Bilobalide attenuates lipopolysaccharide-induced HepG2 cell injury by inhibiting TLR4-NF-κB signaling via the PI3K/Akt pathway

SHUMEI MAO[1*], JINPENG YAO[2*], TENG ZHANG[1], XIANG ZHANG[1], WEI TAN[3] and CHENGDE LI[4]

[1]Department of Pharmacology, Weifang Medical University, Weifang, Shandong 261053; [2]Department of Cardiology, Yantai Kaifaqu Hospital, Yantai, Shandong 264006; [3]Department of Respiratory Medicine, Weifang People's Hospital, Weifang, Shandong 261041; [4]Department of Clinical Pharmacy, Key Laboratory of Applied Pharmacology in Universities of Shandong, Weifang Medical University, Weifang, Shandong 261053, P.R. China

Received January 22, 2021; Accepted September 23, 2021

DOI: 10.3892/etm.2023.12312

Abstract. Inflammation is involved in the pathological process underlying a number of liver diseases. Bilobalide (BB) is a natural compound from *Ginkgo biloba* leaves that was recently demonstrated to exert hepatoprotective effects by inhibiting oxidative stress in the liver cancer cell line HepG2. The anti-inflammatory activity of BB has been reported in recent studies. The major objective of the present study was to investigate whether BB could attenuate inflammation-associated cell damage. HepG2 cells were cultured with lipopolysaccharide (LPS) and BB, and cell damage was evaluated by measuring cell viability using MTT assay. The activity of the NF-κB signaling pathway was assessed by measuring the levels of IκBα, NF-κB p65, phosphorylated (p)-IκBα, p-p65, p65 DNA-binding activity and inflammatory cytokines IL-1β, IL-6 and TNF-α. A toll-like receptor (TLR)4 inhibitor (CLI-095) was used to detect the involvement of TLR4 in cell injury caused by LPS. In addition, the PI3K/Akt inhibitor LY294002 was applied to explore the involvement of the PI3K/Akt axis in mediating the effects of BB. The results demonstrated that LPS induced HepG2 cell injury. LPS also elevated the levels of p-IκBα, p-p65, p65 DNA-binding activity and inflammatory cytokines. However, CLI-095 significantly attenuated the LPS-induced cell damage and inhibited the activation of NF-κB signaling. BB also dose-dependently attenuated the LPS-induced cell damage, activation of NF-κB signaling and TLR4 overexpression. Furthermore, it was observed that LY294002 diminished the cytoprotective effects of BB on cell injury, TLR4 expression and NF-κB activation. These findings indicated that BB could attenuate LPS-induced inflammatory injury to HepG2 cells by regulating TLR4-NF-κB signaling.

Correspondence to: Professor Chengde Li, Department of Clinical Pharmacy, Key Laboratory of Applied Pharmacology in Universities of Shandong, Weifang Medical University, 7166 Baotongxi Street, Weifang, Shandong 261053, P.R. China
E-mail: lichde@wfmc.edu.cn

Professor Wei Tan, Department of Respiratory Medicine, Weifang People's Hospital, 151 Guangwen Road, Weifang, Shandong 261041, P.R. China
E-mail: wfweitan@126.com

*Contributed equally

Key words: bilobalide, hepatoprotective effect, toll-like receptor 4, nuclear factor-κB, PI3 kinase/Akt

Introduction

Liver diseases have become a serious public health concern worldwide. Inflammation is involved in the pathological process underlying a number of hepatic disorders, such as sepsis, hepatitis, alcoholic and autoimmune liver diseases (1). Hepatic inflammation can induce structural and functional liver damage, ultimately resulting in liver failure or even cancer (2). Anti-inflammatory treatment has been considered as an important therapeutic strategy in the management of several hepatic disorders (3,4).

Hepatic inflammation is characterized by the excessive release of inflammatory cytokines (5). Hepatic cells are the targets of inflammatory injury; however, following specific stimulation they may also contribute to the production of cytokines (5,6). Hepatic inflammation may be triggered by a variety of stimuli, such as viral infection, alcohol and toxins (7,8). Lipopolysaccharide (LPS) can induce the production of inflammatory mediators, initiating inflammatory damage to hepatic cells (9). Since LPS can induce inflammation in various animal species and cell lines, and can mimic the typical pathophysiological process of inflammation, LPS stimulation has been widely used in a number of experimental models to investigate the implicated mechanisms and possible treatments of inflammatory diseases (10).

Toll-like receptors (TLRs) are considered to be important components of the innate immune response (11). They are expressed by both hepatocytes and Kupffer cells and serve important roles in hepatic inflammation (12-14). LPS,

as a TLR ligand, can bind to TLRs and trigger the release of multiple inflammatory mediators, leading to activation of the inflammatory cascade and liver injury (15). Among the several TLRs, TLR4 has been widely investigated in a variety of diseases, such as pneumonia (16), hepatitis (17) and diabetes mellitus (18). Activation of TLR4 by LPS may activate inflammatory signaling via the NF-κB pathway (19). NF-κB is expressed in nearly all types of cells (10). Under normal conditions, NF-κB is inactivated in the cytoplasm by binding to IκB. The activation of TLR4 by LPS can trigger the degradation of IκB through phosphorylation, resulting in the translocation of NF-κB into the nucleus, where it can trigger the expression of specific inflammatory genes (20). Therefore, NF-κB activation regulated by TLR4 serves a key role in regulating inflammation (21). Several agents, such as aucubin (22) and vildagliptin (23), exhibit hepatoprotective effects by interfering with TLR4-NF-κB signaling.

Medicinal plants have a history of medical use in hepatic diseases. A number of natural products, such as sea buckthorn polysaccharide (24), artesunate (25) and annona squamosa seed extract (26), have been indicated to exhibit hepatoprotective effects in several hepatic cellular and animal models. Bilobalide (BB) is a sesquiterpene trilactone compound extracted from the leaves of *Ginkgo biloba* that has been reported in the literature to exhibit anti-inflammatory and cytoprotective properties (27,28). A previous study has demonstrated that BB abated inflammation in 3T3-L1 adipocytes, partially via inhibiting NF-κB signaling (29). Another study reported that BB attenuated oxygen-glucose deprivation/reoxygenation (OGD/R) injury in BV2 microglia cells via regulating TLR4 signaling (30). However, the role of BB in LPS-induced inflammatory response and cell injury in the liver and the role of TLR4-NF-κB signaling in this process remain unclear.

Thus, the present study was undertaken to investigate the cytoprotective effects of BB in HepG2 cells and elucidate the underlying mechanism.

Materials and methods

Reagents and antibodies. BB (purity≥99%) was purchased from Shanghai Winherb Medical Technology Co., Ltd. and dissolved in DMSO as a stock solution. MTT was purchased from Sigma-Aldrich; Merck KGaA. DMEM was purchased from Cytiva and FBS was from Gibco; Thermo Fisher Scientific, Inc. BCA assay kit (cat. no. CW0014S) and RIPA buffer (cat. no. CW2334S) were from CoWin Biosciences. IL-6 (cat. no. CSB-E04638h), IL-1β (cat. no. CSB-E08053h) and TNF-α (cat. no. CSB-E04740h) ELISA kits were obtained from CUSABIO TECHNOLOGY LLC. β-actin (cat. no. 4970S), IκBα (cat. no. 4812S), NF-κB p65 (cat. no. 8242S), phosphorylated (p)-IκBα (cat. no. 2859S) and p-p65 (cat. no. 3033S) antibodies were purchased from Cell Signaling Technology, Inc. Horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (cat. no. BA1054) was purchased from Wuhan Boster Biological Technology, Ltd. TransAM NF-κB p65 kit (cat. no. 40096) was obtained from Active Motif, Inc. The chemiluminescent horseradish peroxidase substrate kit was purchased from MilliporeSigma. TRIzol® reagent was purchased from Invitrogen; Thermo Fisher Scientific, Inc. The ReverTra Ace qPCR Kit (cat. no. FSQ-101) was obtained from Toyobo Life Science. CLI-095 and LY294002 were obtained from MedChemExpress and dissolved in DMSO. QuantiNova SYBR Green PCR kit (cat. no. 208054) was purchased from Qiagen GmbH. Primers were purchased from Sangon Biotech Co., Ltd. LPS (cat. no. S11060) from *Escherichia coli* O55:B5 was purchased from Shanghai Yuanye Bio-Technology Co., Ltd.

