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Licochalcone A attenuates oxidative stress and inflammation in carbon tetrachloride-instigated acute hepatotoxicity in the mouse

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Abstract

Background and Objective: Liver disorders are associated with high rate of morbidity and mortality. Carbon tetrachloride (CCL4)-instigated model of ALI is a valid model for exploring liver damage. Licochalcone A is a bioflavonoid which is primarily isolated from roots of Glycyrrhiza species. In this study, the effect of this flavonoid in CCl4 mouse model of acute liver injury (ALI) was assessed.

Materials and Methods: For induction of ALI, CCl4 (10 ml/kg body weight, 0.175% in olive oil) was intraperitoneally injected and licochalcone A was orally administered at doses of 10 or 50 mg/kg. Functional markers of liver dysfunction were determined in addition to hepatic analysis of oxidative stress and inflammatory factors.

Results: Licochalcone A pretreatment at a dose of 50 mg/kg significantly and notably decreased level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), reactive oxygen species (ROS), tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and myeloperoxidase (MPO) and significantly improved total antioxidant capacity (TAC) and superoxide dismutase (SOD) activity and with no significant effect on interleukin-1β (IL-1β). In addition, these beneficial effects were not obtained for licochalcone A at a dose of 10 mg/kg in CCL4-injured group.

Conclusion: These findings show beneficial property of licochalcone A following CCL4-induced liver injury that is exerted via its regulation of oxidative and inflammatory processes and upregulating antioxidant power.

Keywords: Acute liver injury, Hepatotoxicity, Carbon tetrachloride, Licochalcone A, Oxidative stress, Inflammation

1. Introduction

Liver disorders are associated with high rate of morbidity and mortality. Acute liver injury (ALI) is the main cause of liver disease. Liver tissue is the largest bodily which actively participates in degradation of toxicants and maintenance of normal metabolism. When attacked by various pathogens or non-infectious chemicals, hepatocytes are injured and abnormal liver function and ALI occurred. When liver injury is not promptly managed, it may cause acute liver failure, cirrhosis, and even other diseases which endangers affected patients. Several predisposing factors such as viruses, drugs, and toxins are associated with ALI. ALI is marked by hepatic oxidative stress, inflammation, and apoptosis (1, 2).

Carbon tetrachloride (CCL4)-instigated model of ALI is a valid model for exploring liver damage. CCL4 in the liver is metabolized into trichloromethyl radicals by cytochrome P450 with final formation of reactive and damaging trichloromethyl peroxy radicals. Such agents result in altered lipid metabolism and reduction of membrane permeability which leads to degeneration and necrosis of hepatic cells (3).

Many herbal products can prevent and even protect against liver damage which may be used in clinical practice. Licochalcone A (molecular formula, C21H22O4) is a bioflavonoid which is primarily isolated from roots of Glycyrrhiza species (4). Licochalcone A can be effective in treatment and prevention of various diseases. Licochalcone A has

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attracted the global attention of pharmacologists. Significant advancements for licochalcone have been obtained over the past decades (4).

Licochalcone A can inhibit the proliferation of carcinoma and sarcoma cells. Licochalcone A also demonstrated various pharmacological effects including antibacterial, anti-inflammation, anti-oxidative, neuroprotective, and dermoprotective besides its regulation of glucose and lipid metabolism (4,5). In this study, advantageous effect of licochalcone A in CCL4 model of ALI was studied.

2. Materials and Methods

2.1. Animals

Male mice (NMRI, 20-25 g) were obtained from Razi Institute (Karaj, Iran). All mice had adaption for 1 week with controlled conditions for temperature at 21-23°C, humidity at about 47% and with 12/12 photoperiods). They had also free access to diet and water. Mentioned procedures were approved by Ethics Committee of Iran University of Medical Sciences (no. IR.IUMS.FMD.REC.1398.003).

