# Biochemical and histopathological responses of Wistar rats to oral intake of microcystins and cyanobacterial biomass

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## Abstract

**OBJECTIVES:** Cyanobacteria are producers of potent and environmentally abundant microcystins, representing an emerging global health issue. In the present study, we investigated the impact of pure microcystins and cyanobacterial biomass on laboratory rats (Wistar albino rats, males, 30 days old) under different exposure scenarios. **METHODS:** The rats were fed diets containing fish meat with microcystins in various concentrations and forms (cyanobacterial biomass and isolated microcystins) for 28 days. **RESULTS:** Although considerable amounts of microcystins (MCs) were administered to the rats, all levels of MCs in the liver were close to the detection limit (3–5 ng/g fresh weight) using liquid chromatography – tandem mass spectrometry. Only rats exposed to cyanobacterial biomass had clearly higher hepatic and splenic somatic indexes while markers of oxidative stress (glutathione-S-transferase, glutathione reductase, lipid peroxidatio) were significantly increased in the group exposed to the high dose of MCs. Most of the analysed biochemical parameters did not show clear differences among groups. Levels of bilirubin and lipases were significantly increased only after exposure to cyanobacterial biomass and MCs, respectively. Considering microscopic findings in the liver, kidney, thymus, spleen and brain, histopathology was dominated by alterations in the hepatic parenchyma and renal cortical tubular system.
**CONCLUSIONS:** The present study demonstrates that oral exposure to MCs and cyanobacterial biomass may induce biochemical and detoxification responses associated with damage to liver and kidneys and in the laboratory rat.



ORIGINAL ARTICLE

**Abbreviations:**

| | |
|---|---|
| ACP | - acid phosphatase |
| Adda | - 3-amino-9-methoxy-2,6,8-trimethyl-10-fenyldeka-4,6-dien acid |
| ALB | - albumin |
| ALT | - alanine aminotransferase |
| ALP | - alkaline phosphatase |
| AST | - aspartate aminotransferase |
| AMP | - adenosine monophosphate |
| AMS | - amylase |
| ANOVA | - analysis of variance |
| BIL | - bilirubin |
| CE | - collision energy |
| CK | - creatine kinase |
| CRE | - kreatinine |
| EDTA | - ethylenediaminetetraacetic acid |
| ESI | - electrospray ionization |
| GGT | - γ-glutamyltransferase |
| GLU | - glukose |
| GST | - glutatione-S-transferase |
| GSH | - glutathione |
| GPX | - glutation peroxidase |
| GR | - glutatione reductase |
| HPLC | - high performance liquid chromatography |
| CHE | - cholinesterase |
| CHOD-PAP | - enzymatic colorimetric determination of serum cholesterol |
| CHOL | - cholesterol |
| IFCC | - international federation of clinical chemistry |
| LACT | - lactate |
| LC-MS/MS | - liquid chromatography with double mass spektrometry |
| LDH | - lactate dehydrogenace |
| LIP | - lipase |
| LPO | - lipid peroxidation |
| LSD test | - the least significant difference test |
| MC, MCs | - microcystin, microcystins |
| MDL | - method detection limit |
| MPK | - mono potassium phosphate |
| MRM | - multiple reactions monitoring mode |
| NADH | - nicotinamide adenine dinucleotide |
| NADPH | - nicotinamide adenine dinucleotide phosphate |
| PBS | - phosphate buffer saline |
| TBA | - thiobarbituric acid |
| TBARS | - thiobarbituric acid reactive substances |
| TCA | - trichloracetic acid |
| TFA | - trifluoroacetic acid |
| TP | - total protein |
| TRIS-HCl buffer | - 2-amino2-hydroxymethyl-propan-1,3-diol with hydrochloric acid |
| TRIG | - triglycerides |
| UA | - uric acid |

## INTRODUCTION

Hepatotoxic microcystins (MCs) are cyclic heptapeptides produced by several cyanobacterial genera. Cyanobacteria are widely distributed throughout the world in freshwater bodies and pose a serious threat to public health. MCs are potent inhibitors of protein phosphatase 1 and 2A that cause increased protein phosphorylation, which is directly related to their cytotoxic effects and tumour-promoting activity (Hooser *et al.* 1989). They primarily harm liver, but they also exhibit nephrotoxic, genotoxic and possibly neurotoxic effects in mammals (Kuiper-Goodman *et al.* 1999; Zegura *et al.* 2004). Besides well-recognised cyanotoxins (e.g. microcystins), cyanobacteria produce a wide range of structurally diverse secondary metabolites which are able to significantly contribute to the toxicity of cyanobacteria (Welker & von Dohren 2006; Berry *et al.* 2009). Recent studies document metabolites other than MCs present in cyanobacterial water blooms that may also be linked to tumor promotion and chemical carcinogenesis (Blaha *et al.* 2010; Novakova *et al.* 2011).

To our knowledge, there are only limited studies on effects of oral exposure to cyanobacterial biomass in mammals. Existing studies provide mostly data on effects after intraperitoneal application of MCs which is an environmentally irrelevant route of exposure. Chronic oral exposure to a low dose of microcystins has adverse effects on different target organs such as the liver, resulting in hemorrhages, apoptosis of hepatocytes, and even liver tumors. It can also affect the gut, kidneys, testes, lungs and blood as well as oxidative stress parameters (Zikova & Kopp 2008). Although articles about MCs exposure exist, there is a general lack of research involving mammals exposed orally to cyanobacterial biomass.

