Protective Role of Anthocyanain and Taurine Against Microcystin Induced Pancreatic and Testicular Toxicity in Balb/C Mice

Ali Saad, R. Murabat, A. Omari and S. Al-Jassabi

1Jordanian University of Science and Technology
2Faculty of Science, Enaba University, Algeria
3Faculty of Health Sciences, Taif, KSA
4Royal College of Medicine Perak, Ipoh, Malaysia

Abstract: Microcystins (MC-LR) are hepatotoxic cyanotoxins produced mainly by cyanobacteria family especially Microcystis sp. This hepatotoxin is reported as potent tumor promoter by inhibiting protein phosphatase activity. Oxidative stress has been implicated as one of the possible mechanisms in MC-LR induced toxicity. Although several studies on the protective role of numerous antioxidants used as antidotes have been reported, to date, there is no effective chemoprotectant against the MC-LR induced toxicity. In the present study, MC-LR was isolated from cyanobacterial blooms from Simemia Lake, Malaysia and intraperitonially injected (34.5mg of MC-LR/kg bw) to BALB/C mice in order to assess MC-LR effect on pancreas and testis. Biochemical studies were conducted to explore the protective effect of orally pretreatment for 10days of mice with 32.1mg/kg bw of anthocyanin extracted from pomegranate and 100mg/kg bw of taurine separately against the induced toxicity in those organs. MC-LR toxicity in testis and pancreas was confirmed by the inhibition of phosphatase activity. The biotoxin induced significant increase in marker of cytotoxicity levels (lipid peroxidation end product and protein carbonyl content formation) in those organs. Hyperglycemia, raised serum α-amylase activity, increased liver glucokinase activity and decreased pancreatic and liver pyruvate kinase activity suggested a pancreatic injury which could induce diabetes. Also, alteration in the activity of testicular enzymes superoxide dismutase, lactate and sorbital hydrogenases were reported in MC-LR treated groups. Data showed an important protection against MC-LR toxicity following the separate preadministration of antioxidants (taurine and anthocyanin) to animals. Comparing the antioxidant capacity of the used chemoprotectants, anthocyanin afforded a better protection than taurine against MC-LR injury.

Key words: Microcystis Aeruginosa • Microcystin- LR • Anthocyanin • Taurine • Antioxidants

INTRODUCTION

Toxic cyanobacterial blooms (TCBs) often referred to as blue green algae, are a worldwide problem, causing serious water pollution and public health hazard to humans and livestock. However, as a result of the enhancement of eutrophication in superficial freshwater bodies: rivers, lakes, dams and water supply reservoirs typically experience TCBs [1, 2].

Microcystins are a group of monocyclic heptapeptide hepatotoxins produced by various cyanobacterial secondary metabolites [3]. They attract special attention not only due to their ability to cause acute poisoning, but also to their cancer promotion potentially by chronic exposure of humans to lower concentrations in drinking water [4].

Taurine, the end product of L-cystiene metabolism has shown capacity in protecting from various free radicals associated with pathological conditions. This conditionally essential aminoacids is also a documented potent scavenger of the hydroxyl radical, a membrane stabilizing agent and as a powerful detoxifying agent [5].

Number of beneficial effects of the pomegranate extracts consisting in the inhibition of lipoprotein oxidation and protection against prostate cancer, was attributed to the antioxidative properties of pomegranate polyphenols and anthocyanins [6].

Anthocyanin belongs to flavonoid family was found to be a strong chemopreventive agent. Dietary supplementations of this last molecule demonstrated high anti-inflammatory, anticarcinogenic and anti-oxidative activities [7].

Corresponding Author: S.Al-Jassabi, Royal College of Medicine Perak/ Ipoh/ Malaysia.
Tel: (++6) 0173067907.
The aim of the study was to assess the toxicity of MC-LR extracted from algal material on Balb/c mice pancreas and testis then to investigate the protective effect of taurine and anthocyanin extracted from pomegranate.

**MATERIALS AND METHODS**

**Chemicals:** All chemicals and standards used in this study were purchased from Sigma Chemicals Inc. Other reagents were obtained from Roche diagnostics.

**Animals:** Adult male Balb/c mice (5-7 weeks old) weighing about 30g were used in this study. The mice were housed in cages with hardwood chip bedding at 22-25°C. They were kept under ideal laboratory conditions during the process of experimentation, maintaining on standard pellet diet and water ad libitum except during anthocyanin supplementation.

