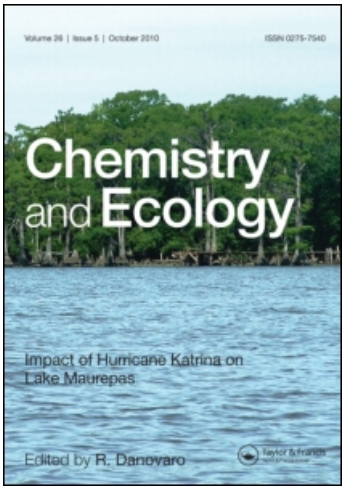

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Biochemical studies on the role of lycopene in the protection of mice against microcystin toxicity

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Biochemical studies on the role of lycopene in the protection of mice against microcystin toxicity

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The presence of cyanobacterial toxins in drinking and recreational waters represents a potential risk to public health. Microcystin-LR (MC-LR) is a potent cyclic heptapeptide hepatotoxin produced by the blue-green alga *Microcystis aeruginosa.* Chemoprotectant studies suggest that membrane-active antioxidants may offer a protection against microcystin toxicity. The aim of this study is to investigate the potential benefits of dietary supplementation of lycopene as antioxidants on microcystin toxicity in mouse liver. A group of Balb/c was pre-treated for 2 weeks with lycopene (10 mg/mouse/d) before an intraperitoneal injection (i.p.) of MC-LR. The potential benefits of lycopene were evaluated based on lipid peroxidation, alanine transaminase (ALT), glutathione peroxidase (GPX) and glutathione-*S*-transferase (GST) levels. Therefore, lycopene supplied as a dietary supplement may have a protective effect against chronic exposure to MC-LR.

Keywords: Chemoprotection; Toxicity; Microcystin; Lycopene

1. Introduction

The contamination of aquatic ecosystems as a consequence of human activities is a well-established fact. In many cases, the direct effects of pollution processes are cyanobacterial blooms, some of which are characterized by the production of toxins [1, 2]. These toxins are secondary metabolites from cyanobacteria, and it has been hypothesized that these are used in chemical defence against predators [3, 4].

Microcystin-LR (MC-LR), is one of the toxins produced by cyanobacteria [5, 6]. This toxin is produced principally by *Microcystis aeruginosa* and is a potent hepatotoxin [7], transported specifically into the liver by multispecific bile acid transporters [8, 9], thereby inducing severe intrahepatic haemorrhaging necrosis and apoptosis [10, 11]. MC-LR specifically inhibits serine/threonine protein phosphatases (PP1 and PP2A), resulting in the disruption of many important cellular processes [12–15]. MC-LR causes oxidative stress and increased reactive oxidative species (ROS), as well as lipid peroxidation concomitant with an increase in lactate dehydrogenase (LDH) release from primary rat hepatocytes [16, 17]. Later work indicated a biphasic response in cellular glutathione levels in primary rat hepatocytes exposed

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to MC-LR [18]. Hermansky *et al.* [19] showed that hydrophobic antioxidants provide some protection against lethal doses of MC-LR. Lycopene was defined as a non-vitamin active carotenoid that has a high oxygen-radical scavenging and quenching capacity [20]. It is very useful in living tissues to reduce the risk of adverse oxidative reactions that produce hydroxy radicals and peroxides [14, 21].

The aim of this study is to investigate the role of lycopene pre-treatment in protection against MC-LR-induced liver injury in mice.

2. Materials and methods

2.1 *Animals*

Five- to 7-week-old male albino Balb/c mice (average weight 30 g) were used in this study.

2.2 *Microcystis cells*

Microcystis aeruginosa cells were collected from selected sites of King Talal Reservoir in Jordan during June, July, August and September of 2004 (twice a month). Isolated cells were cultivated in a culture medium as recommended by Lehtimaki [22]. Microcystin was extracted from the freeze-dried cells using the method suggested by Mazur and Plinski [7], and the LD_{50} of the toxin extract was determined according to the Fawell *et al.*'s up–down method [23] to reduce the number of mice used and quantity of toxin required.