Our study investigates and compares effects of microcystins and cyanobacterial biomass in a simple model of food chain. Experimental rats were fed food with fish meat, which contained isolated microcystins as well as toxic cyanobacteria *Microcystis*. Using the purified hepatotoxic microcystins and cyanobacterial biomass, we evaluated and compared subchronic biochemical and histopathological responses of laboratory rats *Rattus norvegicus* var. *alba* as well as tissue levels of MCs.

## MATERIALS AND METHODS

*Experimental design and animals*

The experiment was performed in compliance with the law for the protection of animals against cruelty as approved by the Expert Committee of the Mendel University Brno, Czech Republic.

Experimental Wistar albino rats (males, 30 days old) were purchased from the commercial breeding company Anlab s.r.o. (Prague, Czech Republic), and acclimated for one week prior to the study under laboratory conditions. Animals kept in the experimental facility (23 °C, 12h light /12h dark, 60% humidity) were supplied *ad libitum* with an optimal diet for rats (i.e., mixture of wheat and starch, vitamins and minerals, lysine and soya oil meat).

The study aimed at investigation of impacts of microcystins by simulating a simple food chain model. Therefore, 25% (based on wet weight) of fish meat (carp muscle) were added to the feed. Preliminary experiments showed that the 25% content of fish meat did not affect food consumption by experimental animals. The complete feeding ration was formed on the basis of data on optimal nutrition of laboratory rats. The high content of nitrogen compounds in fish meat allowed

for a maximum supplement of 25% of fish meat. The standard feeding ration was composed of wheat flour 33%, fish muscle 25%, starch 37.8%, mono potassium phosphate (MPK) 3%, mixture of vitamins 0.2%, and lysine 1%. Control feeding rations without fish meat contained a higher proportion of wheat and were lower in starch, however the diet was supplemented with soya in order to maintain the nutritional value.

The following different exposure variants were investigated: A) Blank control (rats fed with optimal commercial diet without fish meat); B) Control (rats fed with commercial diet with 25% of fish from the locality with no occurrence of cyanobacteria and microcystins); C) Rats fed with commercial diet with 25% of fish from the locality with no occurrence of cyanobacteria and microcystins enriched with cyanobacterial bloom biomass (1% based on dry weight), which contained the final total nominal MCs concentration of 25 000 μg/kg of food (analyzed total concentration was 26 572 μg/kg containing MC-LR 8 829 μg/kg, MC-RR 15 425 μg/kg, MC-YR 872 μg/kg, MC-LF 671 μg/kg and MC-LW 775 μg/kg; it means 3033 μg MCs/kg of body weight daily); D and E ) Rats fed with commercial diet with 25% of fish from the locality with no occurrence of cyanobacteria and microcystins enriched with externally added microcystins in two nominal doses corresponding with 700 and 5 000 μg of total MCs per kg feed wet weight (analyzed MCs content was 698 and 4902 μg/kg, respectively; it means 136 μg MCs and 928 μg MCs/kg of body weight daily, respectively); F) Rats fed with commercial diet with 25% of fish (common carp muscle) from the locality with heavy cyanobacterial bloom.

A total of 10 rats per exposure were kept in cages each containing 5 animals. On a random basis of tossing cage numbers, rats were allocated to exposure variants (two cages per treatment, randomly placed to avoid positional effects). Diet and drinking water were provided *ad libitum*. Food consumption was recorded daily. The study lasted 28 days. At the end, animals were weighed, blood sampled and sacrificed by decapitation in Isoflurane anesthesia (Isoflurane, Abbott Laboratories, U.S.A.). Tissues were also collected for further analyses. The blood was taken by cardiac puncture using heparinised syringes at a regular time (7:00 a.m.) to avoid pre-analytical variations due to the circadian rhythm. Weights of major organs (liver, kidney, brain, thymus, spleen and testes) were measured.

*Microcystin mixture preparation and analysis of MCs in biomass*

The microcystin mixture for dosing of feed was prepared from a natural cyanobacterial bloom (dominated by *Microcystis aeruginosa*, collected during summer 2003 at the Nové Mlýny reservoir, Czech Republic). The freeze-dried material was repeatedly extracted with 50% methanol using sonication followed by solid-phase extraction on ODS cartridge (SepPak 35c c 10 g C-18 cartridge, Waters, Millford, MA, USA). The final solution contained 139.6 μg of total MCs/mL with three dominant MC variants (MC-LR 47.5 μg/L (34%), MC-RR 86.6 μg/L (62%) and MC-YR 5.6 μg/L (4%)), and it was stored at –18 °C until use.

Concentrations of microcystins in cyanobacterial samples used for the preparation of feed rations were analyzed as described previously using high performance liquid chromatography HPLC (Agilent 1100 Series, Agilent Technologies, Waldbronn, Germany) with Supelcosil ABZ Plus column (150×4.6 mm, 5 μm; Supelco) at 30 °C with UV detection (Palikova *et al.* 2012). The binary gradient of the mobile phase consisted of (A) water and (B) acetonitrile. Both A and B contained 0.1% TFA. We used a linear gradient increase with 20% B at 0 min to 59% B at 30 min using the flow rate of 1 mL/min. Chromatograms at 238 nm were recorded with an Agilent 1100 Series PDA detector and MCs were identified by retention time and characteristic UV absorption spectra (200–300 nm). Quantification was based on external calibrations.

