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TOXIC EFFECTS OF *MICROCYSTIS* CELL EXTRACTS CONTAINING MICROCYSTIN-LR ON THE BLOOD OF MICE

LI Xiao-Yu, LIANG Jun-Bo and HUANG Pei-Pei
(College of Life Science, Henan Normal University, Xinxiang 453007, P.R. China)

Abstract In the present study, toxic effects of *Microcystis* cell extracts containing microcystin-LR on the treated-mice blood were investigated. Mice in three experimental groups received microcystin-LR by intraperitoneal injection of the cell extracts for 14 days at three sublethal doses of 2.4, 4.8 and 9.6 μg microcystin-LR/kg body weight. Effects of microcystin-LR on hematological parameters and serum biological levels of the treated mice were evaluated after two weeks of administration. Mouse hematopoietic tissues, the bone marrow and the spleen were also collected for histopathological examination. Remarkable differences in liver and spleen body weight ratios between the treatment and the control were discovered. Furthermore, activities of alanine transaminase, aspartate transaminase, alkaline phosphatase, and lactate dehydrogenase in 9.6 μg/kg group markedly increased while the levels of serum total protein, albumin and albumin/globulin ratio significantly decreased compared to the control, indicating that microcystin-LR caused damages to the liver of the treated mice. In addition, severe hypoglycemia was observed in all treated mice, which was probably due to acute failure of hepatic gluconeogenesis as well as altered glucose mobilization and metabolism. In comparison to the control group, a remarkable decrease in white blood cell count in 9.6 μg/kg group was observed by hematological test. Histopathological observations revealed that obvious impairment in the spleen was found in 9.6 μg/kg group besides severe pathological lesions in the liver. Results presented in this paper clearly revealed that *Microcystis* cell extracts containing microcystin-LR had severe toxic effects on the blood of mice.

Key words: *Microcystis* cell extracts; Microcystins; Mouse; Hematology; Histopathology
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The occurrence of toxic cyanobacterial blooms has been reported in many regions of the world and it presents a serious public health problem[1, 2]. Particularly, public health concerns associated with cyanobacteria arise from their ability to produce a variety of toxins that can be classified into three groups: dermatotoxins, neurotoxins, and hepatotoxins according to the toxic effects on animals[3]. Microcystins are a family of potent hepatotoxins produced by species of freshwater cyanobacteria, primarily *Microcystis aeruginosa*[4-5], which is commonly involved in freshwater blooms world-wide.

Microcystins are concentrated into hepatocytes via multi-specific bile acid transporters where they can potently inhibit serine/threonine protein phosphatase 1 and 2A[6-8]. The consequent protein phosphorylation imbalance disrupts the liver cytoskeleton, which causes massive hepatic haemorrhages that is the cause of animal death[5]. There have been many reports attributing the death of animals[2, 9, 10] and human illness[11-13] to microcystin-exposure. It also has been reported that microcystins are potent tumor promoters in laboratory animals[14-16]. In humans, epidemiological studies have related the presence of microcystins in drinking water to the high incidence of liver cancer in some areas of China[17, 18].

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Corresponding author: LI Xiao-Yu, Tel: +86-373-3329390; Fax: +86-373-3329390; E-mail: lixiaoyu65@263.net

Microcystins are primarily hepatotoxic, but damage to other organs, such as kidney, lung and intestine, has also been reported[19]. However, little information has been available in the present lectures about the effects of microcystins on blood and blood-forming tissues. In the present study, we aimed to evaluate the toxic responses of the blood and blood-forming tissues to microcystin-LR in Kunming mice following 14 days of intraperitoneal exposure to *Microcystis* cell extracts containing microcystin-LR.

1 Materials and methods

1.1 Chemicals Diluent for hematological test was purchased from Zhuhai Baso Diagnostic Inc. (China). Diagnostic reagent kits for serum biochemical assay were obtained commercially from Shanghai Kehua Bioengineering Co. Ltd (China). Other chemicals were purchased from Sigma (St. Louis, MO, USA).

1.2 Batch culture of *Microcystis aeruginosa* Samples of algae were collected from the Nanwan Reservoir using a 64μm mesh plankton net. *Microcystis aeruginosa* was isolated, purified and then identified according to the method of Watanabe (1996)[20]. Cultures were maintained and grown in MA medium[21] at (25±1)℃.