Cell line. Liver cancer cell line HepG2 (cat. no. CBP60199; CoBioer Biosciences Co., Ltd.) were cultured with DMEM containing 10% FBS and 1% penicillin-streptomycin at 37˚C with 5% CO_2. HepG2 cells were precultured in DMEM complete medium for 24 h, then subjected to different treatments in the subsequent experiments.

Experimental design. The study consisted of four experiments as follows: Experiment 1 aimed to exclude the possible cytotoxicity of BB. HepG2 cells were seeded in 96-well plates ($5x10^3$ cells/well). Cells were grown in DMEM for 24 h, followed by incubation with various concentrations of BB (0, 5, 10, 20 and 40 μM) for 2, 24, 48 and 72 h. The viability of HepG2 cells was detected using an MTT assay. Experiment 2 aimed to detect whether BB could protect HepG2 cells against LPS-induced damage. HepG2 cells were incubated with BB (10 and 20 μM) for 2 h, then stimulated with LPS at the concentration of 50 μg/ml (31-34) for 24 h. Cell viability was detected using an MTT assay. The levels of TLR4 mRNA, p-IκBα, p-p65, p65 DNA-binding activity, IL-1β, IL-6 and TNF-α were measured. Experiment 3 aimed to explore whether TLR4 was involved in mediating LPS-induced cell injury and inflammatory response in HepG2 cells. HepG2 cells were incubated with LPS and the TLR4 inhibitor CLI-095 (10 μM) for 24 h. Cell viability and inflammatory response were assessed as aforementioned. Finally, experiment 4 aimed to confirm the role of the PI3K/Akt pathway in mediating the effects of BB. Cells were pretreated with BB (20 μM) with the presence of the PI3K/Akt inhibitor LY294002 (20 μM) for 2 h, followed by a 24-h LPS incubation. All incubations were performed at 37˚C.

MTT assay. Following each treatment, cells were incubated with 20 μl MTT solution (5 mg/ml) for 4 h at 37˚C. Subsequently, 100 μl DMSO were added to dissolve the formazan crystals after removing the supernatant from the wells. The absorbance of each well was measured at 570 nm using a microplate reader (Bio-Rad Laboratories, Inc.). Cell viability was expressed as a percentage of the control cells (considered to be 100%).

Measurement of inflammatory cytokines. Following each aforementioned treatment, the culture medium was collected. The concentrations of IL-1β, IL-6 and TNF-α were measured using commercially available ELISA kits according to the manufacturer's instructions.

Western blot assay. HepG2 cells were harvested separately, washed in cold PBS and lysed using RIPA buffer. The protein concentrations of the samples were detected via BCA assay. Briefly, 25 μl of each sample and BSA standard solution (0, 0.125, 0.25, 0.5, 1 and 2 μg/μl) from the BCA assay kit were

added to 96-well plates. Then, 25 μl BCA solution was added to each well and the plates were incubated at 37˚C for 30 min. The absorbance of each well was measured at 570 nm using a microplate reader. Ultimately, the protein concentration of the samples was calculated according to the standard curve. After the measurement of the protein concentrations, 40 μg protein/sample were loaded on 10% SDS-PAGE gels, separated and subsequently transferred to PVDF membranes. Following blocking with 5% skimmed milk buffer for 1 h at 37˚C, the membranes were incubated overnight at 4˚C with rabbit antibodies (p65, p-p65, IκBα, p-IκBα and β-actin) diluted 1:1,000. Subsequently, the membranes were incubated with 1:5,000 HRP-conjugated anti-rabbit IgG secondary antibody for 1 h at room temperature. Protein expression was visualized with a chemiluminescent HRP substrate kit. The intensity of the bands was measured using ImageJ software v1.52a (National Institutes of Health). The ratio of phosphorylated/total protein was evaluated.

Reverse transcription-quantitative PCR (RT-qPCR) analysis. Total RNA was obtained from HepG2 cells with TRIzol reagent and the extracted RNA in each group was reversely transcribed into cDNA using the ReverTra Ace qPCR kit according to the manufacturer's instructions. qPCR was then carried out on a Real Time system machine using the cDNA samples and QuantiNova SYBR Green PCR kit. The PCR amplification conditions were as follows: 95˚C for 30 sec, followed by 40 cycles at 95˚C for 10 sec and at 60˚C for 60 sec. The primer sequences use for the qPCR were as listed follows: TLR4 forward, 5'-TGAGCAGTCGTGCTGGTATC-3' and reverse, 5'-CAGGGCTTTTCTGAGTCGTC-3'; β-actin forward, 5'-CACACTGTGCCCATCTACGA-3' and reverse, 5'-CTCAGTGAGGATCTTCATGAGGTAGT-3'. The expression of the target gene was determined using the $2^{-\Delta\Delta Cq}$ method (35) and was normalized to the endogenous control β-actin.

Measurement of p65 DNA-binding activity. Nuclear extracts were obtained from HepG2 cells and used to measure p65 DNA-binding activity with TransAM NF-κB p65 kit strictly according to the manufacturer's protocol. Following completion of the reactions, the absorbance of each sample was measured at 450 nm using a plate reader. The DNA-binding activity was expressed as a percentage of the control cells (considered to be 100%).

Statistical analysis. Data are presented as the mean ± SEM and were analyzed with SPSS software version 21.0 (IBM Corp.). Differences between groups were compared using one-way ANOVA followed by Tukey's honestly significant difference test. All the experiments were performed in triplicates and repeated three times. $P<0.05$ was considered to indicate a statistically significant difference.

Results

BB exerts no significant toxic effects on HepG2 cells. In experiment 1, there were no significant differences in the viability of HepG2 cells incubated with different concentrations of BB (0, 5, 10, 20 and 40 μM) for 2, 24, 48 and 72 h. This indicated that BB at concentrations of 0-40 μM exerted no significant cytotoxic effects (Fig. 1).

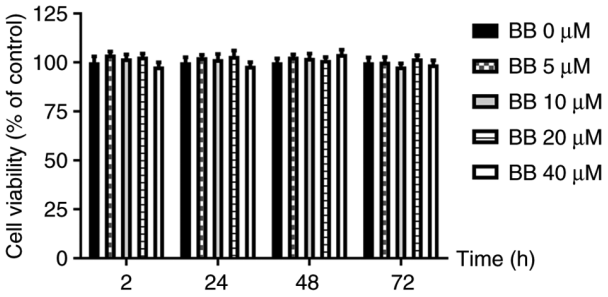

Figure 1. BB exerts no significant cytotoxic effects on HepG2 cells. MTT assay results revealed that BB (0-40 μM) exerted no significant toxic effects on HepG2 cells. The results are presented as the mean ± SEM. All the experiments were performed in triplicate and repeated three times. DMSO as solvent was used for the 0 μM BB treatment. BB, bilobalide.

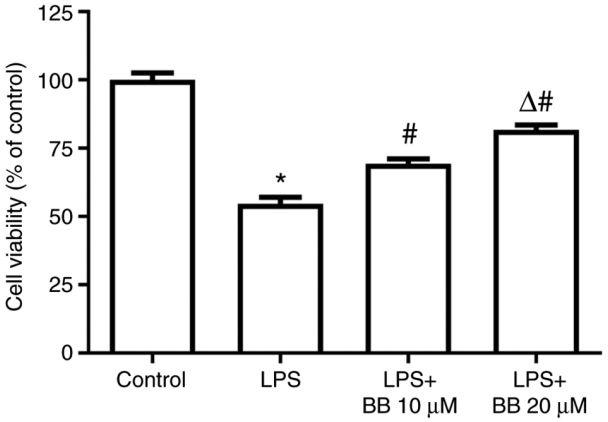

Figure 2. BB attenuates LPS-induced HepG2 cell injury. MTT assay results revealed that LPS significantly decreased the viability of HepG2 cells. Preincubation with BB (10 and 20 μM) markedly increased the viability of LPS-stimulated HepG2 cells in a dose-dependent manner. The results are presented as the mean ± SEM. All the experiments were performed in triplicate and repeated three times. DMSO was used as the control. $^{*}P<0.05$ vs. control; $^{\#}P<0.05$ vs. LPS; $^{\Delta}P<0.05$ vs. LPS + BB 10 μM. BB, bilobalide; LPS, lipopolysaccharide.

BB attenuates LPS-induced HepG2 cell injury. In experiment 2, the MTT assay results revealed that LPS stimulation significantly decreased the viability of HepG2 cells, but BB (10 and 20 μM) dose-dependently increased the viability of the LPS-stimulated HepG2 cells, indicating that BB may protect HepG2 cells from LPS-induced injury (Fig. 2).