*Analysis of microcystin in tissue and prepared feed*

Prepared feed and rat tissues were analysed by LC-MS/MS method according to recent study (Kohoutek *et al.* 2010). Tissue (frozen sample; 0.5 g fresh weight) was homogenised three times with methanol (3 mL), sonicated in an ultrasonic bath for 30 min, and centrifuged at 2 900 *g* for 10 min. Supernatants were pooled and extracted repeatedly (3 times) with hexane (1 mL) to remove lipids. Extract was evaporated at 50 °C, and the residue was dissolved in 300 μL of 50% aqueous methanol (v/v) and used for LC-MS/MS analyses.

Analyses were based on liquid chromatography mass spectrometry (MS/MS) with MRM using the HPLC apparatus Agilent 1200 series (Agilent Technologies, Waldbronn, Germany), which consisted of a vacuum degasser, a binary pump, an autosampler, and a thermostatted column compartment kept at 30 °C. The column was a Supelcosil ABZ+Plus RP-18 endcapped (5 μm) 150×4.6 mm i.d. (Supelco). A SecureGuard C18 (Phenomenex, Torrance, CA, USA) guard column was used. The mobile phase consisted of 5 mM ammonium acetate in water, pH 4 (A) and acetonitrile (B). The binary pump gradient was linear (increase from 20% B at 0 min to 59% B at 30 min, then 90% B for 15 min); the flow rate was 0.4 mL/min. 20 μL of individual sample was injected for the analyses. The mass spectrometer was an Agilent 6410 Triple Quad mass spectrometer (Agilent Technologies, Waldbronn, Germany) with electrospray ionisation (ESI). Ions were detected in the positive mode. The ionisation parameters were as follows: capillary voltage, 5.5 kV; desolvation temperature, 350 °C; desolvation gas flow, 11 L/min. The transitions from the protonated molecular ion to a fragment of amino acid Adda (unusual aminoacid present only in microcystins and related nodularins – (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) at m/z 135.2 and fragment at m/z 127.1

were monitored in MRM mode. Collision energies (CE, V) used for fragmentation were 50 V for MC-RR and respective conjugates, and 40 V for MC-YR and -LR and respective conjugates. Quantification of analyses was based on external standards of MC-RR, MC-YR, MC-LR in matrix (final extract of microcystin-free fish tissue). Method detection limit (MDL; per gram of tissue, fresh weight) was 3 ng/g in MRM mode.

*Sampling and measuring biochemical parameters*

Rat blood was collected by cardiac puncture using heparinised syringes. Heparin at a concentration of 50 I.U. per 1 ml was used for blood stabilisation. The blood was centrifuged at 400 g for 15 min at 4 °C, and the resulting plasma was stored at –80 °C until the day of analyses. Biochemical analyses were performed by the ADVIA 1650 automatic analyser (Siemens, USA) using commercially available reagents. Following biochemical parameters were measured as described previously (Kopp *et al.* 2009): alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), acid phosphatase (ACP), cholinesterase (CHE), γ-glutamyltransferase (GGT), creatine kinase (CK), total serum protein (TP), glucose (GLU), calcium (Ca), magnesium (Mg), phosphorus (P), total bilirubin (BIL), iron (Fe), lactate (LACT), albumin (ALB), urea (UREA), cholesterol (CHOL), electrolyte levels (Na, K, Cl), amylase (AMS), triglycerides (TRIG), lipase (LIP), uric acid (UA), creatinine (CRE).

*Determination of biomarkers of oxidative stress*

The tissue of liver were homogenized on ice in phosphate buffer saline (PBS, pH 7.2) using mechanical homogeniser, 100 mg of tissue in 1 mL of PBS; post-mitochondrial supernatant was collected after centrifugation (15 min at 10 000 *g* at 4 °C) and stored frozen at –80 °C until biochemical analyses. All biochemicals and enzymes were purchased from Sigma-Aldrich (Prague, CR), other chemicals used for preparation of buffers were of the highest commercial grade available. Biomarkers of oxidative stress such as glutathione-S-transferase (GST), reduced glutathione, glutathione peroxidase (GPX), glutathione reductase (GR) and the level of lipid peroxidation in tissues assessed as total thiobarbituric acid (TBA) reactive species (TBARS) and the protein concentrations, respectively, were measured as described in Paskova *et al.* (2008).

*Histopathological examination*

Samples of liver, kidney, thymus, spleen and brain were collected and placed in 10% buffered formalin during necropsy. They were treated using a routine histological technique and embedded in paraffin. Sections of 5 µm thickness were made from the paraffin blocks, and these were stained with haematoxylin and eosin. The samples were then screened and photographed using Olympus BX 51 and the technique of shading and phase contrast.

*Statistical analysis*

Statistica for Windows® 7.0 (StatSoft, Tulsa, OK, USA) was used to compare differences among treatment groups using one-way analysis of variance (ANOVA) and post-hoc analysis of means by the LSD test. The homogeneity of variances was tested by Levene´s test. In these cases, the non-parametric Kruskall-Wallis and Mann-Whitney tests were used for the comparison of treatment groups. Values of $p<0.05$ and $p<0.01$ were considered statistically significant and highly significant, respectively, for all tests.

