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N-acetylcysteine as a therapeutic extract for cardiac, lung, intestine and spleen injuries induced by microcystin-LR in mice

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ABSTRACT

N-acetylcysteine (NAC) is antioxidant product that improves its function by scavenges of free oxygen radical and production of reduced glutathione (GSH). It is used in treatment of different inflammatory diseases and cancers.

Subjects and methods: 60 male Balb/c mice aged 5–7 weeks old classified into; control (C), two toxin control groups (M6 and M12), NAC control group (S) and two toxin NAC groups SM6 and SM12. From each mouse a blood samples were collected for estimation serum transaminases. Lungs, cardiac, intestinal and splenic homogenates were used for measurement of protein carbonyl content (CC), reduced glutathione, lipid peroxidation products (LPO), protein phosphatase I (PPI) activity, and methylglyoxal (MG).

Results: Both ALT and AST were showed a significant statistical difference between all groups. In cardiac homogenate both reduced glutathione and methylglyoxal showed significant statistical differences between six groups ($P < 0.05$). In intestinal homogenate protein phosphatase I activity, methylglyoxal, and lipid peroxidation content showed significant statistical differenced between all groups ($P < 0.05$).

Conclusion: Microcystin-LR has cytotoxic effect of different tissue and induces oxidative stress in these tissues. In addition, n-acetylcysteine has antioxidant activity in different tissue by induction of protein phosphatase activity and intracellular level of reduced glutathione.

1. Introduction

Microcystin is a hepatotoxin produced by algae called cyanobacteria. Water supplies such as lakes and dams are main habitat of this type of algae (Li and Han, 2012). Cyanobacteria grow in several countries around the world. Previous study found a growth of cyanobacteria in different dams and blooms in south area of Saudi Arabia (Mohamed, 2008). Microcystin-LR is a well define and common toxin variant that cause toxicity in different countries (Zholobenko and Modriansky, 2014). N-acetylcysteine (NAC) is a thiol mucolytic compound which plays an important role as an antioxidant molecule (Balsamo et al., 2010). It performs antioxidant activity directly by its reaction with few oxidants such as nitric oxide and hypochlorous acid (Soldini et al., 2005). NAC also act as a precursor of reduced glutathione which acts as antioxidant with wide range of reactive oxygen species (ROS) (Zhang et al., 2018). This reduced glutathione induces activity of catalase, mitochondrial superoxide dismutase and glutathione peroxidase. These enzymes play a significant role in antioxidant system (McGuckin et al., 2015). Moreover, NAC produces its antioxidant activity by breaking disulfide bridges in different proteins such as mucin (Deponte, 2013). Oral dose of NAC is completely absorbed and this gives him a beneficial in medication use. Based on previous mentioned properties of NAC, it used as a therapeutic agent in condition characterized by induction of oxidative stress such as cardiovascular diseases, inflammatory reaction and cancers (Kerksick and Willoughby, 2007). In chronic bronchitis and other lung mucinous disorders, NAC used as mucolytic agent (Persinger et al., 2002). Oxidative stress contributed as a factor for cardiovascular diseases. NAC reduces homocysteine resulting from oxidative stress which responsible for ischemic heart diseases (Talasaz et al., 2011). Previous study concluded that NAC has a protective effect in intestinal mucosa by reducing oxidative stress and maintain normal distribution of intestinal normal flora Korey (Shieh et al., 2019). This study was done to demonstrate therapeutic effect of NAC in pulmonary, cardiac, splenic and intestinal injuries induce by microcystin-LR.

