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Ethanol extracts of *Scutellaria baicalensis* protect against lipopolysaccharide-induced acute liver injury in mice

Hai Nguyen Thanh[1], Hue Pham Thi Minh[2], Tuan Anh Le[1], Huong Duong Thi Ly[1], Tung Nguyen Huu[1], Loi Vu Duc[1], Thu Dang Kim[1], Tung Bui Thanh[1*]

[1]*School of Medicine and Pharmacy, Vietnam National University, 144 Xuan Thuy, Cau Giay, Ha Noi, Viet Nam*

[2]*Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Ha Noi, Viet Nam*

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ABSTRACT

Objective: To investigated the protective potential of ethanol extracts of *Scutellaria baicalensis* (*S. baicalensis*) against lipopolysaccharide (LPS)-induced liver injury.

Methods: Dried roots of *S. baicalensis* were extracted with ethanol and concentrated to yield a dry residue. Mice were administered 200 mg/kg of the ethanol extracts orally once daily for one week. Animals were subsequently administered a single dose of LPS (5 mg/kg of body weight, intraperitoneal injection). Both protein and mRNA levels of cytokines, such as tumor necrosis factor alpha, interleukin-1β, and interleukin-6 in liver tissues were evaluated by ELISA assay and quantitative PCR. Cyclooxygenase-2, inducible nitric oxide synthase, and nuclear factor-κB protein levels in liver tissues were analyzed by western blotting.

Results: Liver injury induced by LPS significantly increased necrosis factor alpha, interleukin-1β, interleukin-6, cyclooxygenase-2, inducible nitric oxide synthase, and nuclear factor-κB in liver tissues. Treatment with ethanol extracts of *S. baicalensis* prevented all of these observed changes associated with LPS-induced injury in liver mice.

Conclusions: Our study showed that *S. baicalensis* is potentially protective against LPS-induced liver injury in mice.

1. Introduction

Liver diseases are a major problem throughout the world. Some toxins from environment can cause liver injury, but until now there is not any treatment for liver diseases which could resolve the problems caused by these toxins. Although there is an increasing demand of quality drug to protect the liver injury, there is no existence of a perfect candidate drug for liver protection. That is the reason why researchers are continuing to search in numerous plant medicines for reliable the treatment of liver diseases [1].

The liver is a main organ which realizes metabolic functions. When liver cells are attacked by substance, this can lead to liver inflammation. Lipopolysaccharide (LPS), a major constituent of the outer membrane of Gram-negative bacteria, may lead to hepatitis by increasing inflammation and chemotactic cytokine production and subsequent inflammatory cell sequestration in liver tissues. Injection of LPS into experimental animals has been a model for inducing a production of tumor necrosis factor-α (TNF-α) and other inflammatory mediators [interleukin (IL)-1β, IL-6, cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)].

Scutellaria baicalensis Georgi (*S. baicalensis*) is a perennial herb of Lamiaceae family, cultivated in China, Japan, Korea, and Vietnam. It is widely used in traditional oriental medicines. Its roots have been used for anti-inflammation, anticancer, antiviral of the respiratory and the gastrointestinal tract and antibacterial. Some flavonoids include baicalin, baicalein, wogonin, and wogonosid, which possess antioxidant and anti-inflammatory activity, were isolated from *S. baicalensis* [2,3]. However, the mechanism of this plant *in vivo* has not been investigated, showing the liver's protective efficacy. So, in this study, we aim to examine the effects of ethanol extract from *S. baicalensis* in a model of LPS-induced acute hepatic injury in mice.

*Corresponding author. School of Medicine and Pharmacy, Vietnam National University, Office 506, Building Y1, 144 Xuan Thuy, Cau Giay, Ha Noi, Viet Nam.
Tel: +84 4 85876172
Fax: +84 0437450188
E-mail: tungasia82@yahoo.es
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2. Materials and methods

2.1. Preparation of S. baicalensis extract

The root of *S. baicalensis* (3 kg) was purchased in July 2014 from a market in Ha Noi, Vietnam and identified by one of our members, Prof. Hai Nguyen Thanh (School of Medicine and Pharmacy, Vietnam National University, Hanoi). A voucher specimen (No. SMP-2014-0015) was deposited in the Herbarium of School of Medicine and Pharmacy, Vietnam National University. The root of *S. baicalensis* (3 kg) was extracted with ethanol (8 L × 3 times) for 24 h at room temperature on a platform shaker. After filtration, the combined ethanol extract was then concentrated to yield a dry residue (105 g) and the extract was stored at −20 °C.

2.2. Animals and feeding regimens

A total of 30 eight-week-old male C57BL/6J mice were used in our study. Animals were housed into enriched environmental conditions in groups of 8 animals per polycarbonate cage in a colony room under a 12 h light/dark cycle (12:00 AM–12:00 PM) under controlled temperature (22 ± 3) °C and humidity. All animals were maintained accordingly to a protocol approved by the Ethical Committee of the Vietnam National University and following the international rules for animal research. Just before starting, animals were randomly divided in three groups: Control, LPS and (LPS + Ex) groups. Animals received water *ad libitum* as vehicle and standard diet administration (AIN-93M). Group (LPS + Ex) mice were preadministered with 200 mg/kg/body weight of ethanolic extracts of *S. baicalensis* root orally by gastric tube, in the form of aqueous suspension once daily for 7 successive days. One hour after the last administration, LPS and (LPS + Ex) groups received LPS (5 mg/kg, *i.p.*). After 1 h, all mice were sacrificed. Liver tissues were dissected and frozen in liquid nitrogen, and stored in −80 °C until analysis.