## RESULTS

*Tissue concentrations of MCs*

Both liver and muscle tissues were analysed for the content of MCs. No MCs were found in the rat muscle and only low levels (in all cases below the limit of quantification) were detected in the liver of 6 animals (out of 10) from group C and 3 animals from the group E (Table 1).

**Tab. 1.** Concentration of total microcystin (MC) and its variants (MC-RR, MC-LR) in muscle and liver of rats exposed to cyanobacterial biomass (group C) or pure microcystins (groups D, E) after 28 days of exposure.

| Group | Muscle | Liver | | |
|---|---|---|---|---|
| | total MC | MC-RR | MC-LR | total MC |
| A | n.d. | n.d. | n.d. | n.d. |
| B | n.d. | n.d. | n.d. | n.d. |
| C | n.d. | 5 ng/g fw (N=6/10[a]) | n.d. | ~ 5 ng/g |
| D | n.d. | n.d. | n.d. | n.d. |
| E | n.d. | 3 ng/g fw (N=3/10[a]) | n.d. | ~ 3 ng/g |
| F | n.d. | n.d. | n.d. | n.d. |

n.d. – not detected (<3 ng/g fresh weight)
[a] – number of rats with positive detection - values above the detection limit (>3 ng/g fw)

*Somatic indexes*

For some of the basic parameters (organ weights, organ-somatic indexes), statistically significant differences among groups were observed. For fish-meat containing groups, liver seemed to weigh less than others (Figure 1A). However, statistical significance was observed only between A>F groups (LSD $p=0.006$, M-W test 0.01). Interestingly, group F also had lower weight of the spleen in comparison with groups A and B (both ANOVA + LSD and nonparametric M-W test significant, Figure 1B). We did not observe any significant differences in the kidney, brain, or thymus.

Some of the observed differences mentioned above were confirmed by analysis of organ-body weight indexes. Hepato-somatic index was clearly higher in