2.3 *Toxicological studies*

Forty mice were divided into four groups (10 mice of each group). Group 1 was the control group with mice that did not receive toxins and lycopene supplementation; group 2 received lycopene supplementation (10 mg/mouse/d) for 2 weeks prior to sacrifice according to Djuric and Powell [24]; group 3 received a single i.p. dose of toxin (75 μg/kg) according to Al-Jassabi and Khalil [9]; group 4 received toxin (the same amount) after 2 weeks of lycopene supplementation (the same amount given to group 2). All toxin-treated mice were sacrificed 24 h after receiving the toxin. The blood was collected and the serum stored at −70 °C for alanine transaminase (ALT) determination, while the liver was perfused with Hanks buffered saline to remove excess blood. Liver tissues were homogenized using (IKA Ultra-Turrax homogenizer) in buffer (0.01 M Tris–HCl pH 7.8; 0.2 mM DTT and protease inhibitors: 7.5 mM PMSF, 2.5 mM EDTA, 3.25 μM bestatin, 2.5 μM leupeptin and 0.75 μM apoprotinin). The homogenate was centrifuged and the supernatant stored at −70°C for the GST and GPX assays according to the method by Bark and Chung [25]. All chemicals used in this study were purchased from Sigma Co. (St. Louis, MO), unless otherwise indicated. All the chemicals were of analytical grade.

2.3.1 Alanine aminotransferase (ALT) assay. ALT was carried out for the serum sample according to the method recommended by Gehringer *et al.* [26].

2.3.2 Lipid peroxidation. The thiobarbaturic acid (TBA) method recommended by Gehringer *et al.* [17] was used to determine the lipid peroxidation in the liver sample.

2.3.3 Glycogen assay. A portion of liver (0.5 g) was homogenized in 1 ml of distilled water [27]. The homogenate was diluted and added to 0.2% anthrone reagent, boiled for 10 min and read at 620 nm according to Pyo and Lee [28].

2.3.4 Protein phosphatase (PP1) activity. Protein phosphatase (PP1) activity was determined by measuring the rate of colour production from the dephosphorylation of *para*-nitrophenolphosphate (ρNPP) substrate as a function of time using the microtitre plate reader according to An and Carmichael's method [29].

3. Results

In this study, our results showed that the liver weight increased by 6.68% of body weight in toxin-treated mice (group 3, without lycopene supplementation) when compared with non-toxin-treated controls (groups 1 and 2) and also group 4. The weight decreased significantly in the group that was toxin-treated with lycopene supplemented (group 4) as shown in table 1. It was found that the approximate intraperitoneal LD_{50} of the microcystin (from *M. aeruginosa* of King Talal Reservoir in Jordan) extract for the Balb/c mice was 56 μg toxin/kg mouse.

The average serum ALT levels (per millilitre) were calculated and are reported in table 1. The average ALT value for the experimental group 3 which received toxin without any supplementation showed a significant increase (3.16 U/ml), while the level of this enzyme decreased in group 4 (0.42 U/ml) which received toxin with lycopene supplementation (table 1).

All livers of control groups (groups 1 and 2) contained normal levels of glycogen, while in the toxin-treated group (group 3) the liver glycogen level significant decreases. For group 4, in which mice received toxins and lycopene, the amount of glycogen in the liver was 20.22 mg/g liver. This value was higher than that observed in mice of group 3 (7.66 mg/g liver) which did not receive lycopene, as shown in table 1.

The average thiobarbaturic acid (TBA) values were calculated and are reported in table 1. The average TBA value for group 3 (treated with toxin) increased significantly in lipid peroxidation levels, when the value was compared with groups 1 and 2. In contrast, the value for the toxin-treated group (group 4 with a supply of 10 mg/mouse/d lycopene) was significantly lower than that for group 3 (table 1).

The level of GST activity was significantly higher (46.66 U/ml) in toxin-treated mice than values reported for all other groups (20.05, 38.08 and 36.89 U/ml for group 1, 2 and 4, respectively). As shown in table 1, in the lycopene-treatment group (group 2), the GPX activity level was significantly higher (7.88 U/ml) than the value reported for the control group (group 1: 1.84 U/ml). In group 3 (toxin treatment), the GPX (1.27 U/ml) was lower than that reported

Table 1. Summary of results of the effect of lycopene supplementation on mice receiving single lethal toxin dose.