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2. Materials and methods

This study included 60 Male Balb/c mice aged 5–7 weeks with 30 g average weight 30 g. These mice were classified into 6 groups: Group 1; a control group consisted of 10 mice (C), this group had no silymarin and microcystin-LR supplements. The second and third groups consisted of 20 mice injected intraperitoneally with microcystin-LR about 19 mg/kg mouse body weight and were assigned as toxin control groups (M6 and M12). The ten mice of M6 were killed after 6 h the ten mice of M12 were killed after 12 h. The fourth group was assigned as the NAC control group (N) consisted of ten mice supplemented orally with 250 mg NAC/kg mouse body weight/day for 10 days, then killed. Both fifth and sixth groups consisted of ten mice each. Mice in both groups supplemented orally with 250 mg NAC/kg mouse body weight/day for ten days. Moreover, both groups were injected intraperitoneally with 19 mg microcystin/kg mouse body weight. Ten mice in the fifth group were killed after six hours (NM6) while in the sixth group were killed after 12 h (NM12). From each group a blood sample was collected immediately after death and serum was isolated and stored at −20 °C for biochemical tests. From each mouse heart, two lungs, intestine and spleen were isolated 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 3000 g for 30 min. The supernatant was removed and stored at −20 °C. All chemicals used were purchased from (Sigma-Aldrich, USA). For each mouse protein phosphatase I (PPI) activity and levels of reduced glutathione (GSH), protein carbonyl derivative (CC), lipid peroxidation products (LPO) and toxic methylglyoxal (MG) were measured in cardiac, lungs, intestine and spleen homogenates supernatant. In addition, serum alanine aminotransferase (ALT) and aspartate aminotransferase were measured. All parameters were measured spectrophotometrically by using (UV-1800, Shimadzu) (Al-hazmi et al., 2019).

3. Statistical analysis

All data in five table were expressed as mean ± SD. The comparison between the mean of all six groups were evaluated by one-way analysis of variance (ANOVA). The level of significance was set at $P < 0.05$. In this project the SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

4. Results

This study was done on 60 mice classified into six groups. Table 1 represents serum ALT and AST activities in all groups. ALT activity showed a significant statistical difference in all groups. Higher enzyme activity was present in M12 group (2005.3 ± 64.21) compared with control group (554.5 ± 7.20), while lower enzyme activity was present in NC group (503.8 ± 2.89) ($P < 0.01$). The induction of ALT activity in both M6 and M12 (1311.7 ± 5.73 and 2005.3 ± 64.21) groups were significantly reduced after oral administration of NAC (894.4 ± 1.37 and 1544.3 ± 62.22) respectively ($P < 0.01$). Moreover, AST activity showed a significant statistical difference between all groups. The lower activity was present in NC group (0.312 ± 0.007), while M12 group had a higher activity (3.666 ± 0.027) compared with control group (0.433 ± 0.011) ($P < 0.05$). Administration of NAC reduced AST in both M6 and M12 (1.986 ± 0.041 and 3.666 ± 0.027) to be (0.566 ± 0.005 and 1.222 ± 0.007) respectively ($P < 0.01$). Table 2 represent PPI activity, and levels of LPO, GSH, CC and MG of all six group in cardiac homogenate. The NC group had a higher GSH level (2.44 ± 0.130) compared with control group (1.21 ± 0.008) ($P < 0.05$). NAC induced GSH levels in both M6 and M12 (0.71 ± 0.054 and 0.56 ± 0.033) to became (1.50 ± 0.141 and 1.35 ± 0.035) respectively ($P < 0.05$). The MG levels significantly high in M12 (4.44 ± 0.031) compared with control group (1.21 ± 0.008), while the NC group had a lower level of MG (0.85 ± 0.004) ($P < 0.01$). High MG levels represented in both M6 and M12 (2.66 ± 0.019 and 4.44 ± 0.031) were reduced by NAC administration (1.66 ± 0.011 and 1.88 ± 0.014) respectively ($P < 0.01$). Biochemical parameters in pulmonary homogenate were represented in Table 3. The GSH was significantly higher in NC group (2.32 ± 0.112), while its level was lower in M12 group (0.58 ± 0.099) compared with control group (1.82 ± 0.462) ($P < 0.05$). Moreover, its levels were induced in M6 and M12 (0.83 ± 0.043 and0.58 ± 0.099) by NAC administration (1.56 ± 0.066 and 1.28 ± 0.077). Biochemical parameters of intestinal homogenate were represented in Table 4. The PPI activity was higher in NC group (6.44 ± 0.055) compared with control group (4.67 ± 0.018) ($P < 0.05$). In addition, NAC administration induced PPI activity (2.30 ± 0.139 and 2.01 ± 0.161) to be (4.02 ± 0.081 and 3.79.08 ± 0.028) respectively ($P < 0.05$). The LPO levels is parallel with PPI activity and LPO level is higher in M12 group (789.10 ± 2.69) compared with control group (429.89 ± 1.24), and administration of NAC reduced its levels

