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Role of α-lipoic acid in LPS/D-GalN induced fulminant hepatic failure in mice: Studies on oxidative stress, inflammation and apoptosis

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ABSTRACT

This study investigated the protective effect of α-lipoic acid (LA) on lipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced fulminant hepatic failure in mice. First, we found that LA markedly reduced LPS/D-GalN-induced increases in serum ALT and AST activities, which were supplemented with histopathological examination, suggested that LA has a protective effect on this model of hepatic damage. Livers challenged with LPS/D-GalN exhibited extensive areas of vacuolization with the disappearance of nuclei and the loss of hepatic architecture. On the contrary, these pathological alterations were ameliorated by LA treatment. Next, we found that ROS and TBARS levels were increased in LPS/D-GalN treated liver homogenates, which were attenuated by LA administration. Consistently, decreases in hepatic CAT and GPx activities were observed in LPS/D-GalN group and were significantly restored by LA administration. Moreover, pretreatment with LA markedly reduced LPS/D-GalN-induced iNOS, COX-2, TNF-α, NF-κB, IL-1β and IL-6 expressions. Furthermore, our data showed that TUNEL-positive cells increased in LPS/D-GalN-treated mice liver which was counteracted by LA administration. LPS/D-GalN induced apoptosis of hepatocytes, as estimated by caspase 3, caspase 8 and caspase 9 activations. Also, the increasing of Bax and the decreasing of Bcl-2 expressions also supported LPS/D-GalN induced apoptosis. Interestingly, LA marked relieved these apoptotic features. Taking together, our results indicated that LA plays an important role on LPS/D-GalN-induced fulminant hepatic failure through its antioxidant, anti-inflammatory and anti-apoptotic activities.

1. Introduction

Fulminant hepatic failure is a dramatic clinical syndrome which has a very high mortality, there have been no effective preventives and therapies currently [1]. Fulminant hepatic failures are normally associated with Gram-negative bacterial endotoxins [2,3], lipopolysaccharide (LPS), the main pathogenic constituent of Gram-negative bacteria, is believed to play an important role on the initiation of endotoxic injury. In addition, D-galactosamine (D-GalN), a liver-specific toxin, sensitizes the toxic effects of LPS and augments fulminate hepatic failure within a few hours. D-GalN depletes uridine nucleotides selectively, then inhibits mRNA and protein synthesis in hepatocytes [4]. D-GalN increases the lethal effect of LPS more than 1000-fold [5]. Take the advantage of D-GalN, the lethal effect of LPS in D-GalN-sensitized mice is usually used as an experimental model of septic fulminant hepatic failure [6–8].

LPS/D-GalN activates Kupffer cells and produces various cytokines, e.g., interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which lead to hepatic necrosis, the decreasing of antioxidant enzyme activities and so on [9]. TNF-α is not only the activator of caspase cascade and downstream apoptotic signals, but also the principle mediator in response of endotoxin shock [10]. In fact, previous study reported that TNF-α-mediated hepatocyte apoptosis plays a central role on LPS-induced liver injury [11]. In addition, LPS can stimulate the production of the inflammatory mediators, nitric oxide (NO)

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; Bax, Bcl-2 associated X; Bcl-2, B-cell lymphoma 2; CAT, catalase; CDNB, 1-chloro-2, 4-dinitrobenzene; COX-2, cyclooxygenase-2; DAB, 3, 3′-diaminobenzidine; DCFH-DA, 2′, 7′-dichlorodihydrofluorescein diacetate; D-GalN, D-galactosamine; GPx, glutathione peroxidase; GSH, glutathione; IL-1β, interleukin-1beta; iNOS, inducible nitric oxide synthase; LA, α-lipoic acid; LPS, lipopolysaccharide; NF-κB, nuclear factor-kappa B; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-alpha; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

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and prostaglandin E2 (PGE_2), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), respectively [12]. Theoretically, the inhibition of pro-inflammatory mediators is a potential strategy for the treatment or prevention of LPS/D-GalN-induced fulminant hepatic failure.

α-Lipoic acid (LA) is a disulfide derivative of octanoic acid and it has been proved to be beneficial in the prevention of inflammation-mediated pathological conditions [13,14]. Subsequent studies have shown that LA not only scavenges hydroxyl radicals, chelates transition metals [14], but also participates in the recycling of Vitamin C, Vitamin E and GSH [15,16]. In addition, another interesting feature of LA is that it is both fat and water soluble and therefore quenches ROS in both hydrophilic and lipophilic environments [17].

Since adequate data suggested that LA has the ability to improve antioxidant defenses and inhibit inflammatory response, here, we explored the protective effect of LA on LPS/D-GalN-induced oxidative stress, inflammation and apoptosis.

2. Material and methods

2.1. Chemicals

LA, D-GalN and 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Aladdin Reagent Database Inc. (Shanghai, China). LPS (Escherichia coli 055:B5) was purchased from Sigma-Aldrich Co. LLC. (Shanghai, China). Diagnostic kits used for the determination of catalase (CAT), glutathione peroxidase (GPx), inducible nitric oxide synthase (iNOS), aspartate transaminase (AST) and alanine transaminase (ALT) activities and thiobarbituric acid reactive substances (TBARS) content were obtained from the Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Rabbit IL-6, NF-κB (p65), Bax, caspase 3, caspase 8, caspase 9, Lamin B, β-actin, mouse TNF-α polyclonal primary antibodies and goat anti-mouse IgG-HRP-conjugated secondary antibody were obtained from Proteintech (Wuhan, China). Rabbit IL-1β, Bcl-2 polyclonal antibodies, goat anti-rabbit IgG-HRP-conjugated secondary antibody and total protein extraction kit were obtained from Sangon Biotech Co. Ltd (Shanghai, China). TUNEL apoptosis detection kit was from Roche (Mannheim, Germany). All other chemicals used were of highest commercial grade.

2.2. Animals and treatment

All animal experiments were based on the guideline of the Animal Care Committee of Southwest University. Male Kunming mice (20 ± 2 g) were obtained from Chongqing Academy of Chinese Materia Medica. Animals were maintained in an air-conditioned room with 12 h dark and light cycle. They were fed standard rodent chow and had free access to water, acclimatized for at least one week prior to use. Mice were randomly divided into four groups with 10 mice in each group. Control group, administered with normal saline. LA group, administered with LA (60 mg/kg body weight/day, prepared immediately before use by dissolving 6 mg/mL LA in 120 mM Tris-buffer and adjusting pH to 7.4) for 5 continuous days. LPS/D-GalN group, received saline once daily for 5 continuous days. One hour after final saline injection, mice were treated with LPS/D-GalN (LPS, 10 μg/kg body weight, D-GalN, 700 mg/kg body weight, dissolved in saline). LA + LPS/D-GalN group, injected LA in Tris-buffer at 60 mg/kg body weight/day for 5 continuous days. One hour after final LA treatment, mice were injected with LPS/D-GalN (LPS, 10 μg/kg body weight, D-GalN, 700 mg/kg body weight, dissolved in saline). All injections were executed intraperitoneally. Animals were sacrificed 6 h after LPS/D-GalN administration.