1.3 Preparation of *Microcystis* cell extracts and high performance liquid chromatograph analysis Batch cultures were harvested by centrifugation and stored at −20℃. *Microcystis* cell extracts from the cultures were prepared as previously described[22]. Briefly, cells were incubated in boiling water for 15 min, and then samples were removed, cooled on ice, and centrifuged at 12 000 rpm for 10 min. Supernatants (representing the *Microcystis* cell extracts) were pooled together and stored at −20℃.

The cell extracts was analyzed by high performance liquid chromatography (HPLC) to determine the quantity and composition of toxins according to the method of Harada et al. (1988)[23].

1.4 Animals Healthy Kunming mice, both sexes, were purchased from the Experimental Animal Center of Henan Province, China. The animals were handled following the guidelines in the China Law for Animal Health Protection and Instructions for Granting Permit for Animal Experimentation for Scientific Purposes (ethics approval No. SCXK (YU) 2005-0001). The mice were housed in plastic cages containing sawdust bedding and were provided with pellet food and water *ad libitum*. Their room was maintained at 22—24℃ with a relative humidity of 50%—60% and kept on a 12h light/dark cycle.

1.5 LD_{50} determination of the cell extracts The intraperitoneal (i.p.) LD_{50} of *Microcystis* cell extracts in mouse was determined according to the up-down method of Fawell et al. (1999)[24].

1.6 Experimental design After one week of acclimatization, mice were divided into three experimental groups and one control group, with 10 mice in each group at random. The mice in experimental groups were administered with *Microcystis* cell extracts by i.p. injection at doses equivalent to 2.4, 4.8 or 9.6μg microcystins/kg body weight daily for 14 days. The doses were chosen based on the i.p. LD_{50} of *Microcystis* cell extracts in mice, approximately 1/20, 1/10 and 1/5 of the LD_{50}, respectively. Mice in the control group were injected with the same volume of sterilized 0.9% saline solution instead of the cell extracts. After 24h of the exposure, the animals were euthanized by ether inhalation and body weights were measured. The blood was collected from ophthalmic veins. A small portion of the blood sample was taken for hematological test, the remainder was used for serum biological assay. After the blood collection, mice were sacrificed and their liver, kidney, spleen and thighbone were immediately removed. The weight of liver, kidney and spleen was recorded and the organ weight ratio was calculated. Portions of the tissue were fixed for histopathological examination.

1.7 Hematological test 20μL of whole blood was removed from the blood sample and added into 50μL diluent. Then, the diluted sample was tested immediately. The hematological parameters, including white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), haematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), red blood cell volume distribution width (RDW), platelet count (PLT), plateletocrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) were determined by an automatic hematology analyzer

(MEK-6108K, Nihon Kohden, Japan).

1.8 Serum biochemical assay The serum was obtained by centrifugation of the blood sample at 3000 rpm for 15 min. The levels of total protein (TP), albumin (ALB), globulin (GLO), albumin/globulin ratio (A/G), total bilirubin (TBIL), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glucose (GLU), cholesterol (CHO), triacyglycerol (TG), blood urea nitrogen (BUN), creatinine (CR) and uric acid (UA) in serum were analyzed by an automatic biochemical analyzer (AU640, Olympus, Japan).

1.9 Histopathological examination A small piece of liver, kidney and spleen was fixed in Bouin's solution for 24h, and then dehydrated, embedded into paraffin, sectioned at 5μm thick. The sections were stained with hematoxylin and eosin (H&E) and examined by light microscopy.

To evaluate the effects of microcystins on bone marrow, mouse thighbones were also removed and fixed in Bouin's solution. After proper fixation, samples were decalcified in 10% nitric acid, and then dehydrated, embedded and sectioned with the same method as described above.

1.10 Statistical analysis Results were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 11.5 for Windows by one-way ANOVA followed by Dunnett t-test or Tamhane test when appropriate. Differences were considered to be statistically significant at $p < 0.05$.

2 Results

2.1 Microcystin concentration and LD_{50} of *Microcystis* cell extracts

HPLC analysis showed that the main component of *Microcystis* cell extracts was microcystin-LR, and other kinds of microcystins were below the limits of detection by HPLC analysis. Therefore, the total concentration of the toxin was 110.4 μg microcystin-LR/mL. The i.p. LD_{50} of the cell extracts in Kunming mice was determined to be 48 μg microcystin-LR/kg body weight.

