ELSEVIER

Toxicon 51 (2008) 449–456

TOXICON

www.elsevier.com/locate/toxicon

Dose-dependent effects of extracted microcystins on embryonic development, larval growth and histopathological changes of southern catfish (*Silurus meridionalis*) ☆

Xuezhen Zhang[a], Ping Xie[b,a,*], Weimin Wang[a], Dapeng Li[a,b], Li Li[a], Rong Tang[a,b], Hehua Lei[b], Zechao Shi[c]

[a]*Fisheries College of Huazhong Agricultural University, Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Wuhan 430070, PR China*

[b]*Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China*

[c]*Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Jinzhou 434000, PR China*

Received 27 May 2007; received in revised form 5 November 2007; accepted 6 November 2007
Available online 17 November 2007

Abstract

A laboratory toxic experiment was conducted to examine dose-dependent effects of extracted microcystins (MCs) on embryonic development, larval growth and histopathological changes of southern catfish (*Silurus meridionalis*). Fertilized eggs were incubated in solutions with four concentrations of MCs (0, 1, 10, 100 μg MC-LReq l^{-1}). Higher MCs retarded egg development (2–10 h delays) and larval growth, reduced hatching rate (up to 45%), and caused high malformation rate (up to 15%) and hepatocytes damage (characterized by disorganization of cell structure and a loss of adherence between hepatocytes, cellular degeneration with vacuolar hepatocytes and marginal nuclei, even hepatocellular necrosis). A 10 μg MC-LReq l^{-1} is close to a high concentration in natural cyanobacterial blooms, suggesting a possible existence of such toxic effects in eutrophic waters.

Keywords: Microcystins; Crude extract; Southern catfish; Embryonic development; Larval growth; Histopathological changes

☆ *Ethical statement*: This is an original research paper examining the dose-dependent effects of extracted microcystins on embryonic development, larval growth and histopathological changes of southern catfish (*Silurus meridionalis*). Submission of this paper implies that it has not been published previously, that it is not under consideration for publication elsewhere, and that if accepted it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the publisher.

*Corresponding author. Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China. Tel./fax: +86 27 68780622.
E-mail address: xieping@ihb.ac.cn (P. Xie).

0041-0101/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2007.11.005

1. Introduction

In the past decades, occurrence of cyanobacterial blooms and the associated cyanotoxins has been documented in many eutrophic inland waters worldwide (Paerl et al., 2001). Among cyanotoxins, the hepatotoxic microcystins (MCs) such as MC-LR and MC-RR are considered to be one of the most dangerous groups (Zimba et al., 2001). MCs are potent inhibitors of protein phosphatases 1 and 2A (Falconer and Yeung, 1992) with liver as the target organ, due primarily to toxin uptake by the bile acid transport system (Runnegar et al., 1993). There have been many toxicological experiments to examine MC bioaccumulation in organs of fish and to evaluate the toxic effect of MCs on physiological status and histopathology of fish. MCs accumulate mainly in liver, but also in gonad, kidney, gill and intestine, causing damages to these organs (Kotak et al., 1996; Fischer and Dietrich, 2000; Xie et al., 2004; Soares et al., 2004; Li et al., 2005; Ding et al., 2006; Zhang et al., 2007).

In recent years, several studies attribute the death of wild and domestic animals to exposure to MCs (Duy et al., 2000; Zimba et al., 2001; Mohamed et al., 2003; Bu et al., 2006), and MCs have also been considered to be responsible for human illnesses (Dawson, 1998; Codd et al., 1999; Carmichael et al., 2001). In 1996, death of over 50 patients resulted from the presence of MCs in the dialysis water in Brazil (Jochimsen et al., 1998; Carmichael et al., 2001; Azevedo et al., 2002). Epidemiological investigations have demonstrated that MCs cause stomach and intestinal inflammation, liver cancer, and disease of the spleen in humans who drink water containing MCs (Ding et al., 2006). Yu (1995) reported that the high incidence of liver cancer in humans in some areas of China was suspected to be related to drinking water containing MCs. However, little is known on the potential risk of MC on reproduction of mammals including human.