BB inhibits LPS-induced activation of NF-κB signaling. In experiment 2, LPS markedly increased the phosphorylation of IκBα and p65 and elevated p65 DNA-binding activity. In addition, LPS promoted the production of TNF-α, IL-1β and IL-6. However, preincubation with BB diminished the NF-κB activation and inflammatory cytokine release induced by LPS (Fig. 3).

BB inhibits LPS-induced TLR4 mRNA expression. In experiment 2, LPS markedly increased TLR4 mRNA expression, whereas BB abolished the LPS-induced TLR4 mRNA increase in a dose-dependent manner (Fig. 4).

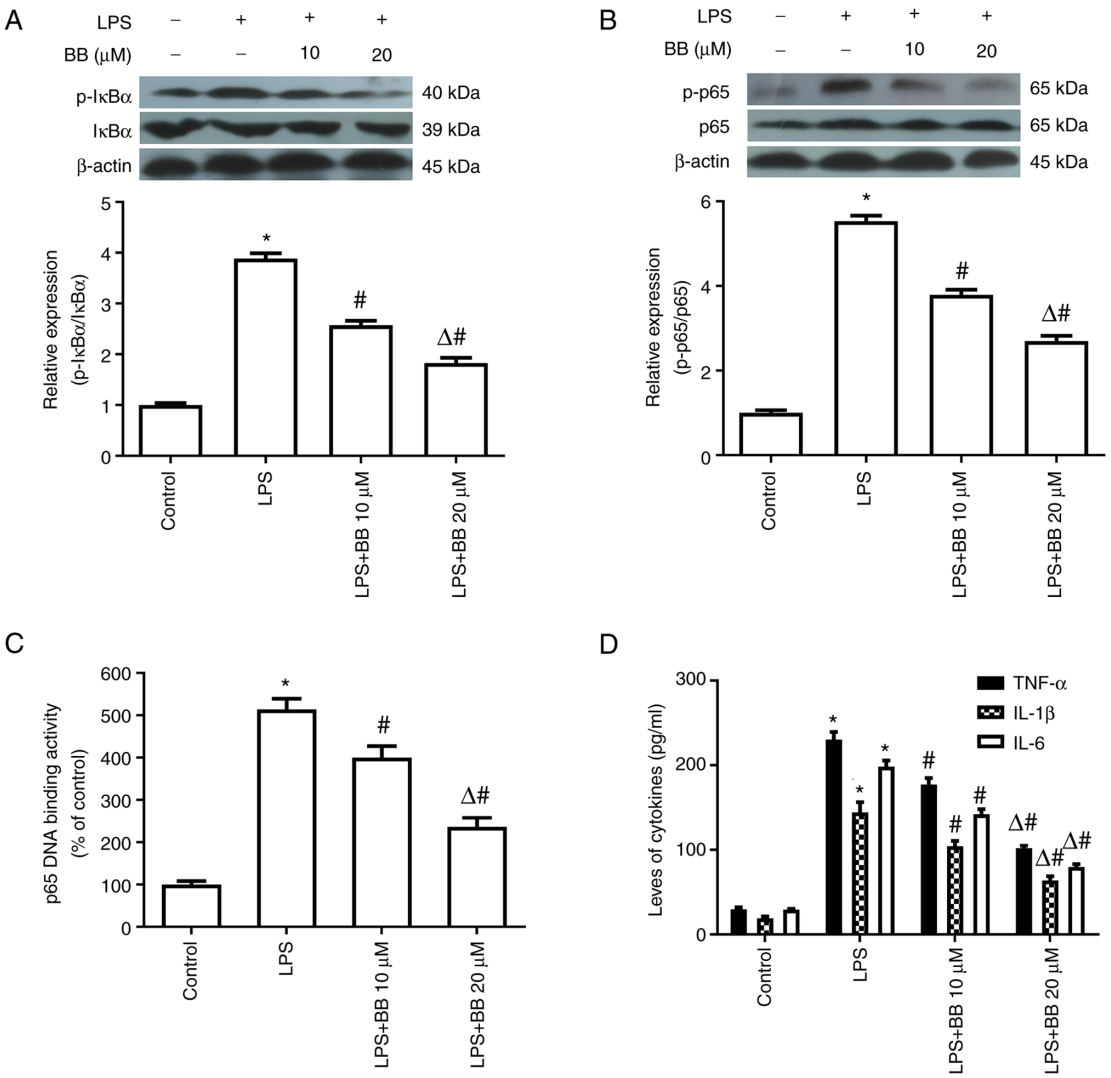

Figure 3. BB inhibits LPS-inducted NF-κB activation and inflammatory cytokine production. LPS induced NF-κB activation and the release of inflammatory cytokines in a dose-dependent manner. LPS stimulation significantly increased the phosphorylation of (A) IκBα and (B) p65, (C) elevated p65 DNA-binding activity and (D) promoted the production of IL-1β, IL-6 and TNF-α. Preincubation with BB (10 and 20 μM) markedly diminished the effects of LPS. The results are presented as the mean ± SEM. All the experiments were performed in triplicate and repeated three times. DMSO was used as the control. *P<0.05 vs. control; $^{\#}$P<0.05 vs. LPS; $^{\Delta}$P<0.05 vs. LPS + BB 10 μM. BB, bilobalide; LPS, lipopolysaccharide; p-, phosphorylated.

TLR4 serves an important role in LPS-induced HepG2 cell injury, NF-κB activation and inflammatory cytokine release. In experiment 3, the TLR4 inhibitor CLI-095 (10 μM) significantly inhibited the LPS-induced increase in the phosphorylation of IκBα and p65, p65 DNA-binding activity and the production of inflammatory cytokines. CLI-095 also attenuated the LPS-induced decrease in cell viability. These results suggested that TLR4 may be involved in LPS-induced cell injury and NF-κB activation in HepG2 cells (Fig. 5).

BB inhibits LPS-induced TLR4 mRNA expression, NF-κB activation and inflammatory cytokine release through the PI3K/Akt pathway. The results of experiment 4 demonstrated that the PI3K/Akt inhibitor LY294002 abolished the inhibition of TLR4 mRNA by BB in the LPS-stimulated HepG2 cells (Fig. 6A). Consistently, LY294002 also abolished the inhibitory effects of BB on the phosphorylation of IκBα and p65, p65 DNA-binding activity (Fig. 6B-D) and inflammatory cytokine release (Fig. 6E) in LPS-stimulated HepG2 cells. Moreover,

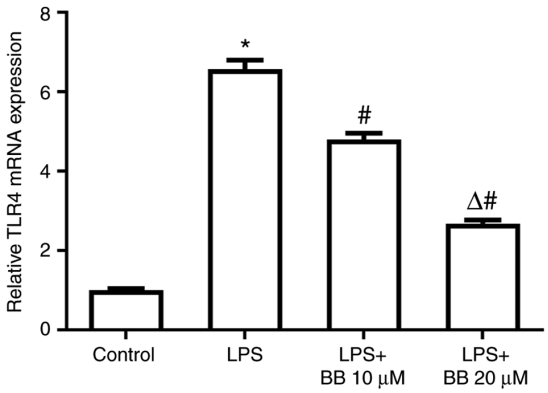

Figure 4. BB inhibits LPS-induced TLR4 mRNA expression in HepG2 cells. LPS stimulation for 24 h markedly increased the expression of TLR4 mRNA, whereas preincubation with BB (10 and 20 μM) partially abolished the LPS-induced increase in TLR4 mRNA expression in a dose-dependent manner. The results are presented as the mean ± SEM. All the experiments were performed in triplicate and repeated three times. DMSO was used as the control. *P<0.05 vs. control; $^{\#}$P<0.05 vs. LPS; $^{\Delta}$P<0.05 vs. LPS + BB 10 μM. BB, bilobalide; LPS, lipopolysaccharide.