**Isolation and Identification of Microcystis aeruginosa:** Identified cells were cultured on BG-11 medium then harvested after 3 weeks according to the method recommended by Mazur and Plinski [8]. Harvested cells (the toxin source) were lyophilized and stored in dark bottles at -20°C.

The lyophilized cells (1g) was mixed with 100ml final volume of water: methanol: n-butanol (70:25:5, v: v: v) by continuous stirring using a shaker for 30min at room temperature. The toxin was extracted from the mixture by sonication bath for 20min then centrifugation at 4000g for another 20min. The supernatant was lyophilized and stored in dark bottles at -20°C.

**LD₅₀ Determination:** LD₅₀ is frequently used as a general indicator of a substance’s acute toxicity. LD50 of the extracted toxin was determined according to up-down method reported by Fawell et al. [10].

**Extraction of Anthocyanin from Pomegranate:** Sweet pomegranates (P. granatum) were manually peeled and liquefied using a blender. The juice sample (pH 5.2) was centrifuged for 2 minutes at 10000rpm and filtered through 0.45µm filter. Anthocyanin was extracted by acidified methanol according to Miguel et al. [11], filtered through a sintered glass funnel and concentrated in vacuo at 37°C, stored in dark containers and frozen at -20°C.

**Determination of Total Anthocyanin Content (TA):** Total Anthocyanin contents were determined according to the pH differential method reported by Elisia et al. [7] and calculated from the difference between absorbance at 510 and 700nm as in the following.

\[ TA \text{ (mg/lit)} = \frac{AX \text{ Molecular Weight X Dilution Factor X 1000}}{\text{Molar extinction coefficient X pathlength}} \]

Here \( A = (A_{510nm} - A_{700nm}) \text{ pH 1.0} - (A_{510nm} - A_{700nm}) \text{ pH 4.5} \)

**Free Radical Scavenging Activity (FRSA) of the Antioxidants:** The free radical scavenging activities of the extracted anthocyanins and taurine was measured, separately by the decrease in the absorbance of methanol solution of 1, 1 diphenyl-2 picryl-hydrazyl (DPPH). Fresh Anthocyanin juice and/or freshly prepared taurine (0.1ml) was mixed with 0.9ml of Tris-HCl buffer (100mM, pH7.4) to which 1ml of 500µM DPPH was added. After 30min, absorbance (A) was measured spectrophotometrically at 517nm [6]. The antioxidant activity was calculated using the following equation.

\[ \text{FRSA} \% = \frac{A \text{ sample 517nm} - A \text{ control 517nm}}{} \times 100 \]

**Experimental Design:** Animals were randomly divided into 7 groups (10 mice each group).

**Group I (C):** Normal control mice

**Group II (TC):** Received the extracted toxin MC-LR (34.5mg/kg b.w) by intraperitoneal (i.p.) injection.

**Group III (ALL C):** Received alloxan monohydrate (180mg/kg b.w.) by i.p. at an interval of 24h. Group IV (TAU C): received taurine (100mg/kg b.w.) per orally once a day for 10days.

**Group V (ANTH C):** Received extracted Anthocyanin (32.1mg/kg b.w) per orally once a day for 10days.

**Group VI (TAU+T):** Received taurine (100mg/kg b.w.) per orally once a day for 10days followed by i.p injection of MC-LR (34.5mg/kg b.w).

**Group VII (ANTH+T):** Received Anthocyanin (32.1mg/kg b.w) per orally once a day for 10days followed by i.p injection of MC-LR (34.5mg/kg b.w).
After 11th day, all mice were sacrificed and blood was immediately collected. The sera of each group were separated and stored at -20°C for further biochemical analysis. Liver, pancreas and testis were excised immediately and kept in the ice cold phosphate buffer. All tissues were homogenized separately at 16,000rpm for 30min and supernatant was taken and stored at 20°C for further biochemical analysis. The following biochemical tests were carried out in the serum as well as tissue homogenates according to the appropriate methods.

Alanine aminotransferase [12], glucose [13], serum α-amylase [14], alkaline phosphatase [15] activities were assessed. Lipid peroxidation assessed in terms of Thiobarbituric acid reactive substances (TBARS) [16], protein carbonyl content [17], Glutathione-S-Transferase [19], glucokinase [20], sorbitol dehydrogenase [21], Lactate dehydrogenase and superoxide dismutase [22] respectively.