2.2 Growth inhibition

Abnormal growth and behavior of mice in the 9.6μg/kg group were caused by 14 days of toxin-exposure. These mice became anorexia, weak and thin during the period of experiment. As showed in Tab.1, body weight gain of the treated mice in 9.6μg/kg group was about 25% lower than the control group after 14 days of administration ($p < 0.01$). However, no significant differences were found in 2.4 and 4.8 μg/kg groups when compared to the control group.

Tab.1 Effects of *Microcystis* cell extracts on the body weight gain of the toxin-treated mice

	Control	2.4	4.8	9.6
Before administration				
Female (g)	22.88±1.25	22.78±1.29	22.87±0.81	22.98±0.74
Male (g)	23.29±1.44	24.03±1.07	23.44±1.22	22.90±1.57
14 days after administration				
Female (g)	28.28±1.67	28.25±0.90	26.29±1.92	21.88±1.85**
Male (g)	32.48±3.74	32.41±1.12	31.21±2.37	24.04±1.76**

Note: The treated mice were exposed to toxin by daily i.p. injection of *Microcystis* cell extracts at three sublethal doses of 2.4, 4.8 or 9.6μg microcystin-LR/kg body weigh for 14 days; Data are expressed as mean ± standard deviation; Asterisk denotes significant difference from the control group (** $p < 0.01$); The same as follows

2.3 Effects of microcystins on organ weight ratio

After 14 days of administration, the liver body weight ratio of mice in 9.6μg/kg group markedly increased ($p < 0.05$) compared to the control. In contrast, the spleen body weight ratios significantly decreased in 4.8 and 9.6μg/kg groups ($p < 0.05$ and $p < 0.01$, respectively) although there was only a slight increase in 2.4μg/kg group ($p > 0.05$). Additionally, no difference was found in the kidney body weight ratios between the treated groups and the control group (Tab.2).

Tab. 2 Effects of *Microcystis* cell extracts on the organ weight ratios of the toxin-treated mice

Dose(μg/kg bw)	Organ weight ratios		
	Liver	Kidney	Spleen
Control	5.35±0.51	1.55±0.18	0.76±0.16
2.4	5.53±0.55	1.53±0.18	0.85±0.26
4.8	5.20±0.47	1.54±0.19	0.56±0.13*
9.6	6.02±0.53*	1.63±0.18	0.50±0.15**

2.4 Changes of hematological parameters

The results presented in Tab. 3 showed that the level of WBC in 9.6μg/kg group remarkably decreased ($p < 0.01$) while the level of MCHC increased ($p < 0.05$). On the other hand, other hematological parameters of the toxin-treated mice maintained unchanged.

Tab. 3 Effects of *Microcystis* cell extracts on the hematological parameters of the toxin-treated mice

Parameters	Control	2.4	4.8	9.6
WBC (10^9/L)	7.3±1.2	7.2±1.2	7.2±1.5	4.9±1.3**
RBC (10^{12}/L)	6.0±0.6	5.6±0.5	5.8±0.8	5.7±0.8
HGB (g/L)	134±7	133±9	134±12	144±10
HCT	0.298±0.031	0.279±0.025	0.288±0.039	0.280±0.038
MCH (10^{-12}g)	22.7±2	23.9±2	23.3±2	25.5±3
MCHC (g/L)	455±44	481±48	470±41	520±61*
RDW	0.167±0.015	0.162±0.012	0.155±0.019	0.149±0.014
PLT (10^9/L)	820±128	880±251	940±165	810±159
PCT	0.005±0.001	0.004±0.001	0.006±0.003	0.004±0.001
MPV (10^{-15}L)	6.6±1.0	5.3±1.4	6.1±1.8	5.4±1.1
PDW	0.164±0.005	0.161±0.009	0.167±0.005	0.162±0.005

2.5 Serum biochemical levels

Effects of microcystin-LR on the serum biochemical levels of the treated-mice are given in Tab. 4. These data clearly showed that activities of ALT, AST, ALP and LDH in 9.6μg/kg group were significantly higher than that of the control group ($p < 0.01$). However, levels of TP, ALB, A/G ratio, TG and GLU at the top dose markedly decreased when compared to the control ($p < 0.01$). In addition, a significant increase of UA level in 4.8 and 9.6μg/kg groups was also found when compared with the control.

