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Silymarin as a therapeutic extract for intestinal and splenic injuries induced by microcystin-LR in mice

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ABSTRACT

Introduction: Microcystin-LR is a toxic compound produced by Cyanobacteria and responsible for to destruction of different types of human and animal tissues. These damages are treated by different flavonoids such as silymarin. Silymarin is a Silybum marianum extract that has antioxidant, anti-inflammatory and anticancer functions.
Materials and methods: Sixty male Balb/c mice 5–7 weeks-old classified into six groups; control (C), two toxin control groups (M6 and M12), silymarin control group (S) and two toxin silymarin groups SM6 and SM12. Blood samples were collected from each mousefor serum transaminases measurement. Intestinal and splenic homogenates were used for measurement of reduced glutathione (GSH), protein carbonyl derivative oxidation (CC), lipid peroxidants (LPO) and methylglyoxal (MG).
Results: Transaminases showed significant statistical differences between all six groups. Moreover, significant statistical differences were observed in PPI, GSH, LPO, CC and MG between six groups in Intestinal tissue. On splenic tissue, significant statistical differences were observed only in PPI and CC.
Conclusion: Silymarin has an antioxidant by induction of protein phosphatase I activity and increases reduced glutathione in microcystin-LR induced intestinal and splenic injuries.

1. Introduction

Cyanotoxins a group of toxins produced by blue-green algae called cyanobacteria. These algae grow in different water supplies such as dams, blooms and lakes (Hitzfeld et al., 2000). Cyanotoxins are responsible for health problems and may cause death in different ways (Lakshmana Rao et al., 2002). Microcystin-LR is a most toxic variant of most common cyanotoxin group called microcystin produced mainly by species called Microcystis aeruginosa. Microcystin-LR is the well define microcystin that causes cell death in different organs of human (Botha et al., 2004). This toxin causing cell death through a different mode of action. It inhibits both protein phosphatase I (PPI) and protein phosphatase 2A (PP2A) important in dephosphrylation of different types of proteins responsible for cell integrity and cytoskeleton. Hyperphosphrylation of these proteins causing cells deformity and degradation (Dawson, 1998). It also induces production of reactive oxygen species (ROS) that causing oxidative stress of the cell followed by cell death (Lankoff et al., 2004). The other mode of microcystin-LR action an induction of apoptosis by activation of caspases pathway and modulation of the immune system (Fladmark et al., 2002). The common symptoms of microcystin-LR intoxication include abdominal pain, diarrhea, vomiting, skin irritation and sore throat (Christoffersen and Kaas, 2000). Microcystin-LR induces apoptosis of gastrointestinal lining epithelial and inhibits its digestive enzymes leading to gastrointestinal lesions in mice (Rohrlack et al., 2005). Microcystin-LR was detected in dams, blooms and groundwater wells in different areas such as southern and western areas (Mohamed and Al Shehri, 2009). Silymarin is an active component of Silybum marianum (Milk thistle), a Carduus marianum member used for long periods for treatment of liver diseases (Morishima et al., 2010). It also used for the treatment of gastrointestinal and biliary tract diseases. Moreover, silymarin has a therapeutic effect in cardiac, lung, kidney and central nervous system disorders (Gharagozloo et al., 2010). It acts as anti-oxidant through free radical scavenger and increasing reduced glutathione and superoxide

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dismutase cellular level. Moreover, it works as an anti-inflammatory via inhibiting T-cells proliferation and pro-inflammatory cytokines (Lankoff et al., 2004). In addition, it inhibits production of IL-2, IL-4, interferon gamma (IFN-y) and natural factor kB (NFkB), while induces B-cell proliferation (Fladmark et al., 2002). This study was aimed to detect the toxic effect of microcystin-LR on intestine and spleen. In addition, to observed therapeutic action of silymarin on splenic and intestinal injuries induced by microcystin-LR (Metcalf et al., 2001).