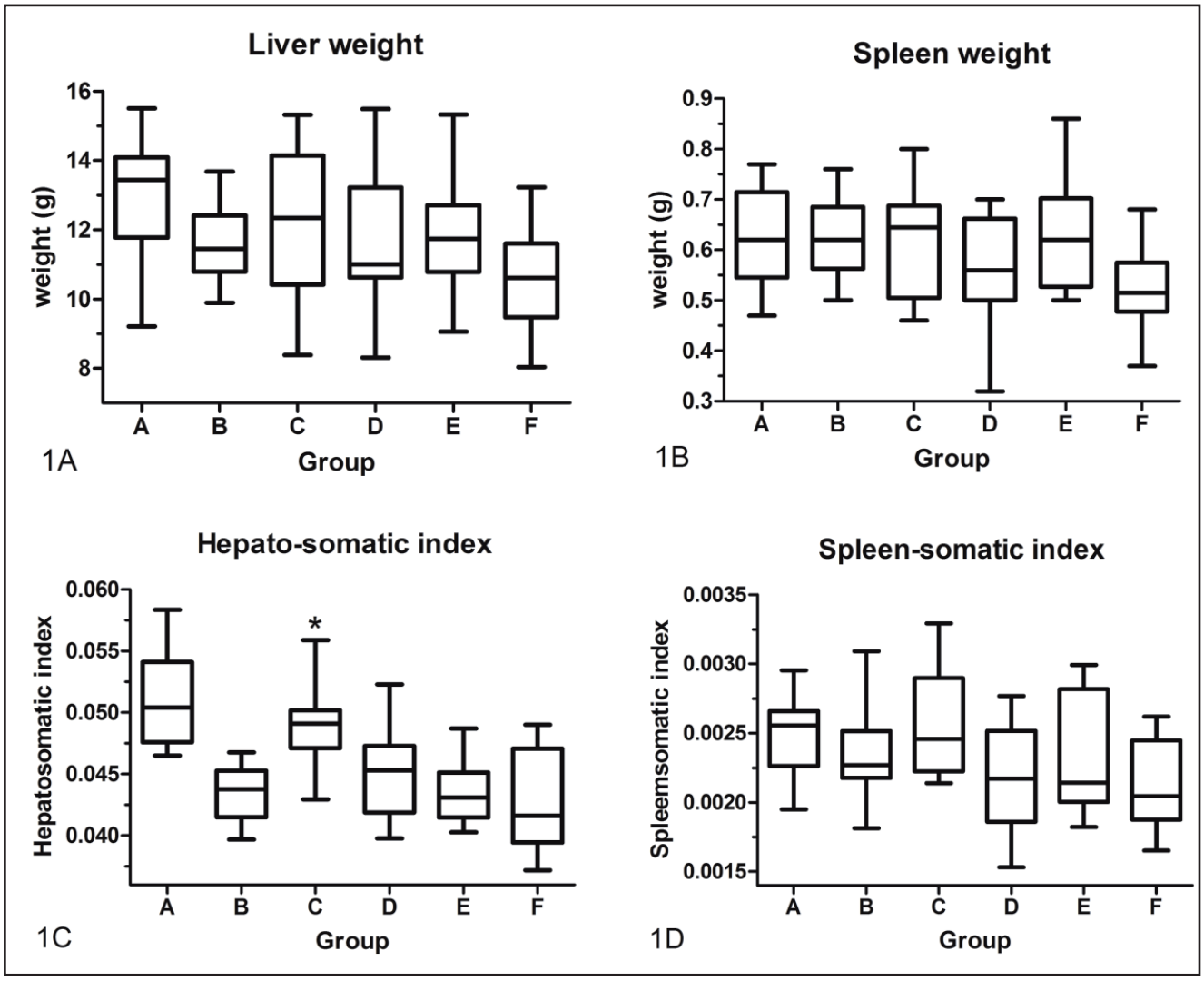


**Fig. 1.** Values of liver weight (1A), spleen weight (1B), hepato-somatic index (1C) and spleen-somatic index (1D) of rats after 28 days of subchronic exposure for experimental groups A–F. Graphs represent min to max box blot with median (N=10). * statistically significant difference (ANOVA+LSD and nonparametric M-W test) in comparison with respective control (group B).

groups A and C which did not differ from each other (Figure 1C). Groups B, D-F did not statistically differ from each other. The only significant was the difference between C and B (Figure 3C; both ANOVA + LSD and nonparametric M-W test). Also spleen-somatic/body index confirmed difference between group A>F and also C>F (Figure 1D). Interestingly, also gonado(testes)-somatic index was slightly higher in groups A and C while groups B and D had the lowest values (data are not shown). Nevertheless, statistical analysis confirmed significant differences only among groups A, C (did not differ from each other) from groups B and D. Other indexes (kidney/body, brain/body and thymus/body) did not statistically differ among groups.

*Biomarkers of oxidative stress*

Differences in some of the biomarker responses were observed between group "B" (clean fish) and "E" (clean fish with externally added microcystin at higher dose). Group E had significantly elevated activities of GST (enzyme involved in detoxification of MC by conjugating with glutathione) and GR (responsible for regeneration of oxidized glutathione) (Figure 2A, B). Slight but significant (t-test, $p$=0.04) increase in lipid peroxides was also observed in liver of rats from group E (Figure 2C).

Other parameters, i.e. levels of GSH (Figure 2D) and induced LPO as well as enzymatic activities of GPX were not statistically different in comparison with their respective control.

*Biochemical parameters of blood*

Most of the analysed biochemical parameters did not show distinct differences among groups. The enzyme activity of ALT (Figure 3A) was suppressed in groups exposed to MCs (D, E) in comparison with the con-