2.3. Tissue homogenization

Frozen liver tissues were weighed and homogenized in ice-cold buffer (50 mmol/L Tris–HCl, pH 7.5, 8 mmol/L $MgCl_2$, 5 mmol/L ethylene glycol bis (2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 0.5 mmol/L ethylene diamine tetraacetic acid, 0.01 mg/mL leupeptin, 0.01 mg/mL pepstatin, 0.01 mg/mL aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride and 250 mmol/L NaCl). Homogenates were centrifuged (10 881 r/min, 15 min, 4 °C) and the supernatants were collected and stored at −80 °C. For liver nuclear extracts, liver tissue was washed with phosphate-buffered saline and homogenized, then centrifuged at 3 141 r/min for 10 min at 4 °C, and the supernatant was centrifuged at 8 310 r/min for 10 min. The pellet was washed and resuspended in the homogenization buffer. Liver nuclear extracts were stored at −80 °C for further analysis. Protein concentration was determined by Bradford's method.

2.4. Measurement of cytokines

Analysis of cytokine IL-1β, IL-6 and TNF-α were performed using a sandwich ELISA method. Briefly, 96-well plates were coated overnight at 4 °C with 100 μL of monoclonal antibody against IL-1β (2.0 μg/mL) or IL-6 (2.01 μg/mL) or TNF-α (1.0 μg/mL) in phosphate buffered saline (PBS 1×) buffer (pH 7.2). The plate was then washed four times with wash buffer (PBS 1× + 0.05% Tween-20), blotted dry, and then incubated with blocking solution (PBS 1× + 1% bovine serum albumin) for 1 h. The plate was then washed and 100 μL of each homogenate sample or standard was added. Then the plate was incubated at room temperature for 2 h, followed by washing, and addition of 100 μL of detection antibody IL-1β (0.5 μg/mL) or IL-6 (0.5 μg/mL) or TNF-α (0.25 μg/mL). The antibody was incubated at room temperature for 2 h. Following additional washing, 100 μL of avidin-horseradish peroxidase conjugated (1:2 000) was added to each well, followed by a 30 min incubation. After thorough washing, plate development was performed using 2,2′-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt liquid substrate solution. Then the plate was incubated at room temperature for color development and the color was monitored using a microplate reader at 405 nm with wavelength correction set at 650 nm. The standard curve for the ELISA was established by using murine standard IL-1β or IL-6 or TNF-α diluted in PBS 1× buffer. All standard curves obtained an r^2 value between 0.98 and 1. Results were normalized to total protein content in the liver samples, determined by Bradford's method. Data are reported as cytokine per milligram protein. All samples were run in triplicate.

2.5. Western blot analysis

Equal amounts of protein homogenates (50 μg) were separated on 10% acrilamide gel by sodium dodecyl sulfatepolyacryamide gel electrophoresis and transferred onto a nitrocellulose membrane. Then, membranes were blocked with 5% skim milk dissolved in 0.5 mmol/L Tris–HCl (pH 7.5), 150 mmol/L NaCl, and 0.1% Tween-20 for 1 h at room temperature. The membranes were subsequently incubated with the primary antibodies anti-COX-2, anti-iNOS, anti–NF–κB (Cell Signaling, USA). After three washes with Tris buffered saline with 0.1% Tween-20 (TBST), blots were incubated with secondary horseradish peroxidase conjugated goat anti-rabbit antibody (Calbiochem, Germany) in TBST with 5% skim milk at a 1:10 000 dilution for 1 h at room temperature. Membranes were then washed three times in TBST and developed using an

Table 1
qRT-PCR primer sequences used with SYBRGreen Master Mix.

Primers	Forward sequence (5′–3′)	Reverse
IL-1β	AGTTGACGGACCCCAAAAG	TTTGAAGCTGGATGCTCTCAT
TNF-α	CTGTAGCCCACGTCGTAGC	TTTGAGATCCATGCCGTTG
IL-6	TGATGGATGCTACCAAACTGG	TTCATGTACTCCAGGTAGCTATGG
β-Actin	TGACCGAGCGTGGCTACAG	GGGCAACATAGCACAGCTTCT

enhanced chemiluminescence detection substrate Immobilon TMWestern Chemiluminescent HRP sustratte (Merk Millipore, Vietnam). Protein levels were visualized by the ChemiDoc™ XRS + system and compiled with Image Lab™ 4.0.1 software (Bio-Rad Laboratories, USA) for quantification. To control equal loading, the membranes were analyzed for β-actin expression using an anti-β-actin antibody (Sigma–Aldrich, Singapore). All samples were run in triplicate.