2.2. Experimental design and treatments

Mice were randomly divided into 5 groups consisting of control, licochalcone A 50-treated control, CCL4, licochalcone A 10-treated CCL4, and licochalcone A 50-treated CCL4. Mice in CCl4 group were IP injected with CCl4 (10 ml/kg, 0.175% in olive oil) (6) 1 h after the last treatment of licochalcone A. Treatment groups received oral licochalcone A daily, begun 1 week till 1 h before CCl4 injection. Dose of licochalcone A was according to its antioxidant and chemopreventive effects in an in vivo system (7). After 1 day, mice were sacrificed after deeply anesthetizing with ketamine (120 mg/kg) and blood samples through the heart and liver tissues were collected for further biochemical analysis.

Blood samples were taken from the heart under ketamine anesthesia. The blood samples were kept at room temperature for 30 min and were then centrifuged at 3000 rpm for 15 min to separate serum. Serum activity of ALT and AST was measured using its kits from Pars Azmun Co. (Tehran, Iran).

2.3. Hepatic evaluation of oxidative stress

After preparing liver homogenate in 150 mM Tris-HCl lysis buffer (pH 7.4) and centrifuging them, its supernatant was used for measurement of oxidative stress factors. Level of MDA as a known index of lipid peroxidation was measured using MDA assay reagent (SigmaAldrich, USA) (8,9). ROS level was measured using DCF-DA with its conversion into dichlorofluorescein in the presence of ROS (10). Activity of SOD was determined using its kit from Cayman Chemical (USA). TAC level was determined using its specific kit from Kiazist (Iran). Bradford method was used for determination of total protein level (11). Measurement of MPO activity as an index of neutrophil infiltration was done in accordance to earlier studies (12,13).

Liver levels of inflammatory factors were determined by means of sandwich Elisa protocol (antibodies for TNF-α were obtained from SigmaAldrich (USA) and antibodies for other factors were obtained from Santa Cruz Biotechnology, Inc. (USA)).

2.4. Statistical analysis

Findings are brought as means□SEM. After verification of normal distribution of data using Shapiro-Wilk test, data analysis was performed by one-way ANOVA and Tukey post-test. In all tests, p value less than 0.05 was taken as significant.

3. Results

3.1. The effect of licochalcone A on serum activity of ALT and AST

Measurement of serum activity of ALT (Fig. 1A) and AST (Fig. 1B) in different groups showed that administration of licochalcone A at a dose of 50 mg/kg to the control group is associated with significant changes of ALT and AST ($p>0.05$). In addition, CCL4 group had higher activity of ALT ($p<0.001$) and AST ($p<0.001$) at a significant level. Such significant increase was also observed in CCL4 group treated with licochalcone A at a dose of 10 mg/kg for ALT ($p<0.001$) and AST ($p<0.001$) as compared to the CCL4 group. In contrast, CCL4 group treated with licochalcone A at a dose of 50 mg/kg had significantly lower activity of ALT ($p<0.001$) and AST ($p<0.001$) versus the CCL4 group.

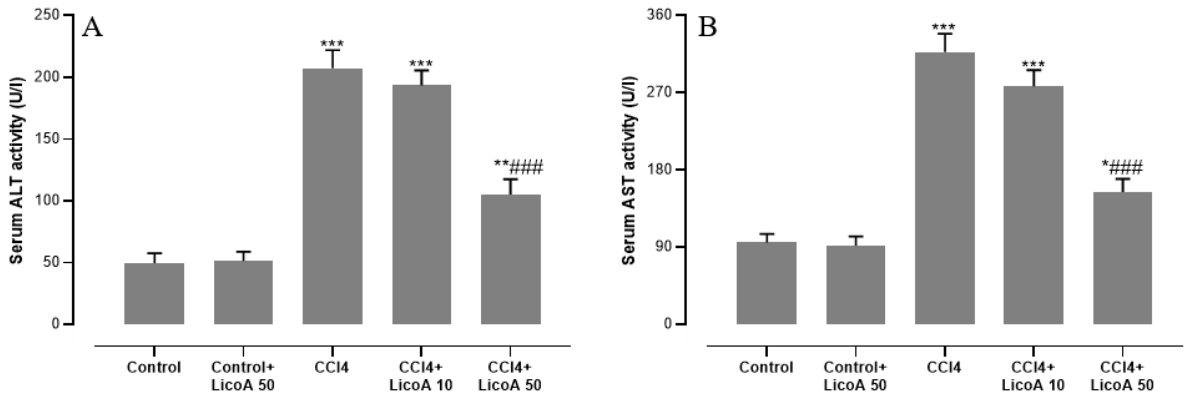

Fig. 1: The effect of licochalcone A on serum activity of ALT (A) and AST (B). * p<0.05, ** p<0.01 vs. control group, *** p<0.001 vs. CCL4 group. Results are presented as mean ± SEM (n = 7 in each group).