Early-life development is usually the most sensitive stage to toxins during the ontogenesis of aquatic organisms and thus is valuable in monitoring environmental pollutants (Burýšková et al., 2006). In recent years, there have been several studies to examine the toxic effect of MCs on the embryonic development and larval growth of aquatic organisms (Oberemm et al., 1997, 1999; Wiegand et al., 1999; Liu et al., 2002; Wang et al., 2005; Wright et al., 2006; Malbrouck and Kestemont, 2006; Palíková et al., 2007). Oberemm et al. (1999) report that the early life stage of fish or amphibians may be more vulnerable to cyanobacterial toxins than juveniles or adults due to lower mobility to avoid contamination. Toxic effects of MCs on embryonic development are stage-specific (Liu et al., 2002; Jacquet et al., 2004). MCs can result in retarded development, low hatching rate and high malformation (Oberemm et al., 1997, 1999; Wiegand et al. 1999; Liu et al., 2002; Jacquet et al., 2004; Wang et al., 2005). MCs are believed to affect larval or juvenile development with high mortality, growth inhibition and histopathological changes (Oberemm et al., 1997; Palíková et al., 2004). Some studies report that in embryonic or larval stages, MCs damage liver with disorganization of cell structure, deformation of nucleus, and moving of nuclei to the side of the nuclear membrane (Oberemm et al., 1997, 1999; Liu et al., 2002; Malbrouck et al., 2003; Malbrouck and Kestemont, 2006).

Many studies report that pure MC-LR can result in severe damage in embryos using a microinjection technology, and they think that external exposure to MCs is a limitation to fish embryonic development (Jacquet et al., 2004; Wang et al., 2005). However, some experiments using crude (cells) extracts as toxicants observed prominent inhibition effects of MCs on development of embryos, which were incubated in toxicant solution (Oberemm et al., 1997, 1999; Palíková et al., 2004, 2007; Burýšková et al., 2006). Compared with the effect of pure MC-LR, the effects of crude extracts were much more evident, possibly due to a high uptake rate of the toxin. Oberemm et al. (1997) report that substances of the crude extracts may increase the uptake rate of toxins, and synergistic actions of toxins and unknown substances existing in freshwater cyanobacteria could increase toxicity.

In eutrophic waters, surface blooms of cyanobacteria may accumulate near the shore where many vertebrates such as fish spend their early life stages. In China, heavy cyanobacterial blooms often occur regularly in some large shallow, eutrophic lakes (e.g. Dianchi Lake, Taihu Lake and Chaohu Lake), and in fish ponds. Southern catfish (*Silurus meridionalis* Chen), also called as large mouth catfish, is a large, obligatory carnivorous, endemic species to China, and is widely cultured in Yangtze and Zhujiang rivers and lakes in southern China. In natural environments, the progenitive time of southern catfish (during May and July) overlaps with the period of cyanobacterial blooms in southern China,

so fertilized eggs are more likely exposed to MCs. Southern catfish often have their fertilized eggs (3–4 mm in diameter) on aquatic plants or reefs, and the larger egg size as well as the hatchery circumstance increase potential risk by MCs. Moreover, hatching of fertilized eggs takes only about 2 days and larvae develop also fast. The speedy developments of southern catfish are useful for examining toxic effects of MCs as a model fish.

In view of the above facts, we chose southern catfish as test fish species to conduct a laboratory experiment with an attempt to model the natural circumstances of cyanobacterial blooms. The major objectives of this study are: (1) to examine the effects of crude extracts of MCs on embryonic development, emphasizing on hatching rate, malformation rate, development retardation and larval growth of southern catfish; and (2) to monitor histopathologic changes in liver tissue of the southern catfish. The concentrations of MCs were set according to both literatures (Oberemm et al., 1997, 1999; Palíková et al., 2004) and levels of dissolved MCs in natural waters in China (Sui et al., 2004; Xie, 2006).

2. Materials and methods

2.1. Toxin

The cyanobacterial material used in this experiment was collected from surface blooms (phytoplankton cells) of Lake Dianchi, Yunnan in China during May and June, 2006. According to microscopic examinations, the predominant species was *Microcystis aeruginosa*. Freeze-dried crude algae were extracted three times with 75% (V/V) methanol. The extract was centrifuged and the supernatant was applied to a C18 reversed phase cartridge, which had been preconditioned by washing with methanol and distilled water. The cyanobacterial material was analyzed for MCs content via reverse-phase high-performance liquid chromatography (HPLC, LC-10A, Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan) equipped with an ODS column (Cosmosil 5C18-AR, 4.6×150 mm, Nacalai, Japan) and a SPA-10A UV–vis spectrophotometer set at 238 nm. Crude extract concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR and MC-RR, Wako Pure Chemical Industries, Japan). The MCs content was 1.41 mg g^{-1} dry weight (DW), among which MC-RR, -LR and -YR were 0.84, 0.50 and 0.07 mg g^{-1} DW, respectively. Extracted crude MCs were finally suspended in distilled water.

