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*Original Article*

# Lycopene attenuates LPS-induced liver injury by inactivation of NF-κB/COX-2 signaling

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**Abstract:** Aim: This study aimed to investigate the effect of lycopene on LPS-induced liver injury in mice and its mechanisms. Methods: Male C57bl/6 mice were randomly assigned to three groups: sham control group (S-C), LPS control group (L-C), lycopene treatment group (L-T). The mice from the L-T were treated with lycopene for 2 weeks, and the remaining mice with solvent. Afterwards, the mice from the L-C and the L-T received an intraperitoneal injection of LPS (20 mg/kg, dissolved in sterile saline), and the S-C mice were injected with sterile saline. Serum levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) were determined for analysis of liver function. Levels of inflammatory cytokines including tumor necrosis factor (TNF)-α and interleukin (IL)-6, malondialdehyde (MDA) content, and the activity of superoxide dismutase (SOD), were detected in serum. Liver tissues were operated for morphologic analysis and determination of protein by western blot. Results: Pretreatment with lycopene significantly decreased levels of ALT, AST, and TNF-α and IL-6, reduced MDA content, and increased activity of SOD in serum compared with the L-C mice. Lycopene increased expression of nuclear factor-erythroid 2 related factor 2 (Nrf2), and reduced expression of cyclooxygenase (COX)-2, and phosphorylation of nuclear factor-kappa B (NF-κB) and extracellular regulated protein kinases 1/2 (ERK1/2). Conclusion: The results showed that lycopene attenuates LPS-induced liver injury by reducing NF-κB/COX-2 signaling by upregulation of Nrf2/HO-1 activation.

**Keywords:** Lycopene, LPS, liver injury

## Introduction

Sepsis is a severe clinical inflammatory response syndrome with high mortality [1]. Infection caused by Gram-negative bacteria is a leading cause of sepsis. Various studies showed that lipopolysaccharide (LPS), an important component in cell wall of Gram-negative bacteria, triggers release of pro-inflammatory cytokines and reactive oxygen species (ROS) which damage to multiple tissues and organs including liver, heart, and kidney [2, 3]. Liver diseases are increasingly becoming a worldwide problem, and there are still few effective treatments for severe hepatic failure. Increasing evidence confirmed that LPS plays a critical role in the development and progression of hepatic injury [4-6]. Clinical and animal experiments suggested that level of LPS in plasma is associated with fibrosis and cirrhosis [7, 8]. Inflammatory mediators such as TNF-α and IL-6 induced by LPS contributes to liver injury by depleting intracellular antioxidants, and causing lipid peroxidation and oxidative damage [9, 10]. Antioxidants such as vitamin E and tocopherols have been reported to play an important role in protection from oxidative damage [11].

Lycopene, a natural lipophilic carotenoid with no provitamin A activity, is synthesized in tomatoes. Increasing evidence showed that lycopene and its metabolites exhibit various important biological functions [12-14]. Lycopene possesses many conjugated double bonds, so its ability to quench singlet oxygen is about 10 times as much as that of vitamin E [15, 16]. Lipophilic lycopene affects lipid metabolism and protects lipid from peroxidation [17-20]. Further, lycopene reduces DNA oxidative damage induced by ROS, and protects endothelial function from oxidative stress [21-23]. Animal studies showed that lycopene reduces synthesis of C-reactive protein (CRP) secreted by the liver [24, 25], and inflammation [26, 27].

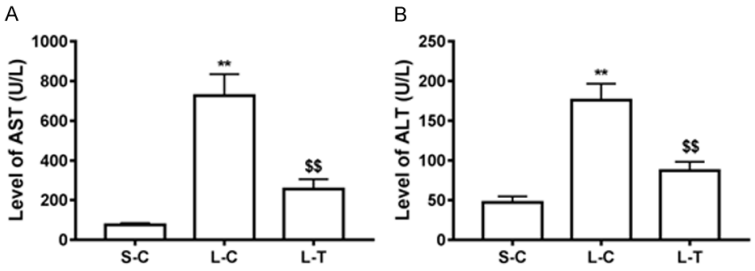


**Figure 1.** Effect of lycopene on liver function. Activity of AST (A) and ALT (B) in serum was measured. Levels of AST (A) and ALT (B) are shown as means and standard deviation. $^{**}P<0.01$ vs. S-C; $^{\$\$}P<0.01$ vs. L-C.

