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# *In vivo* effects of microcystins and complex cyanobacterial biomass on rats (*Rattus norvegicus* var. *alba*): Changes in immunological and haematological parameters

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ABSTRACT

Toxic cyanobacteria represent a serious health and ecological problem in drinking and recreational waters worldwide. Some previous toxicological studies investigated effects of isolated microcystins on laboratory rodents including mice and rats. However, much less attention has been paid to more realistic exposure situations such as the effects of MCs accumulated in food. The objectives of the present study were to provide a simple model simulation of the food chain in order to evaluate impacts of microcystins (MCs) on rat immune and haematologicalparameters. Impacts of feeding experimental rats with a diet containing fish meat with and without microcystins and complex toxic biomass have been studied during a 28 day exposure. Red blood cell parameters (RBC counts, haematocrit values, MCH, MCV and MCHC) showed significant differences in experimental groups ($p \leq 0.05$, $p \leq 0.01$) in comparison with the control group. We also detected an immunomodulatory effect in the experimental groups. NK cells and γδ+ T lymphocytes were significantly increased in peripheral blood in the group exposed to isolated microcystin in the food. Significant change in the ratio of CD4+ and CD8+ cells (increase of CD4+ and a drop in CD8+) was found in the group with added cyanobacterial biomass with low concentration of MCs. The greatest changes in lymphoid organs were observed in the same groups. There was an increase of spleen subpopulations of γδ+ T lymphocytes as well as of IgM+ lymphocytes (B lymphocytes) and CD8+ T lymphocytes. Indeed, the modulation of CD4+ and CD8+ of peripheral lymphocytes was associated with similar changes in thymic lymphocytic subpopulations. In summary, food containing fish meat with considerable doses of microcystins (or toxic cyanobacterial biomass) induces significant changes in RBC parameters and influence preferably innate part of the immune system represented by NK cells and by gamma-delta T cells, which are known to play role as a bridge between adaptive and innate immune response.



## 1. Introduction

Toxic cyanobacteria represent a serious health and ecological problem in drinking and recreational waters

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worldwide (Kuiper-Goodman et al., 1999). Besides their impact on overall water quality, cyanobacteria produce a range of bioactive and toxic metabolites with microcystins being the most widely studied (Welker and von Dohren, 2006). Bioaccumulation of microcystins in aquatic food chains has been discussed as well as possible human health impacts related to the presence of toxins in edible fish (Adamovsky et al., 2007). Various documented toxic effects of microcystins include chronic hepatocarcinogenicity (Eriksson et al., 1990), oxidative stress (Paskova et al., 2011) as well as modulations of hepatological parameters (Zikova and Kopp, 2008).

The immune system plays a major role in the overall health of both higher and lower vertebrates. Lankoff et al. (2004) using human and chicken peripheral blood lymphocytes, showed that microcystin-LR influences the production of IL-2 and IL-6 and decreases proliferation of T as well as B lymphocytes. Likewise, Hernandez et al. (2000) showed that small concentrations of cyanobacterial hepatotoxins, i.e. microcystin and nodularin, are able to significantly enhance the early adherence of resting human peripheral polymorphonuclears. Yea et al. (2001) showed that MC-LR, MC-YR and nodularin inhibit immune functions as *in vitro* polyclonal antibody forming cell (AFC) response and mitogen-induced lymphoproliferation in mice lymphocytes. In their study, the immunosuppressive effect was also demonstrated, and nodularin caused greater cellular effects on T lymphocytes. The immunosuppression was mediated, at least partly, through decreased IL-2 mRNA stability. Immunosuppression in mice was demonstrated also by Shen et al. (2003). These authors recorded the reduction of phagocytosis evaluated using the phagocytic index of peripheral phagocytes, the inhibition of LPS-induced lymphoproliferation and the dose-dependent decrease in the counts of antibody-forming cells after intraperitoneal injection of the cyanobacterial bloom extract containing MCs. In the later study, Chen et al. (2004) focused on *in vitro* determination of the effects of MC-LR on nitric oxide (NO) generation and some mice macrophage-related cytokines. The results demonstrated that NO production, mRNA levels of induced nitric oxide synthase, IL-1β, TNF-α were down-regulated by MC-LR in a dose-dependent manner and mRNA levels of GM-CSF and IFN-γ were also decreased. Shi et al. (2004) reported data on modulations of multiple cytokines following *in vivo* exposure of mice to the crude cyanobacterial extract. The results showed significantly decreased mRNA levels of TNF-α, IL-1β (pro-inflammatory cytokines) and IL-2, IL-4 and IL-10 (Th1/Th2-related cytokines), while the IL-6 level was unaffected. Teneva et al. (2005) investigated the influence of microcystin-LR on mouse B and T-lymphocyte subpopulations *in vitro*. These authors demonstrated clearly that MC-LR specifically induces apoptosis in mouse B cells, while the T cells were not affected. Kujbida et al. (2006) observed affection of human polymorphonuclear leukocytes after *in vitro* exposure of MC-LR even at concentrations lower than those recommended by the WHO as safety levels for chronic exposure. In their study, MCs influenced the direct chemotactic activity of polymorphonuclear cells, induced the oxidative burst in these cells and increased phagocytosis and killing of *Candida albicans*. Microcystins increased interleukin-8, cytokine-induced neutrophil chemoattractant-2αß in human and rat neutrophils, as well as extracellular reactive oxygen species (ROS) levels (Kujbida et al., 2008). Reactive oxygen species play a central role in the host defence and removal of cell debris. However, they can also cause tissue damage and injury (Jaeschke et al., 2002), and accumulation of activated neutrophils in the liver can greatly contribute to the pathogenesis of hepatic damage (Jaeschke and Hasegawa, 2006).

Some previous toxicological studies investigated effects of isolated microcystins on laboratory rodents including mice and rats (Zikova and Kopp, 2008). However, much less attention has been paid to more realistic exposure situations such as the effects of MCs accumulated in food such as fish meat. The objectives of the present study were to provide a simple model simulation of the food chain in order to evaluate impacts on Wistar laboratory rat physiology under different exposure scenarios. Impacts of feeding experimental rats with a diet containing fish meat with and without microcystins and complex toxic biomass have been studied during a 28 day exposure. The present study is focused on outcomes of haematological and immunological parameters.

## 2. Material and methods

### 2.1. Experimental animals and design

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, 12 h light/12 h 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).

As the study aimed at investigation of impacts of microcystins by simulating a simple food chain model, 25% (based on wet weight) of fish meat (carp muscle) was 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, decrease in starch but the diet was supplemented with soya in order to maintain the nutritional value.

Following, different exposure variants were investigated according to feed, i.e.

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);