2.6. Real-time PCR analysis

Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and treated with RNase-free DNase (Deoxyribonuclease 1 Amplification Grade, Sigma–Aldrich) to remove genomic DNA. Briefly, about 50 mg tissues were homogenized in 1 mL TRIzol and then extracted with chloroform by vortexing. A small volume (1.2 mL) of aqueous phase after chloroform extraction of the TRIzol homogenate was adjusted to 35% ethanol and loaded onto an RNeasy column. The column was washed and RNA was eluted. RNA quantity and purity was determined using the UV Spectrophotometer NanoDrop® ND-1000 (Thermo Scientific) and the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

For the synthesis of cDNA, DNase-treated total RNA (0.5 μg) was reverse transcribed using iScript™ cDNA Synthesis (Bio-Rad) kit. The reaction was realized in the iCycler Thermal Cycler (Bio-Rad), following the protocol: 5 min at 25 °C, 45 min at 42 °C, 5 min at 85 °C and 5 min at 4 °C. Real-time PCR primers were generated using Beacon Designer software (BioRad), purchased from Eurofins MWG Synthesis GmgH and listed as in Table 1.

Quantitative RT-PCR was conducted in a CFX Connect™ Real-Time PCR Detection System using 1 μL of cDNA mix, 500 nmol/L of sense and antisense primers and 5 μL of iTaq™ Universal SYBR® Green Supermix (2×) (BioRad) in 10 μL final volumes. PCR program began with 10 min of incubation at 95 °C. The reactions consisted of 45 cycles, using a denaturation temperature of 95 °C for 15 s and annealing and extension at 60 °C for 30 s and 72 °C for 30 s to determine the threshold cycle (Ct) value. A melt curve was performed for all reactions to check for product integrity and primer–dimer formation. Standard curves were generated for each gene of interest using dilutions of purified PCR products at known concentrations and all PCR primers had efficiencies of >95%. Quantitative PCR using primers for β-actin mRNA was conducted in each plate to provide a normalization reference. The Ct for all genes was normalized to the Ct of β-actine. Quantification of relative gene expression was calculated by the comparative Ct method ($2^{-\Delta\Delta Ct}$), and the data are presented as fold change over the control. All real-time RT-PCR were conducted at least three times from independent RNA preparations. Distillated H_2O served as a negative control. All samples were run in triplicate.

2.7. Statistical analysis

All results are expressed as mean ± SEM. Serial measurements were analyzed by using Two-way ANOVA with Tukey's post hoc test using SigmaStat 3.5 program and figures were performed by using SigmaPlot 10.0 program (Systat Software Inc). The critical significance level α was 0.050 and, then, statistical significance was defined as $P < 0.05$.

3. Results

3.1. Lipid peroxidation

Free radical attacks polyunsaturated fatty acids and leads to the formation of highly reactive electrophilic aldehydes, including malondialdehyde (MDA) and 4-hydroxy-2-nonenal

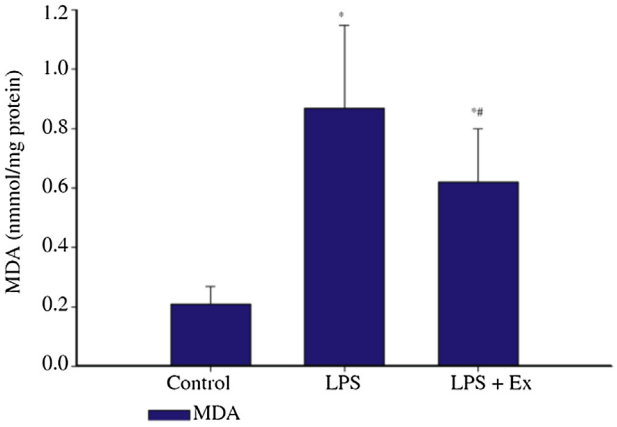

Figure 1. Effects of *S. baicalensis* extract on LPS-induced hepatic lipid peroxidation.
The bars represent the mean ± SEM ($n = 8$). *: Significantly different from control mice ($P < 0.05$); #: Significantly different from LPS-treated mice ($P < 0.05$).