Statistical Analysis: Values were presented as means±S.D. Data were analyzed using analysis of variance (ANOVA) and group means were compared with Duncan’s multiple range test (DMRT) using Statistical Package for Social Science (SPSS).

RESULTS

Microcystin LR was identified by its characteristic absorption spectrum with a maximum absorbance between the interval 230-240nm.

LD₅₀ Determination: Acute toxicity of the extracted toxin was an LD₃₀ (i.p. mice) for 34.5mg/kg of mouse body weight. The mice revealed weakness, increased respiratory rate, abdominal contraction and death.

Total Anthocyanin Measurement: Results of rapid quantification of total anthocyanins utilizing the pH differential analysis method [7] showed that the pomegranate extract contained 96.3±9mg/L of total anthocyanins.

Free Radical Scavenging Activity of the Used Antioxidants: The change in colorization from violet to yellow and subsequent fall in absorbance of the stable radical DPPH due to the addition of the antioxidant solutions (taurine and anthocyanin separately) was measured at 517nm. The results were 37.2±0.23% as FRSA of the taurine solution and 65.5±0.57% as FRSA of the anthocyanin juice reflecting a greater antioxidant activity of the last compound.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alanine transaminase (U/L)</th>
<th>Amylase (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>13.07±0.0238</td>
<td>452.00±1.41</td>
</tr>
<tr>
<td>TC</td>
<td>61.99±0.0141</td>
<td>1195.00±4.24</td>
</tr>
<tr>
<td>TAU C</td>
<td>6.01±0.0212</td>
<td>683.00±4.24</td>
</tr>
<tr>
<td>TAU + T</td>
<td>28.91±0.0212</td>
<td>872.00±4.24</td>
</tr>
<tr>
<td>ANTH C</td>
<td>15.00±0.0071</td>
<td>497.00±2.83</td>
</tr>
<tr>
<td>ANTH + T</td>
<td>23.98±0.0212</td>
<td>560.95±1.48</td>
</tr>
</tbody>
</table>

Values are given as mean ±S.D. for groups of ten animals each. Values not sharing a common superscript (a-e) differ significantly at p < 0.05, Duncan’s multiple range test (DMRT).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Postprandial blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>97.56±0.62</td>
<td>116.80±1.13</td>
</tr>
<tr>
<td>TC</td>
<td>141.00±1.41</td>
<td>157.00±2.83</td>
</tr>
<tr>
<td>ALL C</td>
<td>210.99±1.43</td>
<td>287.50±0.71</td>
</tr>
<tr>
<td>TAU C</td>
<td>104.39±2.28</td>
<td>123.00±1.04</td>
</tr>
<tr>
<td>TAU+T</td>
<td>115.80±0.28</td>
<td>136.50±3.54</td>
</tr>
<tr>
<td>ANTH C</td>
<td>97.95±0.07</td>
<td>119.40±0.57</td>
</tr>
<tr>
<td>ANTH+T</td>
<td>105.45±2.05</td>
<td>127.35±0.78</td>
</tr>
</tbody>
</table>

Values are given as mean ±S.D. for groups of ten animals each. Values not sharing a common superscript (a-e) differ significantly at p < 0.05, Duncan’s multiple range test (DMRT).

Effect of MC-LR Administration on ALT and Amylase Activity: ALT and amylase activities are depicted in Table-1. Injection of MC-LR to the mice elevated ALT about five fold. The pharmacological treatment with taurine and anthocyanin respectively prevented these elevations significantly. It seems that anthocyanin afforded a better protection against ALT leakage from liver than it was given by taurine treatment. Its activity and level was reduced to normal value in ANTH + T and TAU + T groups.

The serum amylase activity was markedly increased (1.6 times the control mean) in the T C groups. A statistically decrease in blood amylase level was absorbed in ANTH + T and TAU + T groups comparing to the T C groups. The serum activity of amylase anthocyanin/MC-LR treated group was closer to the control range than it was in TAU + T group.

