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DNA Fragmentation Induced by Microcystin-RR in Mice Liver

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Abstract

Microcystins are cyclic heptapeptides synthesized by various genera of cyanobacteria where *Microcystis* is major toxin producing genera. Several variants (>100) of microcystin have been reported. Microcystins are highly toxic and hepatotoxic in nature. Microcystin intoxication in vertebrates mainly affects the intestine and liver although liver is the maximum affected organ. Microcystin toxicity induces DNA fragmentation as well as apoptosis in mice liver. Few earlier reports evidenced about DNA fragmentation induced by microcystin-LR (a microcystin variant). While for other microcystin variants including microcystin-RR, fewer studies have been done. During present study we have tried to find out the effect of microcystin-RR on DNA in mice liver. Results clearly indicate that microcystin-RR induces DNA fragmentation in mice liver.

Keywords: *Microcystis, hepatotoxin, microcystin-RR, DNA fragmentation*

Introduction

Blooms of cyanobacteria have been reported from all over the world (Carmichael, 1994; Sivonen and Jones, 1999; Tyagi *et al.*, 1999; Rai, 2012). Cyanobacteria (blue green algae) have a number of characteristic features which resulted to their survival in various ecological niches. They can grow as- single cells, single cells in colonies, or single cells in filaments. Cells growing in colonies may be packed in a mucilaginous sheath like *Microcystis* sp.

Microcystis is dominantly occurring genera in almost all cyanobacterial blooms. Cyanobacterial blooms may be hazardous to aquatic organisms because cyanotoxins released to the water after the collapse of cells. Cyanotoxins are generally toxic to both higher animals such as human and lower animals such as rotifers.

Microcystins are cyclic heptapeptides produced by various genera of cyanobacteria including *Microcystis*. More than 100 variants of microcystin have been reported till date (Sivonen and Jones, 1999; Feurstein *et al.*, 2011; Rai, 2012). Microcystins show general structure as cyclo (D-Ala^{1}-X^{2}-D-MeAsp^{3}-Z^{4}-Adda^{5}-D-Glu^{6}-Mdha^{7}) where X and Z are variable L-amino acids (Neilan *et al.*, 1999; McElhiney and Lawton, 2005). Most frequent and studied variant of microcystin is microcystin-LR (MCYST-LR) with the variable amino acids leucine (L) and arginine (R), while in case of microcystin-RR both are arginine (R). Microcystin toxicity in freshwater has been reported globally that showed toxic effects on multi-cellular organisms ranging from planktonic crustaceans to vertebrates such as fish and mammals including human (Carmichael, 1994; Sivonen and Jones, 1999; Dawson, 1998; Singh *et al.*, 2001; Rai and Kumar, 2012). Microcystin intoxication in vertebrates mainly affects the intestine and liver although liver is the maximum affected organ (Dawson, 1998).

Toxicological manifestations of microcystins also lead to DNA fragmentation (Zegura *et al.*, 2003; Chen *et al.*, 2005). DNA fragmentation is cleavage of chromosomal DNA and formation of distinct fragments of oligo-nucleosomal size which occurs generally as a consequence of cellular process termed as apoptosis. Fragmentation of chromosomal DNA into distinct fragments of oligo-nucleosomal size also reflects the authentication of the process of apoptosis (Kerr *et al.*, 1972). DNA fragmentation caused during apoptosis can be categorized in three types; a) inter-nucleosomal DNA cleavage, b) fragmentation into large 50-300 kb lengths, and c) single-strand cleavage events (Bortner *et al.*, 1995). As microcystin induces apoptosis, DNA fragmentation during the process has been studied by some workers (Rao and Bhattacharaya, 1996; Dawson, 1998; Rao *et al.*, 1998; Zegura *et al.*, 2003; Gaudin e*t al.*, 2008). Rao and Bhattacharaya (1996) reported that microcystin induces DNA damage in mouse liver *in vivo*, while Ding *et al.* (1999) observed in cultured rat hepatocytes under *in vitro* condition. Study of DNA fragmentation in kidney cells and mouse embryo primary fibroblasts has been made by Rao *et al.* (1998). Zegura *et al.* (2003) have demonstrated that microcystin-LR induces dose and time dependent DNA strand breaks in human hepatoma cell line HepG2. Gaudin *et al.* (2008) showed DNA-damage induction by microcystin-LR and found that DNA damage was induced by microcystin-LR irrespective of the administration route.

Since maximum work on microcystin toxicity has been made for a variant namely microcystin-LR, while for other variant less studies have been made. During present study, it has been tried to find out the effect of microcystin-RR upon DNA when compared with respect to control untreated conditions.

Materials and Methods

Source of toxin & organisms used

Initial characterization of various genera, if any, present in samples were made by Microscopic observations by putting samples on the clean glass slides and observed under bright field microscope.

Toxin (Microcystin-RR) was extracted from *Microcystis* blooms collected from various ponds water of Varanasi city as per method described by Rai (2012). HPLC purified (~95%) microcystin-RR was used for the further toxicological studies.