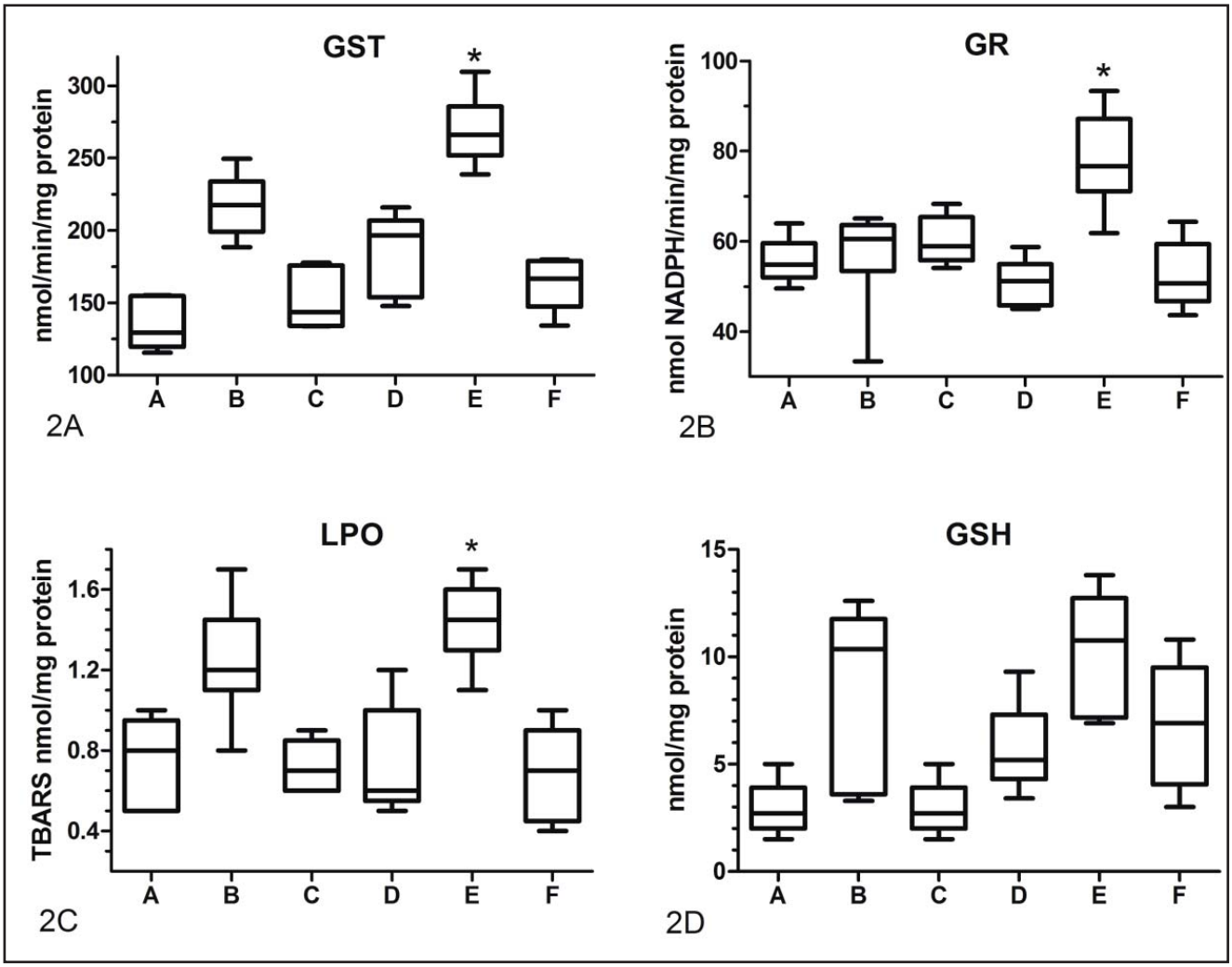


**Fig. 2.** Levels of glutathione-S-transferase (2A), glutathione reductase (2B), lipid peroxidation (2C) and glutathione (2D) in the livers of rats from groups A-F after 28 days of exposure. Graphs represent min to max box blot with median. Each group is consisted of 5–10 individuals. * statistically significant difference (t-test; N=5–10)

trol group (B), but it did not differ from other experimental groups including animals fed by normal food (i.e. group A). Considering bilirubin levels (Figure 3B), group C had significantly higher values than A, D, E and F groups; in group B lower values than C were also observed but they did not differ from each other statistically (C–F). Differences were also found in lipase activities (Figure 3C). Groups A and F had an order of magnitude higher levels than groups B, C, D. Group E was highly variable, and it did not differ from any other group. A similar trend, i.e. difference between rats exposed to higher dose of MCs (group E) and the control (group B) was observed also in iron (Fe) (B<E). However, E was not statistically different from other groups (including group A), Figure 3D. Pronounced effects were observed in parameters of Na and Cl, where animals fed with fish meat were characterized by lower values. Concentrations of Na and Cl in group C were the lowest. Due to the high variability, significant differences were only confirmed between some groups, i.e. A>C, A>B, A>E (Figure 3E, F). Considering urea, group A had lower values than most other groups but only the difference from groups B and C was significant. The only nonsignificant difference in glucose was that of A>C.

*Histopathology*

Microscopic findings were dominated by hepatic parenchyma alterations and damage to the cortical tubular system of kidneys, in particular. The majority of samples of the liver parenchyma were affected by focal or diffuse fatty degeneration of microvesicular or mixed type, frequently associated with loss of the trabecular structure wiping the lobular borders away. Moreover, some specimens from groups C (30%) and E (20%) showed necrotic hepatic lesions due to focal coagulation necrosis (Figure 4B). Alterations to the microscopic renal structure were restricted to the corti-

