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HISTOPATHOLOGY OF LARVAE CARP BIOMARKER OF POLLUTION WITH CYANOBACTERIA OF SHKODRA LAKE WATER

Përmbledhje: Qëllimi i këtij studimi është të përcaktohet se si ndikon ekstrakti i cianobaktereve në zhvillimin larvar të krapit në bazë të testit të toksicitetit dhe të evidentohen ndryshimet histologjike në mëlçinë e larvave të krapit të ekspozuara për 30 ditë ndaj ekstraktit të cianobaktereve me përqëndrim 9.0 µg l–1 (përqendrimi i mesëm i ekstraktit) dhe 0.9 µg l–1 (përqendrim i ulët i ekstraktit) të mikrocistinës LR, RR dhe YR. Eksperimenti zgjati 30 ditë. Vlerësimi i mostrave u bë në bazë të guidës 210 të OECD të vitit 1992 për vlerësimin e kimikateve. Ngjyrosja u bë me haematoxylin-eosin dhe vlerësimi u bë me mikroskop të zakonshëm. U vëzhgua që përqendrimi i mesëm i toksinës shkaktonte rritje të numrit të larvave të keqformuara dhe rritje të vdekshmërisë. Përqendrimi i ulët i toksinës shkakton rritje të numrit të larvave të vdekura. Distrofia vakuolare e hepatociteve e shoqëruar me dëmtim të bërthamës u vu re në grupin e larvave të ekspozuara ndaj përqendrimit të ulët të toksinës. Nekrozë fokale dhe ndryshime të hepatociteve të shoqëruara me vakuolizim dhe dëmtim të bërthamës (piknozë e kariolozë) u gjet në grupin e larvave të ekspozuara ndaj përqendrimit të mesëm të toksinës. Shkalla e dëmtimit të larvave varet nga përqendrimi i ekstraktit.

Fjalë kyçe: *hepatocit, cianotoksina, keqformime, peshk, test i toksicitetit embrion-larvor*

Abstract: The aim of this study was to find out how crude extract of cyanobacteria can influence larval development of carp on the basis of embryo-larval toxicity test and histological changes of liver of larvae carp exposed 30 days to the crude extract of cyanobacteria with the cumulative concentration 9.0 µg l–1 (medium concentration of the extract) and 0.9 µg l–1 (low concentration of the extract) of microcystins LR, RR and YR. The experiments were finished after 30 days. Evaluation of the tests was based on the OECD guideline for testing chemicals, direction 210 from 1992. Liver sections were stained with haematoxylin-eosin and using light microscopy. The extract with medium concentration caused an increase in malformed and dead larvae. The extract with low concentration caused an increase in dead larvae. Vacuolar dystrophy of hepatocytes accompanied by damage of nuclei (pyknosis) was found in the group exposed to the low concentration of the extract. Focal necro-

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ses and dystrophic changes of hepatocytes with vacuolization and nuclei damage (pyknosis, karyolysis) were found in the group exposed to the medium concentration of the extract. The degree of damage depended on the concentration of the extract.

Key words: *Hepatocyte, cyanotoxins, malformations, fish, embryo-larval toxicity test*

INTRODUCTION

Cyanotoxins produced by cyanobacteria pose an environmental problem and influence the health status of both human and aquatic organisms. The most common cyanotoxins are hepatotoxins.

Many authors examined the histopathological findings and the mechanism of influence of microcystins. Deformation of hepatocytes is the most pronounced effect (Falconer & Yeung, 1992). These authors concluded that the mechanism of microcystin toxicity to the hepatocyte is through cytoskeletal damage leading to loss of cell morphology, cell to cell adhesion and finally cellular necrosis. Toxicity of microcystin LR *in vivo* primarily consists in the hepatocellular deformation inducing degenerative changes of the tissue (Eriksson *et al.*, 1989).

Recently, research into this area has also been aimed at the evaluation of effects of cyanotoxins to the early life stages of organisms. The effect of microcystins and the crude extract of cyanobacteria on the development of fish and amphibians were studied (Oberemm *et al.*, 1997, 1999) without description of histopathology of fish embryos and larvae. The level of dissolved microcystins in the Shkodra Lake was measured in south coast where untreated waste water are deposited. The level of dissolved microcystins in the Shkodra Lake was measured at IPH Tirane. The results of embryo-larval tests in carp exposed to crude extract of cyanobacteria containing the known amount of microcystins are presented in this study. Concentrations of microcystins were chosen to compare the results with literature and to be compared with the level of dissolved microcystins in natural waters.