Male mice of Albino Parks strain (weight 20±2 g) were used as test organism. Mice were divided into two set; 1- microcystin-RR treated mice and, 2- untreated control mice. Mice injected with toxins were closely observed over a period of 6 h. Effect of microcystin-RR upon DNA was assayed as per method described following.

DNA Fragmentation Assay

Microcystin-RR treated as well as untreated control mice liver tissue was washed thrice with ice-cold PBS. To check whether DNA was intact or fragmented in toxin treated mice, total genomic DNA was isolated from both type of samples. For DNA isolation approximately 50 mg liver tissue was cut in small pieces and put in normal saline solution. Tissue was manually homogenized under aseptic condition. After proper homogenization, genomic DNA of treated and control samples was isolated employing DNeasy Tissue kit (Qiagen GmbH, Germany) according to the instructions of manufacturer. DNA was run on 1% agarose gel containing ethidium bromide (0.05 mg/mL) and visualized in a gel documentation unit.

Results & Discussion

Microscopic observations

Microscopic observations of bloom samples clearly reflect the presence and dominance of *Microcystis* sp. (Figure 1A). When we observed that at higher magnifications *Microcystis* cells became clearly visible (Figure 1B). These observations are in accordance with earlier reports where *Microcystis* has been reported as major dominating genera in bloom containing water (Carmichael, 1994; Sivonen and Jones, 1999; Tyagi *et al*., 1999). These samples were further employed for microcystin-RR extraction.

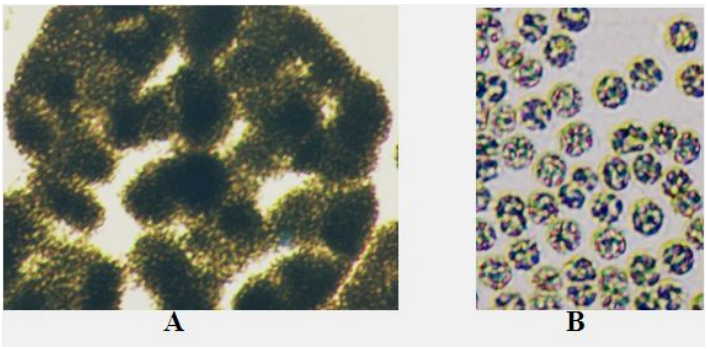

Figure 1 A-B: Microscopic observation of *Microcystis* colony to be involved in toxin biosynthesis (100X magnifications). B-Separate *Microcystis* cells at higher magnifications (400X).

DNA fragmentation induced by microcystin-RR

Our results clearly reflects that DNA fragmentation occurred by microcystin-RR as evidenced from Figure 2. Lane 1 (Figure 2) showing genomic DNA from untreated control mice, while lane 2 showing genomic DNA isolated from microcystin-RR treated mice. In gel photograph it is clearly evidenced that DNA fragmentation occurred in treated condition (lane 2), while in case of untreated control condition (lane 1) fragmentation did not recorded (Figure 2).

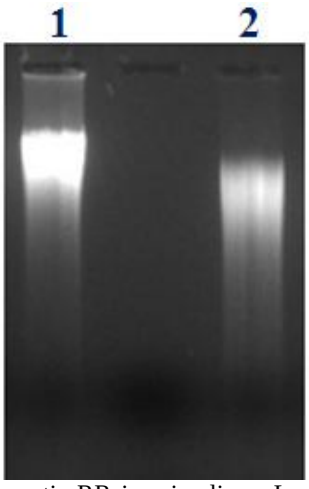

Figure 2: DNA fragmentation induced by microcystin-RR in mice liver. Lane-1 DNA from untreated control mice. Lane- 2 showing fragmented DNA from microcystin-RR treated mice.

Present observation clearly indicates that microcystin-RR induces DNA fragmentations in mice liver as its toxicological manifestations. Similar to our findings, DNA fragmentation in mice liver upon administration of toxin has been reported by Chen *et al*. (2005). They reported microcystin-LR induced apoptosis in mice liver by DNA fragmentation assay. They suggested that microcystin induces apoptosis in hepatocytes which is probably mediated by caspases via Bid-Bax-Bcl-2 pathway. Bortner *et al.* (1995) and Hua and Xu (2000) reported formation of distinct DNA fragments of oligo-nucleosomal size as an evidence of apoptosis in the cells. Our results also showed DNA fragmentation in mice liver and thus support earlier findings.

Apoptosis and related toxicological manifestation induced by microcystin-RR have also been reported by some workers (Huang *et al.*, 2008; Zhang *et al.*, 2008). Huang *et al.* (2008) reported that microcystin-RR induces apoptosis in tobacco BY-2 suspension cells via reactive oxygen species pathway. Zhang *et al*. (2008) suggested that microcystin-RR induces apoptosis in fish lymphocytes by generating reactive oxygen species leading to changes in mitochondria. Based on our present observations we can conclude that microcystin-RR induces DNA fragmentations in mice liver.

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