2. Materials and methods

This study was done in Applied Medical Sciences, Taif university. Sixty mice Male Balb/c mice 5–7 weeks-old with average body's weight 30 g were used were used in this study and classified into six groups as follows: Group 1 consisted of ten mice assigned as control (C) group, no silymarin and microcystin-LR supplements. Both groups 2 and 3 consisted of twenty mice injected with microcystin-LR intraperitoneally about 19 mg/kg mouse body weight and were assigned as toxin control groups (M6 and M12). Ten mice of M6were killed after 6 h and while for M12 were killed after 12 h. Group 4 was the silymarin control group (S) consisted of ten mice supplemented orally with 250 mg silymarin/kg mouse body weight daily for ten days, then they were killed. Both group 5 and group 6 consisted of ten mice each. Mice in both groups supplemented orally with 250 mg silymarin/kg mouse body weight daily for ten days, then both were injected intraperitoneally with 19 mg microcystin/kg mouse body weight. Ten mice in the fifth group were killed after six hours (SM6) while mice in the sixth group were killed after 12 h (SM12). A blood sample was collected immediately after death and serum was isolated and stored at −20C for biochemical tests. Both intestine and spleen were removed immediately after death, perfused with normal saline containing heparin, weighted and homogenized with phosphate buffer saline (pH 7.2) using Ultra Turax homogenizer, centrifuged at 3000g for 30 min. The supernatant was removed and stored at −20 °C.

2.1. Biochemical measurement

All chemicals used for measurement of different parameters were purchased from (Sigma-Aldrich, USA) and all chemical parameters were measured spectrophotometrically by using (UV-1800, Shimadzu). Both serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were measured for each mouse included in this study. Tissue reduced glutathione (GSH) which acts as a protector against reactive oxygen species, protein phosphatase I (PPI), protein carbonyl derivative (CC) resulting from protein oxidation, lipid peroxidants (LPO) resulting from oxidative degradation of polyunsaturated fatty acids in cell membrane and toxic methylglyoxal (MG) were measured by using the same spectrophotometer machine with wavelength ranged 360–550 nm (Gehringer et al., 2004).

2.2. Statistical analysis

It was done by using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). All data were expressed as mean ± SD and all comparisons of total chemical parameters included in this project (ALT, AST, LPO, PPI, GSH, CC and MG) between different groups were done by using one-way analysis of variance (ANOVA). The level of significance was set at $P < 0.05$.