MATERIALS AND METHOD

The carp eggs were obtained by artificial reproduction at the fishery in Laknas (Albania). Fertilised and unsticked carp eggs were divided into three groups till 8 hours from fertilisation, each containing two hundred eggs. The eggs were incubated in glass vials containing 1 L of water. The water was changed every 8 hours including the crude extract of cyanobacteria to keep the concentration of crude extract of cyanobacteria. The conditions in baths were as follows water temperature 21–22 °C, dissolved oxygen 75–100%, i. e. 5.5–10 mgL^{-1} and pH was 8–9. The larvae were fed by commercial food Artemia Premium since the 5 th day. Feeding was performed before 20–30 min water changing intervals. Tests were performed with the crude cell extract obtained from field samples of water bloom (Shkodra Lake). Water samples contained the planktonic species *M. aeruginosa (*90%), *Microcystis ichtyoblabe* (4%) and *Aphanizomenon flos-aquae* (2%). The samples were collected from surface water bloom (0.3 m depth) and concentrated by plankton net 25 μm. The sam-

ples were stored frozen at -20°C. The concentration of microcystins was determined by HPLC according to the method described by Lawton *et al*., 1994. Total microcystin concentration (MC) was 1 056.2 $\mu g \cdot g^{-1}$ dry weight in the biomass. To obtain the crude extract, the material was ultrasonicated for 7 min. and was centrifuged for 20 min at 4 500 rpm. Re-extraction was done twice by standard water. The final concentration of hepatotoxic microcystins in the crude extract used for exposure was 17.3 $\mu g \cdot L^{-1}$ (4.8 $\mu g \cdot L^{-1}$ of microcystin YR, 9.59 $\mu g \cdot L^{-1}$ of microcystin LR, 2.9 $\mu g \cdot L^{-1}$ of microcystin RR).

Experimental treatments

The crude extract of cyanobacteria with known amount of microcystin LR (5 and 0.5 $\mu g \cdot L^{-1}$) was added to the eggs in two concentrations: the first with 0.5 µg·L–1 of microcystin LR (low concentration of the extract) the second with 5 $\mu g \cdot L^{-1}$ of microcystin LR (medium concentration of the extract). The controlled eggs were incubated in toxic free water. The cumulative amount of microcystins was 9 and 0.9 $\mu g \cdot L^{-1}$, respectively. Tests were finished after 30 days. Evaluation of the tests was based on the OECD Direction 210 from 1992.

During the test we observed:

– time of start and the end of hatching;

– numbers of larvae hatching each day;

– numbers of malformed larvae.

After finishing the tests, we evaluated:

– cumulative mortality;

– numbers of healthy fish at the end of the test;

– average total length and body mass (the average total length was determined in 10 larvae and body mass in 20 larvae).

Histology: Five fish from each group were killed, immediately fixed in Bodian solution and processed using standard methods for histology. Tissue sections were stained with haematoxylin-eosin and cells were detected with H&E test. All sections were examined using light microscopy. Liver tissues were examined.

RESULTS

Larvae hatched during three days. In the control group, the majority of larvae hatched in the second day. In the groups with medium and low concentrations of the extract it was similar, the majority of larvae hatched in the first day. Total numbers of hatched larvae were 186 in the control, 187 in the group with low concentration of the extract and 186 in the group with medium concentration of the extract. Results of tests with egg hatching are presented in Table 1. Malformed and dead larvae (numbers of malformed and dead larvae) are presented in Table 2. In the control group 5 malformed larvae were found (2.68% from 186 hatched larvae), 8 in the group with low concentration of the extract (4.27% from 187 hatched larvae) and 10 in the group with medium concentration of the extract (5.37% from 186 hatched larvae) during the experiment.

15 larvae died during the experiment in the control group (8.06% from 186 hatched larvae), 21 in the group with low concentration of the extract (11.23% from 187 hatched larvae) and 36 in the group with medium concentration of the extract 19.35% from 186 hatched larvae).

Cumulative mortality is presented in the Table 2. In the control group 171 larvae survived. In the group with low concentration of the extract 166 larvae survived and in the group with medium concentration of the extract were 150 surviving larvae. Average total length and body mass of surviving larvae (no significant differences in the average total length and body mass were found, see Table 2).