Effect of MC-LR Administration on Blood Glucose Level: In order to induce diabetes in mice, the animals were injected with alloxan at a dose of 180mg/kg of b.w and showed increased fasting blood glucose concentration to 116.80% of control. Increased serum glucose was also observed in T C group. There was significantly difference between all groups. Table 2 demonstrates that all microcystin-treated mice showed elevated blood glucose levels as compared to C group. This increase was...
Table 3: ALP (U/ml) and MDA level (nmol/mg of wet tissue) in the liver, pancreas and testis of all groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Testis</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.177±0.014*a</td>
<td>0.131±0.028*a</td>
<td>0.164±0.056*a</td>
<td>0.770±0.0141*a</td>
<td>0.665±0.0354*a</td>
<td>0.685±0.0212*a</td>
</tr>
<tr>
<td>TC</td>
<td>0.033±0.042*b</td>
<td>0.074±0.037*b</td>
<td>0.053±0.021*b</td>
<td>6.415±0.1202*b</td>
<td>3.160±0.0424*b</td>
<td>4.200±0.1273*b</td>
</tr>
<tr>
<td>TAU C</td>
<td>0.167±0.019*b</td>
<td>0.119±0.021*b</td>
<td>0.124±0.014*b</td>
<td>1.305±0.0212*b</td>
<td>0.920±0.0566*b</td>
<td>1.040±0.0141*b</td>
</tr>
<tr>
<td>TAU+T</td>
<td>0.090±0.021*c</td>
<td>0.087±0.017*c</td>
<td>0.096±0.028*c</td>
<td>3.095±0.0354*c</td>
<td>2.140±0.0707*c</td>
<td>2.045±0.0354*c</td>
</tr>
<tr>
<td>ANTH C</td>
<td>0.187±0.021*c</td>
<td>0.127±0.035*c</td>
<td>0.151±0.028*c</td>
<td>1.135±0.0495*c</td>
<td>0.755±0.0495*c</td>
<td>1.025±0.0354*c</td>
</tr>
<tr>
<td>ANTH+T</td>
<td>0.168±0.021*c</td>
<td>0.110±0.034*c</td>
<td>0.134±0.049*c</td>
<td>2.845±0.0212*c</td>
<td>1.785±0.0071*c</td>
<td>1.415±0.0212*c</td>
</tr>
</tbody>
</table>

Values are given as mean ±S.D. for groups of ten animals each. Values not sharing a common superscript (a-e) differ significantly at \( p < 0.05 \), Duncan’s multiple range test (DMRT).

Table 4: Protein carbonyl contents (mol/mg of wet tissue) and GST (U/ml) estimation in the liver, pancreas and testis of all groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Testis</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.496±0.0021*a</td>
<td>0.403±0.0049*a</td>
<td>0.413±0.0028*a</td>
<td>0.181±0.021*a</td>
<td>0.101±0.014*a</td>
<td>0.121±0.021*a</td>
</tr>
<tr>
<td>TC</td>
<td>2.463±0.0042*b</td>
<td>1.893±0.0049*b</td>
<td>2.055±0.0495*b</td>
<td>0.092±0.021*b</td>
<td>0.065±0.017*b</td>
<td>0.066±0.014*b</td>
</tr>
<tr>
<td>TAU C</td>
<td>0.610±0.0014*a</td>
<td>0.507±0.0014*a</td>
<td>0.835±0.0354*a</td>
<td>0.185±0.035*a</td>
<td>0.098±0.014*a</td>
<td>0.111±0.017*a</td>
</tr>
<tr>
<td>TAU+T</td>
<td>2.114±0.0070*c</td>
<td>1.409±0.0035*c</td>
<td>1.336±0.0057*c</td>
<td>0.111±0.014*c</td>
<td>0.086±0.017*c</td>
<td>0.099±0.019*c</td>
</tr>
<tr>
<td>ANTH C</td>
<td>1.088±0.0162*c</td>
<td>0.743±0.0262*c</td>
<td>0.725±0.0321*c</td>
<td>0.175±0.014*c</td>
<td>0.102±0.035*c</td>
<td>0.113±0.014*c</td>
</tr>
<tr>
<td>ANTH+T</td>
<td>1.872±0.0106*c</td>
<td>1.070±0.0566*c</td>
<td>1.475±0.0212*c</td>
<td>0.155±0.014*d</td>
<td>0.092±0.035*c</td>
<td>0.089±0.014*c</td>
</tr>
</tbody>
</table>

Values are given as mean ±S.D. for groups of ten animals each. Values not sharing a common superscript (a-e) differ significantly at \( p < 0.05 \), Duncan’s multiple range test (DMRT).