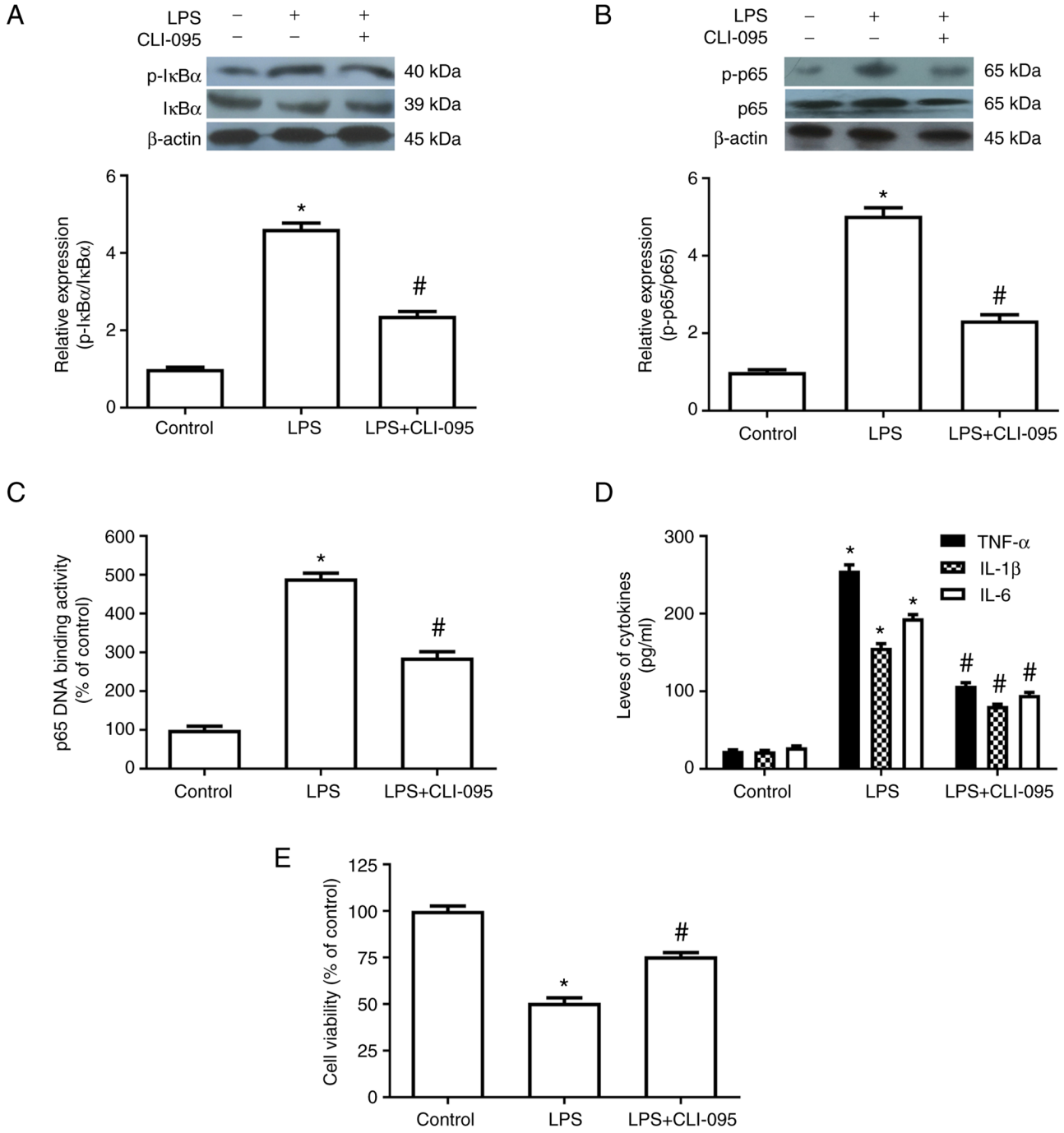

Figure 5. Toll-like receptor 4 is involved in LPS-induced HepG2 cell injury, NF-κB activation and inflammatory cytokine release. CLI-095 significantly decreased LPS-induced increase in (A) IκBα phosphorylation, (B) p65 phosphorylation, (C) p65 DNA-binding activity and (D) IL-1β, IL-6 and TNF-α levels. (E) CLI-095 attenuated LPS-induced cell injury. The results are presented as the mean ± SEM. All the experiments were performed in triplicate and repeated three times. DMSO was used as the control. *P<0.05 vs. control; #P<0.05 vs. LPS. LPS, lipopolysaccharide; p-, phosphorylated.

LY294002 diminished the cytoprotective effects of BB after LPS treatment (Fig. 6F). These results strongly suggested that the effects of BB on LPS-stimulated HepG2 cells may be mediated via the PI3K/Akt pathway.

Discussion

An increasing amount of experimental and clinical studies have focused on the regulation of inflammatory response by natural products (36-39). A number of natural agents have been indicated to exhibit hepatoprotective properties (24-26,40). BB (Fig. 7), which is a sesquiterpene trilactone extracted from the *Ginkgo biloba* leaf, has been indicated to exhibit multiple pharmacological effects (41-43), and it has been investigated as a therapeutic agent for cardiovascular diseases (44), neurological disorders (41,45), ethanol-induced gastric ulcer (46) and cuprizone-induced demyelination (47). Previous studies also demonstrated that BB exerts anti-inflammatory effects (27,28). However, whether BB exhibits beneficial effects on hepatic injury following an inflammatory response has yet to be elucidated.

The liver cancer cell line HepG2 has been widely used in evaluating the hepatoprotective activities of novel agents (48),