	Group 1 (control)	Group 2 (lycopene control)	Group 3 (toxin control)	Group 4 (lycopene + toxin)
Liver (% body wt.)	4.22	4.52	6.68	4.32
ALT (U/ml)	0.68	0.39	3.16	0.42
Glycogen (mg/g)	20.40	23.30	7.66	20.22
TBA values	0.020	0.022	0.052	0.024
GPX activity (U/ml)	1.84	7.88	1.27	2.08
GST activity (U/ml)	20.05	38.08	46.66	36.89

for group 1, whereas the value in the lycopene pre-treatment (group 4) was slightly higher (2.08 U/ml) than that in groups 1 and 2.

4. Discussion

Al-Jassabi and Khalil [9] were the first to collect *M. aeruginosa* from King Talal Reservoir in Jordan, and to culture and use these cyanobacteria as a source for Microcystin-LR (MC-LR). The phosphatase inhibitory activity proved the bioactivity of the toxin, while the HPLC analysis confirmed that the extract was MC-LR. In the present study, the i.p. LD_{50} for this extract when given to Balb/c mice was found to be within the range reported in the literature [10].

The expected signs of toxin-induced injury, such as haemorrhage, cell death and apoptosis, have been analysed previously [2, 11, 17, 23], as have the expected biochemical changes in serum ALT, liver glycogen content, TBA values, GPX and GST activities.

In our results, less severe liver damage was observed in group 4 (supplemented with lycopene) than that which occurred in group 3 (toxin control) where the weight of the liver increased, suggesting the occurrence of liver damage and the accumulation of fluids [11]. Moreover, the ability of lycopene to protect the liver from toxin injury was observed in the positive changes in all biochemical markers investigated (serum ALT, liver glycogen content, TBA values, GPX and GST activities) before the toxin supply.

Ding *et al.* [14] reported that MC-LR was a strong ROS inducer in hepatocytes of rats because of cytoskeletal disruption and mitochondrial alterations. It is well documented that ROS can affect the microtubules' intermediate filaments and microfilament organization owing to their ability to oxidize the cytoskeleton proteins or disturb the intracellular thiol balance [14, 30]. Ding *et al.* [18] showed that the disruption of the cytoskeletone led to ALT leakage, suggesting that the disruption of the cytoskeleton could have played a crucial role in microcysin- induced hepatotoxicity.

Our findings suggested a significant decrease in glycogen in the liver of toxin control mice (group 3) when compared with other groups (table 1). MC-LR can cause depletion of glycogen in the liver, possibly via the direct inhibition of glycogen synthesis and indirect activation of glycogen phosphorylase [31, 32].

In this study, the levels of lipid peroxidation increased in toxin-treated mice (group 3), as reflected by increased TBA values because of the severe liver damage, necrotic changes, haemorrhaging and apoptosis [16, 33, 34]. These levels were decreased in group 4 (supplemented with lycopene), presumably via the ability of lycopene to quench free radicals.

Treatment of mice with microcystin appears to slightly decrease the activity of GPX (1.27 U/ml vs. 1.84 U/ml). However, the effect of lycopene supply on the activity of GPX is quite obvious (approximately three- to fourfold compared with the control). In spite of this, lycopene administration (supplied as a dietary supplement) before 2 weeks of toxin injection improved (almost doubled) the GPX activity. This indicates the protective effect of this supplement against microcystin toxicity.

As previously reported by Gehringer *et al.* [17] and Pietch *et al.* [35], we also measured an increase level of GST activity in toxin-treated mice (group 3). GST is a phase II detoxification enzyme known to be involved in the detoxification of MC-LR [36]. It was found by Harris and Stone [27] that the inhibition of GST occurs via an increase in lipid peroxidation levels, and so our results regarding GST (table 1) may suggest that MC-LR produces its cytotoxic effects through this route. This suggestion is in agreement with previous reports [14, 30]. Stahl and Sies [37] indicated that lycopene is a cyclic carotenoid containing conjugated double bonds arranged in the all-*trans* form, and so it acts as an antioxidant through physical and chemical quenching of singlet oxygen and traps peroxyl radicals.