Table 5: GK (U/ml), PK (U/ml), SDH (U/ml), LDH (U/ml) and SOD (U/ml) activities of various organs of all groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Testis</th>
<th>Liver</th>
<th>Pancreas</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.251±0.0014a</td>
<td>2.910±0.014a</td>
<td>1.425±0.0212a</td>
<td>0.089±0.0007a</td>
<td>0.037±0.0014a</td>
<td>36.35±0.495a</td>
</tr>
<tr>
<td>TC</td>
<td>0.405±0.0056b</td>
<td>0.975±0.007a</td>
<td>0.675±0.0071a</td>
<td>0.011±0.0014b</td>
<td>0.012±0.0070b</td>
<td>83.00±0.141b</td>
</tr>
<tr>
<td>ALL C</td>
<td>0.555±0.0070c</td>
<td>0.570±0.014c</td>
<td>0.585±0.0071c</td>
<td>0.092±0.0026c</td>
<td>0.035±0.0018c</td>
<td>33.56±0.426c</td>
</tr>
<tr>
<td>TAU C</td>
<td>0.221±0.0021d</td>
<td>2.830±0.056d</td>
<td>1.345±0.0636d</td>
<td>0.076±0.0007d</td>
<td>0.040±0.0021d</td>
<td>40.50±0.707d</td>
</tr>
<tr>
<td>TAU+T</td>
<td>0.323±0.0042e</td>
<td>2.180±0.012e</td>
<td>0.940±0.0141e</td>
<td>0.060±0.0021e</td>
<td>0.022±0.0014e</td>
<td>56.10±0.141e</td>
</tr>
<tr>
<td>ANTH C</td>
<td>0.244±0.0021f</td>
<td>3.055±0.063f</td>
<td>1.210±0.0283f</td>
<td>0.087±0.0014f</td>
<td>0.033±0.0018f</td>
<td>39.01±0.057f</td>
</tr>
<tr>
<td>ANTH+T</td>
<td>0.248±0.0021f</td>
<td>2.935±0.007f</td>
<td>1.215±0.0354f</td>
<td>0.067±0.0021f</td>
<td>0.023±0.0014f</td>
<td>50.55±0.629f</td>
</tr>
</tbody>
</table>

Values are given as mean ±S.D. for groups of ten animals each. Values not sharing a common superscript (a-f) differ significantly at \( p < 0.05 \), Duncan’s multiple range test (DMRT).

Attenuated in the groups treated with taurine and anthocyanin separately. In addition, postprandial blood glucose level was higher than normal levels in both alloxan and toxin treated group even after the supplementation of the antioxidant.

**Effect of MC-LR Administration on the Enzymatic Activity of ALP and MDA:** Microcystins have been characterized as potent inhibitors of serine and threonine phosphatases. This characteristic was assessed in the liver pancreas and testis of mice i.p. injected with the extracted cyanotoxin (Table-3). It was obvious that the liver was the target organ with a dramatic decrease in the ALP activity, reaching 5.36 fold. the testis and pancreas ALP activity was inhibited at 2 and 0.77 fold respectively, comparing to the control group. This inhibition was not observed in the groups being supplemented with antioxidants.

Lipid peroxidation was significantly increased in liver (7.33 fold), pancreas (3.75 fold) and testis tissue (5.13 fold) of MC-LR treated mice compared with control groups. However, MDA levels of antioxidant- treated groups (ANTH + T and TAU + T groups) were significantly lower than that of toxin injected groups in all the tissues.

**Determination of Protein Carbonyl Contents and GST:** Values of the protein content, a biomarker of protein oxidation protein carbonyl content were significantly increased in the groups intoxicated with MC-LR in comparison to the controls. The administration of the...
chemoprotectants resulted in a significant reduction on the protein oxidation level in the liver, pancreas and testis of intoxicated mice. The protective effect was significantly demonstrated in the three organs.

It was evident that the toxin control group had the lowest activity of the antioxidant enzymes in the liver, pancreas and testis as compared to their control groups. The protective effects of anthocyanin and taurine in intoxicated groups were significantly demonstrated in the three organs. It was apparent that anthocyanin supplementation to mice afforded better results in protecting against MC-LR toxicity than it was afforded by taurine supplementation.

