural proteins (Stp)	94.22[a]	64.41[c]	48.94[d]	72.19[b]
± SD	2.12	1.81	2.37	1.68
% change	-----	-31.6387	-48.0577	-23.3814
Soluble proteins (Sop)	46.11[a]	41.54[b]	29.89[d]	36.17[c]
± SD	4.39	3.04	1.30	3.07
% change	-----	-9.9111	-35.1768	-21.5571
Sop/Tp	0.325	0.382	0.376	0.329
% change	-----	17.5385	15.6923	1.2308
Stp/Tp	0.665	0.593	0.615	0.658
% change	-----	-10.8271	-7.5188	-1.0526
Sop/Stp	0.484	0.644	0.610	0.50
% change	-----	33.0579	26.0331	3.3058

Means are ± SD (n = 6) for a tissue in a column followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test

Table 2. Protease activity (µM of amino acids/mg protein/h) and free amino acid (mg/g wet wt.) in the liver of the fish, *Labeo rohita* following exposure to sublethal concentration of malathion.

Parameters	Control	Sublethal exposure period in days		
		5	15	25
Protease activity	0.576 [c]	0.592[d]	0.619[a]	0.551[b]
± SD	0.021	0.011	0.019	0.012
% change	----	+2.89	+6.32	-4.33
Free amino acid	5.142[g]	5.243[d]	5.478[a]	5.923[e]
± SD	0.086	0.080	0.072	0.044
% change	----	+1.98	+6.25	+15.19

Means are ± SD (n = 6) for a tissue in a column followed by the same letter are not significantly different (P≤ 0.05) from each other according to Duncan's multiple range (DMR) test.

Table 3. AchE activity (MM of acetyl choline hydrolyzed/mg protein/h) and Ach level (MM/g wet wt.) in the liver of the fish, *Labeo rohita,* following exposure to sublethal concentrations of malathion.

Parameters	Control	Sublethal exposure period in days		
		5	15	25
AchE activity	2.033[d]	1.698[a]	1.797[b]	1.948[c]
± SD	0.008	0.007	0.004	0.005
% change	----	-16.44	-11.57	-4.14
Ach	17.594[a]	17.804[d]	18.111[e]	17.605[f]
± SD	0.0023	0.003	0.005	0.004
% change	----	-1.189	-2.93	-0.005

Means are ± SD (n = 6) for a tissue in a column followed by the same letter are not significantly different (P≤ 0.05) from each other according to Duncan's multiple range (DMR) test.

Decrease in AChE activity and increase in ACh levels is due to the inhibition of AChE activity and consequent accumulation of ACh. Acetylcholinesterase is an enzyme that modulates the amount of neurotransmitter substance at neuron junctions (O' BRIEN, 1967). The inhibition of AChE and elevation in ACh content may be due to the decreased ionic composition in the liver exposed for 5 and 15 d, which is in accordance with the earlier reports, thus supporting the findings of the current investigation (PARMA DE CROUX et al., 2002). Increase in AChE activity and decreased ACh content in 25 d may

be due to rapid detoxification of malathion as the liver is a major site for detoxication, assuming that the pesticide concentration is within the threshold limit. This nominal concentration might be rapidly detoxified leaving less for inhibitory activities.These results reflect the fact that organisms are adapting to the sublethal concentration, which seems to be strategic and adaptive.

Conclusion

Thus it is inferred that exposure to malathion in sublethal doses affects protein metabolism and the normal neural physiology of the liver. But recovery in later periods may be a revitalization phenomenon as every organism strives to overcome stress to prove its existence. Recovery phenomenon may be adaptive and even strategic.

References

ANONYMOUS (1998): American Public Health Association: Washington D.C., Standard methods for the examination of water and waste water.

ANONYMOUS (2005): United States Environmental Protection Agency Technical Report.

ANEES, M. A. (1975): Acute toxicity of 4-organophosphorus insecticides to a freshwater teleost, *Channa punctatus.* Pak. J. Zool. 7, 135-141.

AUGUSTINSON, K. B. (1957): Methods in Biochemical analysis. Vol. 5 (Ed. Glick, D) Interscience Publishers. New York. pp. 5.

BASHAMOHIDEEN, M. D. (1988): Toxicity evaluation of commercial grade malathion on the freshwater fish, *Cyprinus carpio.* Environ. Ecol. 6, 488-490.

BHARGAVA, S., M. RAWAT (1999): Toxicity of some pesticides to the fish, *Heteropneustes fossilis.* Ad. Bios. 181, 23-26.

CARPENTER, P. L. (1975): Immunology and Serology, 3rd ed. W.B. Saunders Co. Philadelphia, London. Toronto. pp. 254.

CHEESEMAN, K. H., J. F. SLATER (1992): An introduction to free radical biochemistry. In: Free Radicals in Medicine. New York, Churchill Livingstone, pp. 481-493.

DAVID, M., S. B. MUSHIGERI, R. SHIVAKUMAR, G. H. PHILIP (2004): Response of *Cyprinus carpio* to sublethal concentration of cypermethrin: Alterations in protein metabolic profiles. Chemosphere 56, 347-352.

DEVA PRAKASH RAJU, B. (2000): Malathion induced changes in the protein metabolism of freshwater fish, *Tilapia mossambica*. Ph.D Thesis. S.K.University, Anantapur, Andhra Pradesh. India.

