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# Protective effects of tenuigenin on lipopolysaccharide and D-galactosamine-induced acute liver injury

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ABSTRACT

Tenuigenin (TEN), a major active component of *polygala tenuifolia* root, has been reported to have a number of biological properties, such as anti-oxidative and anti-inflammatory activities. However, the protective effect of TEN on acute liver injury has not yet been reported. This research aims to detect the protective effect of TEN on lipopolysaccharide (LPS) and D-galactosamine (D-GalN)-induced acute liver injury in mice and to investigate the molecular mechanisms. TEN was administered intraperitoneally 1 h before LPS/D-GalN treatment. The levels of TNF-α, IL-1β, ALT, and AST were measured. The expression of NF-κB, ASK1, MAPKs, Nrf2, and HO-1 were detected by western blot analysis. The results showed that TEN significantly inhibited LPS/D-GalN-induced serum ALT and AST levels. TEN also inhibited LPS/D-GalN-induced TNF-α and IL-1β production. Furthermore, LPS/D-GalN-induced hepatic MDA and MPO activities were also inhibited by TEN. In addition, TEN was found to inhibit LPS/D-GalN-induced ASK1 expression, NF-κB and MAPKs activation and up-regulate the expression of Nrf2 and HO-1. In conclusion, TEN protected against LPS/GalN-induced acute liver injury by suppressing inflammatory and oxidative responses.



## 1. Introduction

Fulminant hepatic failure, caused by virus infection, alcohol or drugs, is a dramatic clinical syndrome characterized by hepatic encephalopathy, severe coagulopathy, jaundice, and hydroperitoneum [1,2]. LPS, an important component of the outer membrane of gram-negative bacteria, has been reported to be an important risk factor in the initiation of endotoxic injury [3]. GalN is a hepatotoxic agent that leads to liver cells necrosis, which has the ability to amplify LPS-induced liver injury in a few hours [4,5]. The mouse model of LPS/GalN-induced acute liver injury is similar to clinical acute hepatic failure and has been widely used in understanding the pathogenesis of clinical hepatitis and developing an effective therapeutic strategy to clinical fulminant hepatic failure [6,7]. LPS can activate TLR4, which subsequently activates NF-κB and leads to the release of inflammatory cytokines such as TNF-α and IL-1β [8]. However, there are no effective preventives and therapies for the disease other than liver transplantation [9]. Therefore, the development of novel therapies for fulminant hepatic failure are imminently needed.

Tenuigenin, known as 'Yuan Zhi', is isolated from the dry roots of *Polygala tenuifolia*. TEN has been reported to have a number of biological properties, such as anti-oxidative and anti-inflammatory activities [10,11]. TEN was found to inhibit LPS-induced inflammatory cytokine production in macrophages. TEN also inhibited LPS-induced acute lung injury in mice. Furthermore, TEN has been reported to protect dopaminergic neurons form inflammation-mediated damage. However, there is little information about the effect of TEN on LPS/GalN-induced acute liver injury in mice. The purpose of this study is to investigate the anti-inflammatory effect and mechanism of TEN on LPS/GalN-induced acute liver injury in mice.

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## 2. Materials and methods

### 2.1. Animals

BALB/c mice, 6–8 weeks old, were provided by the Center of Experimental Animals of Harbin Medical University (Harbin, China). The mice were fed a standard diet and housed under a 12/12 h light/dark cycle at 24 ± 1 °C and 40–80% humidity for at least 3 days to adapt themselves to the environment prior to the experiments. All animal procedures were performed in accordance with NIH guidelines for the care and use of laboratory animals.

### 2.2. Reagents

TEN (purity>98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). LPS and GalN were obtained from the Sigma Chemical Co. (L-2880, St. Louis, MO, USA). MDA and MPO determination kits were purchased from the Jiancheng Bioengineering Institute of Nanjing (Nanjing, Jiangsu Province, China). All ELISA kits were purchased from Biolegend (CA, USA). Rabbit monoclonal antibodies NF-κB p65 (#8242), NF-κB p-p65 (#3033), *p*-IκBα (#9246), IκBα (#9242), NLRP3 (#15101), ASC (#67824), and caspase-1 (#2225) were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). The second antibody was provided by GE Healthcare (Buckinghamshire, UK). All other chemicals were of reagent grade.