**Determination of GK, PK, SDH, LDH and SOD:**
Glucokinase activity measured as the difference between the glucose phosphorylating capacity of the liver homogenate assayed at 100 and 0.5 mM glucose was about 2.2 fold higher in the diabetic group and about 1.6 fold higher in MC-LR treated mice. This over activation was reduced in the groups supplemented with the chemoprotectants. Pretreatment of the animals with taurine lowered the GK activity about 25%. However the effect of the anthocyanin supplementation was better with 40% of comparing to the TC group.

PK activity was assayed in the liver and pancreas tissues. Compared with the control groups, activity of PK was markedly decreased in mice receiving i.p. injection of MC-LR (2.98 times in liver and 2.11 times in pancreas). This decrease was 5 fold and 2.4 fold higher in the liver and the pancreas of the alloxan treated mice. Anthocyanin supplementation increased PK activity about 2.5 fold in the liver and 1.65 fold in the pancreas whereas it was about 2.23 and 1.39 fold in the liver and pancreas of the groups pretreated with taurine respectively.

Microcystin exposure may have induced a decrease in SDH activity. Treatment with the extracted toxin decreased (5.5 fold) SDH activity in comparison to control group and the protection was observed when mice were supplemented with the antioxidants.

The oral administration of taurine to mice increased 3 times the SDH activity in comparison to intoxicated mice. Similar changes were demonstrated with anthocyanin supplementation with raise of 3.46 times in SDH activity.

LDH exhibited a trend toward decreasing in activity in association with the toxin exposure. The enzymatic activity of LDH in LC-MR treated was lowered about three times to that of control group. In the other hand, its activity was positively affected by supplementing taurine and augmented about two times to that of toxin control group. Similar effect was noted with anthocyanin pretreatment of the mice but a lower level.

The SOD activity experienced a significant increase (2.3 fold in the testis of intoxicated group that did not receive the chemoprotectant. By contrast, a significant decrease in SOD activity (0.66 fold) comparing to the CT group was detected the testis of ANTH + T group. The administration of taurine as chemoprotectant was effective too. The decreases in SOD activity was 1.47 fold as change. Exceptionally, the last treatment afforded a slightly better protection than it was given by anthocyanin supplementation.

**DISCUSSION**

MC-LR rapidly accumulates in the liver of mice inhibiting the protein phosphatases causing damage by cytoskeletal disorganization, cell blebbing and cellular disruption followed by intra hepatic haemorrhage that may lead to the death of the organisms [23]. ALT is a cytosolic prevailing enzyme release into the blood following liver damage or necrosis. Liver cytotoxicity was also confirmed by the inhibition of phosphatase alkaline in intoxicated groups and raised level of MDA and protein carbonyl contents. These results are consistent with the findings of previous workers who have established that MC-LR inhibits the catalytic subunits of PP1 and PP2A [24, 25].

The toxins apparently enter non hepatocytes, which lack the multispecific organic anion transporter that enables efficient uptake into hepatocytes, by a non specific route such as pinocytosis as reported by Wickstrom [12]. However testis tissue of intoxicated groups experienced a more adverse effect than it was noted in their pancreas. Oxidative damage might also contribute to the reorganization of actin and disruption of intracellular junctions following microcystin ingestion [26]. At present it has been documented that MC-LR hepatotoxicity is closely associated with intracellular ROS formation and apoptosis in hepatocytes [9]. The microcystins was also known to cause damage to cellular membranes, particularly that of the mitochondria resulting in the release of cytochrome C, calcium ions and inter membrane proteins into cytosol of the cell [18].
Amylase is one of the main enzymes produced in exocrine pancreatic cells, which may be recognised as an adequate indicator of organ’s activity both in physiological and pathological status [27]. It will enhance two to three folds in pancreatic attack. Nevertheless, the separate supplementation of anthocyanin and taurine to the toxin treated groups showed a remarkable protection against alpha amylase leakage from the pancreas. Pyruvate kinase is one of the rate limiting steps of glycolysis. A decrease in the pyruvate kinase activity clearly indicates the overall flux of glycolysis [25]. In the present study, there is a decrement in PK activity in both liver and pancreas in MC-LR treated mice. It was reported by Miguel et al. [11] that PK activity and mRNA expression were found to be decreased both in adipose tissue and in cultural pancreatic islets of diabetic patients, as well as in animal models of insulin deficient diabetes which explain the present results. This decrement was reduced in mice groups pretreated with antioxidants. Glucokinase, one of the key enzymes in the catabolism in the glucose, which phosphorylates glucose and converts into glucose-6-phosphate and that is known to be altered in experimental diabetes [26]. High glucose concentration leads to the dissociation of glucokinase and its inhibitory enzyme glucose regulatory protein and therefore activates glucokinase [28]. Referring to the fact that protein phosphatases are markedly involved in glycolysis [27] and the fact that MCs are potent inhibitors of those phosphatases, the present results concerning PK and GK alterations could be obviously explained. Oxidative stress is one of the mechanisms of action leading to diabetes, due to over production of ROS and decreased efficiency of antioxidant defences [30].