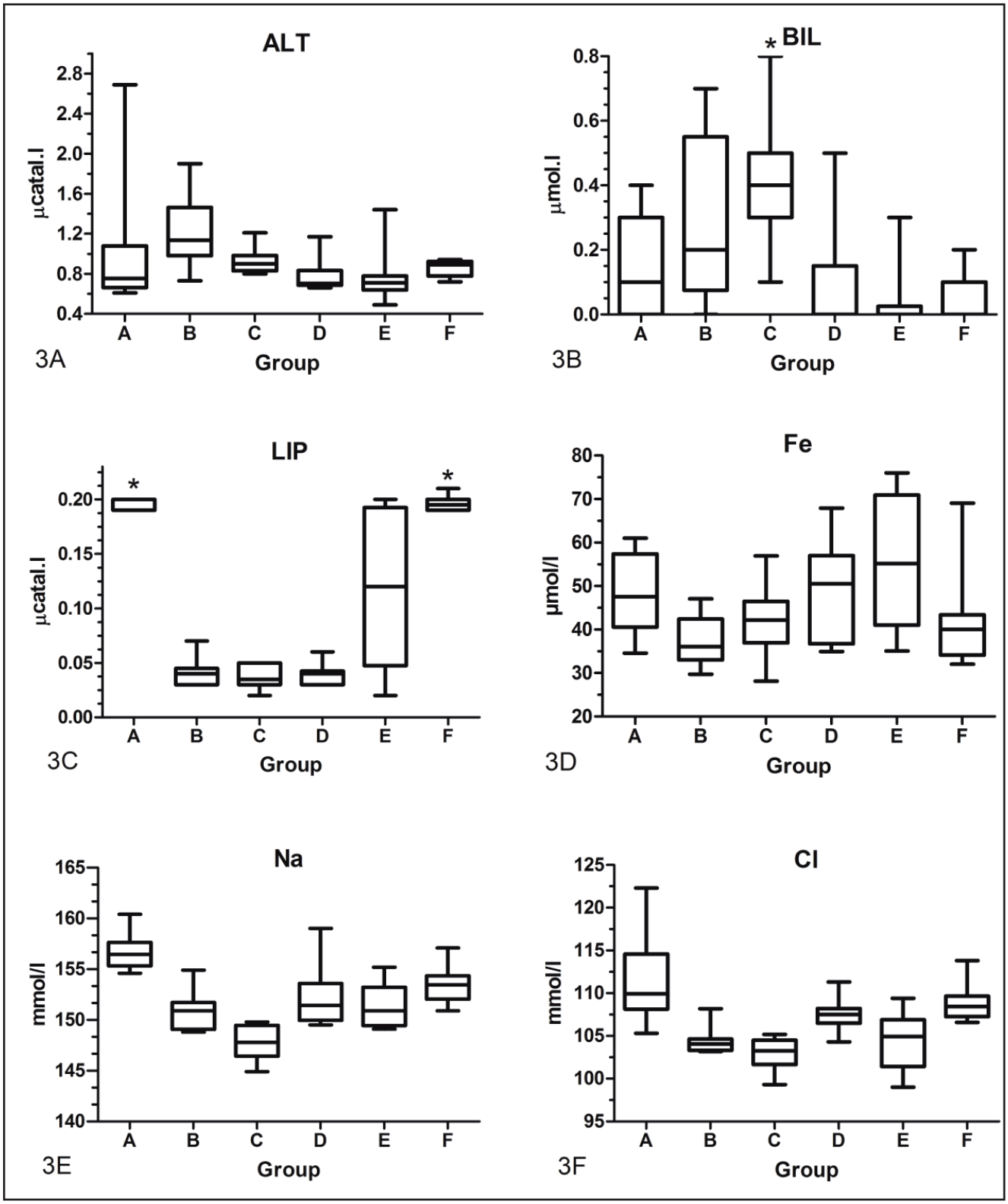


**Fig. 3.** Results of selected blood biochemical parameters. Effect of cyanobacterial biomass (group C) and microcystins (groups D, E) on levels of alanine amino transferase (3A), bilirubin (3B), lipase (3C), ferrum (3D), sodium(3E), chlorine (3F) in blood of rats after 28 days of exposure. Graphs represent min to max box blot, with median. *statistically significant difference between groups (N=10)

cal layer and its tubular system, i.e. the proximal and distal tubules of nephrons. The findings were similar in all specimens examined and included damage to the tubular epithelial layer characteristic for nephrosis (Figure 4A). Thymus, spleen and brain were without pathological findings.

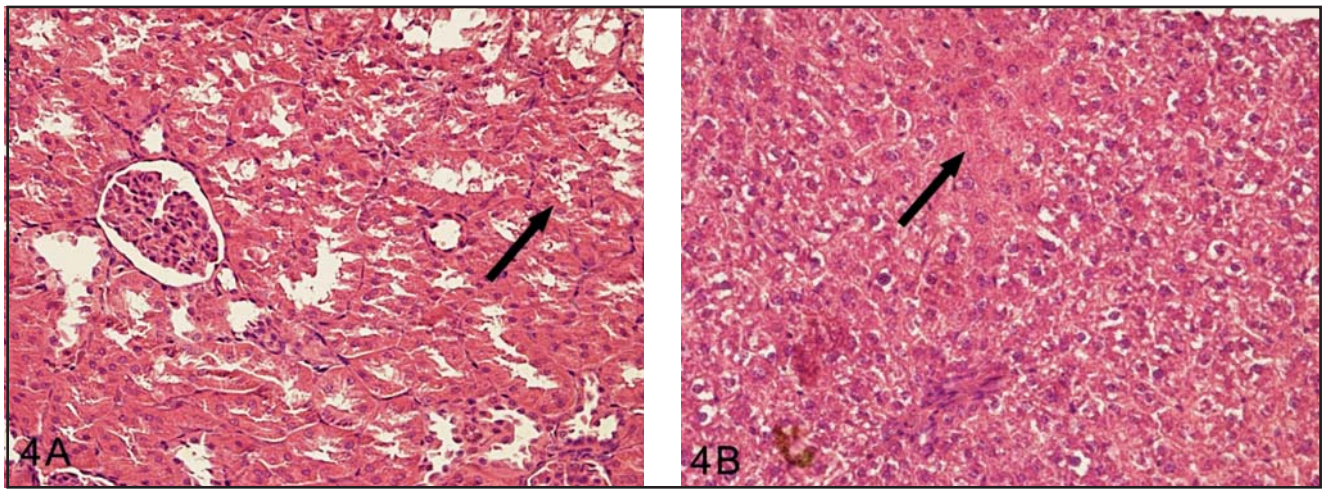


**Fig. 4.** Histopathological findings in rats exposed to microcystins (group E). Diffuse fatty degeneration of microvesicular steatosis in liver parenchyma. Arrow shows a small focus of coagulative necrosis (4B). Alteration of epithelial layer of the cortical tubular system in kidney tissue (4A). Magnification 400x.