DAVIS, N. C., E. L. SMITH (1955): Assay of proteolytic enzymes. Met. Biocem. Anal. 2, 215-257.

DUNCAN, D. M. (1955): Multiple range and multiple tests. Biometrics 42, 1-4.

FINNEY, D. J. (1971): Probit Analysis. 3rd ed., Cambridge University Press, London, UK.

HAI, D. Q., I. S. VARGA, B. MATKOVICS (1995): Effect of organophosphates on the antioxidant systems of fish tissues. Acta. Biol. Hung. 46, 39-50.

HILL, E. F. (2003): Wildlife toxicology of organophosphorus and carbamate pesticides. In: Handbook of Ecotoxicology. (Hoffman, D. J., B. A. Rattner, G. A, Burton Jr., J. Cairns Jr., Eds.), Lewis Publishers, Boca Raton, pp. 281-312.

KAPIL, M., G. RAGOTHAMAN (1999): Mercury, copper and cadmium induced changes in the total proteins in muscle tissue of an edible estuarine fish, *Boleophthalmus dussumeri* (Cuv.). J. Environ. Biol. 20, 231-234.

LOWRY, O. H., N. J. ROSENBROUGH, A. L. FARR, R. J. RANDAL (1951): Proteins estimation by folin phenol method. J. Biol. Chem. 193, 265-275.

MAGARE, S. R., H. T. PATIL (2000): Effect of pesticides on oxygen consumption. Red blood cell count and metabolites of fish, *Puntius ticto.* Environ. Ecol. 18, 891-894.

MALLA REDDY, P. (1987): Modulations in protein profiles of white muscle of *Cyprinus carpio* under malathion stress. Biomed. 7, 21-24.

METCALF, R. L. (1951): Methods in Biochemical Analysis (Glick, D., Ed.), Interscience Publishers, New York. p. 5.

MOORE, S., W. H. STEIN (1954): A modified ninhydrin reagent for the photometric determination of amino acids and treated compounds. J. Biol. Chem. 211, 907-913.

MURTHY, K. S., B. KASI REDDY, K. S. SWAMI, C. S. CHETTY (1984): Changes in respiration and ionic content in tissues of mussel exposure to methyl parathion toxicity. Toxicol. Lett. 21, 287-291.

MUSHIGERI, S. B. (2003): Effect of fenvalerate on the metabolism of Indian major carp *Cirrhinus mrigala.* Ph.D. Thesis, Karnataka University, Dharwad, Karnataka, India.

O' BRIEN, R. D. (1967): Insecticides Action and Metabolism. Academic Press. New York.

ÖZCAN ORUÇ, E., N. ÜNER, Y. SEVGILER, D. USTA, H. DURMAZ (2006): Sublethal effects of organophosphate diazinon on the brain of *Cyprinus Carpio*. Drug Chem. Toxicol. 29, 57-67.

PARMA DE CROUX, M. J., A. LOTESTE, J. CAZENAVE (2002): Inhibition of plasma cholinesterase and acute toxicity of monocrotophos in neotropical fish, *Prochilodus lineatus.* (Pisces, Curimatidae). Bull. Environ. Contam. Toxicol. 69, 356-363.

PRAKASAM, A., S. SETHUPATHY, S. LALITHA (2001): Plasma and RBCs antioxidant status in occupational male pesticide sprayers. Clin. Chim. Acta. 310, 107-112.

RAMALINGAM, K. (1982): Effects of sublethal levels of DDT, malathion and mercury on tissue proteins of *Sarotherodon mossambicus.* Ind. Acad. Sci. 6, 501-505.

SPRAGUE, J. B. (1973): Measurement of pollutant toxicity of fish, utilizing and applying bioassay results. Mars. Res. 4, 3-32.

STORM, J. E., K. R KARL, J. DOULL (2000): Occupational exposure limits for 30 organophosphate pesticides based on inhibition of red blood cell acetylcholinesterase. Toxicol. 150, 1-29.

Received: 12 January 2008
Accepted: 21 December 2008

PATIL, V. K., M. DAVID: Mogućnost hepatotoksičnoga djelovanja malationa u slatkovodnoga teleosta *Labeo rohita* (Hamilton). Vet. arhiv 79, 179-188, 2009.

SAŽETAK

Slatkovodna jestiva riba *Labeo rohita* bila je izložena subletalnoj koncentraciji (0,9 µL/L) komercijalnoga pripravka malationa (50% emulzija) tijekom 5, 15 i 25 dana. Nakon razdoblja izloženosti jetra je bila uzeta za istraživanje biokemijskih poremećaja. Pet i 15 dana nakon izloženosti ustanovljena je povećana koncentracija slobodnih masnih kiselina, pojačana aktivnost proteaza i povećana razina acetilkolina, dok se smanjila ukupna količina proteina i aktivnost acetilkolinesteraze. Nakon 25 dana izloženosti za sve su pokazatelje ustanovljene gotovo normalne vrijednosti. Uspostava normalnih vrijednosti proteinskih frakcija, slobodnih masnih kiselina, aktivnosti proteaza, razine acetilkolina i aktivnosti acetilkolinesteraze nagovješćuje da se nakon 15 dana izloženosti javlja kolebajuća faza u metabolizmu s pojačanom sintezom proteina što dovodi do oporavka i prilagodbe novim uvjetima.

Ključne riječi: *Labeo rohita*, jetra, toksičnost, malation