In conclusion, lycopene supply in dietary form for mice could confer some protections against MC-LR toxicity resulting from contaminated water sources, but further studies are needed to test the efficiency of this type of treatment in other systems.

References

[1] J.M. Monserrat, J.S. Yunes, A. Bianchini. Effect of *Anabaena spiroides* (cyanobacteria) aqueous extracts on the acetylcholinesterase activity of aquatic species. *Environ. Toxicol. Chem.*, **20**, 1228–1235 (2001).

[2] P.J. Oberholster, A.M. Botha, J.U. Grobbelaar. *Microcystis aeruginosa* source of toxic microcystins in drinking water. *Afr. J. Biotechnol.*, **3**, 159–168 (2004).

[3] W.W. Carmichael. Cyaobacterial secondary metabolites. The cyanotoxins. *J. Appl. Bacteriol.*, **72**, 445–459 (1992).

[4] W.W. Carmichael. The toxins of cyanobacteria. *Sci. Am.*, **270**, 78–86 (1994).

[5] R.M. Dawson. The toxicology of microcystins. *Toxicon*, **36**, 953–962 (1998).

[6] S.F. Baldia, C.G. Conaco, T. Nishijima, S. Imanishi, K.I. Harada. Microcystin production during algal bloom occurrence in Laguna de Bay, the Philippines. *Fish. Sci.*, **69**, 110–116 (2003).

[7] H. Mazur, M. Plinski. Nodularia spumigena blooms and the occurrence of hepatotoxin in the Gulf of Gdansk. *Oceanologia*, **45**, 305–316 (2003).

[8] J.E. Eriksson, L. Groberg, S. Nygard, J.R. Slotte, J.A.O. Meriluoto. Hepatocellular uptake of ^{3}H-dihydromicrocystin-LR, a cyclic peptide toxin. *Biochem. Biophys. Acta*, **1025**, 60–66 (1990).

[9] S. Al-Jassabi, A.M. Khalil. First report on the identification of nodularin from King Talal Reservoir (Jordan). *J. Biol. Sci.*, **1**, 559–563 (2004).

[10] T. Yoshida, Y. Makita, S. Nagata, T. Tsutsumi, F. Yoshida, M. Sckijima, S.L. Tamaru, Y. Ueno. Acute oral toxicity of MC-LR, a cyanobacterial hepatotoxin in mice. *Nat. Toxins*, **5**, 91–95 (1997).

[11] W.X. Ding, C.N. Ong. Role of oxidative stress and mitochondrial changes in cyanobacteia-induced apoptosis and hepatotoxicity. *FEMS Microbiol. Lett.*, **220**, 1–7 (2003).

[12] C. Hummert, M. Reichett, B. Luckas. New strategy for the determination of microcystins and diarrhetic shellfish poisoning (DSP) toxins, two potent phosphatase 1 and 2A inhibitors and tumour promoters. *Fresenius J. Anal. Chem.*, **366**, 508–513 (2000).

[13] D. Kearns, M.D. Hunter. Green algal extracellular products regulate antialgal toxin production in cyanobacterium. *Environ. Microbiol.*, **2**, 291–297 (2000).

[14] W.X. Ding, H.M. Shen, C.N. Ong. Critical role of reactive oxygen species formation in microcystin-induced cytoskeletone disruption in primary cultured hepatocytes. *J. Toxicol. Environ. Health*, **64**, 507–519 (2001).

[15] J.A. Baker, B. Entsch, B.A. Neilan, D.B. McKay. Monitoring changing toxigenicity of a cyanobacterial bloom by molecular methods. *Appl. Environ. Microbiol.*, **68**, 6070–6076 (2002).

[16] W.X. Ding, H.M. Shen, H.G. Zhu, C.N. Ong. Studies on oxidative damage induced by cyanobacterial extract in primary cultured rat hepatocytes. *Environ. Res.*, **78**, 12–18 (1998).

[17] M.M. Gehringer, K.S. Downs, T.G. Downing, R.J. Naude, E.G. Shephard. An investigation into the effect of selenium supplementation on microcystin hepatotoxicity. *Toxicon*, **41**, 1–8 (2003).

[18] W.X. Ding, H.M. Shen, H.G. Zhu, C.N. Ong. Microcystic cyanobacteria extract induces cytoskeletal disruption and intracellular glutathione alteration in hepatocytes. *Environ. Health Perspect.*, **108**, 605–609 (2000).

