HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.07.007

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*

ARTICLE INFO

Article history:
Received 20 Apr 2015
Received in revised form 8 May 2015
Accepted 10 Jun 2015
Available online 12 Aug 2015

Keywords:
Scutellaria baicalensis
Lipopolysaccharide
Liver injury
Cytokine
Nuclear factor-κB

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
Peer review under responsibility of Hainan Medical University.

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