Alloxan is commonly used for diabetogenic agents, can destroy insulin producing beta cells by free radical inducing cellular damage [26]. Alloxan can impair enzymatic activity in pancreatic beta cells, it could be hypothesised that MC’s diabetogenic effect in the pancreas is similar to that of alloxan, considering that both have the same mechanism of action. The cyanotoxin also affected mice testis by causing oxidative stress indicated by the significantly increased levels of lipid peroxidation and protein carbonyl contents after exposure. Mammalian testis cell membranes are rich in polyunsaturated fatty acids and are sensitive to oxygen induced damage mediated by lipid peroxidation [25].

SOD is one of the testicular enzymatic ROS scavengers. Spermatogenic cells are highly susceptible to oxidative injury. Experiments revealed that sertoli cells could produce not only cytosolic SOD, but also an extra cellular form of SOD [31]. It is well known that SDH and LDH in testicular tissue are associated with the maturation of germinal epithelial layer of seminiferous tubules [21]. The considerable decrease of LDH and SDH suggest that the toxic exposure could cause deterioration of germinal epithelium and damaging the testis. The enhanced lipid peroxidation in testis may result in the disintegration of the mitochondrial membrane ultra structure which in turn affects the membrane bound LDH function.

In fact, uncontrolled and excessive production of ROS plays a vital role as one of the major factors leading to an infertile status and causes oxidative stress resulting in decreased sperm motility, viability, increased sperm capacitation and acrosome reaction defects [21]. Administration of anthocyanin and taurine in MC-LR treated mice afforded a significant protection against MC-LR induced toxicity reflected in restoration of testicular LDH, SDH and SOD levels and activities to normal values. GSTs are enzymes of detoxication systems found in animals and plants. GST involvement in the in vivo MC-LR detoxication pathways has been shown in several organisms (Best et al., 2002). The present data found that the MC-LR provoked the formation of considerable amount of ROS in mice and the alteration of antioxidant enzymatic activity. GST activity was lower in liver, pancreas and testis of MC-LR treated group. GST is involved in eliminating peroxides that are formed during metabolism. Decreased GST activity may be due to the depletion of GSH level in response to MC-LR toxicity [7, 25], which is necessary component in the enzymatic conjugation of GST.

Several studies have been well documented as the protective effect of antioxidants against the toxic action of MCs [31-33]. Also the present study, the separate pre-supplementation of taurine and anthocyanin to the MC-LR intoxicated groups showed a remarkable protection against oxidative stress meeting the finding previously published by Tas et al. [34] and Jaina et al. [35]. This supplementation may have a role in scavenging hydroxyl and peroxy radicals generated by MC-LR. These results suggest that both taurine and anthocyanin administration prior to the toxin injection to mice reduces lipid peroxidation possibly by decreasing free radical formation, increasing antioxidant and associated enzymes content with a significant lowering in blood glucose level. Also antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves [36].
Taurine (2-aminoethane sulfonic acid), widely present in high intracellular concentrations in most mammalian tissues, is taken in a normal diet and can be derived from methionine or cysteine. It has various biological and physiological functions such as being an antioxidant and reducing the cellular damage caused by free radicals [34]. Anthocyanins are naturally occurring polyphenolic pigments widely distributed in the plant kingdom. In vivo and in vitro studies indicate that anthocyanin have several salutary effects such as improving lipid profile, anti-inflammatory and antioxidative activities [37].

REFERENCES