## DISCUSSION

No mortality or behavioural changes were observed during the entire exposure period of 28 days, although experimental rats received up to 3 mg MCs/kg b.w./day (group C). This dose is close to $LD_{50}$ previously observed by Fawell *et al.* ($LD_{50}$ 5 mg/kg b.w.) after oral administration of MC-LR. Microcystins are 30–100 times less toxic via oral ingestion in rats than via intraperitoneal injection (Fawell *et al.* 1999), thus i.p. application can overestimate the risk assessment of microcystins or cyanobacterial biomass for mammals. Even if the dose was relatively high, only low levels of MCs were found in liver tissue of exposed animals (up to 5 ng/g f.w.). Accumulation experiments of microcystins with rats are rare, but some studies with terrestrial animals show that the levels of microcystins in the liver are 2–3 times higher than in our study (Paskova *et al.* 2008). Results of the present study concerning the toxin concentration in tissues of experimental rats following oral administration cannot be compared with other papers owing to the lack of published data. The present experimental data documents the fact, that despite relatively high toxin concentrations in the feed of rats, the accumulation of toxins within individual tissues amounted to very low levels and most of MCs were just slightly above the limit of detection. Low concentrations of MCs (i.e. 5 and 3 ng/g f.w.) were only detected in the liver tissue of rats from groups C and E that received the highest doses of toxins.

Calculation of organ-somatic indexes shows differences from the control (group B) only in the group of experimental rats exposed to cyanobacterial biomass. Our study confirms adverse effects of cyanobacterial biomass on liver and spleen of rats. Increased hepato-somatic and spleen-somatic indexes in group C corresponded well with previous studies with mice (Gupta *et al.* 2003; Moreno *et al.* 2003). Results from different studies clearly demonstrated that intraperitoneal injection of mice or rats caused the most efficient adverse impact of MC-LR on various target organs, in particular the liver, such as haemorrhage, necrosis and apoptosis in liver and liver tumours. These effects can increase the liver weight and the hepato-somatic index in the rat. A similar trend, i.e. differences between rats exposed to cyanobacterial biomass (group C) and controls (B) were observed also in the gonado(i.e. testicular)-somatic index. This increased index points to alteration of testis and potential adverse impacts on spermatogenesis as described previously in another study with MCs by Ding *et al.* (2006).

Our study also provides evidence on activation of the detoxification system *in vivo*. Several important markers of oxidative stress such as activities of GST, GR and lipid peroxidation were significantly elevated in the group exposed to high doses of microcystins. This observation clearly indicates that oral exposure of rats to high doses of microcystins in the diet induced detoxification activities by stimulating phase II enzymes GST and GR. These observations are in agreement with previous studies, which documented formation of GSH-conjugates of MCs as well as GST modulations (Jayaraj *et al.* 2006). Elevated lipid peroxidation also indicates direct damage to cellular membranes as shown by Rao *et al.* (2005) in their studies with mice.

A large set of biochemical blood parameters were observed to evaluate markers of health status and effects on liver such as levels of bilirubin, activities of alanine amino transferase and lipase, and elements including ferrum, sodium and chlorine. These parameters revealed differences among groups exposed to pure microcystins and cyanobacterial biomass. Interestingly,

activity of ALT was generally decreased after exposure with MCs or cyanobacterial biomass. This cytosolic enzyme is released into the blood following hepatocellular damage or necrosis (Clark *et al.* 2007). However, some studies indicate, that ALT could be lower due to a decrease of ALT synthesis after MCs exposure (Solter *et al.* 2000). Cyanobacterial biomass modulates several biochemical blood markers such as the level of bilirubin, sodium and chlorine in comparison with controls. However levels of sodium and chlorine are insignificant, lower levels of these ions can result in changes in blood and osmotic pressure or signal transduction which depends on sodium ion motion. Increased levels of bilirubin indicate predominantly an interference with normal liver function. Higher levels of bilirubin in blood after exposure to cyanobacterial biomass can result from inflammation. High dosage of pure microcystins (group E) revealed systematically different pattern among biochemical blood parameters. Most affected markers were lipase, ferrum and chlorine. Modulation of lipase levels demonstrates the impact of microcystins on liver and pancreas. Increased blood levels of the pancreatic enzymes amylase and lipase is generally a consequence of inflamed pancreas.

Considering the histopathology, findings of hepatic parenchyma alterations and damage to the cortical tubular system of kidneys are non-specific and can be induced by a variety of aetiological factors such as some nutritional components, individual tolerance of the feeding ration, and nutritional deficiencies of vitamins. Effects of microcystins can be responsible for the necrotic hepatic lesions due to focal coagulation necrosis observed only in groups C and E, i.e. rats with the highest toxin exposure. Microcystins were previously confirmed to cause histopathological liver damage in various organisms including birds (Kral *et al.* 2012). The authors describe dose dependent histopathological liver damage. The range of morphological changes in liver varied from mild vacuolar dystrophy to focal liver necroses. The most severe damage was observed in the group of birds treated with 2LD50. Diffuse vacuolar dystrophy of hepatocytes and scattered foci of necrotic hepatocytes were observed. The difference in pathological findings may be due to the dose of MCs which birds and rats were exposed to on a daily basis. While birds received an extremely high single dose, the sum of MCs in rats was higher than in birds but it was divided into lower amounts of MCs over a 28-day period. Therefore, rats might have adapted to the toxin exposure through detoxification mechanisms (Palikova *et al.* 2004).

In conclusion, this study compared and comprehensively assessed the toxicity of natural cyanobacterial biomass and pure microcystins in laboratory rats as a model organism for trophic toxin transfer. It is clear that exposure to cyanobacterial toxins disrupts several organs such as the liver and kidney. Changes were also observed in somatic indexes and in histopathology of these organs. The animals also suffered from oxidative stress observed in the group exposed to pure microcystins, in particular. Therefore, reported data can have implications for public health.

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