2.2. Embryo collection

Southern catfish were artificially propagated with injection of human chorionic gonadotropin (HCG), 4000 and 2000 IU kg^{-1} for females and males, respectively. Fertilized eggs (with strong stickiness) were collected by 12 pieces of nylon nets and transported to the laboratory in the College of Fishery, Huazhong Agricultural University. The eggs from the 12 pieces of nylon nets were divided into 12 groups, each with 200 eggs. The eggs were incubated in 12 aquaria and each aquarium contained 15 l pond water (MC-free). The experiment was conducted at 23–27 °C with dissolved oxygen values between 5.0 and 7.1 mg l^{-1} by continuously aerating, in a 14-h/10-h, light/dark photoperiod.

2.3. Embryo exposure

The embryonic exposure experiment was conducted from the fertilization stage to the end of the embryonic period. Fertilized eggs were incubated in the test solution with three concentrations (1, 10,100 μg MC-LReq l^{-1}), expressed as low-, medium-, high-dose groups, and the control (with only MC-free pond water). Each concentration had three replicates. Exposure solutions were changed every 24 h until fry hatched. Dead embryos were recorded and removed. At the end of the embryonic development, fries were counted, and malformations were recorded. MC exposure expired when fry hatched. Hatching time in each group was noted on the basis of time when 90% survival eggs hatched.

2.4. Larval development

Fifty larvae sampled randomly from each aquarium were reared in toxin-free pond water (20 l) with nauplius for a period of 14 days to monitor larval development. Feeding was administered 3 days after hatching when vitellus almost disappeared, and equivalent food was provided twice everyday in each group. At the end of the experiment, 10 larvae were batch measured for whole body length and weight noted. Five fish from each group were killed, immediately fixed in Bouin solution for 24 h, dehydrated and embedded in paraffin. Sections of

6 μm were cut with a microtome and stained with hematoxylin-eosin (HE). All sections were examined using light microscopy, and photographs were taken with a video camera linked to computer image analysis software (Image-Pro Express C, USA). Experiments were performed according to the guidelines of the Ethical Committee for Animal Experiments at Huazhong Agricultural University, Wuhan, China.

2.5. Statistical analysis

Experimental values are expressed as means ± SD. The data were analyzed by one-way analysis of variance (ANOVA) and Dunnett's post test using STATISTICA software package (Version 6.0, Statsoft, Inc.). Differences were measured against control values and considered to be statistically significant at $P<0.05$.

3. Results

In the present study, alteration of the hatching time in surviving embryos was observed in both the high- and medium-dose groups (100, 10 μg l^{-1}) with, respectively, 10 and 2 h delays compared with the control (52 h). However, there was no significant difference in hatching duration between the low-dose group and the control. MCs exposure significantly reduced the hatching rates of the high-dose group (up to 45% with $P<0.01$) and the medium-dose group (up to 22% with $P<0.05$) compared with the control. Marked toxic effects ($P<0.01$) on malformation of larvae were observed after exposure of MCs, with as high as 15% and 9% malformation rates in the high- and medium-dose groups (Fig. 1), while there was not significant difference ($P>0.05$) between the malformation rates of the low-dose group (up to 4%) and the control (up to 3%). The malformed larvae were mainly characterized by curved body and tail, as well as abnormal mouth with longer mandible compared with the normal larvae.

After being cultured in the toxin-free pond water for 14 days, compared with the control, larvae showed significant reductions ($P<0.01$) in both body length (up to 56%) and weight (up to 63%) in the high-dose group, and marked reductions ($P<0.05$) in both larvae body length (up to 29%) and weight (up to 41%) in the medium-dose group were also observed (Fig. 2). On the other hand, histopathological changes were observed in the livers of the larvae hatching from embryos treated with the medium and high doses of MC, especially in the high-dose group. However, no obvious pathological changes were present in livers of the low-dose group and the control. Liver damages in the high-dose group were characterized by disorganization of cell structure and a loss of adherence between hepatocytes, vacuolar hepatocytes with pyknotic and marginal nuclei, even hepatocellular necrosis (Fig. 3). Larvae of high- and medium-dose groups exhibited depression, lethargy and sluggish swimming, and dodged in the corner of the aquarium with weak desires for food. However,