[19] S.J. Hermansky, S.J. Stohs, Z.M. Eldeen, V.E. Roche, K.A. Mereish. Evaluation of potential chemoprotectants against microcystin-LR hepatotoxicity in mice. *J. Appl. Toxicol.*, **11**, 65–74 (1991).

[20] G.R. Beecher. Nutrient content of tomatoes and tomato products. *Proc. Soc. Exp. Biol. Med.*, **218**, 98–100 (1998).

[21] S. Agarwal, A.V. Rao. Tomato lycopene and its role in human health and chronic diseases. *Can. Med. Assoc. J.*, **163**, 739–744 (2003).

[22] J. Lehtimaki. Characterization of cyanobacterial strains originating from Baltic sea with emphasis on *Nodularia* and its toxin, nodularin. Acad. PhD dissertation, University of Helsinki, Helsinki (2000).

[23] J.K. Fawell, R.E. Mitchell, D.J. Everett, R.E. Hill. The toxicity of cyanobacterial toxins in the mouse: microcystin-LR. *Hum. Exp. Toxicol.*, **18**, 162–167 (1999).

[24] Z. Djuric, L.C. Powell. Antioxidant capacity of lycopene-containing food. *Int. J. Food Sci. Nutr.*, **52**, 143–149 (2001).

[25] Y.Y. Bark, A.S. Chung. Induction of some glutathione-*S*-transferases in rat liver by selenium. *Korean Biochem. J.*, **22**, 61–67 (1989).

[26] M.M. Gehringer, E.G. Shephard, T.G. Downing, C. Weigand, B.A. Neilan. An investigation into the detoxification of microcystin-LR by the glutathione pathway in Balb/c mice. *Int. J. Biochem. Cell Biol.*, **36**, 931–941 (2004).

[27] C.M. Harris, W.L. Stone. The effects of in vitro lipid peroxidation and on the activity of liver microsomal GST from rats supplemented or deficient in antioxidants. *Life Sci.*, **42**, 415–420 (1998).

[28] D. Pyo, S. Lee. Rapid purification of MC-LR using supercritical fluid extraction and flash chromatography. *Anal. Lett.*, **35**, 1591–1602 (2002).

[29] J. An, W.W. Carmichael. Use of colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystin and nodularin. *Toxicon*, **32**, 1495–1507 (1994).

[30] C. Fiorentini, L. Falzano, R. Rivabene, A. Fabbri, W. Malorni. *N*-Acetylcysteine protects epithelial cells against the oxidative imbalance due to *Clostridium dillicite* toxins. *FEBS Lett.*, **453**, 124–128 (1999).

[31] W. Wera, B.A. Hemmings. Serine/threonine protein phosphatases. *Biochem. J.*, **311**, 17–29 (1995).
[32] W.X. Ding, H.M. Shen, C.N. Ong. Calpain activation after mitochondrial permeability transition in microcystin-induced cell death in rat hepatocytes. *Biochem. Biophys. Res. Commun.*, **291**, 321–331 (2002).
[33] S. Takenaka. Covalent glutathione conjugation to cyanobacterial hepatotoxin microcystin-LR by F344 rat cytosolic and microsomal GST. *Environ. Toxicol. Pharmacol.*, **9**, 135–139 (2001).
[34] I.R. Falconer. Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. *Phycologia*, **4**, 228–233 (2002).
[35] C. Pietch, C. Wiegand, M.V. Ame, A. Nicklisch, D. Wunderlin, S. Pflugmacher. The effect of cyanobacterial crude extract on different aquatic organisms: evidence for cyanobacterial toxin modulating factors. *Environ. Toxicol.*, **16**, 535–542 (2001).
[36] S. Pflugmacher, C. Wiegand, A. Oberemm, K.A. Beattie, E. Krause, G.A. Codd, C.E.W. Steinberg. Identification of enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin MC-LR; the first step in detoxification. *Biochem. Biophys. Acta*, **1425**, 527–533 (1998).
[37] W. Stahl, H. Sies. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am. J. Clin. Nutr.*, **62**, 1366–1378 (1996).