3. Results

This study included sixty male Balb/c mice classified into six groups each group contains ten mice. Table 1 represents transaminases levels in all groups. Serum ALT activity showed significant statistical differences between all six groups. Microcystin-LR induces ALT in both M6 and M12groups (1495.6 ± 14.52and 2110.4 ± 53.98) respectively compared with the control group (513.5 ± 6.09) ($P < 0.01$). Silymarin reduced ALT activity in both SM6 and SM12 (644.4 ± 23.67and901.3 ± 26.31) respectively by two folds compared with M6 and M12 groups. Moreover, microcystin-LR induced serum AST activity in both M6 and M12 (1.800 ± 0.009 and 3.456 ± 0.011) respectively compared with the control group (0.392 ± 0.005) ($P < 0.01$). Silymarin reduced AST activity in both M6 and M12by three folds (0.602 ± 0.006and 1.246 ± 24.24) respectively. Table 2 represents intestinal tissue homogenate; protein phosphatase I (PPI), lipid peroxidation (LPO), reduced glutathione (GSH), protein carbonyl content (CC) and methylglyoxal (MG) levels in all six groups. Microcystin-LR reduced PPI levels as showed in both M6 and M12 (4.50 ± 0.146 and 3.33 ± 0.141) respectively compared with the control group (8.34 ± 0.021). Silymarin induced PPI level in both microcystin-LR groups (5.33 ± 0.060 and 5.88 ± 0.036) respectively ($P < 0.05$). Also, silymarin induced reduced glutathione level after microcystin-LR injection in both M6 and M12 (2.300 ± 0.150 and 1.750 ± 0.283) respectively to become (2.700 ± 0.212 and2.100 ± 0.354) ($P < 0.05$). Protein carbonyl content showed significant statistical differences between all groups ($P < 0.05$). The higher level was within the M12 group (2.000 ± 0.015) compared with the control group (1.010 ± 0.065). Silymarin reduced protein carbonyl in both microcystin-LR groups (1.700 ± 0.026 and 2.000 ± 0.015) to be (1.200 ± 0.002 and 1.310 ± 0.032) respectively ($P < 0.05$). According to methylglyoxal, microcystin-LR induced its levels in bothM6 and M12 (8.030 ± 0.021 and 13.940 ± 0.106) respectively compared with the control group (1.150 ± 0.035) ($P < 0.01$). Silymarin treatment reduced methylglyoxal after microcystin-LR injection to be (3.940 ± 0.014 and 4.340 ± 0.014) respectively ($P < 0.01$). Intestinal tissue lipid peroxidation induced by microcystin-LR injection in both M6 and M12 (710.20 ± 4.31 and 789.10 ± 2.69) respectively compared with the control group (429.89 ± 1.24) ($P < 0.05$). Silymarin treatment reduced these levels into (601.20 ± 2.59 and 710.00 ± 2.16) respectively ($P < 0.05$). Biochemical parameters of six groups in intestinal homogenate also represented in Fig. 1. Table 3 represents splenic tissue homogenate; protein phosphatase I (PPI), lipid peroxidation (LPO), reduced glutathione (GSH), protein carbonyl content (CC) and methylglyoxal (MG) levels in all groups. Protein phosphatase I level reduced by microcystin-LR in both M6 and M12 (2.960 ± 0.042 and 3.33 ± 0.141) respectively compared with the control group (5.330 ± 0.084) ($P < 0.05$). Treatment of these groups with silymarin induced protein phosphatase I in both M6 and M12 (5.155 ± 0.038 and 5.000 ± 0.063) respectively. Microcystin-LR induced protein carbonyl content in both M6 and M12 (4.312 ± 0.230 and 4.693 ± 0.260) compared with control group (1.241 ± 0.180) ($P < 0.01$). Microcystin-LR induced lipid peroxidation and methylglyoxal and theses product reduced by silymarin treatment but the comparisons are not statistically significant. Finally, biochemical parameters of six groups in splenic homogenate also represented in Fig. 2.

4. Discussion

Oxidative stress meaning imbalance between reactive oxygen species and its detoxifying agents called antioxidants. This oxidative stress induces membrane permeability and destruction. Damaging of cell membrane leading to escape of its constituents such

Table 1
Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in all groups.

Parameters	C	M6	M12	SC	SM6	SM12	P value
ALT (U/L)	513.5 ± 6.09	1495.6 ± 14.52	2110.4 ± 53.98	375.2 ± 3.22	644.4 ± 23.67	901.3 ± 26.31	0.005**
AST (U/L)	0.392 ± 0.005	1.800 ± 0.009	3.456 ± 0.011	0.302 ± 0.005	0.602 ± 0.006	1.246 ± 24.24	0.006**

*P < 0.05 **P < 0.01 Control group (C), microcystin6 hours group (M6), microcystin 12 h group (M12), silymarin control group (SC), silymarin microcystin 6 h group and silymarin microcystin 12 h group (SM12).

Table 2
Intestinal tissue lipid peroxidation (LPO), reduced glutathione (GSH), protein carbonyl content (CC) and methylglyoxal (MG) levels in all groups.

Parameters	C	M6	M12	SC	SM6	SM12	P value
PP1 (U/mg)	8.34 ± 0.021	4.50 ± 0.146	3.33 ± 0.141	8.22 ± 0.030	5.33 ± 0.060	5.88 ± 0.036	0.033*
LPO (μM)	429.89 ± 1.24	710.20 ± 4.31	789.10 ± 2.69	399.86 ± 2.36	601.20 ± 2.59	710.00 ± 2.16	0.041*
GSH (nM)	3.200 ± 0.200	2.300 ± 0.150	1.750 ± 0.283	3.00 ± 0.250	2.700 ± 0.212	2.100 ± 0.354	0.021*
CC (nmol)	1.010 ± 0.065	1.700 ± 0.026	2.000 ± 0.015	0.920 ± 0.013	1.200 ± 0.002	1.310 ± 0.032	0.041*
MG (μM)	1.150 ± 0.035	8.030 ± 0.021	13.940 ± 0.106	1.010 ± 0.063	3.940 ± 0.014	4.340 ± 0.014	0.003**

*P < 0.05 **P < 0.01 Control group (C), microcystin6 hours group (M6), microcystin 12 h group (M12), silymarin control group (SC), silymarin microcystin 6 h group and silymarin microcystin 12 h group (SM12).