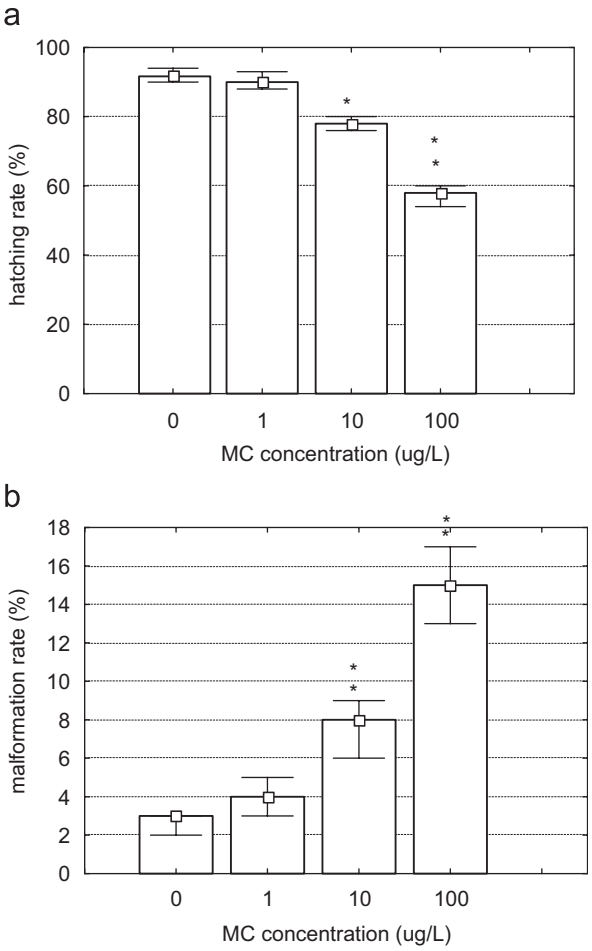

Fig. 1. Effect of crude extracte microcystins on southern catfish embryos hatching rate and malformation rate with 1, 10, 100 μg MC-LReq l^{-1}. Values are expressed as mean ± SD. Compared with the control, significant alterations ($P<0.01$) in hatching rate and malformation rate occurred in high-dose groups, and significant alterations ($P<0.05$) in hatching rate and malformation rate occurred in the medium-dose group. * indicates significant differences at $P<0.05$ between MC-treated groups and the control group.

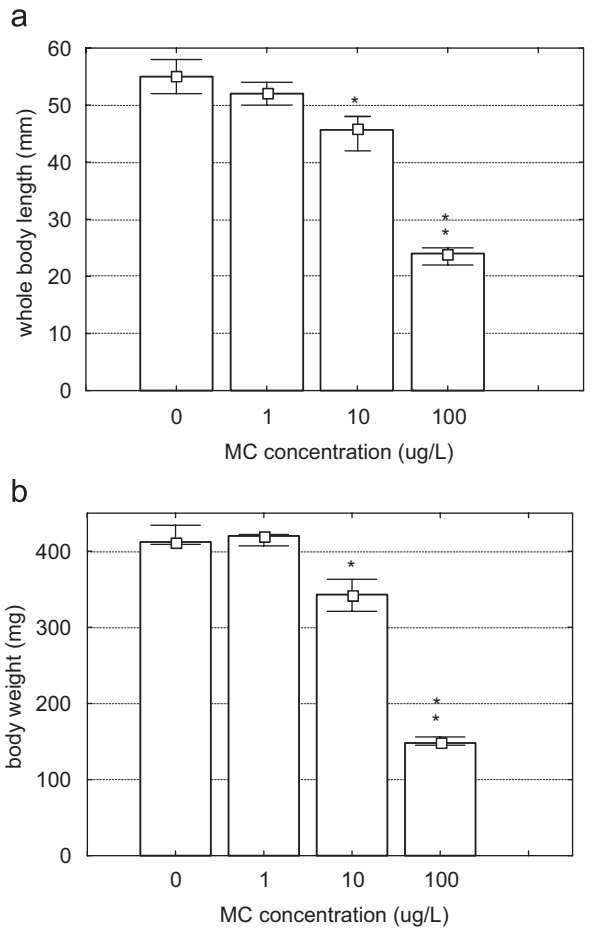

Fig. 2. Observations of southern catfish larval development (hatched from microcystins exposure) treated with toxin-free water. Values are expressed as mean ± SD. Compared with the control, significant alterations ($P<0.01$) in whole body length and body weight occurred in high-dose groups, and significant alterations ($P<0.05$) in these growth indices occurred in the medium-dose group. * indicates significant differences at $P<0.05$ between MC-treated groups and the control group.

difference in mortality was not statistically significant among the four groups.

4. Discussion

In the present study, exposure of eggs with medium and high doses of MCs (10, 100 $\mu g l^{-1}$) retarded egg and larval development of southern catfish. A few researches have also reported similar delay in development of fish eggs caused by MC exposure (Wiegand et al., 1999; Liu et al., 2002; Palíková et al., 2004). These might be due to increased energy demand for detoxification processes. However, some studies show that MC exposure results in precocious or premature phenomena in rainbow trout (*Oncorhynchus mykiss*) or in zebra fish (*Danio rerio*) embryos (Oberemm et al., 1999; Keil et al., 2002; Jacquet et al., 2004). The mechanisms of precocious phenomena still remain unclear.