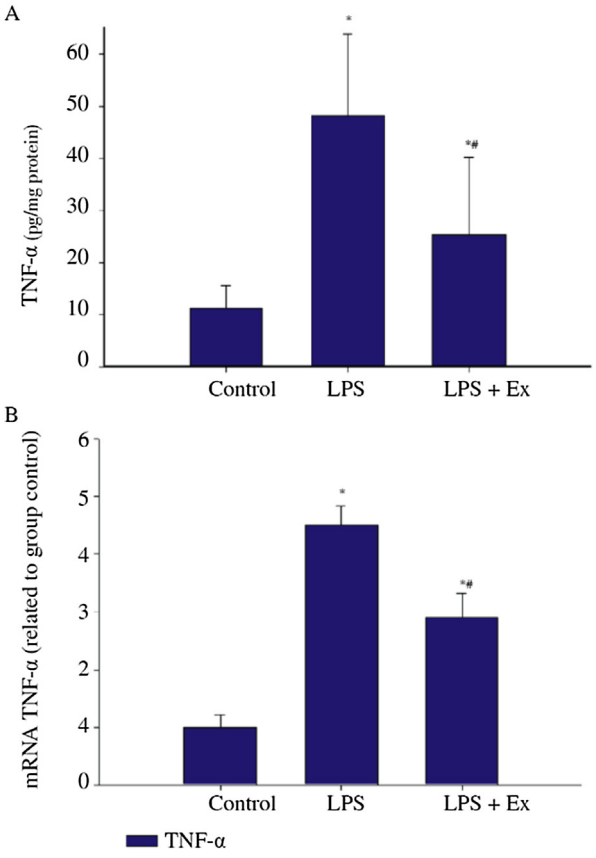

Figure 2. Effects of *S. baicalensis* extract on TNF-α in LPS-induced liver injury.
A: Protein level of TNF-α; B: mRNA of TNF-α. The bars represent the mean ± SEM ($n = 8$). *: Significantly different from control mice ($P < 0.05$); #: Significantly different from LPS-treated mice ($P < 0.05$).

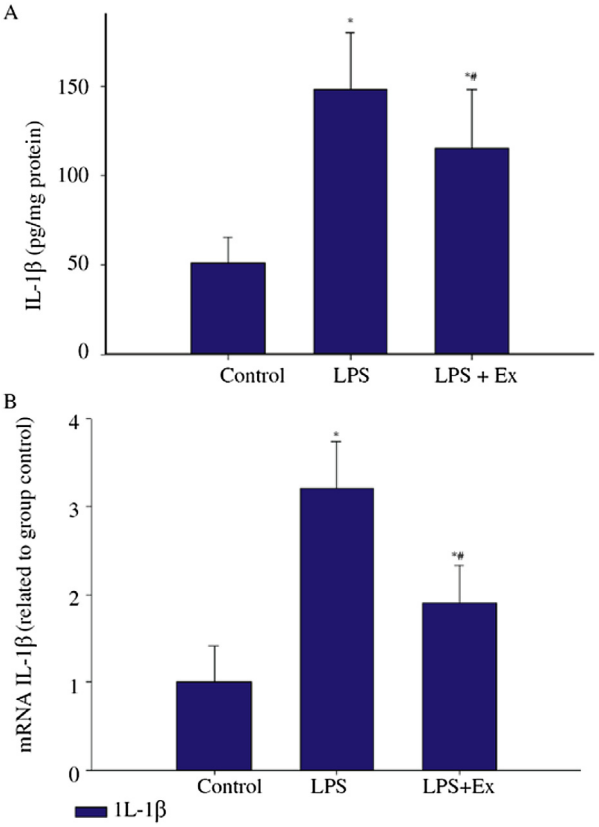

Figure 3. Effects of *S. baicalensis* extract on 1L-1β in LPS-induced liver injury.
A: Protein level of IL-1β; B: mRNA of IL-1β. The bars represent the mean ± SEM (n = 8). *: Significantly different from control mice ($P < 0.05$); #: Significantly different from LPS-treated mice ($P < 0.05$).

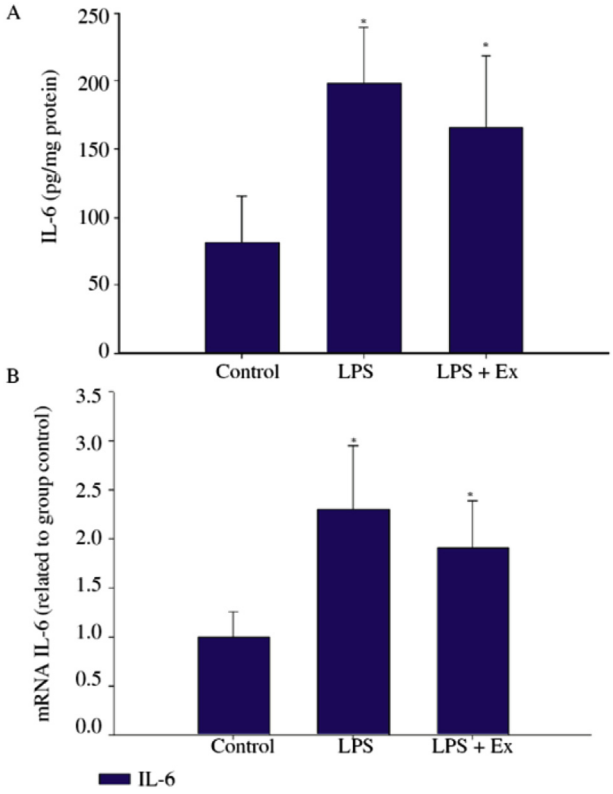

Figure 4. Effects of *S. baicalensis* extract on IL-6 in LPS-induced liver injury.
A: Protein level of IL-6; B: mRNA of IL-6. The bars represent the mean ± SEM (n = 8). *: Significantly different from control mice ($P < 0.05$); #: Significantly different from LPS-treated mice ($P < 0.05$).

[4]. MDA has been used frequently as a standard biomarker of lipid peroxidation *in vivo*. Figure 1 shows the amount of lipid peroxidation in the three groups of mice. There was a significant increase in the levels of MDA in LPS-treated mice. Treatment with extract significantly decreased the elevated levels of MDA in LPS-treated mice.