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

Parameters	C	M6	M12	NC	NM6	NM12	P value
ALT (U/L)	554.5 ± 7.20	1311.7 ± 5.73	2005.3 ± 64.21	503.8 ± 2.89	894.4 ± 1.37	1544.3 ± 62.22	0.007**
AST (U/L)	0.433 ± 0.011	1.986 ± 0.041	3.666 ± 0.027	0.312 ± 0.007	0.566 ± 0.005	1.222 ± 0.007	0.024*

* $P < 0.05$ ** $P < 0.01$ Control group (C), microcystin 6 h 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
Cardiac tissue protein phosphatase I (PPI), lipid peroxidation (LPO), reduced glutathione (GSH), protein carbonyl content (CC) and methylglyoxal (MG) levels in all groups.

Parameters	C	M6	M12	NC	NM6	NM12	P value
PP1 (U/mg)	4.09 ± 0.077	4.87 ± 0.098	5.44 ± 0.098	4.21 ± 0.062	3.81 ± 0.104	3.64 ± 0.111	0.151
LPO (uM)	434.00 ± 1.809	522.21 ± 2.332	584.44 ± 0.512	421.87 ± 4.003	485.33 ± 0.991	478.33 ± 0.420	0.158
GSH (nM)	1.90 ± 0.0.634	0.71 ± 0.054	0.56 ± 0.033	2.44 ± 0.130	1.52 ± 0.109	1.41 ± 0.037	0.048**
CC (nmol)	0.30 ± 0.043	0.44 ± 0.004	0.58 ± 0.047	0.22 ± 0.012	0.31 ± 0.017	0.35 ± 0.011	0.099
MG (uM)	1.21 ± 0.008	2.66 ± 0.019	4.44 ± 0.031	0.85 ± 0.004	1.66 ± 0.011	1.88 ± 0.014	0.041**

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

Table 3
Lung tissue protein phosphatase I (PPI), lipid peroxidation (LPO), reduced glutathione (GSH), protein carbonyl content (CC) and methylglyoxal (MG) levels in all groups.

Parameters	C	M6	M12	NC	NM6	NM12	P value
PP1 (U/mg)	4.54 ± 0.044	4.33 ± 0.022	4.88 ± 0.077	5.33 ± 0.012	5.10 ± 0.122	4.92 ± 0.212	0.183
LPO (uM)	412.00 ± 1.611	423.55 ± 0.008	482.61 ± 0.006	401.77 ± 0.006	422.89 ± 0.005	476.01 ± 0.003	0.065
GSH (nM)	1.82 ± 0.462	0.83 ± 0.043	0.58 ± 0.099	2.32 ± 0.112	1.56 ± 0.066	1.28 ± 0.077	0.046*
CC (nmol)	0.34 ± 0.032	0.66 ± 0.011	0.88 ± 0.00912	0.29 ± 0.004	0.41 ± 0.006	0.51 ± 0.020	0.780
MG (uM)	1.07 ± 0.011	1.31 ± 0.012	1.56 ± 0.017	1.00 ± 0.006	1.19 ± 0.021	1.26 ± 0.007	0.094

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

Table 4
Intestinal tissue protein phosphatase I (PPI), lipid peroxidation (LPO), reduced glutathione (GSH), protein carbonyl content (CC) and methylglyoxal (MG) levels in all groups.