2.3. Tissue preparation

Blood was collected and centrifuged for biochemical parameter analysis. Livers were removed immediately, rinsed with ice-cold physiological saline and homogenized in saline to get a 10% homogenate. All steps were performed at 4 °C.

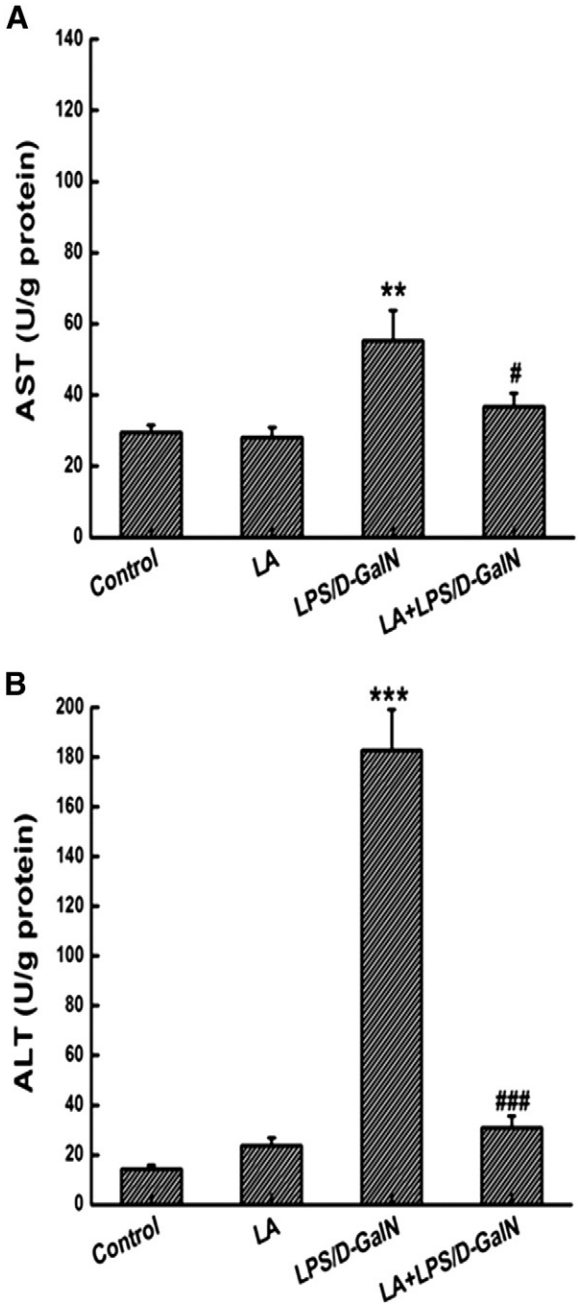

Fig. 1. The effect of LA on serum biochemical parameters after LPS/D-GalN-intoxication. (A) AST and (B) ALT. Each point is the mean ± SD (n = 10). $^{***}p < 0.001$ or $^{**}p < 0.01$, as compared with control group, $^{\#\#\#}p < 0.001$ or $^{\#}p < 0.05$, as compared with LPS/D-GalN group.

2.4. Serum biochemical assays

Serum from individual mice were separated by centrifugation of blood at 600 *g* for 15 min and stored at −20 °C until use. Serum AST and ALT activities were determined spectrophotometrically.

2.5. Histopathological examination

Liver tissues were fixed in 4% paraformaldehyde and dehydrated in a graded series of alcohols, cleared in xylene. They were embedded in paraffin and sectioned at a thickness of 5 μm. After hematoxylin–eosin

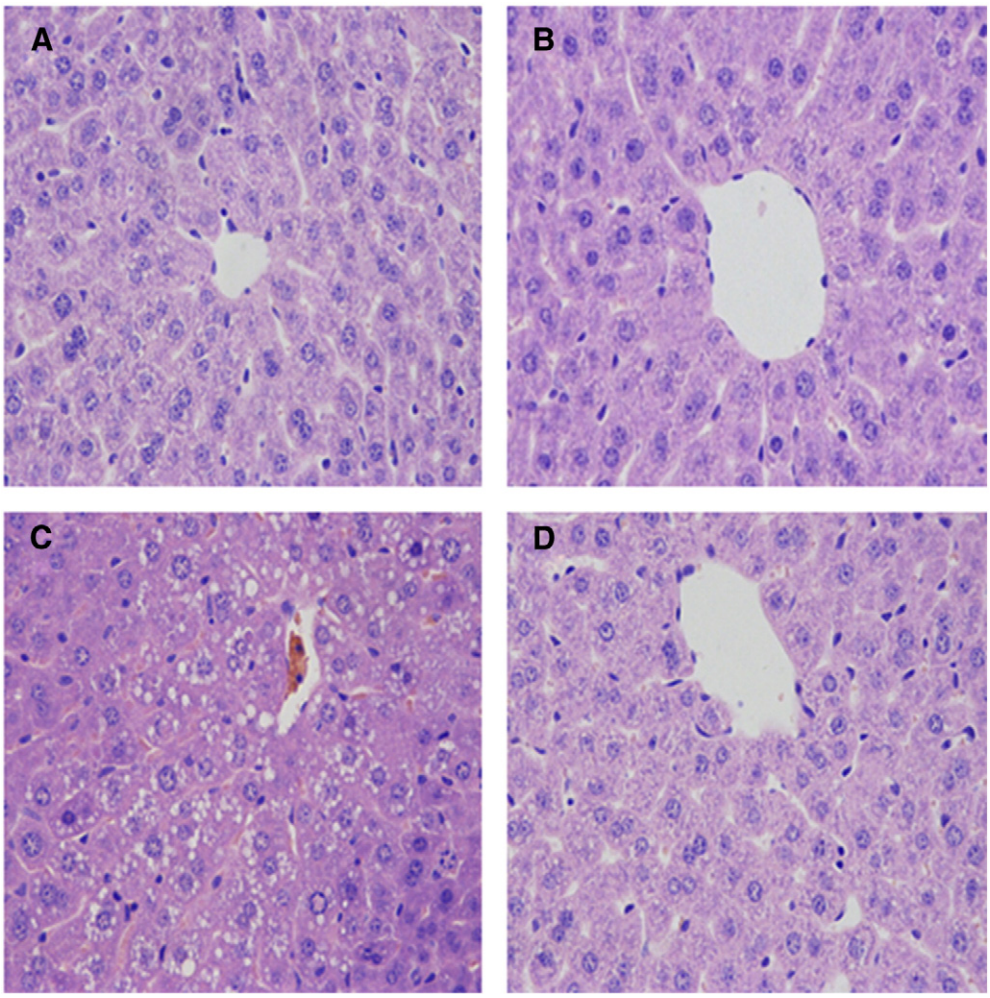

Fig. 2. The effect of LA on the histopathological change after LPS/D-GalN-intoxication. LPS/D-GalN-intoxication caused apparent histological changes in liver tissues, including extensive vacuolization with the disappearance of nuclei and the loss of hepatic architecture. Pretreatment of LA prevented the development of histopathological changes induced by LPS/D-GalN. Original magnifications were 400×.