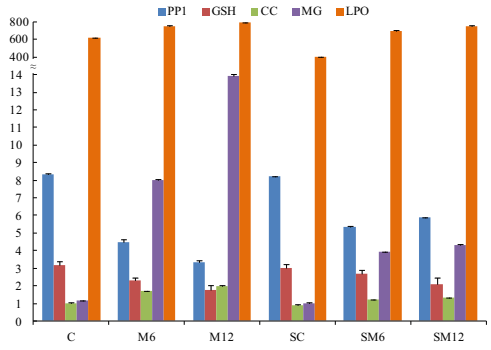

Fig. 1. Biochemical parameters of six groups in intestinal homogenate.

as carponyl protein content, lipid peroxidation products such as methylglyoxal (Chandra et al., 2015). Microcystin-LR is a major and potent toxin produced by cyanobacteria. It defines as a hepatotoxin that causes severe damage of hepatic tissue. Microcystin also induces destruction of intestinal tissue. Cyanobacteria are observed in dams and groundwater in Asir area in our country Saudi Arabia (Mohamed and Al Shehri, 2009). Microcystin-LR uptake is accomplished by bile acid in intestinal lining epithelia and absorbed mainly by small intestine (Ito et al., 2000). The intestinal microflora distributed through duodenum, jejunum, caecum and ilium by the specific species and amount. It plays an important role in gut function and immunity (Turnbaugh et al., 2006). Microcystin-LR affects intestinal through induction of in both intestinal lining epithelia and microflora (Zhao et al., 2006). Shin and his colleague conclude that microcystin-LR induces intestinal lining epithelia damage through increases its microflora death and induction of epithelial cell apoptosis. In present study the microcystin-LR induces intestinal lining epithelial destruction by two ways. Primary it inhibits protein phosphatase I and suppress cell growth and proliferation. Secondary, microcystin-LR increases the level of oxidative stress through suppression of reduced glutathione (The Scientific World Journal, 2018). This oxidative stress promotes membrane permeability and destruction. According to that, the intestinal homogenate of two groups treated only with this toxin contain higher level of carponyl protein content, lipid peroxidation, and methylglyoxal and low level of reduced glutathione and protein phosphatase I compared with control group (Guerrero-Beltrán et al., 2012). Interestingly, microcystin-LR reduced protein phosphatase I and reduced glutathione nearly by half compared with normal control (Christoffersen and Kaas, 2000). Silymarin is an extract of Silybum marianum has an antioxidant activity in various tissues. It creates this process by different ways (Dawson, 1998). Silymarin work as scavenger of free radical such as hydroxyl radical, hydrogen peroxide, and superoxide. It also improves its antioxidant activity by induction of reduced glutathione level and by chelating of metal such as cupper, manganese, and ferrous (Di Meo et al., 2013). Chtourou and his colleagues found that administration of silymarin induces protective effect in manganese induced kidney destruction and reduces oxidative stress in tissue. Antioxidant activity of silymarin also accomplished by induction of antioxidant enzymes such as superoxide dismutase and catalase. Kadir et al., study found that administration of 100 mg/kg silymarin dose induces activity of superoxide dismutase and catalase in rat. Moreover, silymarin reduces activity of enzymes that produce reactive oxygen species such as NADPH oxidase and xanthine oxidase (Pauff and Hille, 2009). In gastrointestinal tract, several studies suggested that silymarin protects the intestinal tract through protection of gastric mucosal nitric oxide and non-protein sulfhydral groups (Ito et al., 2002). It prevents colon damage by reduction of lipid peroxidation and inflammatory cytokines and inhibition of neutrophils infiltration. Previous study showed that silymarin treatment reduces levels of IL-1β, tumor necrosis factor-α (TNF-α) and activity of natural factor kappa B (Mountfort et al., 2005). In addition, it modulates intestinal microbial enzymes (Esmaily et al., 2009).