Parameters	C	M6	M12	NC	NM6	NM12	P value
PP1 (U/mg)	4.67 ± 0.018	2.30 ± 0.139	2.01 ± 0.161	6.44 ± 0.055	4.02 ± 0.081	3.79.08 ± 0.028	0.047*
LPO (uM)	429.89 ± 1.204	710.20 ± 4.31	789.10 ± 2.69	389.00 ± 1.011	603.01 ± 1.32	662.54 ± 1.200	0.020*
GSH (nM)	1.72 ± 0.383	1.17 ± 0.121	1.01 ± 0.222	1.86 ± 0.170	1.44 ± 0.041	1.21 ± 0.021	0.086
CC (nmol)	0.47 ± 0.065	1.51 ± 0.039	1.87 ± 0.076	0.41 ± 0.011	1.21 ± 0.010	1.48 ± 0.009	0.106
MG (uM)	1.05 ± 0.035	4.22 ± 0.049	5.88 ± 0.121	0.71 ± 0.034	2.00 ± 0.049	3.05 ± 0.027	0.009**

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

Table 5
Splenic tissue protein phosphatase I (PPI), lipid peroxidation (LPO), reduced glutathione (GSH), protein carbonyl content (CC) and methylglyoxal (MG) levels in all groups.

Parameters	C	M6	M12	NC	NM6	NM12	P value
PP1 (U/mg)	4.32 ± 0.077	3.88 ± 0.039	3.67 ± 0.066	4.70 ± 0.029	4.01 ± 0.076	4.00 ± 0.039	0.133
LPO (uM)	429.67 ± 1.331	551.89 ± 0.061	599.55 ± 0.097	418.67 ± 0.059	433.00 ± 0.044	470.78 ± 0.045	0.088
GSH (nM)	1.61 ± 0.114	1.22 ± 0.085	1.08 ± 0.144	1.71 ± 0.133	1.52 ± 0.033	1.49 ± 0.069	0.157
CC (nmol)	0.57 ± 0.005	1.31 ± 0.189	1.69 ± 0.098	0.53 ± 0.204	1.11 ± 0.117	1.39 ± 0.281	0.075
MG (uM)	1.09 ± 0.043	1.66 ± 0.299	1.97 ± 0.051	1.01 ± 0.004	1.31 ± 0.011	1.51 ± 0.009	0.172

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

(710.20 ± 4.31 and 789.10 ± 2.69) into (603.01 ± 1.32 and 662.54 ± 1.200) respectively (P < 0.05). Moreover, MG showed a significant statistical difference between all six groups with higher level in M12 (5.88 ± 0.121) and its level is suppressed by NAC administration (4.22 ± 0.049 and 5.88 ± 0.121) into (2.00 ± 0.049 and 3.05 ± 0.027) respectively (P < 0.01). Splenic homogenate did not show any significant statistical difference in all biochemical parameters between all six group. In all four homogenates, NAC reduces LPO, CC and MG levels caused by microcystin-LR toxic effect while induces antioxidant level of both GSH level and PPI activity (Table 5).

5. Discussion

Reactive oxygen species (ROS) such as hydrogen peroxide, nitric oxide and superoxide result from aerobic metabolic reactions occur in our bodies (Li et al., 2008). Excessive ROS leading to oxidative stress involved in several chronic diseases and its complication. In addition, several studied showed that oxidative stress plays a significant role in carcinogenesis (Lushchak, 2014). The mode of action of ROS including oxidation of polyunsaturated fatty acids present in cell membrane leading to formation of lipid peroxidation products (Clarkson and Thompson, 2000). ROS also oxidized membrane proteins leading production of protein carponyl carbon. In addition, ROS can be penetrating nuclear membrane and induces DNA damage and mutation (Hart and Kamm, 2002). Microcystin-LR is the most microcystin associated with multiple organ toxicity (Campos and Vasconcelos, 2010). Previous study done on male Balb/c mice showed that microcystin-LR induces lipid peroxidation resulting from oxidative degradation of lipid. In addition, protein carbonyl derivatives resulting from proteins oxidation is increased in intestinal and splenic tissue after injection of microcystin-LR into these mice (Al-hazmi et al., 2019). Methylglyoxal is a toxic byproduct of threonine catabolism and increased in cardiac, pulmonary and intestinal homogenates of these mice (Kalapos, 2008). Microcystin-LR also decreases the level of reduced glutathione that plays a significant role in antioxidant system. Present study showed that, microcystin-LR induces serum ALT and AST by four folds. In addition, it induces lipid peroxidation products by two folds in all four tissues, but decreases the level of reduced glutathione (Zhang et al., 2018). Moreover, protein carponyl products increased by two folds in intestinal and splenic homogenates. The most oxidative product induced by microsistine-LR is methylglyoxal which increased by ten folds in intestinal and cardiac homogenates (Al-hazmi et al., 2019). NAC is a mucolytic thiol containing tripeptide working as antioxidant through different processes. It works as a scavenger of free radical such as hydroxyl radical or induces intracellular reduced glutathione by its cysteine residue (Chaudière and Ferrari-Iliou, 1999). Since 1970 NAC is widely used as a therapeutic agent in different type of disorders (Bistrian, 2018). In addition to its antioxidant properties it had an anti-inflammatory property by reducing polymorphonuclear neutrophils infiltration (Sheweita, 2005). Previous observational study indicates that, NAC is useful in acute myocardial infarction because it induces reduced glutathione level and reperfusion of myocardial ischemia (Ferrari et al., 1991). In addition, it reduced lipoprotein-a and homocysteine levels