Therefore, this study aimed to investigate the effect of lycopene on LPS-induced liver injury and the relevant mechanisms involving oxidative stress and pro-inflammation.

## Materials and methods

### *Materials*

Lipopolysaccharide (LPS) was purchased from Sigma (St. Louis, USA). Elisa mice interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) kits were purchased from Hefei Bomei Biotechnology CO., LTD (Hefei China). SOD and MDA commercially available kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Primary polyclonal antibodies β-actin, HO-1, TNF-α, IL-6, COX-2, NF-κB, p-NF-κB, ERK1/2, p-ERK1/2 and Nrf2 were purchased from Bio Basic Inc., Canada.

### *Animals*

C57bl/6 mice (20±2 g) were obtained from the Animal Experimental Center in Wannan Medical College. Animal experiments obeyed Chinese Community Guidelines for the use of experimental animals. The mice were raised with a standard animal facility for acclimatization. After 1 weeks, the animals were randomly assigned to three groups (10 mice per group): sham control group (S-C), LPS control group (L-C), lycopene treatment group (L-T). The mice from lycopene treatment group received lycopene treatment by oral administration for 2 weeks, and the others were treated with solvent. Then, the L-C and L-T mice were intraperitoneally injected with LPS (20 mg·kg$^{-1}$) dissolved in sterile saline, and the S-C mice received sterile saline. After 6 hours, animals were anesthetized with sodium pentobarbital (50 mg/kg); the livers were collected, stored in liquid nitrogen and partially fixed with 4% neutral formalin. Blood samples were obtained for biochemical analyses.

### *Estimation of liver function*

To estimate the effect of Lycopene on liver function, aspartate transaminase (AST) and alanine transaminase (ALT) in plasma were detected by an automated biochemical analyzer.

### *Determination of inflammatory cytokines*

Levels of TNF-α, and IL-6 were determined by mice TNF-α and IL-6 specific ELISA kits according to the instructions. Their levels were expressed as ng/L, respectively.

### *Change of antioxidation*

Activity of antioxidases such as superoxide dismutase (SOD), and level of malondialdehyde (MDA) were measured by commercially available kits for assessment of antioxidant effects.

### *Morphological analysis*

Livers fixed in formalin were dehydrated, and then embedded in paraffin. Embedded livers were cut into 5-μm sections, and mounted on glass slides. Sections were stained with hematoxylin and eosin. Morphological examination was performed under a light microscope at magnifications of 400×.

### *Western blot*

Livers were dissected out, homogenized and lysed in lysis buffer (50 mmol/L HEPES, 2 mmol/L EDTA, 100 mmol/L Na4P2O7, 100 mmol/L NaF, and 1% Triton X-100) with 0.2 mmol/L PMSF for 10 min. Homogenates were centrifuged at 13,000 g at 4°C. for 15 min. Equal amounts of protein were separated by SDS-PAGE, and then electrophoretically transferred to nitrocellulose membranes. Subsequently, the membranes were incubated with primary rabbit antibodies including β-actin (1:1000), HO-1 (1:1000), TNF-α, IL-6, p-ERK1/2, ERK, COX-2, NFκB, p-NFκB, and Nrf 2 (Bio Basic