3.2. Effects of S. baicalensis extract on cytokine production

In order to determine if inflammation increased by LPS in mouse liver and the effect of *S. baicalensis* extract treatment we determined the levels of proinflammatory cytokines in whole homogenates.

Our data showed the levels of protein and mRNA of TNF-α increased significantly in the mouse liver by LPS when compared with the control group. Interestingly, *S. baicalensis* extract treatment significantly decreased the production of TNF-α on both levels of protein and mRNA ($P < 0.05$) as shown in Figure 2.

We found IL-1β at both levels of protein and mRNA also significantly increased in mouse liver induced by LPS as shown in Figure 3. Interestingly, *S. baicalensis* extract treatment induced decrease of this cytokine in mouse liver.

We also determined the level of IL-6 in homogenates liver. We found IL-6 at both levels of protein and mRNA also significantly increased in mouse liver induced by LPS as shown in Figure 4. However, we found *S. baicalensis* extract treatment tend to decrease both levels of protein and mRNA.

3.3. Effects of S. baicalensis extract on COX-2 and iNOS expression

The changes of the expression of iNOS and COX-2 by *S. baicalensis* extract in LPS treated mice were investigated. It has been well known that COX-2 is implicated in inflammation via production of various prostaglandins. We determined the changes of protein level of COX-2 induced by LPS with and without *S. baicalensis* extract treatment. The result was shown in Figure 5. We found that LPS increased significantly the level of protein expression of COX-2 as compared with the control group. Interestingly, the protein level expression of COX-2 was decreased significantly by the effect of *S. baicalensis* extract treatment.

Inducible nitric oxide synthase is a key enzyme responsible for the production of NO and it plays an important role in the oxidative stress and inflammation. Immunoblot analysis revealed LPS induced robust expression of iNOS after 1 h. This expression of iNOS was reduced in mice treated with *S. baicalensis* extract as shown in Figure 6.

3.4. Effects of SB extract on NF-κB expression

The transcription factors, NF-κB, regulates TNF-α, IL-1β, iNOS and COX-2 expressions. We found that LPS increased significantly the level of protein expression of NF-κB in nuclei as compared with the control group. Interestingly, we found that the protein level expression of NF-κB was decreased significantly by effect of *S. baicalensis* extract treatment as shown in Figure 7.

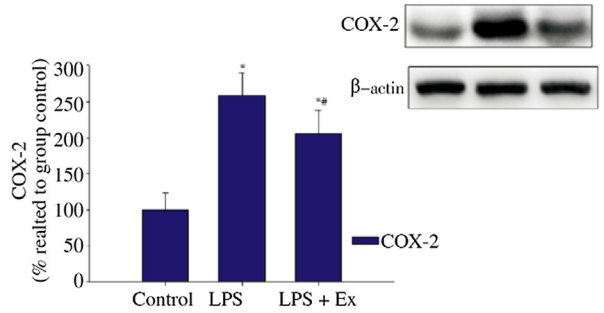

Figure 5. Effects of *S. baicalensis* extract on protein level of COX-2 in LPS-induced liver injury.
The bars represent the mean ± SEM (n = 8). *: Significantly different from control mice ($P < 0.05$); #: Significantly different from LPS-treated mice ($P < 0.05$).

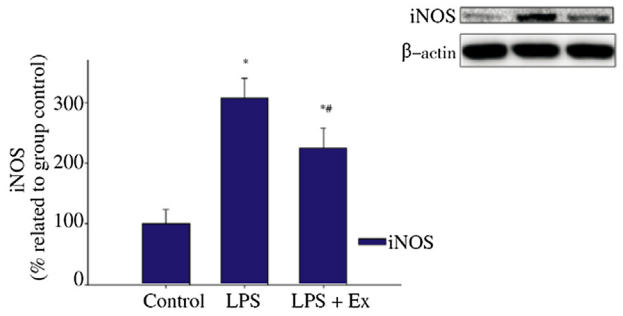

Figure 6. Effects of *S. baicalensis* extract on protein level of iNOS in LPS-induced liver injury.
The bars represent the mean ± SEM ($n = 8$). *: Significantly different from control mice ($P < 0.05$); #: Significantly different from LPS-treated mice ($P < 0.05$).

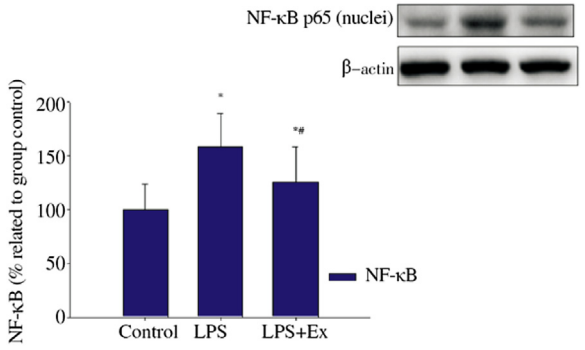

Figure 7. Effects of *S. baicalensis* extract on protein level of NF-κB in LPS-induced liver injury.
The bars represent the mean ± SEM ($n = 8$). *: Significantly different from control mice ($P < 0.05$); #: Significantly different from LPS-treated mice ($P < 0.05$).