3.2. The effect of licochalcone A on oxidants

Measurement of liver tissue levels of MDA as a marker of lipid peroxidation (Fig. 2A) and ROS (Fig. 2B) in different groups indicated that licochalcone A administration at a dose of 50 mg/kg to the control animals did not produce significant changes of MDA and ROS (p>0.05). In addition, CCL4 group had higher liver levels of MDA (p<0.01) and ROS (p<0.001) at a significant level relative to the control group. Such significant increase was also noted in CCL4 group receiving licochalcone A at a dose of 10 mg/kg for MDA (p<0.01) and ROS (p<0.01) versus the CCL4 group. In contrast, CCL4 group treated with licochalcone A at a dose of 50 mg/kg had significantly lower levels of MDA (p<0.01) and ROS (p<0.01) relative to the CCL4 group.

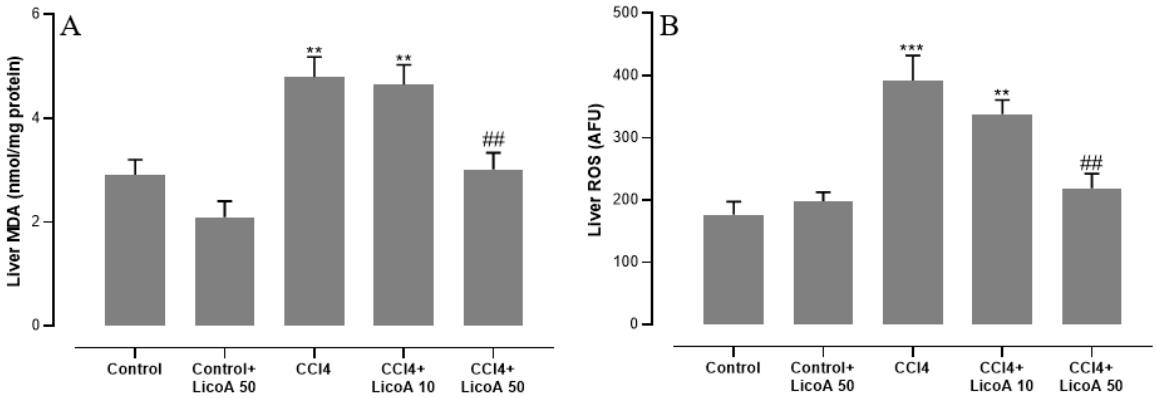

Fig. 2. The effect of licochalcone A on liver tissue levels of MDA (A) and ROS (B). ** p<0.01, *** p<0.001 vs. control group, ## p<0.01 vs. CCL4 group. Results are presented as mean ± SEM (n = 7 in each group).

3.3. The effect of licochalcone A on antioxidants

Measurement of liver tissue levels of antioxidants including TAC (Fig. 3A) and SOD (Fig. 3B) in different groups showed that licochalcone A given at a dose of 50 mg/kg to the control animals did not produce significant changes of TAC and SOD (p>0.05). In addition, CCL4 group had lower liver levels of TAC (p<0.01) and SOD activity (p<0.01) at a significant level relative to the control group. Such significant decrease was also obtained for CCL4 group given licochalcone A at a dose of 10 mg/kg for TAC (p<0.05) and SOD (p<0.05) versus the CCL4 group. Conversely, CCL4 group treated with licochalcone A at a dose of 50 mg/kg had significantly higher levels of TAC (p<0.05) and SOD (p<0.05) relative to the CCL4 group.