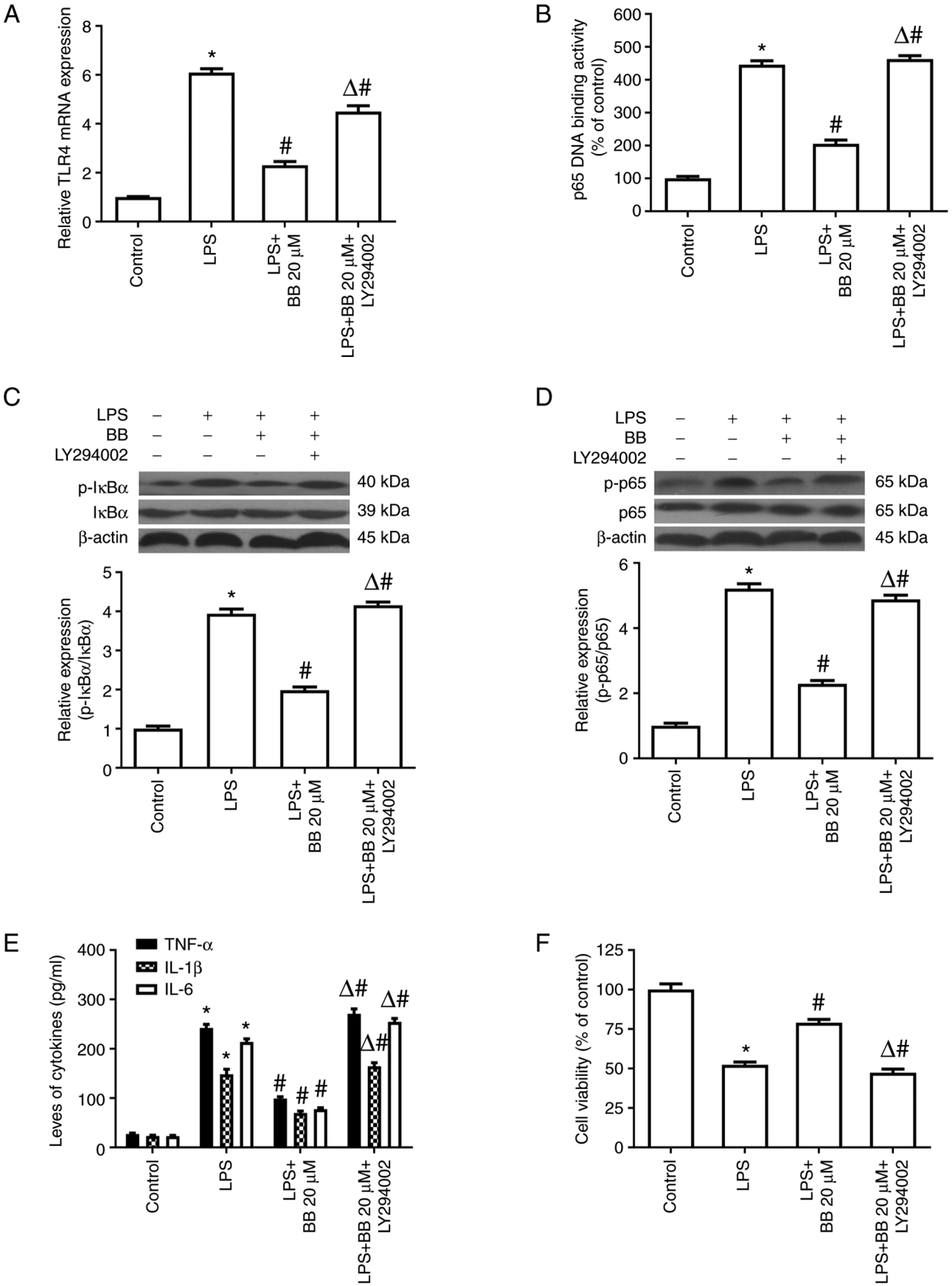

Figure 6. BB inhibits LPS-induced TLR4 mRNA expression, NF-κB activation and inflammatory cytokine release through the PI3K/Akt pathway. (A) LY294002 attenuated the inhibitory effects of BB on TLR4 mRNA expression in LPS-stimulated HepG2 cells. LY294002 abolished the effects of BB on (B) p65 DNA-binding activity, (C) IκBα phosphorylation, (D) p65 phosphorylation and (E) inflammatory cytokine release. (F) The cytoprotective effect of BB was also abolished by LY294002. The results are presented as the mean ± SEM. All the experiments were performed in triplicate and repeated three times. DMSO was used as the control. *P<0.05 vs. control; #P<0.05 vs. LPS; ΔP<0.05 vs. LPS + BB 20 μM. BB, bilobalide; TLR4, toll-like receptor 4; p-, phosphorylated; LPS, lipopolysaccharide.

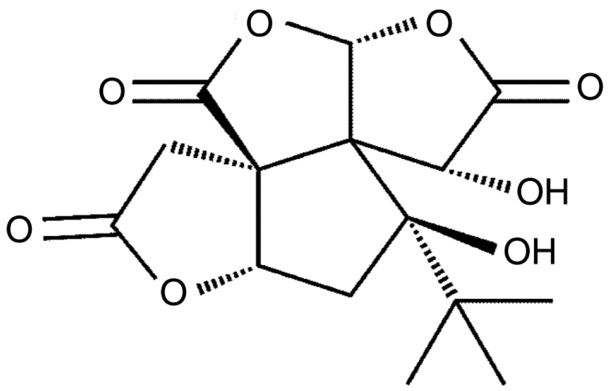

Figure 7. Chemical structure of bilobalide.

as these cells retain several morphological and biochemical characteristics of normal hepatocytes (49). In the present study, the possible cytotoxic effects of BB on HepG2 cells were firstly excluded. HepG2 cells were incubated with various concentrations of BB for 2, 24, 48 and 72 h, and the viability of HepG2 cells was evaluated. The results revealed that incubation with BB at concentrations of 0-40 μM demonstrated no adverse effects on cell viability, indicating that BB was not cytotoxic to HepG2 cells. In order to observe whether BB could protect HepG2 cells from inflammatory damage, HepG2 cells were stimulated with LPS, which has been widely used to induce inflammation in animal models and cell lines (50). The results demonstrated that LPS stimulation resulted in decreased cell viability, indicating that LPS caused HepG2 cell injury, whereas 10 and 20 μM BB treatment dose-dependently attenuated the LPS-induced cell damage. Our previous pilot study demonstrated that 10 and 20 μM BB exhibited a relevant potency on inhibiting LPS-induced damage; therefore, 10 and 20 μM BB were used in the current study (data not shown). Similar to the findings of the present study, the cytoprotective effects of BB have also been demonstrated in other cell lines. Hua *et al* (51) revealed that BB could protect neural cells against aggregated α-synuclein-induced apoptosis. Cao and Li (52) observed that BB ameliorated OGD-induced injury in H9c2 cells. A study by Mao *et al* (53) demonstrated that BB alleviated IL-17-induced inflammatory injury in ATDC5 cells. In addition to the cytoprotective effects of BB, the present study also demonstrated that BB treatment markedly suppressed the production of TNF-α, IL-1β and IL-6 induced by LPS in HepG2 cells. The role of these cytokines in exacerbating hepatocyte apoptosis (54,55), hepatic fibrosis (56,57), hepatic stellate cell activation (58,59) and Kupffer cell activation (60) has been demonstrated in numerous studies. Considering the important role of these cytokines in hepatic injury, inhibiting the overproduction of these cytokines has been regarded as an important therapeutic strategy in the management of hepatic disorders in both clinical and experimental studies (61-63). Therefore, it was hypothesized that the downregulation of inflammatory cytokines by BB should contribute to the attenuation of the LPS-induced HepG2 cell injury in the present study. Consistent with the findings of the current study, BB has been reported to attenuate inflammatory injury in different disease models. In a previous study performed by Zhou *et al* (30), BV2 cells were subjected to oxygen-glucose deprivation/reoxygenation (OGD/R) and treated with BB. The results of the study revealed that OGD/R induced inflammatory response and cellular damage in BV2 cells *in vitro*, but BB treatment suppressed the production of IL-1β, IL-6, IL-8 and IL-10, and attenuated cell damage. In another study by Jiang *et al* (27), BB treatment alleviated brain damage and inhibited the expression of the inflammatory cytokines TNF-α and IL-1β in rats with middle cerebral artery occlusion/reperfusion. Simultaneously, Jiang *et al* (27) observed that BB significantly downregulated TNF-α and IL-1β expression in rat cortical neurons after OGD/R-induced injury *in vitro*. Hui and Fangyu (46) reported that BB decreased TNF-α, IL-6 and IL-1β levels in mice with gastric ulcers. Additionally, other studies also demonstrated the protective effects of BB in several disorders through various mechanisms. For example, Liu *et al* (64) demonstrated that bilobalide acts against cerebral ischemia injury by activating the Akt/Nrf2 pathway *in vitro* and *in vivo*; Qin *et al* (65) reported that bilobalide alleviates neuroinflammation in Alzheimer's disease by upregulating lincRNA-p21; and Zheng *et al* (66) demonstrated that bilobalide inhibits autophagy and promotes angiogenesis following focal cerebral ischemia reperfusion by activating Akt/eNOS.

The regulation of inflammatory gene transcription has been demonstrated to be regulated by specific signaling pathways and transcriptional regulators (67,68). NF-κB is one of most important inflammatory regulators, and serves a key role in determining cytokine expression (69). After being released from its inhibitor, IκB, the activated p65 subunit translocates to the nucleus to regulate the expression of inflammatory genes (70). NF-κB has been considered to be an important therapeutic target in treating inflammatory disorders (71,72). In the present study, it was observed that LPS stimulation activated NF-κB signaling in HepG2 cells by enhancing the phosphorylation of IκBα and p65 and increasing p65 DNA-binding activity. However, BB significantly abolished NF-κB activation induced by LPS. These inhibitory effects on NF-κB activation were in accordance with its effects on inflammatory cytokine release in the present study. In a similar manner, a number of other natural agents, such as raspberry ketone, grape-leaf extract and fraxetin, have also been demonstrated to exert hepatoprotective effects through suppressing NF-κB activation (73-75). Although this is, to the best of our knowledge, the first study to demonstrate the effects of BB on NF-κB activation in HepG2 cells, a recent study by Priyanka *et al* (28) demonstrated that BB abated inflammation and inhibited NF-κB activation in 3T3-L1 adipocytes. In addition, Zhang *et al* (76) also reported that BB attenuated colitis by inhibiting NF-κB signaling in mice.

As one of the receptors of LPS, TLR4 serves a key function in hepatic diseases (77,78). Certain agents, such as raspberry ketone (73) and monotropein (79), have been demonstrated to attenuate liver injury by acting on the TLR4 signaling pathway. In the present study, BB was indicated to abolish LPS-induced TLR4 mRNA overexpression. To confirm the involvement of TLR4 in mediating the LPS-induced alterations in HepG2 cells, the TLR4 inhibitor CLI-095 was used to inhibit TLR4, as described previously (80). The results demonstrated that CLI-095 reduced LPS-induced cell injury and suppressed the

activation of NF-κB signaling. These results suggested the involvement of TLR4 in the LPS-induced damage to HepG2 cells. This is in line with the conclusions of previous studies reporting that LPS primarily induces inflammatory response through TLR4-NF-κB signaling (79,81). Therefore, the inhibition of TLR4 by BB in the present study may contribute to the suppressive effects of BB on NF-κB activation and cytokine release. In addition to the inhibitory effects of BB on TLR4 expression observed in the present study, a previous study also demonstrated that BB inhibited TLR4 expression in BV2 microglia cells (30).