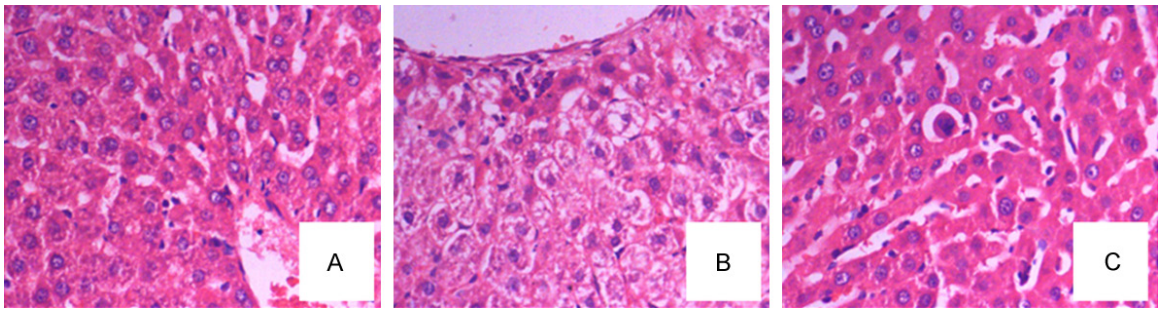


**Figure 2.** Observation of morphology of liver tissues. A. Sham control mice; B. LPS control mice; C. Treated mice with lycopene.

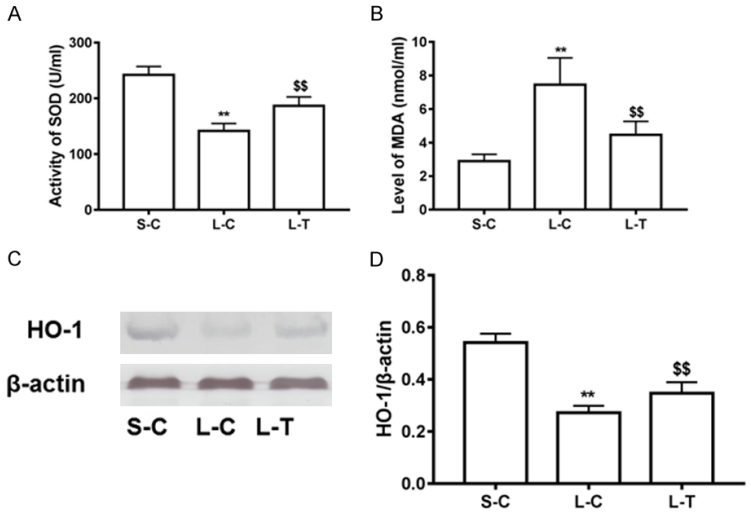


**Figure 3.** Improvement of lycopene on antioxidant effect. Activity of SOD (A) and content of MDA (B) were determined. Protein expression of HO-1 (C) was evaluated in liver tissues with western blot. Relative Level of HO-1 (D) was expressed as means and standard deviation. $^{**}P<0.01$ vs. S-C; $^{\$\$}P<0.01$ vs. L-C.

Inc., Canada) dissolved in TBS-T (10 mmol/L Tis-HCl, 150 mmol/L NaCl, and 1% Tween 20) containing 5% nonfat milk overnight at 4°C, respectively. After incubated with goat anti-rabbit secondary antibody, the membranes were rinsed, and then used to detect the immunoreactive bands by visualization with DAB (Bio Basic Inc., Canada).

*Statistics*

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by SPSS16.0. Statistical difference was analyzed by Tukey's test for unpaired data and one-way Analysis of Variance (ANOVA), followed by Bonferroni's post-test. A value of $P<0.05$ was considered significant.

## Results

*Lycopene attenuates LPS-induced liver injury*

Serum levels of AST and ALT were determined for assessment of liver injury. Levels of AST and ALT in the L-C mice were significantly increased compared with the S-C ($P<0.01$) (**Figure 1**). Preconditioning of lycopene decreased levels of AST and ALT ($P<0.01$) (**Figure 1**).

To further investigate LPS-induced liver injury, morphology of liver tissues was observed. Liver histological sections of the L-C mice showed the infiltration of inflammatory cells, and necrosis of liver cells (**Figure 2**). Lycopene treatment improved architecture of tissue, and reduced congestion in the L-T mice. Fewer inflammatory cells and intact lobular structure were observed in the L-T mice (**Figure 2**).