(H&E) staining, histopathological changes in the liver tissues were observed using fluorescence microscope system (TE2000, Nikon, Japan). The sections were evaluated in nonconsecutive, randomly chosen 400× histological fields and representative images were presented.

2.6. Determination of ROS level

Hepatic ROS level was measured using DCFH-DA as a probe. In brief, assay buffer contained 20 mM Tris–HCl (pH 7.4), 20 mM NaH_2PO_4, 30 mM glucose, 130 mM KCl, 5 mM $MgCl_2$ and 5 μM DCFH-DA. The mixture was incubated at 37 °C for 15 min and reaction was terminated by adding into 1 μM H_2O_2. Fluorescence was measured in a HITACHI F7000 fluorescence spectrophotometer with an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

2.7. Determination of antioxidant and inflammatory biomarkers

CAT, GPx and iNOS activities and TBARS content were determined using commercial assay kits. CAT activity was determined by measuring the decrease of absorbance at wavelength of 240 nm after H_2O_2 decomposition. GPx activity was measured by measuring the absorbance at 412 nm based on the oxidation of NADPH with H_2O_2. iNOS activity was measured by measuring the absorbance at 530 nm based on the formation of colored compound L-citrulline. TBARS content was measured by following the absorbance at 532 nm based on the condensation of malondialdehyde using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

2.8. Western blotting assay

The liver tissue lysate was separated using 10% or 12.5% SDS-PAGE and transferred onto nitrocellulose membranes. Then, blots were blocked in 37 °C water bath for 1.5 h and incubated with appropriate primary antibodies overnight at 4 °C. Blots were incubated at room temperature for 1.5 h with secondary IgG-HRP-conjugated antibodies. Target proteins were visualized and representative images were presented. Densitometric analysis of blots was performed with ImageJ software using β-actin as an internal standard.

2.9. Immunohistochemistry staining

Paraffin-embedded liver sections were de-paraffinized, rehydrated and endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol. Antigen retrieval was performed with a 1 mM EDTA buffer (pH = 9.0) in a microwave for 3 min. The following steps were performed according to the instructions of Histostain™-Plus and DAB substrate Kits. The sections were incubated with NF-κB, COX2 or iNOS antibodies. After washing with PBS, sections were incubated with IgG-HRP-

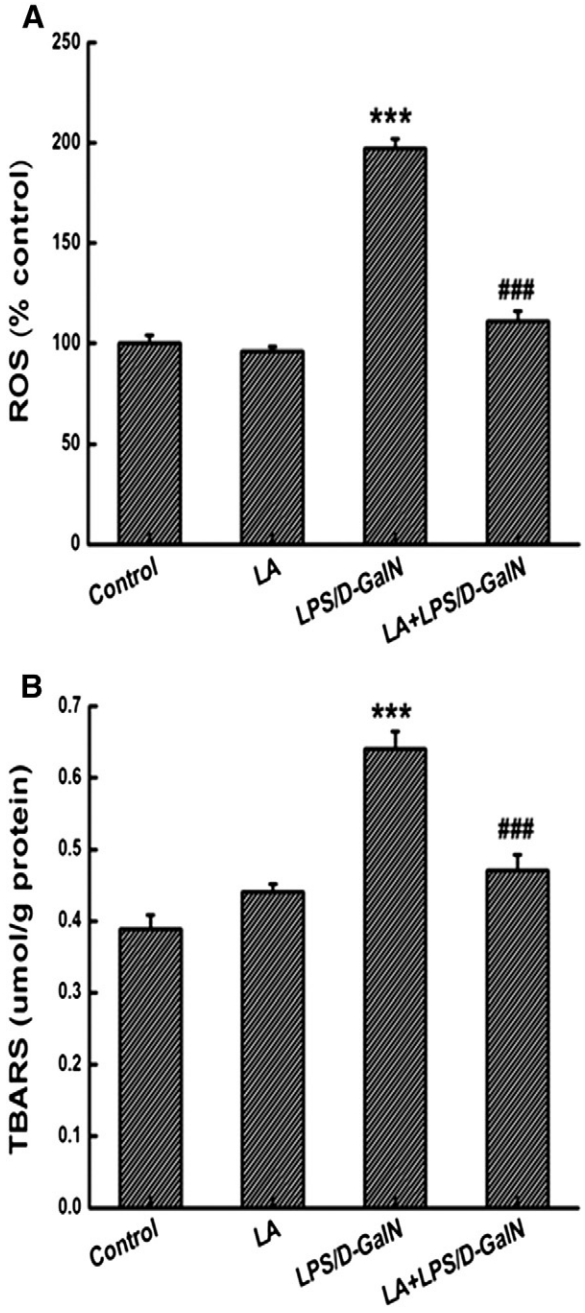

Fig. 3. The effect of LA on ROS and TBARS level after LPS/D-GalN-intoxication. (A) ROS and (B) TBARS level. Each point is the mean ± SD (n = 10). $^{***}p < 0.001$, as compared with control group, $^{###}p < 0.001$, as compared with LPS/D-GalN group.