4. Discussion

The liver is the main organ responsible for many critical functions within the body. When the liver becomes injured, the loss of those functions can cause significant damage to the body. Inflammation shows an important and complex feature in liver disease [5]. Continuing on our program for searching medicinal plant to prevent liver disease, we found that *S. baicalensis* is one of promising plants for preventing and treating liver inflammation. It is well known that *S. baicalensis* is an important herbal in traditional medicine for the treatment of hepatosis because of its high content of flavonoids. Extract of the dry root of *S. baicalensis* have been shown to contain high amount of flavonoids and phenolic compounds, mainly baicalin, baicalein, wogonosid, and wogonin [6]. However, the effects of *S. baicalensis* on proinflammatory mediators in liver had not been fully studied. In the present study, we investigated the anti-inflammatory effects of *S. baicalensis* using the mouse model induced by LPS.

LPS is a principal component of all Gram-negative bacteria. It is noted that LPS is as a major factor contributing to the pathogenesis of bacterial infections. LPS induces the activation and the transcription of receptor NF-κB and subsequently increases the production and release of some cytokines by various polymorphonuclear [7]. Also LPS is thought to activate reactive oxygen species (ROS) through NF-κB activation pathways. LPS can disturb the normal redox state of cells, leading to oxidative stress condition. Oxidative stress is produced when the process of ROS generation overcomes the cellular antioxidants. Free radical in oxidative tress state can damage all components of the cell, including proteins, lipids, and DNA. In the present study, we have assessed the liver damage by measurement of level of MDA, as an indicator of liver injury, and TNF-α, IL-6, IL-1β and COX-2, iNOS and NF-κB levels as indicators of inflammation.

MDA is formed when ROS attack polyunsaturated fatty acids, leading to cell membrane damage. Lipid peroxidation caused by LPS plays an important role in the production of septic shock [8]. The enhanced production of liver MDA observed in our experiments by LPS injection are in agreement with previous study which reported that LPS increased dose-dependently extracellular MDA level [7]. In addition, we have shown *S. baicalensis* could reduce the level of MDA in liver tissues. Our data are in line with study of Kong *et al.*, which have shown total flavonoids of *S. baicalensis* stem-leaf extract could decrease MDA content in the brain against cerebral ischemia/reperfusion injury in model of Sprague-Dawley rat [9]. Additionally, Sang *et al.* have reported that baicalin can reduce the levels of lipid peroxidation and serum aminotransferases in mice [10]. Therefore, our data suggested that root extract of *S. baicalensis* could protect hepatocytes from the oxidative damage caused by LPS. The protective mechanism of *S. baicalensis* may relate to its capacity to increase the antioxidant level in cells. In fact, Zhang *et al.* have shown that flavonoids from *S. baicalensis* could increase the content of superoxide dismutase, the main antioxidant enzyme that catalyzes the conversion of superoxide anion (O^{2-}) to H_2O_2 [11]. This mechanism needs to be studied in deep.

LPS is well known to provoke signaling cascade for inflammatory mediator expression such as TNF-α and IL-6, iNOS and COX-2, and NF-κB [12]. IL-1β, IL-6, and TNF-α are proinflammatory cytokines. TNF-α is one of the most important mediators of the host cell in response to LPS and Gram-negative bacteria. TNF-α is a pleiotropic cytokine with a lot of functions, which is produced by a variety of cell types, including activated macrophages and lymphocytes. It stimulates proliferation of normal cells, exerts cytolytic or cytostatic activity against tumor cells, and causes inflammatory, antiviral and immunoregulatory effects [13]. IL-1β, a pro-inflammatory cytokine, is released by activated macrophages and neutrophils and contributes to systemic inflammatory manifestations [14]. IL-6, a multifunctional cytokine, is considered as a B-cell differentiation factor that

regulates immune responses, hematopoiesis, acute phase response, and inflammation [15]. Increased IL-6 levels have been shown in several diseases, include rheumatoid arthritis, systemic-onset juvenile chronic arthritis, osteoporosis, psoriasis, polyclonal plasmacytosis, malignant plasmacytoma and Crohn's disease [16]. NF-κB have pivotal role in the regulation of TNF-α transcription stimulated by LPS. When cells were exposed to LPS, NF-κB is translocated to the nucleus and binds to NF-κB promoter sites on DNA and activates gene transcription of cytokines, including TNF-α, IL-1β, IL-6, inducing liver injury [17]. Thus, inhibitors of proinflammatory cytokines may be useful in the treatment of inflammatory diseases. In the present study, *S. baicalensis* exhibited down regulatory effects on LPS-induced TNF-α, IL-1β and IL-6 in mouse liver. Our results are in agreement with the study of Kim *et al.* which reported that *S. baicalensis* extract could attenuate the level of these cytokines in LPS-treated Raw 264.7 cells [18]. Also Kim *et al.* have shown that baicalin, an active component extracted from *S. baicalensis*, attenuates the serum alanine aminotransferase activity, TNF-α, and IL-6 levels in alcoholic fatty liver in rats [19]. Furthermore, Sang *et al.* have shown that baicalin protects hepatocytes from the oxidative damage caused by CCl_4 through increasing the hepatic glutathione content in mice liver [10]. So, we suggest that *S. baicalensis* could prevent increase of proinflammatory cytokines in different conditions that damage the liver.