Tab. 4 Effects of *Microcystis* cell extracts on the serum biological parameters of the toxin-treated mice

Parameters	Control	2.4	4.8	9.6
ALT (U/L)	58±14	57±10	63±16	152±36**
AST (U/L)	103±23	103±17	116±28	184±42**
ALP (U/L)	211±45	182±38	249±47	353±96**
LDH (U/L)	303±129	364±116	426±98*	502±108**
TP (g/L)	56.7±2.8	57.6±3.3	58.4±3.8	51.0±3.6**
ALB (g/L)	30.8±1.7	30.5±1.3	30.4±1.5	24.6±1.9**
GLO (g/L)	25.9±1.4	27.1±3.1	28.0±2.7	26.4±3.1
A/G	1.19±0.06	1.14±0.14	1.09±0.08	0.95±0.13**
TBIL (μmol/L)	1.2±0.3	1.2±0.6	0.8±0.4	1.6±0.5
CHO (mmol/L)	2.73±0.44	2.51±0.33	2.84±0.43	2.26±0.63
TG (mmol/L)	1.21±0.24	1.00±0.26	0.80±0.15**	0.48±0.10**
GLU (mmol/L)	9.85±0.83	8.64±1.11*	6.49±1.26**	4.54±0.94**
BUN (mmol/L)	9.50±0.85	10.28±1.47	10.35±1.35	10.60±1.95
CR (μmol/L)	32.0±7.8	35.6±5.2	37.4±6.3	35.3±5.9
UA (μmol/L)	147±38	181±34	220±40**	208±50**

2.6 Histopathological alternation

Severe damages of mouse liver in 9.6μg/kg group were found by histopathological examination, presenting the histopathological changes such as hydropic degeneration of hepatocytes, neutrophil infiltration and loss of adherence between hepatocytes (Fig. 1, B). Abnormal white pulp morphology, unclear circumscription between white pulp and red pulp and increased macrophages in red pulp (Fig. 1, D, F, H) in the spleen lesion of the treated mice exposed to microcystin-LR at dose of 9.6μg/kg were also observed. On the contrary, no histopathological alternation was found in kidney and bone marrow of the toxin-treated mice.

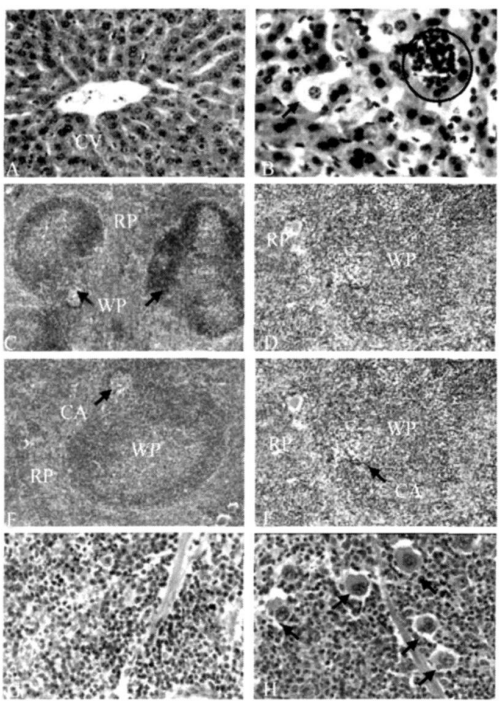

Fig. 1 Liver and spleen histology of mice exposed to microcystin-LR by daily i.p. injection of *Microcystis* cell extracts at a dose of 9.6μg microcystin-LR /kg for 14 days

Panels on the left are controls and panels on the right are treatments; A. Control liver showing normal hepatic cord pattern of lobules, hepatocytes and central vein (H&E, 200×); B. Exposed liver showing hydropic degeneration (arrow), neutrophilic infiltration (circle) and a loss of adherence between hepatocytes (H&E, 200×); C. Control spleen showing parenchyma with normal white pulp and red pulp (H&E, 50×); D. Exposed spleen showing parenchyma with abnormal white pulp morphology (H&E, 50×); E. Control spleen showing clear circumscription between white pulp and red pulp (H&E, 50×); F. Exposed spleen showing unclear circumscription between white pulp and red pulp (H&E, 50×); G. Control spleen showing normal red pulp (H&E, 100×); H. Exposed spleen showing increased macrophages (arrows) in red pulp (H&E, 100×)