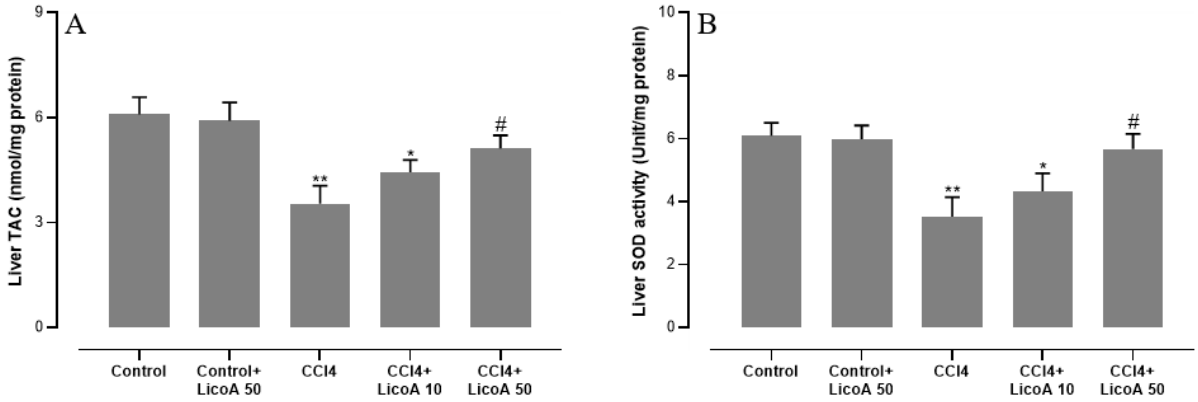

Fig. 3. The effect of licochalcone A on liver tissue levels of TAC (A) and SOD activity (B). * p<0.05, ** p<0.01 vs. control group, ## p<0.01 vs. CCL4 group. Results are presented as mean ± SEM (n = 7 in each group).

3.4. The effect of licochalcone A on inflammation and neutrophil infiltration

Measurement of liver tissue levels of inflammatory factors including TNFα (Fig. 4A), IL-6 (Fig. 4B), and IL-1β (Fig. 4C) in addition to determination of MPO activity as a marker of neutrophil infiltration (Fig. 4D) showed that licochalcone A administration at a dose of 50 mg/kg to the control animals did not produce significant changes of IL-1β, MPO activity, TNFα, and IL-6 (p>0.05). In addition, CCL4 group had higher liver levels of MPO activity (p<0.01), TNFα (p<0.01) and IL-6 (p<0.01) at a significant level relative to the control group with no significant change of IL-1β. Such significant increase was also noted in CCL4 group receiving licochalcone A at a dose of 10 mg/kg only for TNFα (p<0.05) versus the CCL4 group. In contrast, CCL4 group treated with licochalcone A at a dose of 50 mg/kg had significantly lower levels of MPO activity (p<0.05), TNFα (p<0.01) and IL-6 (p<0.01) relative to the CCL4 group and with no significant change of IL-1β.