*Effect of lycopene on oxidative stress*

To assess the effect of lycopene on oxidative stress, we first determined level of MDA, and activity of SOD in serum. Level of MDA was increased in the L-C mice compared with the S-C ($P<0.01$) (**Figure 3**), while activity of SOD was reduced in the L-C mice ($P<0.01$) (**Figure 3**). However, administration of lycopene prior to PLS decreased level of MDA, and increased activity of SOD compared to LPS alone ($P<0.01$) (**Figure 3**). Further, we measured expression of antioxidase such as HO-1, and observed that

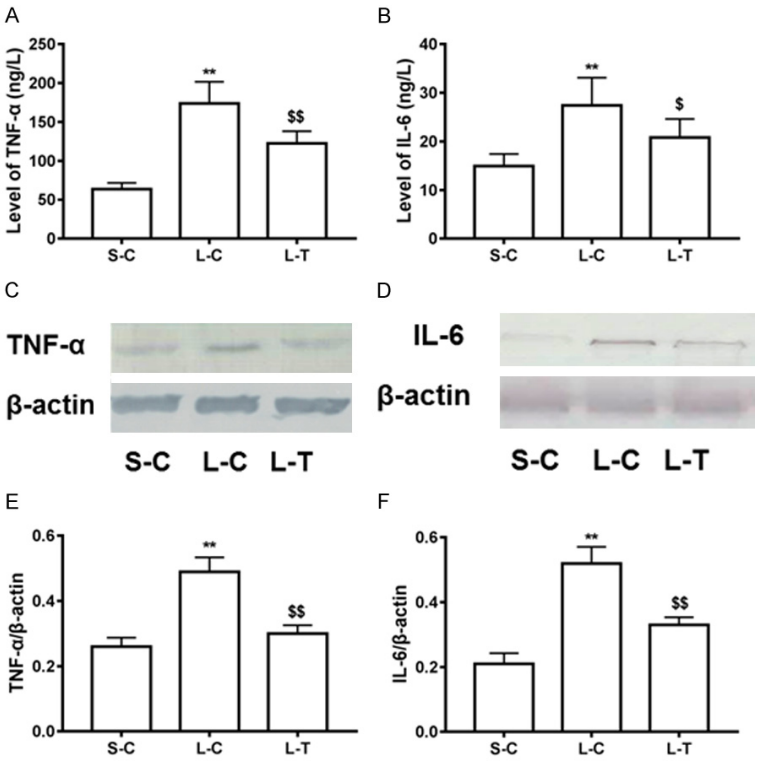


**Figure 4.** Effect of lycopene on inflammatory cytokines. Levels of TNF-α (A) and IL-6 (B) in serum were measured. Protein expression of TNF-α (C) and IL-6 (D) were determined in liver tissues by western blot. Relative levels of TNF-α (E) and IL-6 (F) were expressed as means and standard deviation. $^{**}P<0.01$ vs. S-C; $^{\$}P<0.05$ and $^{\$\$}P<0.01$ vs. L-C.

expression of HO-1 was significantly increased in the L-T mice compared with the L-C (P<0.01) (**Figure 3**).

*Lycopene decreases levels of pro-inflammatory cytokines*

Pro-inflammatory cytokines play a pivotal role in LPS-induced liver injury, thus we investigated the effect of lycopene on production of pro-inflammatory cytokines. The results showed that levels of pro-inflammatory cytokines such as TNF-α and IL-6 in serum were significantly increased in the L-C mice compared with the S-C (P<0.01) (**Figure 4**). Pretreatment of lycopene reduced the increase in levels of TNF-α and IL-6 (P<0.01) (**Figure 4**). Second, expression of TNF-α and IL-6 in liver tissues was significantly decreased in the L-T mice compared with the L-C (P<0.01) (**Figure 4**).

*Effect of lycopene on p-ERK1/2, COX-2, p-NFκB, and Nrf 2 protein expression*

To further explore the mechanism of lycopene treatment in LPS-induced liver injury, we determined expression of COX-2 and Nrf 2 protein, and phosphorylated levels of NF-κB and ERK1/2. The result showed that expression of COX-2, and phosphorylated levels of NF-κB and ERK1/2 were increased in the L-C mice compared with the S-C (P<0.01) (**Figure 5**), while expression of Nrf 2 was reduced (P<0.01) (**Figure 5**). Pretreatment by lycopene increased expression of Nrf 2, and decreased expression of COX2 and phosphorylated levels of NF-κB and ERK1/2 (P<0.01) (**Figure 5**).