WP: white pulp; CV: central vein; RP: red pulp; CA: central artery

3 Discussion

There have been many reports that indicated microcystins are potent inhibitors of phosphatase 1 and 2A[6, 8, 25]. Acute toxicosis of animals follbwing microcystin administration is characterized by rapid disorganization of the hepatic architecture, causing intrahepatic hemorrhage and even death in mammals[4, 5, 26]. Chronic uptake of microcystins resulted in generalized degeneration, single-cell necrosis, fibrosis and neutrophil infiltration of hepatocyte[27, 28]. Gross lesions in livers of the treated-mice were observed in the present study, which is consistent with those described in mammals following exposure to microcystins[26, 29, 30].

We also found a significantly decreased weight gain of the treated mice in 9.6μg/kg group when compared to the control group in addition to hepatic damage. A similar phenomenon in rats was reported by Solter et al. (1998)[29]. This growth inhibition was assumed to result from severe liver damage due to the toxin, which was verified by serum biochemical changes and histopathological observations.

As a terminal indicator, the organ weight ratio is commonly used by both acute and chronic toxicity tests. An increased liver body weight ratio of mice in 9.6μg/kg group was found in this study, probably due to severe damage of hepatocytes. By organ weight ratio assays, we also discover that the spleen is very sensitive to microcystin-exposure. The increased spleen body weight ratio of mice in 2.4μg/kg group was observed due to the harmful effects of microcystin-LR on spleen. On the other hand, this index decreased in 4.8 or 9.6μg/kg groups compared to the control group, and this maybe result from spleen impairment caused by toxin, which is verified by histopathbgical examination (Fig. 1, D, F, H).

ALT activity is often measured along with that of AST, ALP and LDH to evaluate whether liver is damaged or diseased. When liver is in dysfunction, activities of these enzymes will increase. In this study, activities of ALT, AST, ALP and LDH in 9.6μg/kg group significantly increased when compared to the control, indicating that microcystins caused damages to the liver of toxin-treated mice. Similar changes of certain serum

enzyme activities associated with liver damage were also reported in mammals[26 30—32]. However, Guzman and Solter (1999)[33] found that in rats, ALT activity in serum decreased after subchronic exposure to microcystin-LR that was induced by decreased hepatic ALT synthesis.

Additionally, results of the present study showed that levels of TP, ALB and A/G ratio of mice in the 9.6μg/kg group were remarkably lower than that of the control while GLO level maintained unchanged. Decreases in serum TP and A/G ratio may be attributed to decreased ALB level because no change was observed in GLO level. Because ALB is only generated in liver, the hypoalbuminemia could be partly caused by liver dysfunction in protein synthesis[34 35]. Our results are inconsistent with the findings obtained by Gupta et al. (2003)[30] who failed to find any change of the serum TP, ALB and A/G ratio when mice were exposed acutely to microcystins. Different duration of exposure may explain the discordance.

Beasley et al. (2000)[31] reported that acute toxicosis of microcystin-LR caused severe hypoglycemia in swine, which was probably due to acute failure of hepatic gluconeogenesis as well as altered glucose mobilization and metabolism. Similar result was obtained in this study when mice were exposed to toxin even at a low dose of 2.4μg/kg. A significant decrease in serum TG of mice in 4.8 or 9.6μg/kg groups was observed, with the likely reason that TG metabolism was altered by toxic effects of microcystin-LR. In addition, microcystin-induced decrease in WBC counts observed in this test may be related to the decreased hematopoiesis of bone marrow, although no obvious change in bone marrow was found by histopathological examination.