COX-2 and iNOS are two enzymes involved in the inflammatory processes. COX-2, a key enzyme of prostanoid biosynthesis, is an inducible cyclooxygenase and its production is stimulated by LPS, proinflammatory cytokines and many other mediators. COX-2 plays a central role in modulating liver inflammation [20]. NO plays an important role in host immune defense, vascular regulation, neurotransmission, and other systems under normal conditions. It has been shown that LPS induced a significant increase in the levels of nitrate and nitrite in plasma [21]. iNOS, which produces large amounts of NO, is induced in macrophages and microglia in response to inflammatory mediators such as LPS and cytokines [22]. The overproduction of iNOS is especially related to various human diseases, such as Alzheimer's, Parkinson's, cardiovascular, autoimmune and chronically inflammatory diseases [23]. Our data have shown that the injection of LPS in mice increased the level of iNOS and COX-2, and those levels in animals treated with the extract could be decreased. Our results are consistent with similar studies reported by other investigators in different cell lines [24,25]. Zhang *et al.* have reported that *S. baicalensis* extract has been demonstrated to inhibit COX-2 activity and expression on head and neck squamous cell carcinoma [24]. They also pointed out that *S. baicalensis* inhibits phenyl glycidyl ether 2 synthesis via suppression of COX-2 expression, which could explain anticancer activity of *S. baicalensis* [24]. Additionally, Chen *et al.* have shown that three compounds-baicalin, baicalein, and wogonin isolated from *S. baicalensis* could inhibit iNOS and COX-2 gene expressions in Raw 264.7 macrophages on LPS-induced NO production in a concentration-dependent manner [25].

In normal condition, NF-κB stays in the cytoplasm as an inactivated dimer composed of p65 and p50 subunits. In response to inflammatory stimuli, IκB is phosphorylated and degraded, and NF-κB is released and translocated into the nucleus. NF-κB modulated many inflammatory genes, including iNOS, COX-2, and IL-1β, Il-6, TNF-α by binding to its specific promoter regions [26]. It is believed that the molecular mechanism of LPS-induced inflammation mainly involves the NF-κB signaling pathway, leading to acute or chronic inflammation and increase the production of proinflammatory cytokines. In the present study, we have demonstrated that the injection of LPS in mice increased the levels of NF-κB and mice fed with extract can inverse significantly this level to that in the control group. Our data are in agreement with previous studies which showed that baicalein, a major bioactive flavonoid component of dried roots of *S. baicalensis*, suppressed LPS-induced activation of NF-κB [27,28].

Our study has demonstrated the protective effects of the *S. baicalensis* extract on LPS-induced liver inflammation. The anti-inflammatory and hepatoprotective effect of *S. baicalensis* in our study may be mediated by the inhibition of cytokine, COX-2, iNOS and NF-κB. Our study suggests that the *S. baicalensis* root extract would be a promising therapeutic for liver disease.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

[1] Park J, Kim HY, Lee SM. Protective effects of Moutan Cortex Radicis against acute hepatotoxicity. *Afr J Tradit Complement Altern Med* 2011; **8**: 220-5.
[2] Long HL, Xu GY, Deng AJ, Li ZH, Ma L, Lu Y, et al. Two new flavonoids from the roots of *Scutellaria baicalensis*. *J Asian Nat Prod Res* 2015; http://dx.doi.org/10.1080/10286020.2014.999048.
[3] Gasiorowski K, Lamer-Zarawska E, Leszek J, Parvathaneni K, Yendluri BB, Błach-Olszewska Z, et al. Flavones from root of *Scutellaria baicalensis* Georgi: drugs of the future in neurodegeneration? *CNS Neurol Disord Drug Targets* 2011; **10**: 184-91.
[4] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* 2014; **2014**: 360438.
[5] Duval F, Moreno-Cuevas JE, González-Garza MT, Maldonado-Bernal C, Cruz-Vega DE. Liver fibrosis and mechanisms of the protective action of medicinal plants targeting inflammation and the immune response. *Int J Inflam* 2015; **2015**: 943497.
[6] Chan E, Liu XX, Guo DJ, Kwan YW, Leung GP, Lee SM, et al. Extract of *Scutellaria baicalensis* Georgi root exerts protection against myocardial ischemia-reperfusion injury in rats. *Am J Chin Med* 2011; **39**: 693-704.
[7] Sebai H, Ristorcelli E, Sbarra V, Hovsepian S, Fayet G, Aouani E, et al. Protective effect of resveratrol against LPS-induced extracellular ipoperoxidation in AR42J cells partly via a Myd88-dependent signaling pathway. *Arch Biochem Biophys* 2010; **495**: 56-61.
[8] Bhattacharyya J, Datta AG. Studies on the effects of lipopolysaccharide on lipid peroxidation of erythrocyte and its reversal by mannitol and glycerol. *J Physiol Pharmacol* 2001; **52**: 145-52.
[9] Kong X, Kong W, Miao G, Zhao S, Chen M, Zheng X, et al. Pretreatment with *Scutellaria baicalensis* stem-leaf total flavonoid protects against cerebral ischemia/reperfusion injury in hippocampal neurons. *Neural Regen Res* 2014; **9**: 2066-73.
[10] Park SW, Lee CH, Kim YS, Kang SS, Jeon SJ, Son KH, et al. Protective effect of baicalin against carbon tetrachloride-induced acute hepatic injury in mice. *J Pharmacol Sci* 2008; **106**: 136-43.