In the present study, MCs exposure through submersion significantly reduced hatching rates and increased the malformation rate of southern catfish. Similar results were also reported for common carp with exposure of crude extract of cyanobacteria (*Cyprinus carpio*) (Palíková et al., 2004). However, Jacquet et al. (2004) report that effects by ambient exposure through submersion of embryos and fry in contaminated water might be variable because the chorion of the medaka (*Oryzias latipes*) and zebra fish (*D. rerio*) act as a barrier to these toxins. Wang et al. (2005) report that sensitivity to MCs exposure is variable among fish species, and the most plausible explanation is the difference in membrane permeability of MCs. The egg of zebra fish has a diameter of 1 mm and a developing duration of 80 h, and is often used as a model to monitor aquatic pollution (Wang and Yin, 2004; Zhang et al., 2006). The egg of southern catfish has relatively larger size and thinner chorion (1.98–2.97 μm), as well as speedy development (Zhang et al., 2004). These traits are important factors contributing to the obvious toxic effects on the embryonic development by MCs in the present study. In fact, this is the first development of toxicity experiments using catfish fertilized eggs to investigate toxic effects of MCs on fish embryos. Similarly, it is known that fish egg size, chorion structure and developing duration affect uptake of toxicants and embryotoxic effect (Routray et al., 2002; Zhang et al., 2004; Finn, 2007).

On the other hand, it is reported that toxic effects of crude extracted MCs are much more evident than pure MC-LR, probably due to the facts that substances (as-yet-unidentified components) of the crude extracts might increase the uptake rate of toxins, and that synergistic actions of toxins and unknown substances could increase toxicity (Oberemm et al., 1997, 1999; Burýšková et al., 2006; Palíková et al., 2007; Falconer, 2007). For instance, lipopolysaccharides in cell extracts might enhance the toxicity of MCs (Best et al., 2002; Ding et al., 2006). In our experiment, significant toxic effects on early life stages of southern catfish exerted by crude extracts of cyanobacterial blooms might be due to both MCs and some unknown synergistic toxins.