It has been demonstrated that the PI3K/Akt pathway is involved in regulating TLR4 expression (82,83). It has been reported that PI3K/Akt activation can inhibit LPS-induced IL-6 and TNF-α production from microglia and bone marrow macrophages (84,85). Notably, a previous study demonstrated that BB could activate PI3K/Akt signaling in SH-SY5Y cells (86). Therefore, it was hypothesized that the effects of BB on HepG2 cells may be mediated via the PI3K/Akt pathway. To confirm this hypothesis, the PI3K/Akt inhibitor LY294002 was applied to block the PI3K/Akt pathway. The use of specific inhibitors is considered to be the optimal method for assessing the pharmacological action of a drug. When assays with inhibitors are used, three possible results may be observed: No alteration, total inhibition or partial inhibition. The results of the present study demonstrated that LY294002 partially abolished the downregulation of TLR4 mRNA expression by BB. In addition, LY294002 significantly diminished the effects of BB on NF-κB activation and cytokine release. Furthermore, the beneficial effects of BB on cell viability were also abolished by LY294002. These results suggested that the PI3K/Akt pathway was involved in mediating the effects of BB on HepG2 cells, as most of the aforementioned effects were entirely inhibited by LY294002. Similar to BB, other agents such as lithium, hypaphorine and ginkgolide A have also been indicated to inhibit TLR4-associated cytokine release through the PI3K/Akt pathway in microglia and human vascular endothelial cells (85,87,88).

In conclusion, the results demonstrated that BB may attenuate LPS-induced HepG2 cell injury by regulating TLR4/NF-κB signaling through the PI3K/Akt pathway. Therefore, BB may be of value as a potential agent for the treatment of inflammation-associated hepatic injury.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Natural Science Foundation of Shandong Province (CN) (grant nos. ZR2020MH381 and ZR2015CL029), the Shandong Provincial Education Department (grant nos. J15LL54 and J16LM11) and Weifang Science and Technology Bureau (grant no. 2021GX062).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WT and CL designed the study and confirm the authenticity of all the raw data. SM, JY, TZ and XZ performed the experiments. SM and CL were major contributors to the writing of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Weifang Medical University (approval no. 2019SDL029; Weifang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Szabo G and Csak T: Inflammasomes in liver diseases. J Hepatol 57: 642-654, 2012.
2. Yu S, Wang Y, Jing L, Claret FX, Li Q, Tian T, Liang X, Ruan Z, Jiang L, Yao Y, *et al*: Autophagy in the 'inflammation-carcinogenesis' pathway of liver and HCC immunotherapy. Cancer Lett 411: 82-89, 2017.
3. Lam P, Cheung F, Tan HY, Wang N, Yuen MF and Feng Y: Hepatoprotective effects of Chinese medicinal herbs: A focus on anti-inflammatory and anti-oxidative activities. Int J Mol Sci 17: 465, 2016.
4. Li H, Huang MH, Jiang JD and Peng ZG: Hepatitis C: From inflammatory pathogenesis to anti-inflammatory/hepatoprotective therapy. World J Gastroenterol 24: 5297-5311, 2018.
5. Seki E and Schwabe RF: Hepatic inflammation and fibrosis: Functional links and key pathways. Hepatology 61: 1066-1079, 2015.
6. Liu J, Abate W, Xu J, Corry D, Kaul B and Jackson SK: Three-dimensional spheroid cultures of A549 and HepG2 cells exhibit different lipopolysaccharide (LPS) receptor expression and LPS-induced cytokine response compared with monolayer cultures. Innate Immun 17: 245-255, 2011.
7. Ju C and Mandrekar P: Macrophages and alcohol-related liver inflammation. Alcohol Res 37: 251-262, 2015.
8. Park BJ, Lee YJ and Lee HR: Chronic liver inflammation: Clinical implications beyond alcoholic liver disease. World J Gastroentero 20: 2168-2175, 2014.
9. Wang Y, Zhou X, Zhao D, Wang X, Gurley EC, Liu R, Li X, Hylemon PB, Chen W and Zhou H: Berberine inhibits free fatty acid and LPS-induced inflammation via modulating ER stress response in macrophages and hepatocytes. PLoS One 15: e0232630, 2020.
10. Lu S, Duan M, Guo Z, Zhou Y, Wu D, Zhang X, Wang Y, Ye C, Ju R, Li J, *et al*: Carboxyamidotriazole exerts anti-inflammatory activity in lipopolysaccharide-induced RAW264.7 macrophages by inhibiting NF-κB and MAPKs pathways. Exp Ther Med 20: 1455-1466, 2020.
11. Huang XY, Ansari AR, Huang HB, Zhao X, Li NY, Sun ZJ, Peng KM, Zhong J and Liu HZ: Lipopolysaccharide mediates immuno-pathological alterations in young chicken liver through TLR4 signaling. BMC Immunol 18: 12, 2017.
12. Zhang J, Wieser A, Lin H, Li H, Hu M, Behrens IK, Schiergens TS, Gerbes AL and Steib CJ: Kupffer cell activation by different microbial lysates: Toll-like receptor-2 plays pivotal role on thromboxane A2 production in mice and humans. Eur J Immunol 50: 1988-1997, 2020.
13. Zhang X, Jiang W, Zhou AL, Zhao M and Jiang DR: Inhibitory effect of oxymatrine on hepatocyte apoptosis via TLR4/PI3K/Akt/GSK-3β signaling pathway. World J Gastroenterol 23: 3839-3849, 2017.