involved in cardiomyocyte destruction during cardiac ischemia (Skovsted et al., 2017). In present study, microcystin-LR reduces level of reduced glutathione in cardiac homogenate. Administration of NAC orally induces level of reduced glutathione by two folds in cardiac homogenate of these mice. Moreover, methylglyoxal induced by microcystin-LR was reduced by two folds after NAC administration. NAC also reduced both lipid peroxidation and protein carbonyl content in microcystin-LR injected mice in cardiac homogenate but insignificantly. In chronic bronchitis, Arfesten and his colleague found that administration of 1200/kg NAC did not improve reduced glutathione in lung (Arfsten et al., 2004). On the other hand, Behr and his workers found that administration of 600 mg NAC three times per day for about 12 weeks for fibrosing alveolitis patients induces GSH and pulmonary function tests (Behr et al., 1997). In mucinous lung diseases, oxidation of cysteine residue of mucin causing cross-linked of this residue and production of hardly transported mucus and airway obstruction. Mucolytic property of NAC reduces the cross-link and formation of easily transported mucin (Yuan et al., 2015). Our results showed that NAC induces GSH level in lung tissue after cytotoxic effect of microcystin-LR. This induction maintains normal level of GSH and prevent more damage caused by oxidative stress caused by this toxin. NAC also induces PPI activity which improves cell renewal and growth, while reduces protein carbonyl content, methylglyoxal and lipid peroxidation products but insignificantly. High NAC doses had anti-inflammatory effect by reducing neutrophils infiltration and myeloperoxidase (MPO) activity. Colitis usually associated with increase neutrophils infiltration MPO activity with reduce levels of both GSH and nitric oxide (NO) (Uraz et al., 2008). Akgun et al., 2005 found that, in vivo rat induced colitis, administration of NAC reduced MPO activity and restore GSH and NO levels (Akgun et al., 2005). Other study done by Ocal and his colleagues found that administration of NAC increases reduced glutathione level and decreases level of lipid peroxidation products and MPO activity in thermal induced intestinal injuries (Ocal et al., 2004). Our results were parallel with two previous studies mentioned before. NAC administration induces GSH levels while decreases both lipid peroxidation products and methylglyoxal in microcystin-LR induced intestinal injuries. It also induces protein phosphatase I activity but insignificantly. Protein phosphatase I induces both cellular growth and differentiation and restore intestinal tissue. About effect of NAC on spleen, Neal et al., suggested that NAC treatment is useful for protection of spleen of mice against radiation energy (Neal, 2003). Results of present study didn't show any significant effect of NAC either in reduction of lipid peroxidation products, protein carponyl content and methylglyoxal or of protein phosphatase I activity or reduced glutathione level.

6. Recommendation and conclusion

This study was done on Balb/c mice, so in vitro study is recommended to determine the effect of N-acetylcysteine on cell and the intracellular signal induced by this compound. We conclude that microcystin-LR has cytotoxic effect of different tissue and induces oxidative stress in these tissues. In addition, n-acetylcysteine has antioxidant activity in different tissue by induction of protein phosphatase activity and intracellular level of reduced glutathione.

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Conflict of interest

There is no conflict of interest.

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