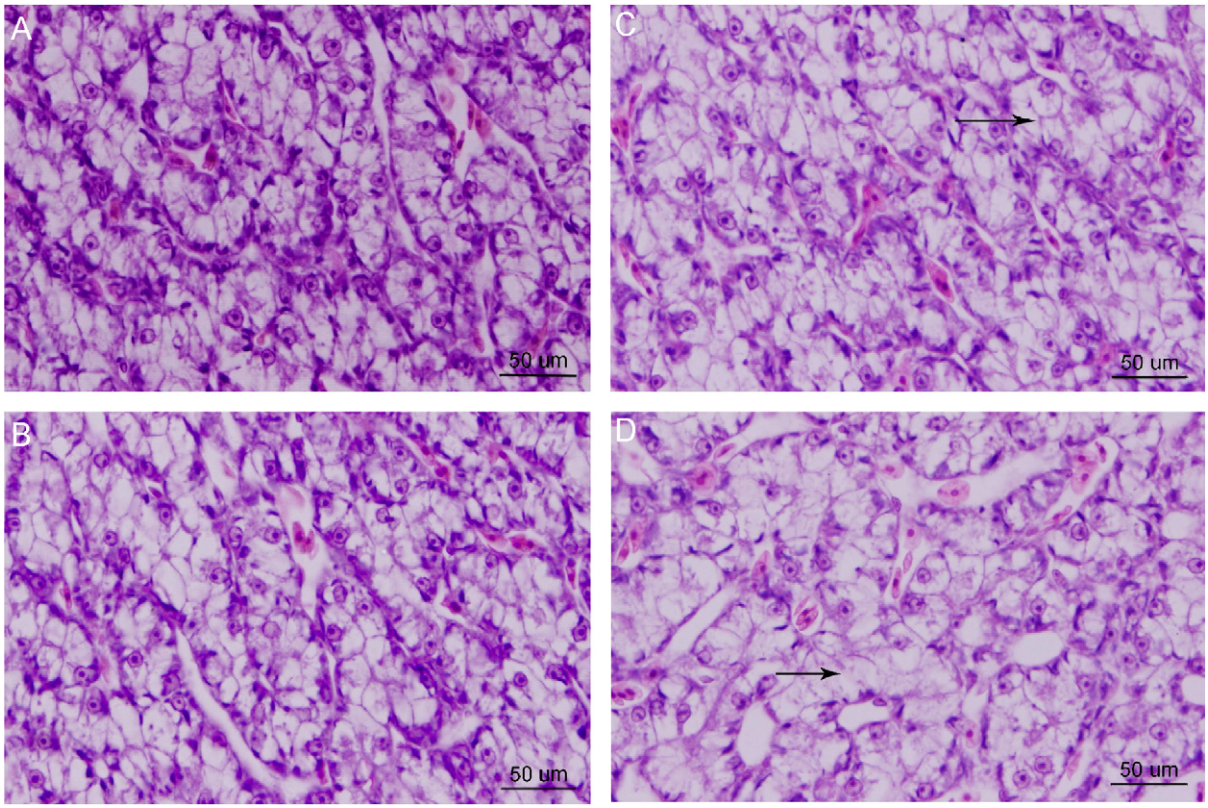

Fig. 3. Hepatic lesions in larva of southern catfish hatched from microcystins exposure and the control. (A) Liver of the control with normal hepatic structure (magnification 400 ×). (B) Liver of the low-dose group without any obvious alterations (magnification 400 ×). (C) Liver of the medium-dose group with vacuolar hepatocytes and marginal nuclei (magnification 400 ×). (D) Liver of the high-dose group with remarkable disorganizations of cell structure, vacuolar hepatocytes and marginal nuclei, even hepatocellular necrosis (magnification 400 ×).

Similarly, Oberemm et al. (1999) also observed much more pronounced effects during embryonic development of zebra fish when embryos were exposed to crude extracts (*M. aeruginosa*), while no effects could be detected with pure MC-LR. They also strongly suggest significant effects of cyanobacterial blooms by direct action of released toxins on early life stages of fish.

Some studies show that larvae treated with MCs exhibited growth inhibitions and organ impairments (Oberemm et al., 1997, 1999; Malbrouck et al., 2003; Malbrouck and Kestemont, 2006; Palíková et al., 2004). In the present experiment, after hatching from MC-treated embryos, the southern catfish larvae were reared in toxin-free water for 14 days, and we observed a marked growth inhibition (obvious reductions in body length and weight) in the medium- and high-dose groups. It appears that damnification by MC exposure during hatching can exert significant toxic effects on larval growth, although the larvae are reared in MC-free water. The clinical signs of depression, lethargy and weak desires for food of larvae in both high- and medium-dose groups also indicated the significant growth inhibition. Moreover, in the present study, growth inhibition was accompanied with hepatocytes damage characterized by disorganization of cell structure and a loss of adherence between hepatocytes, cellular degeneration with vacuolar hepatocytes and marginal nuclei, even hepatocellular necrosis. Similar histopathological alterations are also described by Palíková et al. (2004) in carp larvae. However, Liu et al. (2002) report that when loach larvae hatching from MC-exposure eggs were transferred to toxin-free dishes and cultured for 30 days, no obvious difference in either body weight or length was observed between the MC-treated groups and the control. Compared with loach juvenile, southern catfish larvae have faster development and growth, which is one of the important

reasons for their significant growth inhibition. Varying conditions, use of other species and variation of exposure in life stage were factors for the different effects of MCs on embryonic and larvae development (Liu et al., 2002). Actually, this is the first study using southern catfish larvae (hatched from MCs-treated fertilized eggs) to determine growth inhibition and liver histopathological changes caused by MCs exposure, although the larvae were cultured in toxin-free water.

In summary, an exposure of the fertilized eggs of southern catfish to crude extracted MCs (10–100 μg MC-LReq l^{-1}) obviously retarded egg and larval development, reduced hatching rate, caused high malformation rate and hepatocytes damage in larvae. A dose of 10 μg MC-LReq l^{-1} is close to a high concentration in natural cyanobacterial blooms, strongly suggesting a possible existence of such toxic effects in eutrophic waters. The potential risk of MC on reproduction of mammals should also be evaluated in future studies.

Acknowledgments

We would like to express our sincere thanks to Dr. Alan Harvey and two anonymous reviewers for their useful comments and suggestions on the manuscript. This study is supported by a fund for lecture professor from the Huazhong Agricultural University and the National Natural Science Foundation of China (30530170).

References

Azevedo, S.M.F.O., Carmichael, W.W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R., Eaglesham, G.K., 2002. Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. Toxicology 181–182, 441–446.

Best, J.H., Pflugmacher, S., Wiegand, C., Eddy, F.B., Metcalf, J.S., Codd, G.A., 2002. Effects of enteric bacterial and cyanobacterial lipopolysaccharides, and of microcystin-LR, on glutathione-*S*-transferase activities in zebra fish (*Danio rerio*). Aquat. Toxicol. 60, 223–231.