References

[1] Haider S, Naithani V, Viswanathan P N, *et al*. Cyanobacterial toxins: a growing environmental concern [J]. *Chemosphere*, 2003, **52**: 1—21

[2] Codd G A, Morrison L F, Metcalf J S. Cyanobacterial toxins: risk management for health protection [J]. *Toxicol. Appl. Pharmacol.*, 2005, **203**(3): 264—272

[3] de Figueiredo D R, Azeiteiro U M, Esteves S M, *et al*. Microcystin-producing blooms-a serious global public health issue. Ecotoxicol [J]. *Environ. Saf.*, 2004, **59**: 151—163

[4] Carmichael W W. Cyanobacteria secondary metabolites-the cyanotoxins [J]. *J. Appl. Bacteriol.*, 1992, **72**: 445—459

[5] Dawson R M. The toxicology of microcystins [J]. *Toxicon*, 1998, **36**: 953—962

[6] Eriksson J E, Toivola D, Meriluoto J A O, *et al*. Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases [J]. *Biochem. Biophys. Res. Commun.*, 1990, **173**: 1347—1353

[7] Yoshizawa S, Matsushima R, Watanabe M F, *et al*. Inhibition of protein phosphatases by microcystins and nodularin associated with hepatotoxicity [J]. *J. Cancer Res. Clin. Oncol.*, 1990, **116**: 609—614

[8] Runnegar M T, Kong S, Berndt N. Protein phosphatase inhibition and *in vivo* hepatotoxicity of microcystins [J]. *Am. J. Physiol.*, 1993, **265**: 224—230

[9] Duy T N, Lam P K S, Shaw G R, *et al*. Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water [J]. *Rev. Environ. Contam. Toxicol.*, 2000, **163**: 113—185

[10] Zimba P V, Khoo L, Gaunt P S, *et al*. Confirmation of catfish mortality from *Microcystis* toxins [J]. *J. Fish Dis.*, 2001, **24**: 41—47

[11] Bell S G, Codd G A. Cyanobacterial toxins and human health [J]. *Rev. Med. Microbiol.*, 1994, **5**: 256—264

[12] Codd G A, Bell S G, Kaya K, *et al*. Cyanobacterial toxins, exposure routes and human health [J]. *Eur. J. Phycol.*, 1999, **34**: 405—415

[13] Chorus I, Falconer I R, Salas H J, *et al*. Health risks caused by freshwater cyanobacteria in recreational waters [J]. *J. Toxicol. Environ. Health*, 2000, **3**: 323—347

[14] Falconer I R. Tumor promotion and liver injury caused by oral consumption of cyanobacteria [J]. *Environ. Toxicol. Water Qual.*, 1991, **6**: 177—184

[15] Nishiwaki-Matsushima R, Ohta T, Nishiwaki S, *et al*. Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR [J]. *J. Cancer Res. Clin. Oncol.*, 1992, **118**: 420—424

[16] Ito E, Kondo F, Terao K, *et al*. Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin LR [J]. *Toxicon*, 1997, **35**(9): 1453—1457

[17] Yu S Z. Primary prevention of hepatocellular carcinoma [J]. *J. Gastroenterol. Hepatol.*, 1995, **10**: 674—682

[18] Ueno Y, Nagata S, Tsutsumi T, *et al*. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay [J]. *Carcinogenesis*, 1996, **17**: 1317—1321

[19] Kuiper-Goodman T, Falconer I, Fitzgerald J. Human health aspects [A]. In: Chorus I, Bartram J (Eds.), Toxic Cyanobacteria in Water [M]. Geneva: World Health Organization, 1999, 113—153

[20] Watanabe M F. Production of microcystins [A]. In: Watanabe M F, Harada K, Carmichael W W, *et al*. (Eds.), Toxic *Microcystis* [M]. New York: CRC Press, 1996, 35—40

[21] Ichimura T. Media for blue-green algae [A]. In: Nishizawa M,

Chihara M (Eds.), Methods in Algology [M]. Kyoritsu Tokyo. 1979, 294

[22] Metcalf J S, Codd G A. Microwave oven and boiling water bath extraction of hepatotoxins from cyanobacterial cells [J]. *FEMS Microbiol. Lett.*, 2000, **184**: 241—246

[23] Harada K I, Suzuki M, Dhalem A M, *et al*. Improved method for purification of toxic peptides produced by cyanobacteria [J]. *Toxicon*, 1988, **26**: 433—439

[24] Fawell J K, Mitchell R E, Everett D J, *et al*. The toxicity of cyanobacterial toxins in the mouse: I Microcystin-LR [J]. *Human Exp. Toxicol.*, 1999, **18**: 162—167