## Discussion

In present study, we investigated the effect of lycopene on LPS-induced liver injury. Our results that lycopene attenuated LPS-induced acute liver injury by reducing oxidative stress and inflammatory response. Further, lycopene increased expression of Nrf2, and decreased expression of COX-2 and phosphorylation of NF-κB and ERK1/2.

Sepsis resulting from infection is a leading cause of mortality. Liver damage is a contributor of mortality caused by sepsis. LPS is found to play an important role in the pathogenesis of infection [28], and the study confirmed that a small dose of LPS led to fatal liver damage in mice [29]. LPS result in tissue injury by excessive inflammation, the elevation of oxidative stress and mitochondrial impairment [30]. Küpffer cells activated by LPS by binding with Toll-like receptor 4 (TLR-4) can excessively release pro-inflammatory cytokines and generate a tremendous amount of ROS, which triggers apoptosis of liver cells, even necrosis [31]. Therefore, oxidative stress and inflammation are involved in LPS-induced liver injury [2]. Some studies showed that various antioxidants can prevent LPS-induced liver injury and oxidative stress [2, 32-34].

Oxidative stress features an imbalance between oxidants and antioxidants such as excessive consumption of antioxidants and overpro-

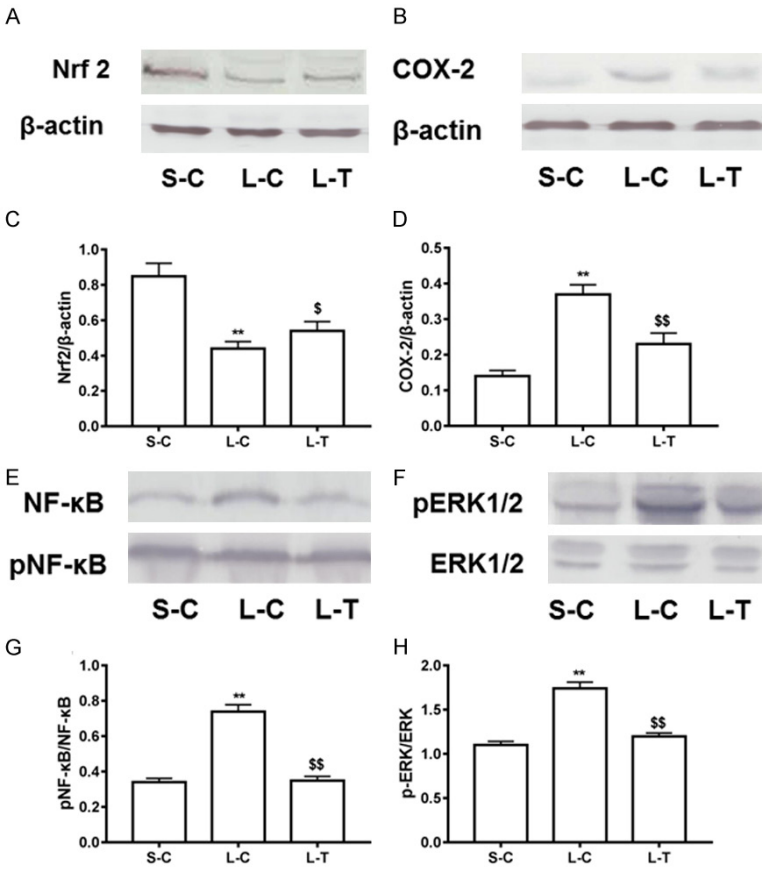


**Figure 5.** Regulation of lycopene on inflammation and antioxidant signaling. Protein levels of Nrf 2 (A) and COX-2 (B), and phosphorylated levels of NF-κB (E) and ERK (F) were detected in liver tissues by western blot. Relative expression levels Nrf 2 (C) and COX-2 (D), and relative phosphorylation levels of NF-κB (G) and ERK (H) were expressed as means and standard deviation. $^{**}P<0.01$ vs. S-C; $^{\$\$}P<0.01$ vs. L-C.

duction of ROS [35]. Superoxide, one of the most common ROS, is transformed to hydrogen peroxide catalyzed by SOD, then further to hydrogen peroxide to water by peroxidases such as catalase and glutathione peroxidases [36]. Depletion of antioxidant enzyme such as SOD, catalase and glutathione peroxidase results in overproduction of ROS including superoxide, which damage macromolecules and increase lipid peroxide [37].