Bu, Y.Z., Li, X.Y., Zhang, B.J., Chung, I.K., Lee, J.A., 2006. Microcystins cause embryonic toxicity in mice. Toxicon 48, 966–972.

Burýšková, B., Hilscherová, K., Babica, P., Vršková, D., Maršálek, B., Bláha, L., 2006. Toxicity of complex cyanobacterial samples and their fractions in *Xenopus laevis* embryos and the role of microcystins. Aquat. Toxicol. 80, 346–354.

Carmichael, W.W., Azevedo, S.M.F.O., An, J.S., Molica, R.J., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ. Health Perspect. 109, 663–668.

Codd, G.A., Bell, S.G., Kaya, K., Ward, C.J., Beattie, K.A., Metcalf, J.S., 1999. Cyanobacterial toxins, exposure routes and human health. Eur. J. Phycol. 34, 405–415.

Dawson, R.M., 1998. The toxicology of microcystins. Toxicon 36, 953–962.

Ding, X.S., Li, Y.Y., Duan, H.Y., Chung, I.K., Lee, J.A., 2006. Toxic effects of *Microcystis* cell extracts on the reproductive system of male mice. Toxicon 48, 973–979.

Duy, T.N., Lam, P.K.S., Shaw, G.R., Connell, D.W., 2000. Toxicology and risk assessment of freshwater cyanobacterial (blue–green algal) toxins in water. Rev. Environ. Contam. Toxicol. 163, 113–185.

Falconer, I.R., 2007. Cyanobacterial toxins present in *Microcystis aeruginosa* extracts—more than microcystins. Toxicon 50, 585–588.

Falconer, I.R., Yeung, D.S.K., 1992. Cytoskeletal changes in hepatocytes induced by *Microcystis* toxins and their relation to hyperphosphorylation of cell proteins. Chem. Biol. Interact. 81, 181–196.

Finn, N.R., 2007. The physiology and toxicology of salmonid eggs and larvae in relation to water quality criteria. Aquat. Toxicol. 81, 337–354.

Fischer, W.J., Dietrich, D.R., 2000. Pathological and biochemical characterization of microcystins-induced hepatopancreas and kidney damage in carp (*Cyprinus carpio*). Toxicol. Appl. Pharmacol. 164, 73–81.

Jacquet, C., Thermes, V., de Luze, A., Puiseux-Dao, S., Bernard, C., Joly, J.S., Bourrat, F., Edery, M., 2004. Effects of microcystins-LR on development of medaka fish embryos (*Oryzias latipes*). Toxicon 43, 141–147.

Jochimsen, E.M., Carmichael, W.W., An, J.S., Cardo, D.M., Cookson, S.T., Holmes, C.E.M., Antunes, M.B.D., de Melo, D.A., Lyra, T.M., Barreto, V.S.T., Azevedo, S.M.F.O., Jarvis, W.R., 1998. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. New Engl. J. Med. 338, 873–878.

Keil, C., Forchert, A., Fastner, J., Szewzyk, U., Chorus, I., Krätke, R., 2002. Toxicity and microcystins content of extracts from a *Planktothrix* bloom and two laboratory strains. Water Res. 36, 2133–2139.

Kotak, B.G., Semalulu, S., Fritz, D.L., Prepas, E.E., Hrudey, S.E., Coppock, R.W., 1996. Hepatic and renal pathology of intraperitoneally administered microcystins-LR in rainbow trout (*Oncorhynchus mykiss*). Toxicon 34, 517–525.

Li, L., Xie, P., Chen, J., 2005. In vivo studies on toxin accumulation in liver and ultrastructural changes of hepatocytes of the phytoplanktivorous bighead carp i.p.-injected with extracted microcystins. Toxicon 46, 533–545.

Liu, Y.D., Song, L.R., Li, X.Y., Liu, T.M., 2002. The toxic effects of microcystins- LR on embryo–larval and juvenile development of loach, *Misguruns mizolepis* Gunthe. Toxicon 40, 395–399.

Malbrouck, C., Kestemont, P., 2006. Effects of microcystins on fish. Environ. Toxicol. Chem. 25, 72–86.

Malbrouck, C., Trausch, G., Devos, P., Kestemont, P., 2003. Hepatic accumulation and effects of microcystins-LR on juvenile goldfish *Carassius auratus* L. Comp. Biochem. Physiol. C 135, 39–48.

Mohamed, Z.A., Carmichael, W.W., Hussein, A.A., 2003. Estimation of microcystins in the freshwater fish *Oreochromis niloticus* in an Egyptian fish farm containing a *Microcystis* bloom. Environ. Toxicol. 18, 137–141.