[25] Honkanen R E, Ziller J, Moore R E, *et al*. Characterization of microcystin LR, a potent inhibitor of type 1 and type 2A protein phosphatases [J]. *J. Biol. Chem.*, 1990, **265**: 19401—19404

[26] Rao PVL, Bhattacharya R, Pant S C, *et al*. Toxicity evaluation of in vitro cultures of freshwater cyanobacterium *Microcystis aeruginosa*: I Hepatic and histopathological effects in rats [J]. *Biomed. Environ. Sci.*, 1995, **8**: 254—264

[27] Falconer I R, Burch M D, Steffensen D A, *et al*. Toxicity of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment [J]. *Environ. Toxicol. Water Qual.*, 1994, **9**: 131—139

[28] Dietrich D, Hoeger S. Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): a reasonable or misguided approach [J]? *Toxicol. Appl. Pharmacol.*, 2004, **203**: 273—289

[29] Solter P, Liu Z, Guzman R. Decreased hepatic ALT synthesis is an outcome of subchronic microcystin-LR toxicity [J]. *Toxicol. Appl. Pharmacol.*, 2000, **164**: 216—220

[30] Gupta N, Pant S C, Vijayaraghavan R, *et al*. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice [J]. *Toxicology*, 2003, **188**: 285—296

[31] Beasley V R, Lovell R A, Holmes K R, *et al*. Microcystin-LR decreases hepatic and renal perfusion, and causes circulatory shock, severe hypoglycemia, and terminal hyperkalemia in intravascular dosed swine [J]. *J. Toxicol. Environ. Health*, 2000, **61**(4): 281—303

[32] Rao P V L, Gupta N, Jayaraj R, *et al*. Age-dependent effects on biochemical variables and toxicity induced by cyclic peptide toxin microcystin-LR in mice [J]. *Comp. Biochem. Physiol. C*, 2005, **140**: 11—19

[33] Guzman R E, Solter P F. Hepatic oxidative stress following prolonged sub lethal microcystin-LR exposure [J]. *Toxicol. Pathol.*, 1999, **27**: 582—588

[34] Miura G A, Robinson N A, Lawrence W B, *et al*. Hepatotoxicity of microcystin-LR in fed and fasted rats [J]. *Toxicon*, 1991, **29**: 337—346

[35] Rabergh C M I, Bylund G, Eriksson J E. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) Microcystis aeruginosa, on common carp (*Cyprinus carpio* L.) [J]. *Aquat. Toxicol.*, 1991, **20**: 131—146

微囊藻细胞抽提液对小鼠血液的亚慢性毒性

李效宇 梁俊波 皇培培

(河南师范大学生命科学学院, 新乡 453007)

摘要: 本文研究了注射含微囊藻毒素的微囊藻细胞抽提液对小鼠血液以及免疫系统的亚慢性毒性作用。实验分为3个处理组和1个对照组(每组10只昆明小鼠, 雌雄各半), 采用腹腔注射的染毒方法对3个处理组进行暴露, 剂量分别为2.4, 4.8和9.6 μg microcystin-LR/kg body weigh, 对照组注射等量的生理盐水, 连续注射14d。实验结果表明, 14d染毒后, 小鼠的肝体比和脾体比都明显增大($p < 0.05$), 同时在9.6 μg/kg处理组, 血清丙氨酸转移酶、天冬氨酸转移酶、乳酸脱氢酶和碱性磷酸酶活性与对照相比明显升高, 但血清总蛋白、白蛋白和白蛋白/球蛋白比率下降。这些指标的变化说明, 含微囊藻毒素的微囊藻细胞提取液对处理组小鼠肝脏造成了损伤, 肝组织学观察也印证了这个结果, 在处理组小鼠肝组织有明显的水样变性。另外, 9.6 μg/kg处理组小鼠血液白细胞数量比对照组明显减少。组织细胞学观察发现, 处理组小鼠脾脏也有明显的损伤。该实验结果说明, 含微囊藻毒素的微囊藻细胞抽提液对小鼠的血液和免役系统都产生了一定程度的损伤。

关键词: 微囊藻细胞抽提液; 微囊藻毒素; 小鼠; 血清学; 组织病理学