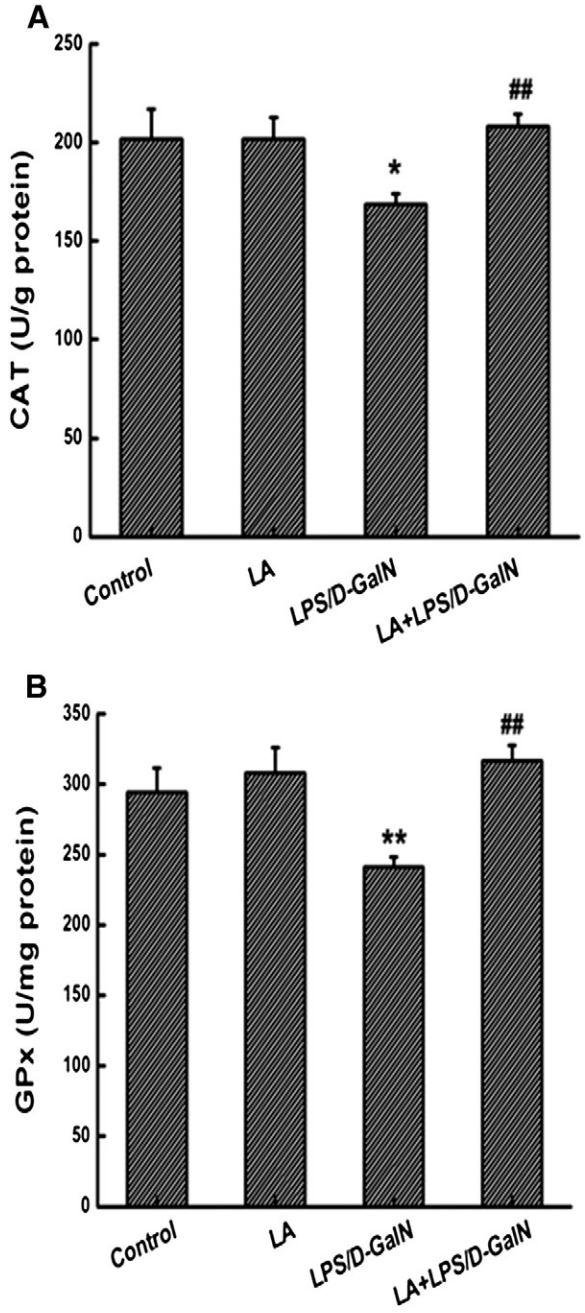

Fig. 4. The effect of LA on antioxidant enzymes activities after LPS/D-GalN-intoxication. (A) CAT and (B) GPx level. Each point is the mean ± SD (n = 10). $^{**}p < 0.01$ or $^{*}p < 0.05$, as compared with control group, $^{##}p < 0.01$, as compared with LPS/D-GalN group.

conjugated antibodies at room temperature for 1 h. Sections were developed with 3, 3′-diaminobenzidine (DAB) solution and were counterstained with hematoxylin. Images were taken by a light microscopy (magnification, 400×, Nikon Eclipse Ti-SR) and representative images were presented.

2.10. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

Paraffin-embedded sections were rehydrated and permeabilized. Tissue sections were incubated with biotinylated nucleotide mix, recombinant terminal deoxynucleotidyl transferase, and equilibration for 1 h at 37 °C. After incubation, tissue sections were immersed in 0.3% H_2O_2 in phosphate-buffered saline to block endogenous peroxidase, then incubated with peroxidase solution for 30 min at room temperature. Tissue sections were stained with DAB substrate and counterstained with hematoxylin. Images were taken by a light microscopy (magnification, 400×, Nikon Eclipse Ti-SR) and representative images were presented.

2.11. Statistical analysis

Data were presented as means ± S.D. Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc analysis of group differences was performed by least significant

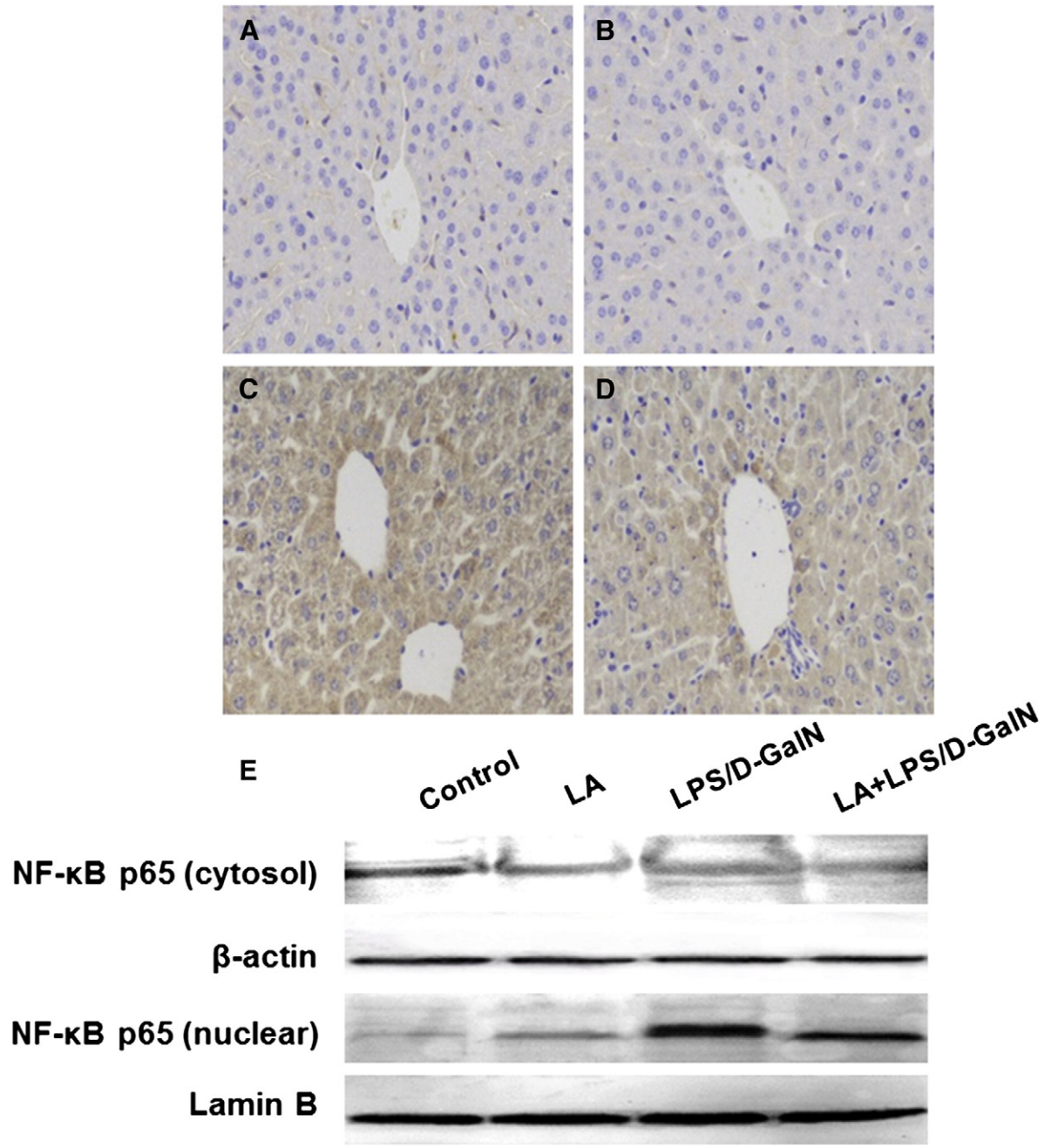

Fig. 5. The effect of LA on the expression/translocation of NF-κB (p65) after LPS/D-GalN-intoxication. (A) Control, (B) LA, (C) LPS/D-GalN and (D) LA + LPS/D-GalN indicated the representative photomicrographs of NF-κB immunoreactivity, Original magnifications were 400×. (E) Nuclear and cytosolic NF-κB (p65) protein expression levels were determined.

difference (LSD) test using SPSS 18.0 software. $p < 0.05$ was considered to be significant.