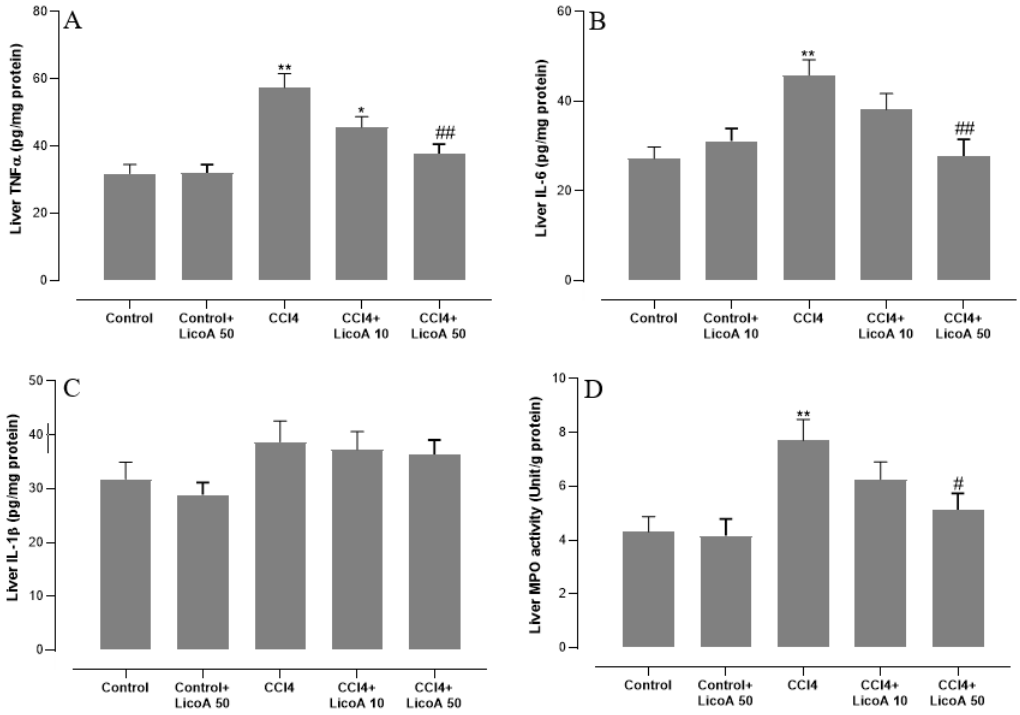

Fig. 4. The effect of licochalcone A on liver tissue levels of TNFα (A), IL-6 (B), IL-1β (C), and MPO activity (D). * p<0.05, ** p<0.01 vs. control group, # p<0.05, ## p<0.01 vs. CCL4 group. Results are presented as mean ± SEM (n = 7 in each group).

4. Discussion

Our results demonstrated that licochalcone A pretreatment dose-dependently can prevent CCL4-provoked liver dysfunction.

In this regard, pretreatment with this flavonoid lowered liver oxidative stress and inflammation. It has been reported that CCl4 is converted to trichloromethyl free radical (CCl3) and trichloromethyl peroxy radical (OOCCl3) by the action of cytochrome P450. Such free radicals released during CCl4 exposure can attack unsaturated fatty acids in the cell membrane which lead to lipid peroxidation and generation of oxygen lipid radicals (2, 3).

A marked increase in serum ALT and AST is a sign of liver damage due to CCL4. Our results revealed that licochalcone A pretreatment could prevent CCl4-induced liver damage. Such beneficial effect of licochalcone A has been shown in mice on a high-fat diet via promotion of Sirt-1/AMPK pathway (14). Production of free radicals due to CCl4 increases ROS and MDA as an indicator of lipid peroxidation. A marked increase of hepatic ROS and MDA following CCL4 show oxidative damage in the liver tissue and failure of the antioxidant defensive system to scavenge free radicals (2, 3). Significant reduction of these factors was noted after the administration of licochalcone A which showed that this flavonoid can protect the liver against CCL4-induced oxidative stress. Of related significance, it has been shown that licochalcone A can improve airway hyperresponsiveness and oxidative stress in a mouse model of asthma, as revealed by lower tissue levels of oxidative and inflammatory factors (15). In addition, neuroprotective effect of licochalcone A against oxygen-glucose deprivation/reperfusion injury has been shown in rat cortical neurons through attenuating oxidative stress and inflammatory events (16). Hepatocyte damage is associated with inflammatory responses. ROS produced by CCl4 activates Kupffer cells and innate immune system. Activated Kupffer cells release different proinflammatory factors. TNF-α which is an inflammatory mediator stimulates immune-related cells to generate various cytokines including IL-6 and IL-1β (2, 3).

Licochalcone A administered at a dose of 50 mg/kg to CCL4 group was significantly effective to lower liver inflammation, as shown lower levels of TNFa, MPO, and IL-6. Anti-inflammatory activity of this flavonoid has been shown in LPS-induced inflammation and acute lung injury by binding to myeloid differentiation factor 2 (MD2) (5). In addition, it has been shown that licochalcone A can exert inhibitory effect on inflammation through targeting TLR4 pathway (17). Similarly, anti-inflammatory effect of licochalcone A was also observed in our CCL4 model of liver injury.

Conclusion

To conclude, findings of the present study showed beneficial property of licochalcone A following CCL4-induced liver injury that is exerted via its regulation of oxidative and inflammatory processes and upregulating antioxidant power.

Acknowledgment

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Compliance with ethical standards

All experimental procedures of this research study were approved by Iran University of Medical Sciences (no. IR.IUMS.FMD.REC.1398.003).

Conflict of interest

The authors declare that they have no competing interest.

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