[11] Zhang Y, Wang X, Wang X, Xu Z, Liu Z, Ni Q, et al. Protective effect of flavonoids from *Scutellaria baicalensis* Georgi on cerebral ischemia injury. *J Ethnopharmacol* 2006; **108**: 355-60.
[12] Rossol M, Heine H, Meusch U, Quandt D, Klein C, Sweet MJ, et al. LPS-induced cytokine production in human monocytes and macrophages. *Crit Rev Immunol* 2011; **31**: 379-446.
[13] Tung BT, Rodríguez-Bies E, Talero E, Gamero-Estévez E, Motilva V, Navas P, et al. Anti-inflammatory effect of resveratrol in old mice liver. *Exp Gerontol* 2015; **64**: 1-7.
[14] Szabo G, Csak T. Inflammasomes in liver diseases. *J Hepatol* 2012; **57**: 642-54.
[15] Akira S, Hirano T, Taga T, Kishimoto T. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). *FASEB J* 1990; **4**: 2860-7.
[16] Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine Growth Factor Rev* 2002; **13**: 357-68.
[17] Lin M, Rippe RA, Niemelä O, Brittenham G, Tsukamoto H. Role of iron in NF-kappa B activation and cytokine gene expression by rat hepatic macrophages. *Am J Physiol* 1997; **272**: G1355-64.
[18] Kim EH, Shim B, Kang S, Jeong G, Lee JS, Yu YB, et al. Anti-inflammatory effects of *Scutellaria baicalensis* extract via suppression of immune modulators and MAP kinase signaling molecules. *J Ethnopharmacol* 2009; **126**: 320-31.
[19] Kim SJ, Lee SM. Effect of baicalin on toll-like receptor 4-mediated ischemia/reperfusion inflammatory responses in alcoholic fatty liver condition. *Toxicol Appl Pharmacol* 2012; **258**: 43-50.
[20] Cui L, Feng L, Zhang ZH, Jia XB. The anti-inflammation effect of baicalin on experimental colitis through inhibiting TLR4/NF-kappaB pathway activation. *Int Immunopharmacol* 2014; **23**: 294-303.
[21] Takano H, Yoshikawa T, Yamaguchi T, Hongo H, Fujii T, Boku Y, et al. Effects of aminoguanidine on systemic inflammatory response syndrome induced by platelet activating factor and by lipopolysaccharide in rats. *Eur J Pharmacol* 1997; **323**: 107-10.
[22] Sierra A, Navascués J, Cuadros MA, Calvente R, Martín-Oliva D, Ferrer-Martín RM, et al. Expression of inducible nitric oxide synthase (iNOS) in microglia of the developing quail retina. *PLoS One* 2014; **9**: e106048.
[23] Amin AR, Attur M, Abramson SB. Nitric oxide synthase and cyclooxygenases: distribution, regulation, and intervention in arthritis. *Curr Opin Rheumatol* 1999; **11**: 202-9.
[24] Zhang DY, Wu J, Ye F, Xue L, Jiang S, Yi J, et al. Inhibition of cancer cell proliferation and prostaglandin E2 synthesis by *Scutellaria baicalensis*. *Cancer Res* 2003; **63**: 4037-43.
[25] Chen YC, Shen SC, Chen LG, Lee TJ, Yang LL. Wogonin, baicalin, and baicalein inhibition of inducible nitric oxide synthase and cyclooxygenase-2 gene expressions induced by nitric oxide synthase inhibitors and lipopolysaccharide. *Biochem Pharmacol* 2001; **61**: 1417-27.
[26] Baima ET, Guzova JA, Mathialagan S, Nagiec EE, Hardy MM, Song LR, et al. Novel insights into the cellular mechanisms of the anti-inflammatory effects of NF-kappaB essential modulator binding domain peptides. *J Biol Chem* 2010; **285**: 13498-506.
[27] Cheng PY, Lee YM, Wu YS, Chang TW, Jin JS, Yen MH. Protective effect of baicalein against endotoxic shock in rats *in vivo* and *in vitro*. *Biochem Pharmacol* 2007; **73**: 793-804.
[28] Dong SJ, Zhong YQ, Lu WT, Li GH, Jiang HL, Mao B. Baicalin inhibits lipopolysaccharide-induced inflammation through signaling NF-kappaB pathway in HBE16 airway epithelial cells. *Inflammation* 2015; http://dx.doi.org/10.1007/s10753-015-0124-2.