3. Results

3.1. Effect of LA on serum AST and ALT activities

To assess liver injury, serum AST and ALT activities were measured. As shown in Fig. 1, both serum AST and ALT activities in LA group were still low, compared with the control group. Mix dose of LPS and D-GalN caused the increasing of serum AST and ALT activities significantly (control vs LPS/D-GalN group, $p < 0.01$ and $p < 0.001$ respectively), indicated hepatotoxicity in mice. However, pretreatment of LA inhibited LPS/D-GalN-induced AST and ALT activities significantly (LPS/D-GalN vs LA + LPS/D-GalN group, $p < 0.05$ and $p < 0.001$ respectively), suggested that LA ameliorate acute liver injury induced by LPS/D-GalN.

3.2. Effect of LA on LPS/D-GalN-induced hepatic necrosis

Histopathological assessment of liver injury was performed with H&E staining assay, our result supported the findings from biochemical analysis. Liver sections from control and LA groups showed normal liver architecture. Administration of LPS/D-GalN caused apparent histological changes in liver tissue, including extensive vacuolization with the disappearance of nuclei and the loss of hepatic architecture. However,

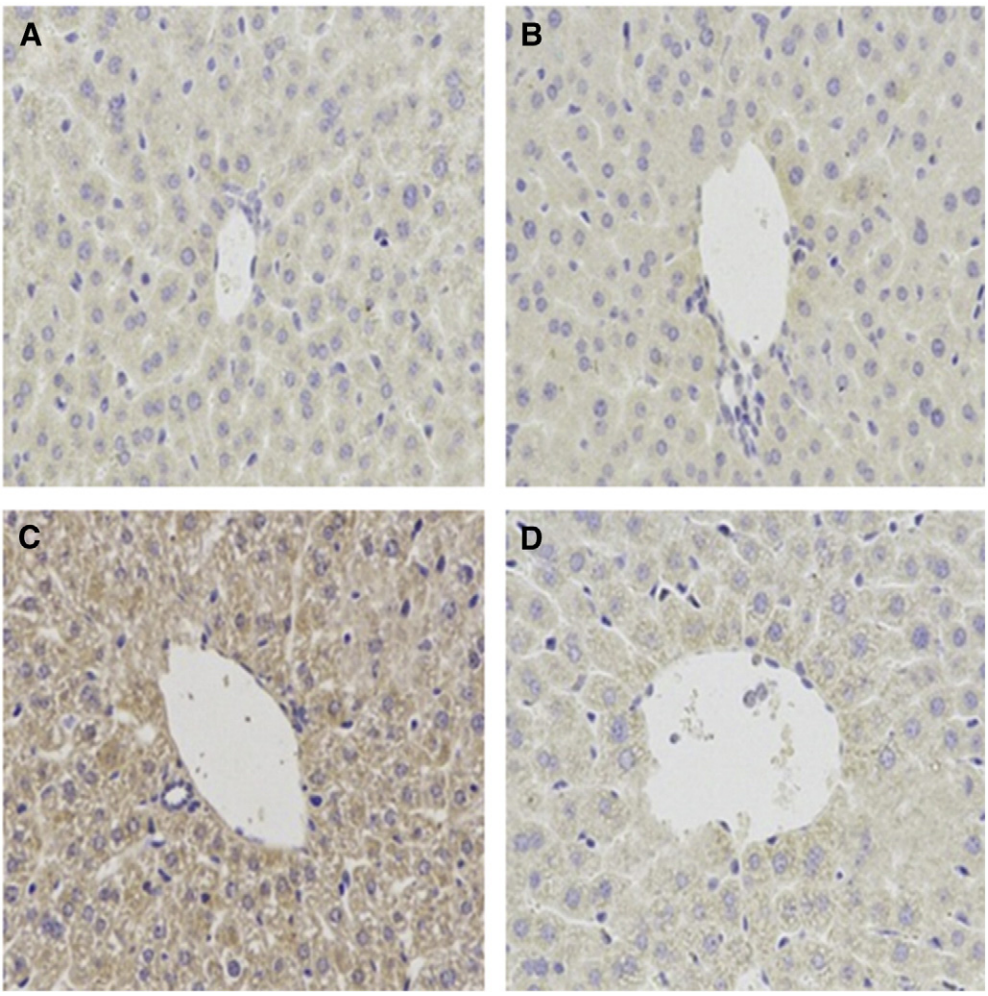

Fig. 6. The effect of LA on the expression of COX-2 after LPS/D-GalN-intoxication. Representative photomicrographs of COX-2 immunoreactivity were shown, Original magnifications were 400×. (A) Control, (B) LA, (C) LPS/D-GalN and (D) LA + LPS/D-GalN.

pretreatment of LA prevented the development of LPS/D-GalN-induced histopathological changes effectively (Fig. 2).

3.3. Effect of LA on LPS/D-GalN-induced oxidative stress

The level of ROS was apparently higher in LPS/D-GalN-treated animals than that in the control group, whereas, the increase of hepatic ROS was significantly prevented in the mice pretreated with LA (Fig. 3A). Our data indicated the pretreatment of LA inhibited LPS/D-GalN-induced oxidative stress.

Lipid peroxidation refers to the oxidative degradation of lipids, which was evaluated by the production of TBARS. LPS/D-GalN-treated mice were observed with the significant elevation of TBARS ($p < 0.001$ vs control). LA administration prevented the degradation of lipids, as evidence with the reduced TBARS level (Fig. 3B).

3.4. Effect of LA on hepatic CAT and GPx activities

The activities of antioxidant enzymes, including CAT and GPx in mouse liver were measured to determine the protective effect of LA on LPS/D-GalN-induced oxidative damages (Fig. 4). Administration of LPS/D-GalN resulted in significant decrease of CAT and GPx activities ($p < 0.05$ and $p < 0.01$ vs control, respectively). However, pretreatment of LA restored CAT and GPx activities, as compared with LPS/D-GalN treatment alone.