14. Kim MS, Lee S, Jung N, Lee K, Choi J, Kim SH, Jun J, Lee WM, Chang Y and Kim D: The vitamin D analogue paricalcitol attenuates hepaticischemia/reperfusion injury through down-regulation of Toll-like receptor 4 signaling in rats. Arch Med Sci 13: 459-469, 2017.
15. Cho HI, Hong JM, Choi JW, Choi HS, Kwak JH, Lee DU, Kook Lee S and Lee SM: β-Caryophyllene alleviates D-galactosamine and lipopolysaccharide-induced hepatic injury through suppression of the TLR4 and RAGE signaling pathways. Eur J Pharmacol 764: 613-621, 2015.
16. Standiford LR, Standiford TJ, Newstead MJ, Zeng X, Ballinger MN, Kovach MA, Reka AK and Bhan U: TLR4-dependent GM-CSF protects against lung injury in Gram-negative bacterial pneumonia. Am J Physiol Lung Cell Mol Physiol 302: L447-L454, 2012.
17. Li Y, Yin S, Chen Y, Zhang Q, Huang R, Jia B, Jie W, Yao K, Wang J, Tong X, *et al*: Hepatitis B virus-induced hyperactivation of B cells in chronic hepatitis B patients via TLR4. J Cell Mol Med 24: 6096-6106, 2020.
18. Tian J, Zhao Y, Wang L and Li L: Role of TLR4/MyD88/NF-κB signaling in heart and liver-related complications in a rat model of type 2 diabetes mellitus. J Int Med Res 49: 300060521997590, 2021.
19. Qu Y, Li X, Xu F, Zhao S, Wu X, Wang Y and Xie J: Kaempferol alleviates murine experimental colitis by restoring gut microbiota and inhibiting the LPS-TLR4-NF-κB axis. Front Immunol 12: 679897, 2021.
20. Mitchell S, Vargas J and Hoffmann A: Signaling via the NFκB system. Wiley Interdiscip Rev Syst Biol Med 8: 227-241, 2016.
21. Sun X, Li P, Qu X and Liu W: Isovitexin alleviates acute gouty arthritis in rats by inhibiting inflammation via the TLR4/MyD88/NF-κB pathway. Pharm Biol 59: 1326-1333, 2021.
22. Zhang S, Feng Z, Gao W, Duan Y, Fan G, Geng X, Wu B, Li K, Liu K and Peng C: Aucubin attenuates liver ischemia-reperfusion injury by inhibiting the HMGB1/TLR-4/NF-κB signaling pathway, oxidative stress, and apoptosis. Front Pharmacol 11: 544124, 2020.
23. Sherif IO and Al-Shaalan NH: Vildagliptin attenuates hepatic ischemia/reperfusion injury via the TLR4/NF-κB signaling pathway. Oxid Med Cell Longev 2018: 3509091, 2018.
24. Liu H, Zhang W, Dong S, Song L, Zhao S, Wu C, Wang X, Liu F, Xie J, Wang J and Wang Y: Protective effects of sea buckthorn polysaccharide extracts against LPS/d-GalN-induced acute liver failure in mice via suppressing TLR4-NF-κB signaling. J Ethnopharmacol 176: 69-78, 2015.
25. Lai L, Chen Y, Tian X, Li X, Zhang X, Lei J, Bi Y, Fang B and Song X: Artesunate alleviates hepatic fibrosis induced by multiple pathogenic factors and inflammation through the inhibition of LPS/TLR4/NF-κB signaling pathway in rats. Eur J Pharmacol 765: 234-241, 2015.
26. Zahid M, Arif M, Rahman MA and Mujahid M: Hepatoprotective and antioxidant activities of Annona squamosa seed extract against alcohol-induced liver injury in Sprague Dawley rats. Drug Chem Toxicol 43: 588-594, 2020.
27. Jiang M, Li J, Peng Q, Liu Y, Liu W, Luo C, Peng J, Li J, Yung KK and Mo Z: Neuroprotective effects of bilobalide on cerebral ischemia and reperfusion injury are associated with inhibition of pro-inflammatory mediator production and down-regulation of JNK1/2 and p38 MAPK activation. J Neuroinflammation 11: 167, 2014.
28. Priyanka A, Nisha VM, Anusree SS and Raghu KG: Bilobalide attenuates hypoxia induced oxidative stress, inflammation, and mitochondrial dysfunctions in 3T3-L1 adipocytes via its antioxidant potential. Free Radic Res 48: 1206-1217, 2014.
29. Priyanka A, Sindhu G, Shyni GL, Preetha Rani MR, Nisha VM, and Raghu KG: Bilobalide abates inflammation, insulin resistance and secretion of angiogenic factors induced by hypoxia in 3T3-L1 adipocytes by controlling NF-κB and JNK activation. Int Immunopharmacol 42: 209-217, 2017.
30. Zhou JM, Gu SS, Mei WH, Zhou J, Wang ZZ and Xiao W: Ginkgolides and bilobalide protect BV2 microglia cells against OGD/reoxygenation injury by inhibiting TLR2/4 signaling pathways. Cell Stress Chaperones 21: 1037-1053, 2016.
31. Norikura T, Mukai Y, Fujita S, Mikame K, Funaoka M and Sato S: Lignophenols decrease oleate-induced apolipoprotein-B secretion in HepG2 cells. Basic Clin Pharmacol Toxicol 107: 813-817, 2010.
32. Wollborn J, Wunder C, Stix J, Neuhaus W, Bruno RR, Baar W, Flemming S, Roewer N, Schlegel N and Schick MA: Phosphodiesterase-4 inhibition with rolipram attenuates hepatocellular injury in hyperinflammation in vivo and in vitro without influencing inflammation and HO-1 expression. J Pharmacol Pharmacother 6: 13-23, 2015.
33. Liu M, Fang G, Yin S, Zhao X, Zhang C, Li J and Liu Z: Caffeic acid prevented LPS-induced injury of primary bovine mammary epithelial cells through inhibiting NF-κB and MAPK activation. Mediators Inflamm 2019: 1897820, 2019.
34. Xiong W, Ma H, Zhang Z, Jin M, Wang J, Xu Y and Wang Z: The protective effect of icariin and phosphorylated icariin against LPS-induced intestinal epithelial cells injury. Biomed Pharmacother 118: 109246, 2019.
35. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
36. Latruffe N: Natural products and inflammation. Molecules 22: 120, 2017.
37. Amaral-Machado L, Oliveira WN, Rodrigues VM, Albuquerque NA, Alencar ÉN and Egito EST: Could natural products modulate early inflammatory responses, preventing acute respiratory distress syndrome in COVID-19-confirmed patients? Biomed Pharmacother 134: 111143, 2021.
38. Beutler JA: Natural products as a foundation for drug discovery. Curr Protoc Pharmacol 86: e67, 2019.
39. LeBlanc JF, Wiseman D, Lakatos PL and Bessissow T: Elderly patients with inflammatory bowel disease: Updated review of the therapeutic landscape. World J Gastroenterol 25: 4158-4171, 2019.
40. Chale-Dzul J, Pérez-Cabeza de Vaca R, Quintal-Novelo C, Olivera-Castillo L and Moo-Puc R: Hepatoprotective effect of a fucoidan extract from Sargassum fluitans Borgesen against CCl_4-induced toxicity in rats. Int J Biol Macromol 145: 500-509, 2020.
41. Feng Z, Sun Q, Chen W, Bai Y, Hu D and Xie X: The neuroprotective mechanisms of ginkgolides and bilobalide in cerebral ischemic injury: A literature review. Mol Med 25: 57, 2019.
42. Bu S, Yuan CY, Xue Q, Chen Y and Cao F: Bilobalide suppresses adipogenesis in 3T3-L1 adipocytes via the AMPK signaling pathway. Molecules 24: 3503, 2019.
43. Li Y, Jiang J, Tong L, Gao T, Bai L, Xue Q, Xing J, Wang Q, Lyu H, Cai M and Sun Z: Bilobalide protects against ischemia/reperfusion-induced oxidative stress and inflammatory responses via the MAPK/NF-κB pathways in rats. BMC Musculoskelet Disord 21: 449, 2020.
44. Maerz S, Liu CH, Guo W and Zhu YZ: Anti-ischaemic effects of bilobalide on neonatal rat cardiomyocytes and the involvement of the platelet-activating factor receptor. Biosci Rep 31: 439-447, 2011.
45. Zhang Y and Zhai H: Bilobalide assuages morphine-induced addiction in hippocampal neuron cells through upregulation of microRNA-101. J Biochem Mol Toxicol 34: e22493, 2020.
46. Hui S and Fangyu W: Protective effects of bilobalide against ethanol-induced gastric ulcer in vivo/vitro. Biomed Pharmacother 85: 592-600, 2017.
47. Sui RX, Miao Q, Wang J, Wang Q, Song LJ, Yu JW, Cao L, Xiao W, Xiao BG and Ma CG: Protective and therapeutic role of Bilobalide in cuprizone-induced demyelination. Int Immunopharmacol 66: 69-81, 2019.
48. Al-Oqail MM, Farshori NN, Al-Sheddi ES, Al-Massarani SM, Siddiqui MA and Al-Khedhairy AA: Petroselinum sativum protects HepG2 cells from cytotoxicity and oxidative stress induced by hydrogen peroxide. Mol Biol Rep 47: 2771-2780, 2020.
49. Dey D, Chaskar S, Bhatt N and Chitre D: Hepatoprotective activity of BV-7310, a proprietary herbal formulation of phyllanthus niruri, tephrosia purpurea, boerhavia diffusa, and andrographis paniculata, in alcohol-induced HepG2 cells and alcohol plus a haloalkane, CCl_4, induced liver damage in rats. Evid Based Complement Alternat Med 2020: 6428906, 2020.
50. Ou TT, Kuo CY, Chyau CC, Lee HJ, Peng JS and Wang CJ: Improvement of lipopolysaccharide-induced hepatic injuries and inflammation with mulberry extracts. J Sci Food Agric 93: 1880-1886, 2013.
51. Hua J, Yin N, Yang B, Zhang J, Ding J, Fan Y and Hu G: Ginkgolide B and bilobalide ameliorate neural cell apoptosis in α-synuclein aggregates. Biomed Pharmacother 96: 792-797, 2017.