### 2.3. Experiment model and experimental protocol

Fifty mice were randomly divided into 5 groups (n = 10/group), including blank control group, LPS/GalN group, LPS/GalN + TEN group (2, 4 and 8 mg/kg). TEN (2, 4 and 8 mg/kg) were administered intraperitoneally 1 h before LPS/GalN treatment. The doses of TEN used in this study were based on our preliminary experiments and a previous study [12]. The blank control group and LPS/GalN group were administered with an equal volume of phosphate buffered saline (PBS). 8 h after LPS/GalN challenge, the blood and liver tissues were collected for subsequent analysis.

### 2.4. Histopathological examination

To detect histopathological changes, liver tissues were collected and fixed in 10% formaldehyde solution. Then the tissues were dehydrated with graded alcohol and embedded in paraffin, immediately following staining with hematoxylin and eosin. Finally, the histopathological changes of the liver tissues were examined under a light microscopy. The extent of histological changes was scored according to previous study [13]. Scores of 1–3 were assigned to cases of minimal liver damage, scores of 4–8 to the mild, scores of 9–12 to moderate, and scores of 13–18 to severe cases.

### 2.5. Biochemical assays

Plasma samples were collected from the mice 8 h after the LPS/GalN injection. Serum levels of ALT and AST were detected by using an automatic biochemical analyzer (OLYMPUS2700, Japan). The levels of MPO and MDA in liver tissues were measured by using test kits (Jiancheng Bioengineering Institute of Nanjing) according to the manufacturer's protocols.

### 2.6. Analysis of cytokine levels

The liver tissues were weighed and homogenized with PBS (1:9, w/v). The supernatants were collected. The levels of TNF-α and IL-1β were detected by ELISA (Biolegend, USA) according to the manufacturer's instructions.

### 2.7. Western blot analysis

Total proteins from liver tissues were extracted by mammalian protein extraction reagent (Thermo, USA). The concentration of the protein was determined by BCA protein assay kit (Thermo, USA). The protein samples (30 mg) were separated through 12% SDS polyacrylamide gels, then transferred to a PVDF membrane. After blocking in 5% skim milk for 2 h at room temperature, the membrane was probed using antibodies against p65, p-p65, *p*-IκBα, IκBα, NLRP3, ASC, and caspase-1. Then the membrane was probed with peroxidase-conjugated secondary antibody (1:50000 dilutions in TBST) at room temperature for 2 h. Antibody-bound proteins were detected with the ECL Plus Western Blotting Detection System (GE Healthcare, Chalfont St Giles, UK) and subjected to densitometric scanning by using an image analyzer and Quantity One software (Bio-Rad).

### 2.8. Statistical analysis

All experimental data were presented as means ± S.E.M. Differences between the mean values of normally distributed data were assessed by one-way ANOVA followed by Tukey-kramer test. Values of $P < 0.05$ were considered to be statistically significant.

## 3. Results

### 3.1. Effects of TEN on LPS/GalN-induced liver histopathological changes

Histological changes in the liver were detected by H&E staining. As shown in Fig. 1, liver tissues of the control group exhibited an integral hepatic lobular architecture and normal hepatocytes. Liver tissues of LPS/D-GalN group showed disturbed architecture, including extensive hemorrhage, necrosis and neutrophil infiltration (Fig. 1B). However, LPS/D-GalN-induced liver histopathological changes were dose-dependently inhibited by treatment of TEN (Fig. 1C, D, E).

### 3.2. TEN inhibits LPS/GalN-induced serum ALT and AST levels

Serum ALT and AST are important markers of liver injury. In the present study, the levels of serum ALT and AST were measured. As shown in Fig. 2, the levels of serum ALT and AST of the control group were low. The levels of serum ALT and AST increased significantly after LPS/GalN treatment. However, treatment of TEN inhibited LPS/GalN-induced serum ALT and AST levels in a dose-dependent manner (Fig. 2).

### 3.3. Effects of TEN on LPS/GalN-induced MDA and MPO content

The effects of TEN on LPS/GalN-induced liver MDA and MPO content were measured in this study. As shown in Fig. 3, the contents of hepatic MDA and MPO of the control group were low. The levels of hepatic MDA and MPO increased significantly after LPS/GalN treatment. However, treatment of TEN inhibited LPS/GalN-induced hepatic MDA and MPO levels in a dose-dependent manner (Fig. 3).

### 3.4. TEN inhibits LPS/GalN-induced serum and hepatic TNF-α and IL-1β production

In this study, we investigated the effects of TEN on inflammatory