3.5. Effect of LA on LPS/D-GalN-induced inflammatory response

Inflammatory mediators/cytokines play important roles in the pathogenesis of fulminant hepatic failure. First, these inflammatory mediators/cytokines were analyzed by immunohistochemistry assay. As we can see in Fig. 5, NF-κB (p65) immunopositive cell numbers were markedly increased in LPS/D-GalN-treated group compared with the control. In addition, western blotting assay clearly showed that NF-κB (p65) in nuclear fraction was significantly increased while no obvious change in cytosol fraction with LPS/D-GalN administration, implied the translocation of NF-κB (p65) after LPS/D-GalN challenge. Consistently, COX-2 (Fig. 6) and iNOS (Fig. 7) immunopositive cell numbers were markedly increased in LPS/D-GalN-treated animals, and LA ameliorated LPS/D-GalN-induced inflammatory response by reduced corresponding immunopositive cell numbers. Moreover, LPS/D-GalN also stimulated iNOS activity in the liver, which was inhibited by the pretreatment of LA (Fig. 8).

In order to further study the effect of LA on LPS/D-GalN-induced inflammation, we studied the expression of inflammatory mediators/cytokines using western blotting. As shown in Fig. 9, the levels of NF-κB, TNF-α, IL-6 and IL-1β proteins were observably up-regulated in

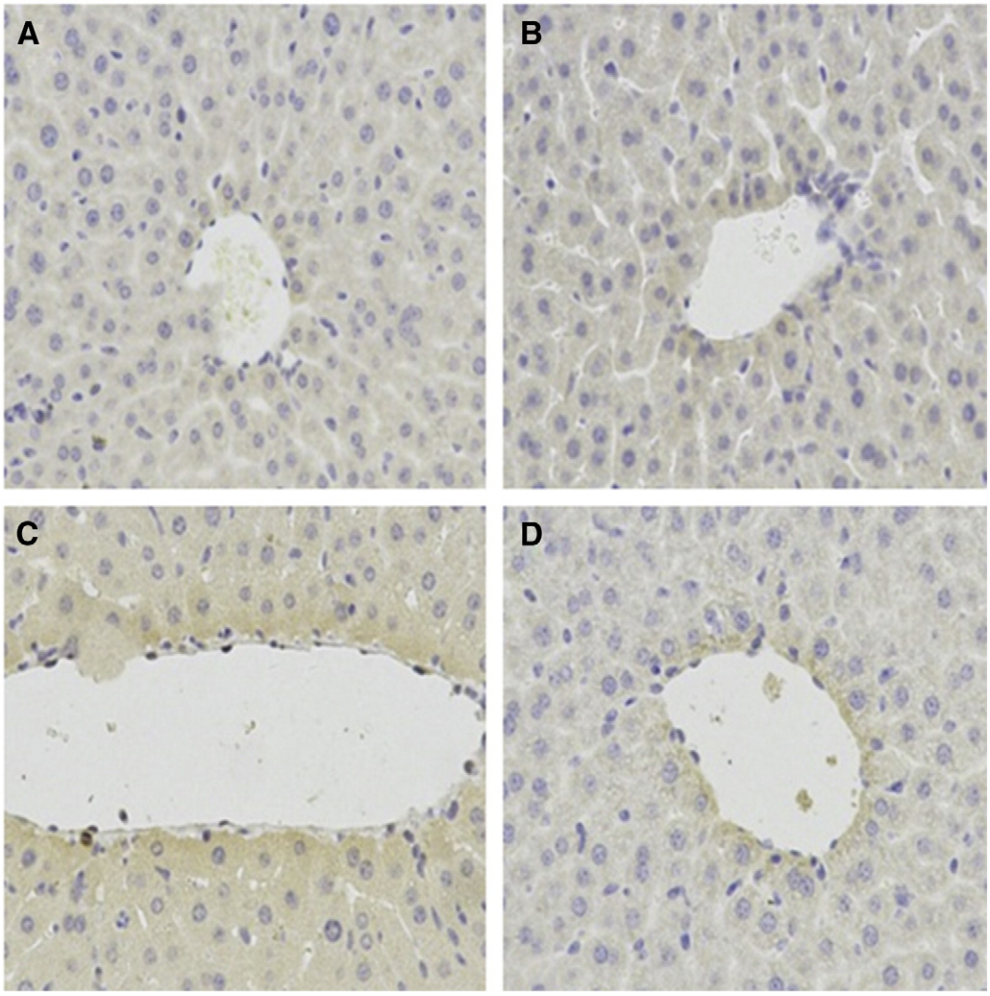

Fig. 7. The effect of LA on the expression of iNOS after LPS/D-GalN-intoxication. Representative photomicrographs of iNOS immunoreactivity were shown, original magnifications were 400×. (A) Control, (B) LA, (C) LPS/D-GalN and (D) LA + LPS/D-GalN.

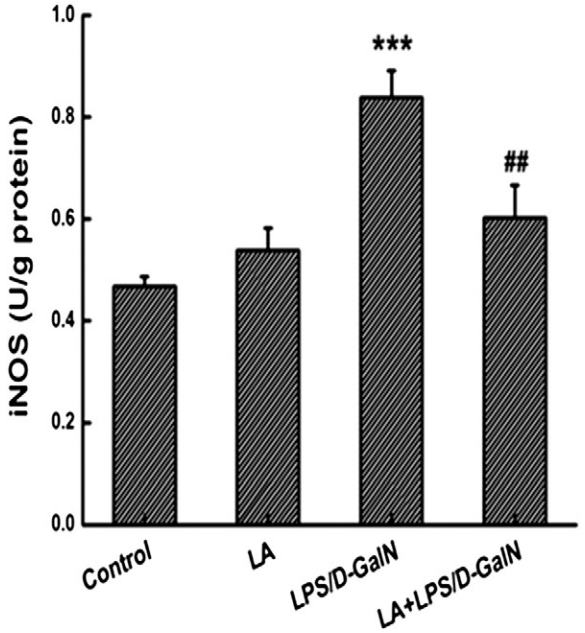

Fig. 8. The effect of LA on iNOS activity after LPS/D-GalN-intoxication. Each point is the mean ± SD (n = 10). $^{***}p < 0.001$, as compared with control group, $^{\#\#}p < 0.01$, as compared with LPS/D-GalN group.

LPS/D-GalN group. However, pretreatment with LA markedly reduced the expression of these proteins, as compared to LPS/D-GalN group.