Table 1. Egg hatching and malformations

Concentration of the extract	Start of hatching	End of hatching	Numbers of hatched larvae for a day			Percentage of malformed larvae
			3 rd day	4 th day	5 th day	
Medium	58	102	156	28	2	5,37
Low	58	102	121	65	1	4,27
Control	58	102	60	125	1	2,68

Table 2. Fry measurements and survival

Concentration of the extract	Cumulative mortality (%)	Average total length (mm ± SD)	Average total body mass (mg ± SD)	Percentage of dead larvae (%)
Medium	25	13,87±2,09	34,17±15,3	19,35
Low	17	14,21±1,41	29,4±12,11	11,22
Control	14,5	14,56±2,1	30,81±12,48	8,06

Histology

No changes in liver were found in the control group. Vacuolar dystrophy of hepatocytes with damage of nuclei (pyknosis) was found in the group exposed to low concentration of the extract (Photo 1). These changes were found in all sampled larvae. Focal necroses and dystrophic changes of hepatocytes with vacuolisation and nucleic damage pyknosis, were found in the group exposed to the medium concentration of the extract (Photo 2). These changes were found in all sampled larvae.

DISCUSSION

The concentrations of crude extract of cyanobacteria (5 and 0.5 μg·L–1 of microcystin LR) had effects even at lower concentrations, in particular, in the group exposed to the medium concentration of the extract. Cumulative mortality was higher and the number of malformed individuals was increased. Neither the total length nor the body weight of larvae was significantly altered. Histopathological changes of liver in our study were similar to the changes described in various papers in young and adult fish (Rodger *et al.*, 1994), which described the histopathological changes

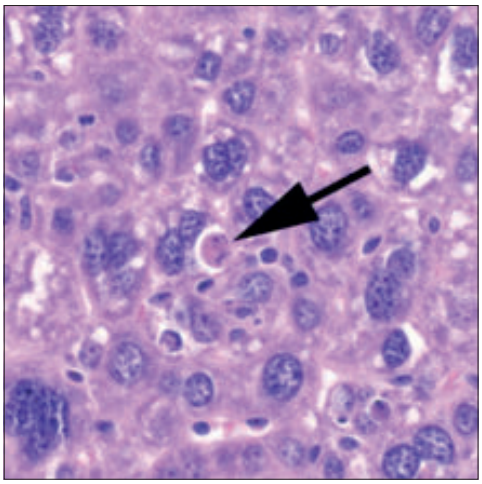

Photo 1. Vacuolar dystrophy of hepatocytes with pyknosis and karyolysis of nuclei in liver of carp with low concentrationof the extract (→H&E × 400).

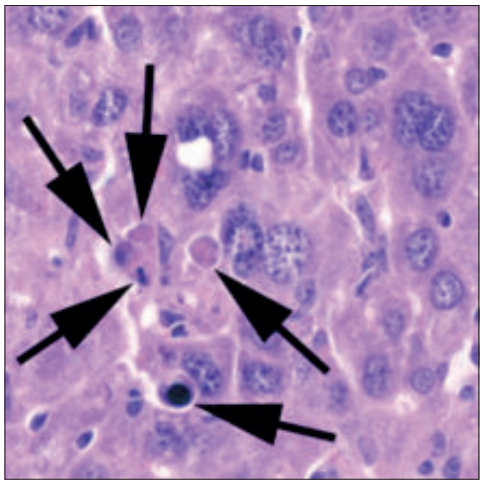

Photo 2. Liver of carp with medium extract concentration. Perivascular focal necrosis (→H&E × 400)

of brown trout (*Salmo trutta*) associated with the death of water blooms of *Anabaena flos-aquae*.

The changes in liver were characterised by confluent necrosis showing cellular degeneration and loss of obvious cell boundaries. Pyknosis and karyorrhexis of hepatocytes are obvious. Similar changes in liver have been described in different fish species by other authors (Carbis *et al*., 1996), which detected histopathological changes in the gills, in liver and kidney of carp exposed to microcystins by gavage, immersion and intraperitoneal administration. Intraperitoneal inoculation caused necrosis or dosedepended degeneration. Gavaging caused changes in the histopathology of the liver and gills. Cellular degeneration and necrosis occurred in the liver, gills and kidneys when carp were introduced to a tank containing 1.7 μg·ml–1 of microcystins. Carbis *et al*., 1997 studied carps exposed to Microcystis aeruginosa at Lake Mokoan (Australia). The total concentration of the microcystins was approximately 4.0 μg·g–1 of the lyophilised scum material. During February, March and April the liver histology was characterised by cytoskeletal collapse, cytoplasmic vacuolization, pyknosis, chromatin margination, eosinophilia and widespread hepatocyte atrophy, particularly in areas close to the arterial blood supply in about 66% of the carp examined. We detected damage of liver in fish. The degree of damage depended on the concentration of the extract.

CONCLUSION

In conclusion we can say that the medium and low concentration of the extract corresponds with the level of dissolved microcystins in lake water. Then it could negatively influence the tissue of larvae of various fish species in natural waters.

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