Table 3
Splenic tissue lipid peroxidation (LPO), reduced glutathione (GSH), protein carbonyl content (CC) and methylglyoxal (MG) levels in all groups:

Parameters	C	M6	M12	SC	SM6	SM12	P value
PP1 (U/mg)	5.330 ± 0.084	2.960 ± 0.042	1.820 ± 0.070	5.245 ± 0.166	5.155 ± 0.038	5.000 ± 0.063	0.028*
LPO (uM)	463.89 ± 0.001	444.89 ± 0.001	450.67 ± 0.002	412.00 ± 0.001	419.67 ± 0.001	435.76 ± 0.001	0.110
GSH (nM)	1.500 ± 0.141	1.400 ± 0.035	1.350 ± 0.106	1.600 ± 0.106	1.430 ± 0.091	1.330 ± 0.070	0.092
CC (nmol)	1.241 ± 0.180	4.312 ± 0.230	4.693 ± 0.260	2.6229 ± 0.150	2.593 ± 0.106	2.157 ± 0.270	0.008**
MG (uM)	1.000 ± 0.244	1.500 ± 0.334	1.570 ± 0.014	0.970 ± 0.070	1.210 ± 0.084	1.440 ± 0.049	0.138

*P < 0.05 **P < 0.01 Control group (C), microcystin6 hours group (M6), microcystin 12 h group (M12), silymarin control group (SC), silymarin microcystin 6 h group and silymarin microcystin 12 h group (SM12).

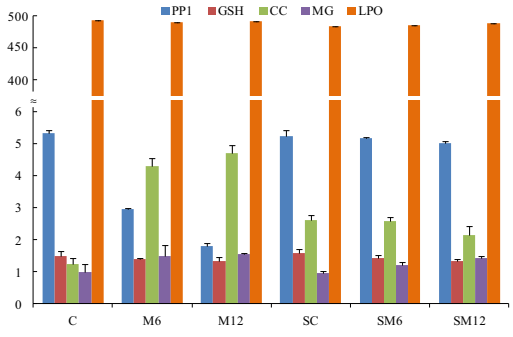

Fig. 2. Biochemical parameters of six groups in splenic homogenate.

Our results showed that silymarin inhibits oxidative stress by induction of reduced glutathione which may reduce lipid peroxidation and protein carponyl content and inhibit methylglyoxal activity in intestinal tissue. Moreover, silymarin induces protein phosphatase I activity which maintain mitosis and cell division. These effects totally result in cells proliferation and injuries cure of this tissue. We know that spleen is a major site for lymphocytes maturation. Microcystin-LR creates its cytotoxic activity in spleen by reduction of lymphocytes proliferation and maturation, cytokines production disturbance and inhibition of phagocytic activity (Lankoff et al., 2004). From our knowledge, there is no any previous study focus on the pathologic effect of microcystin-LR on the spleen. Our results showed that, microcystin-LR induces spleen destruction through inhibition of protein phosphatase I activity in microcystin-LR treated group compared with control group. Oral Silymarin dose maintain normal activity of protein phosphatase I in this tissue. In addition, protein carbonyl content increase in splenic tissue as a result of microcystin-LR injection. After silymarin treatment the protein carponyl content decrease compared with microcystin-LR groups. Reduced glutathione level decreased in microcystin-LR treated groups compared with control group but insignificantly. Silymarin induces reduced glutathione in microcystin-LR treated group but also insignificantly.

Recommendation and conclusion

In vitro study is required to take a broad picture about the therapeutic function of silymarin on cell line and determine the signal proteins involved in these processes. In conclusion, silymarin has an antioxidant by induction of protein phosphatase I activity and increases reduced glutathione in microcystin-LR induced intestinal and splenic injuries.

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Conflict of interest

There is no conflict of interest.

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