Oberemm, A., Fastner, J., Steinberg, C.E.W., 1997. Effects of microcystins-LR and cyanobacterial crude extracts on embryo-larval development of zebra fish (*Danio rerio*). Water Res. 31, 2918–2921.

Oberemm, A., Becker, J., Codd, G.A., Steinberg, C., 1999. Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. Environ. Toxicol. 14, 77–88.

Paerl, H.W., Fulton, R.S., Moisander, P.H., Dyble, J., 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Sci. World J. 1, 76–113.

Palíková, M., Navratil, S., Tichy, F., Sterba, F., Marsalek, B., Bláha, L., 2004. Histopathology of carp (*Cyprinus carpio* L.) larvae exposed to cyanobacteria extract. Acta Vet. (Brno) 73, 253–257.

Palíková, M., Krejčí, R., Hilscherová, K., Babica, P., Navratil, S., Kopp, R., Bláha, L., 2007. Effect of different cyanobacterial biomasses and their fractions with variable microcystin content on embryonal development of carp (*Cyprinus carpio* L.). Aquat. Toxicol. 81, 312–318.

Routray, P., Suzuki, T., Strussmann, C.A., Takai, R., 2002. Factors affecting the uptake of DMSO by the eggs and embryos of medaka, *Oryzias latipes*. Theriogenology 8, 1483–1496.

Runnegar, M.T., Kong, S., Berndt, N., 1993. Protein phosphatase inhibition and in vivo hepatotoxicity of microcystins. Am. J. Physiol. 265, 224–230.

Soares, R.M., Magalhães, V.F., Azevedo, S.M.F.O., 2004. Accumulation and depuration of microcystins (cyanobacteria hepatotoxins) in *Tilapia rendalli* (*Cichlidae*) under laboratory conditions. Aquat. Toxicol. 70, 1–10.

Sui, H., Yan, W., Xu, H., Chen, Y., 2004. Investigation of microcystins pollution on Dong Lake, Wuhan City. J. Hygiene Res. 1, 39–41.

Wang, P.-J., Chien, M.-S., Wu, F.-J., Chou, H.-N., Lee, S.-J., 2005. Inhibition of embryonic development by microcystins-LR in zebrafish, *Danio rerio*. Toxicon 45, 303–308.

Wang, R., Yin, H.W., 2004. Analysis on feasible toxicologic endpoints in embryonic development of *Brachydanio rerio*. J. Environ. Occup. Med. 2, 88–93.

Wiegand, C., Pflugmacher, S., Oberemm, A., Meems, N., Beattie, K.A., Steinberg, C.E.W., Codd, G.A., 1999. Uptake and effects of microcystins-LR on detoxication enzymes of early life stages of the zebra fish (*Danio rerio*). Environ. Toxicol. 14, 89–95.

Wright, A., Olaf Papendorf, O., König, G., Oberemm, A., 2006. Effects of cyanobacterium *Fischerella ambigua* isolates and cell free culture media on zebrafish (*Danio rerio*) embryo development. Chemosphere 65, 604–608.

Xie, L., Xie, P., Ozawa, K., 2004. Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a subchronic toxicity experiment. Environ. Pollut. 127, 431–439.

Xie, P., 2006. Microcystins in Aquatic Animals with Potential Risk to Human Health. Science Press, Beijing.

Yu, S.Z., 1995. Primary prevention of hepatocellular carcinoma. J. Gastroenterol. Hepatol. 10, 674–682.

Zhang, Y.G., Yang, G.Z., Jin, L., 2004. Studies on the developmental ultrastructure of follicle cell of ovary and egg envelope in *Silurus meridionalis* Chen. J. Southwest China Normal Univ. (Nat. Sci. Ed.) 6, 1009–1015.

Zhang, T., Wang, R.Y., Bao, Q.-Y., Rawson, D.M., 2006. Development of a new rapid measurement technique for fish embryo membrane permeability studies using impedance spectroscopy. Theriogenology 66, 982–988.

Zhang, X., Xie, P., Li, D., Shi, Z., 2007. Haematological and plasma biochemical responses of crucian carp (*Carassius auratus*) to intraperitoneal injection of extracted microcystins with the possible mechanisms of anemia. Toxicon 49, 1150–1157.

Zimba, P.V., Khoo, L., Gaunt, P.S., Brittain, S., Carmichael, W.W., 2001. Confirmation of catfish, *Ictalurus punctatus* (Rafinesque), mortality from *Microcystis* toxins. J. Fish Dis. 24, 41–47.