Evidence suggested that lycopene supplements elevated activity of the antioxidant enzymes, decreased lipid peroxidation, and attenuated hepatic steatosis [38, 39]. Further, lycopene and its metabolite regulated transcription systems and cell signaling pathways [40]. In present study, our results showed that injection of LPS increased levels of AST and ALT (markers of liver injury) in serum, and the infiltration of inflammatory cells was observed in section of liver tissues. Consistent with previous study, LPS treatment decreased activity of SOD, and increased content of MDA. Lycopene pretreatment increased activity of SOD, and decreased content of MDA in serum. Further, lycopene increased expression of antioxidant enzymes such as HO-1 in liver.

Excessive release of pro-inflammatory cytokines is implicated in various acute and chronic disease such as trauma, sepsis, and chronic vascular disease [41]. Pro-inflammatory cytokines is involved in pathogenesis of acute and chronic liver injury [5]. In non-alcoholic liver disease, pro-inflammatory cytokines induced by LPS aggravated liver injury by accelerating apoptosis of liver cells, and was closely associated with severity of liver injury [42]. Our study suggested that lycopene pretreatment decreased levels of serum TNF-α and IL-6, and reduced expression of TNF-α and IL-6 in liver. Previous study confirmed that lycopene attenuated inflammation, and reduced secretion of C-reactive protein (CRP) [24, 25].

It is well known that inflammatory response and oxidative stress are closely associated. It has been reported that ROS resulting from oxidative stress regulated inflammatory cytokines by NFκB signaling [43]. NF-κB is a transcription factor which can be activated under oxidative stress status. Activated NF-κB regulated the encoding of various genes such as inflammatory cytokines, and chemokine through upregulation of COX-2, which lead to inflammation [44, 45]. It has been reported that LPS regulates inflammatory response by activating NF-κB [46]. Mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase

(ERK) is involved in LPS-induced inflammation [47]. ERK activated by phosphorylation mediates translocation of NF-κB to nuclear, and COX-2 [48, 49].

Cyclooxygenase 2 (COX2) is critical for regulation of inflammation signaling, and regarded as the target of drugs. COX-2 can catalyze synthesis of prostaglandins [50, 51], which play a vital role in various physiological processes [52]. Some work suggests that prostaglandins are involved in generation of inflammatory cytokines [53]. HO-1, an antioxidant enzyme, can decompose heme into biliverdin, carbon monoxide (CO), and free iron. Catalytic products of HO-1 such as biliverdin and CO have been demonstrated to possess antioxidation [54, 55], and CO can reduce inflammatory response and apoptosis of cells [56, 57]. Further study showed that increased expression of HO-1 attenuates oxidative stress damage to cells and tissue [58-60]. It has been reported that CO exerts anti-inflammatory effect by modulating inflammatory signaling including NK-κB, and attenuates LPS-induced inflammatory response through reduction of NK-κB [61, 62].

Nrf2 is a vital transcription factor which mediates expression of various antioxidases including HO-1 [63, 64]. Under oxidative stress, activated Nrf2 translocates into the nucleus, and modulates expression of antioxidant-related genes at transcriptional level [65]. Nrf2 plays a vital role in antioxidant defense systems by reducing inflammation and oxidative stress [66]. In this study, our data showed that lycopene pretreatment increased Nrf2 expression, and decreased expression of COX-2, and phosphorylation of ERK and NF-κB. These findings suggested that the protective effect of lycopene against LPS-induced liver injury may be associated with suppression of NF-κB/COX-2 by Nrf2/HO-1 activation.

In conclusion, our results suggest that lycopene protected liver against LPS-induced injury. Lycopene exerted its beneficial effect by reducing oxidative stress and inflammation damage, which may be associated with upregulation of Nrf2/HO-1 signaling pathway, and inhibition of LPS-activated inflammatory signaling (NF-κB/COX-2). Although the exact mechanisms called for further elaboration, our results support a preventive effect of lycopene in acute liver injury.

## Acknowledgements

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## Disclosure of conflict of interest

None.

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