3.6. Effect of LA on LPS/D-GalN-induced hepatocellular apoptosis

The protective effect of LA on LPS/D-GalN-induced hepatocellular apoptosis was evaluated by TUNEL assay. After LPS/D-GalN injection

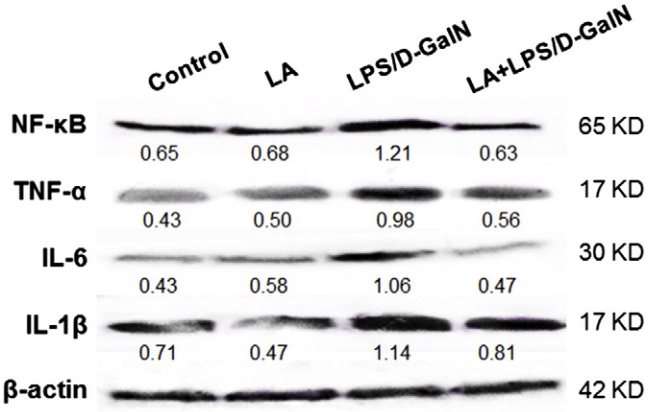

Fig. 9. The effect of LA on NF-κB, TNF-α, IL-6 and IL-1β expressions after LPS/D-GalN-intoxication. Representative immunoblots were shown. Data were normalized to β-actin used as loading control.

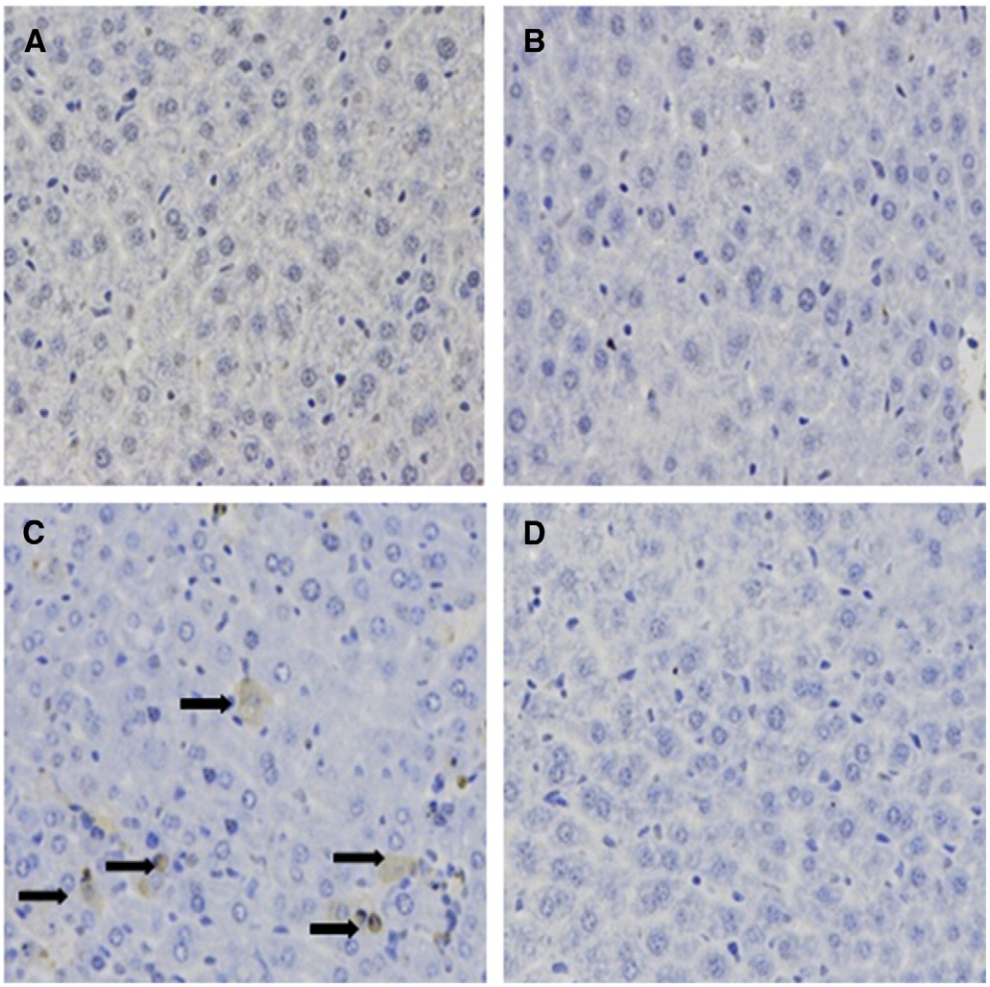

Fig. 10. The effect of LA on the liver apoptosis after LPS/D-GalN-intoxication. Representative photomicrographs of the TUNEL in situ assay corresponding to (A) Control, (B) LA, (C) LPS/D-GalN and (D) LA + LPS/D-GalN. Arrows indicate the TUNEL-positive cells. Original magnifications were 400×.

for 6 h, the number of apoptotic hepatocytes (immunopositive cells) in the liver was significantly increased, compared to the control group (Fig. 10). However, pretreatment with LA reduced the number of apoptotic cells.

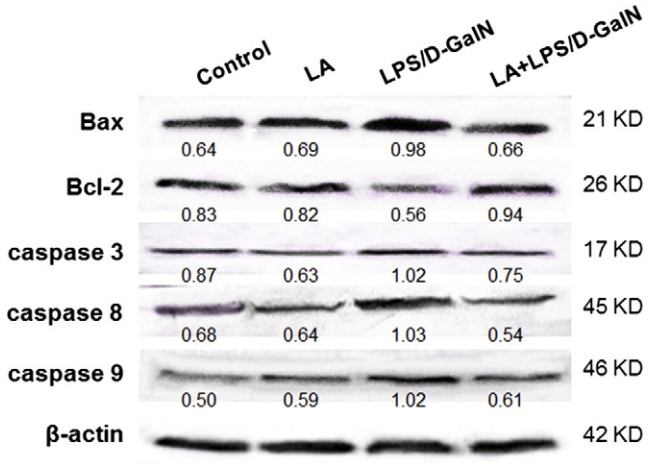

Fig. 11. The effect of LA on Bax, Bcl-2, caspase 3, caspase 8 and caspase 9 expressions after LPS/D-GalN-intoxication. Representative immunoblots were shown. Data were normalized to β-actin used as loading control.

To explore the mechanism of LA on the inhibition of LPS/D-GalN-induced hepatocyte apoptosis, apoptotic-related protein expressions were analyzed by western blotting. As shown in Fig. 11, LPS/D-GalN obviously increased the expression of Bax and decreased the expression of Bcl-2, which indicated apoptosis. On the contrary, down-regulated Bax and up-regulated Bcl-2 were found in LA + LPS/D-GalN group. Furthermore, LPS/D-GalN induced the expressions of caspase family proteins caspase 3, caspase 8 and caspase 9, which were attenuated by LA. These results suggested that LA inhibited LPS/D-GalN-induced hepatocyte apoptosis by affecting the expression of apoptosis-related factors.