52. Cao A and Li X: Bilobalide protects H9c2 cell from oxygen-glucose-deprivation-caused damage through upregulation of miR-27a. Artif Cells Nanomed Biotechnol 47: 2980-2988, 2019.
53. Mao D, Li H, Zhang L, Xu J, Yu C and Zhang Q: Bilobalide alleviates IL-17-induced inflammatory injury in ATDC5 cells by downregulation of microRNA-125a. J Biochem Mol Toxicol 33: e22405, 2019.
54. Zhan X, Zhang J, Chen H, Liu L, Zhou Y, Zheng T, Li S, Zhang Y, Zheng B and Gong Q. Capsaicin alleviates acetaminophen-induced acute liver injury in mice. Clin Immunol 220: 108578, 2020.
55. Du YC, Lai L, Zhang H, Zhong FR, Cheng HL, Qian BL, Tan P, Xia XM and Fu WG: Kaempferol from Penthorum chinense Pursh suppresses HMGB1/TLR4/NF-κB signaling and NLRP3 inflammasome activation in acetaminophen-induced hepatotoxicity. Food Funct 11: 7925-7934, 2020.
56. Del Campo JA, Gallego P and Grande L: Role of inflammatory response in liver diseases: Therapeutic strategies. World J Hepatol 10: 1-7, 2018.
57. Xu R, Zhang Z and Wang FS: Liver fibrosis: Mechanisms of immune-mediated liver injury. Cell Mol Immunol 9: 296-301, 2012.
58. Liu M, Xu Y, Han X, Yin L, Xu L, Qi Y, Zhao Y, Liu K and Peng J: Dioscin alleviates alcoholic liver fibrosis by attenuating hepatic stellate cell activation via the TLR4/MyD88/NF-κB signaling pathway. Sci Rep 5: 18038, 2015.
59. Chen Y, Zeng Z, Shen X, Wu Z, Dong Y and Cheng JC: MicroRNA-146a-5p negatively regulates pro-inflammatory cytokine secretion and cell activation in lipopolysaccharide stimulated human hepatic stellate cells through inhibition of toll-Like receptor 4 signaling pathways. Int J Mol Sci 17: 1076, 2016.
60. Wu G, Yang Q, Yu Y, Lin S, Feng Y, Lv Q, Yang J and Hu J: Taurine inhibits Kupffer cells activation induced by lipopolysaccharide in alcoholic liver damaged rats. Adv Exp Med Biol 975 Pt 2: 789-800, 2017.
61. Song S, Chu L, Liang H, Chen J, Liang J, Huang Z, Zhang B and Chen X: Protective effects of dioscin against doxorubicin-induced hepatotoxicity via regulation of Sirt1/FOXO1/NF-κb signal. Front Pharmacol 10: 1030, 2019.
62. Fu T, Wang S, Liu J, Cai E, Li H, Li P and Zhao Y: Protective effects of alpha-mangostin against acetaminophen-induced acute liver injury in mice. Eur J Pharmacol 827: 173-180, 2018.
63. Lu L, Wu C, Lu BJ, Xie D, Wang Z, Bahaji Azami NL, An YT, Wang HJ, Ye G and Sun MY: BabaoDan cures hepatic encephalopathy by decreasing ammonia levels and alleviating inflammation in rats. J Ethnopharmacol 249: 112301, 2020.
64. Liu Q, Jin Z, Xu Z, Yang H, Li L, Li G, Li F, Gu S, Zong S, Zhou J, *et al*: Antioxidant effects of ginkgolides and bilobalide against cerebral ischemia injury by activating the Akt/Nrf2 pathway in vitro and in vivo. Cell Stress Chaperones 24: 441-452, 2019.
65. Qin YR, Ma CQ, Wang DP, Zhang QQ, Liu MR, Zhao HR, Jiang JH and Fang Q: Bilobalide alleviates neuroinflammation and promotes autophagy in Alzheimer's disease by upregulating lincRNA-p21. Am J Transl Res 13: 2021-2040, 2021.
66. Zheng Y, Wu Z, Yi F, Orange M, Yao M, Yang B, Liu J and Zhu H: By Activating Akt/eNOS Bilobalide B inhibits autophagy and promotes angiogenesis following focal cerebral ischemia reperfusion. Cell Physiol Biochem 47: 604-616, 2018.
67. Adcock IM: Transcription factors as activators of gene transcription: AP-1 and NF-kappa B. Monaldi Arch Chest Dis 52: 178-186, 1997.
68. Mitchell JP and Carmody RJ: NF-κB and the transcriptional control of inflammation. Int Rev Cell Mol Biol 335: 41-84, 2018.
69. Li Q and Verma IM: NF-kappaB regulation in the immune system. Nat Rev Immunol 2: 725-734, 2002.
70. Pahl HL: Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18: 6853-6866, 1999.
71. Freitas RHCN and Fraga CAM: NF-κB-IKKβ pathway as a target for drug development: Realities, challenges and perspectives. Curr Drug Targets 19: 1933-1942, 2018.
72. Lin TH, Pajarinen J, Lu L, Nabeshima A, Cordova LA, Yao Z and Goodman SB: NF-κB as a therapeutic target in inflammatory-associated bone diseases. Adv Protein Chem Struct Biol 107: 117-154, 2017.
73. Fouad D, Badr A and Attia HA: Hepatoprotective activity of raspberry ketone is mediated via inhibition of the NF-κB/TNF-α/caspase axis and mitochondrial apoptosis in chemically induced acute liver injury. Toxicol Res (Camb) 8: 663-676, 2019.
74. Amen Y, Sherif AE, Shawky NM, Abdelrahman RS, Wink M and Sobeh M: Grape-leaf extract attenuates alcohol-induced liver injury via interference with NF-κB signaling pathway. Biomolecules 10: 558, 2020.
75. Wu B, Wang R, Li S, Wang Y, Song F, Gu Y and Yuan Y: Antifibrotic effects of Fraxetin on carbon tetrachloride-induced liver fibrosis by targeting NF-κB/IκBα, MAPKs and Bcl-2/Bax pathways. Pharmacol Rep 71: 409-416, 2019.
76. Zhang H, Cao N, Yang Z, Fang X, Yang X, Li H, Hong Z and Ji Z: Bilobalide alleviated dextran sulfate sodium-induced experimental colitis by inhibiting M1 macrophage polarization through the NF-κB signaling pathway. Front Pharmacol 11: 718, 2020.
77. Takeuchi O and Akira S: Pattern recognition receptors and inflammation. Cell 140: 805-820, 2010.
78. Guven-Maiorov E, Keskin O, Gursoy A and Nussinov R: A structural view of negative regulation of the toll-like receptor-mediated inflammatory pathway: Biophys J 109: 1214-1226, 2015.
79. Chen Y, Lu Y, Pei C, Liang J, Ding P, Chen S and Hou SZ: Monotropein alleviates secondary liver injury in chronic colitis by regulating TLR4/NF-κB signaling and NLRP3 inflammasome. Eur J Pharmacol 883: 173358, 2020.
80. Zhang M, Xue Y, Chen H, Meng L, Chen B, Gong H, Zhao Y and Qi R: Resveratrol inhibits MMP3 and MMP9 expression and secretion by suppressing TLR4/NF-κB/STAT3 activation in Ox-LDL-greated HUVECs. Oxid Med Cell Longev 2019: 9013169, 2019.
81. Tao Y, Wang Y, Wang X, Wang C, Bao K, Ji L, Jiang G and Hong M: Calycosin suppresses epithelial derived initiative key factors and maintains epithelial barrier in allergic inflammation via TLR4 mediated NF-κB pathway. Cell Physiol Biochem 44: 1106-1119, 2017.
82. Ke B, Shen XD, Ji H, Kamo N, Gao F, Freitas MC, Busuttil RW and Kupiec-Weglinski JW: HO-1-STAT3 axis in mouse liver ischemia/reperfusion injury: Regulation of TLR4 innate responses through PI3K/PTEN signaling. J Hepatol 56: 359-366, 2012.
83. Kim SY, Jeong E, Joung SM and Lee JY: PI3K/Akt contributes to increased expression of Toll-like receptor 4 in macrophages exposed to hypoxic stress. Biochem Biophys Res Commun 419: 466-471, 2012.
84. Tsukamoto K, Hazeki K, Hoshi M, Nigorikawa K, Inoue N, Sasaki T and Hazeki O: Critical roles of the p110 beta subtype of phosphoinositide 3-kinasein lipopolysaccharide-induced Akt activation and negative regulation of nitrite production in RAW 264.7 cells. J Immunol 180: 2054-2061, 2008.
85. Dong H, Zhang X, Dai X, Lu S, Gui B, Jin W, Zhang S, Zhang S and Qian Y: Lithium ameliorates lipopolysaccharide-induced microglial activation via inhibition of toll-like receptor 4 expression by activating the PI3K/Akt/FoxO1 pathway. J Neuroinflammation 11: 140, 2014.
86. Shi C, Wu F, Yew DT, Xu J and Zhu Y: Bilobalide prevents apoptosis through activation of the PI3K/Akt pathway in SH-SY5Y cells. Apoptosis 15: 715-727, 2010.
87. Sun H, Zhu X, Cai W and Qiu L: Hypaphorine attenuates lipopolysaccharide-induced endothelial inflammation via regulation of TLR4 and PPAR-γ dependent on PI3K/Akt/mTOR signal pathway. Int J Mol Sci 18: 844, 2017.
88. Zhaocheng J, Jinfeng L, Luchang Y, Yequan S, Feng L and Kai W: Ginkgolide A inhibits lipopolysaccharide-induced inflammatory response in human coronary artery endothelial cells via downregulation of TLR4-NF-κB signaling through PI3K/Akt pathway. Pharmazie 71: 588-591, 2016.