4. Discussion

The combination of LPS and D-GalN was widely used as an experimental model of fulminant hepatic failure. The interaction of LPS with hepatocytes is related to the activation of mediators such as, ROS, NO, and TNF-α [18,19]. Recently, LA has been recognized as a powerful antioxidant in the prevention/treatment of pathological conditions, e.g., oxidative stress, ischemia–reperfusion injury, diabetes and radiation injury [20–22]. The present study was designed to investigate the protective effect of LA against LPS/D-GalN-induced hepatotoxicity. Also, the underlying mechanisms of LA on oxidative stress, inflammatory response and apoptosis signals were investigated.

LPS/D-GalN mixture was given intraperitoneally in a single dose of LPS, 10 μg/kg body weight and D-GalN, 700 mg/kg body weight dissolved in saline. The levels of AST and ALT activities were used as

biochemical markers of early acute hepatic injury. Our results are in agreement with previous observations, where administration of LPS resulted in an increase in serum AST and ALT levels, suggestive of liver injury [23]. Our results were supported by histopathological examination where pathological changes, including the extensive vacuolization, the disappearance of nuclei and the loss of hepatic architecture were observed. These results were in accordance with previous results as well [24,25]. Interestingly, liver function was recovered by the pretreatment with LA, as the evidence of inhibited AST and ALT activities as well as normal hepatic architecture.

LPS/D-GalN-intoxication causes the imbalance of free radical production and the loss of antioxidant capacity in the liver, which bring the consequence of oxidative stress and lipid peroxidation. Oxidative stress is a recognized phenomenon in LPS/D-GalN-induced liver damage [26,27]. Thus, antioxidant enzyme activities, such as SOD and CAT, are decreased upon LPS administration [28]. Previous study also indicated that LPS-treatment decreased the content of reduced thiol group, as well as GSH reductase activity [29]. In the present study, we observed the decrease of CAT and GPx activities, along with the increase of ROS and TBARS level, which is in parallel with their findings. In LPS/D-GalN-intoxicated animals, ROS initiate the lipid peroxidation process and protein oxidation process [30]. Here, LA pretreatment significantly reduced ROS and TBARS accumulation and enhanced total antioxidant activity by restored CAT and GPx activities, compare with LPS/D-GalN challenged group. Our results implied that the antioxidant action of LA involved in the protective mechanisms against LPS/D-GalN-induced liver injury.

In addition, we explored the possible anti-inflammatory effect of LA. Our results illustrated that LA significantly inhibited the release of pro-inflammatory factors. NF-κB plays an important role in LPS/D-GalN-induced inflammatory process. NF-κB activation is triggered by the phosphorylation of IκB and dissociated from inactive cytoplasmic complex, then translocate to nucleus [31]. In the nucleus, NF-κB binds to corresponding sites and regulates the transcription of many pro-inflammatory genes. In the current study, we determined NF-κB levels at 6 h after LPS/D-GalN challenge, our immunohistochemistry staining and western blotting assay both indicated the up-regulation of NF-κB. Consistently, LA reduced NF-κB level which showed a protective role against LPS/D-GalN-induced inflammatory response. Previous study provided evidence that LA attenuated NF-κB-dependent expression of cellular adhesion molecule, intercellular adhesion molecule 1 and monocyte chemoattractant protein 1, then inhibited acute inflammatory responses by activating the PI3K/AKT pathway [32]. Overexpression of inflammatory mediators has been implicated in pathogenesis of inflammatory diseases. However, the inhibition of NF-κB pathway was shown to inhibit the expression of pro-inflammatory cytokines [33]. Here, pro-inflammatory cytokines, including TNF-α, IL-6 and IL-1β, expressions were also down-regulated by the pretreatment of LA. TNF-α has been known as the primary and central cytokine in mediating an early inflammatory response, characterized mainly by neutrophil infiltration and activation [34]. In the present study, it was demonstrated that lipoic acid was able to prevent the LPS-induced increases in TNF-α levels. To further determine the mechanism involved in LA-suppressed inflammation, we evaluated iNOS activity and expression. It is well known that NO level is low under normal condition. However, LPS/D-GalN causes the induction of iNOS, resulting in the production of NO [7]. The large amount of NO in response to LPS/D-GalN plays an important role in inflammatory conditions. We found that iNOS expression and activity were both down-regulated with the pretreatment with LA. Previous study indicated that the inhibition of NF-κB could prevent iNOS expression in a rat model of septic shock [35], this may imply that LA decreased the level of iNOS activity and expression through the suppression of NF-κB activation. Overexpression of COX-2 was connected with the pathophysiology of inflammatory disorders as well [36,37]. We observed that LPS/D-GalN induced COX-2 expressions were inhibited by LA. Thus, the inhibition of iNOS and COX-2 activities has beneficial therapeutic effects on the treatment of LPS/D-GalN-induced inflammatory conditions.

LPS/D-GalN intoxication also induces apoptosis in mouse liver. Hepatocytes are sensitized to TNF-α-mediated toxicity, which result in the activation of caspase family proteins. LPS increases the permeability of cells and induces structural mitochondrial damage with caspase-mediated apoptosis. Using TUNEL assay, we observed a significant increase of immunopositive cells in LPS/D-GalN-intoxicated animal liver section. Hepatocytes are sensitized to TNF-α mediated cytotoxicity, which in turn activates caspase family proteins. Initiators caspase 8 and caspase 9 were activated by a variety of apoptotic signals. Then, activated caspase 8 and caspase 9 can cleave and activate effector caspase 3, in turn cleave a cellular substrate PARP and trigger apoptotic process [38]. In this study, LPS/D-GalN-activated caspase 3, caspase 8 and caspase 9 were significantly inhibited by LA. Bcl-2 family proteins also regulate LPS/D-GalN-induced apoptotic signals and control the fate of cells [38]. Bcl-2 family proteins include two different types, anti-apoptosis proteins (such as Bcl-2) and pro-apoptosis proteins (Bax). The ratio of Bcl-2/Bax determines survival or death fate of cell after an apoptotic stimulus. LPS induces oligomerization of Bax, which causes the release of cytochrome *c*. Our results indicated that administration with LA markedly reduced the D-GalN/LPS-induced Bcl-2/Bax ratio depression.

In summary, our results indicated that LA protects liver from LPS/D-GalN-induced fulminant hepatic failure in mice. The underlying mode of action of LA includes antioxidant, anti-inflammation and anti-apoptosis. In view of these findings, we concluded that LA may be a potential therapeutic agent in the treatment of fulminant hepatic failure.

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