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Saikosaponin a ameliorates lipopolysaccharide and D-galactosamine-induced liver injury via activating LXRα

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ABSTRACT

Saikosaponin a (SSa), one of the major active components of *Bupleurum falcatum*, has antioxidant and anti-inflammatory pharmacological properties. However, the effects of SSa on liver injury have not been reported. In the present study, we evaluated the protective effects and mechanisms of SSa on lipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced liver injury. The mice were pretreated with SSa 1 h before LPS/D-GalN treatment. The liver MPO, MDA, and the serum AST and ALT levels were tested by specific determination kits. The pro-inflammatory cytokines TNF-α and IL-1β were tested by ELISA kits. The expression of NF-κB signaling pathway and LXRα were tested by western blot analysis. The results showed that SSa significantly reduced the levels of liver MPO, MDA, and serum AST, ALT levels induced by LPS/D-GalN. SSa also dose-dependently inhibited LPS/D-GalN-induced pro-inflammatory cytokines TNF-α and IL-1β production. Furthermore, we found that SSa inhibited NF-κB signaling pathway activation induced by LPS/D-GalN. In addition, SSa dose-dependently increased the expression of LXRα. In conclusion, the results demonstrated that SSa had protective effect on liver injury and the anti-inflammatory mechanisms of SSa on LPS/D-GalN-induced liver injury may be due to its ability to increase LXRα expression. SSa might be a potential treatment for liver injury.

1. Introduction

Liver disease is a serious disease which links to high mortality [1]. Liver injury is a life-threatening critical condition without effective treatment strategies [2–4]. There are multiple factors that lead to liver injury, which include dugs, hepatitis virus infections, alcohols, malperfusion, and toxins [5–7]. The mouse model of lipopolysaccharide (LPS) and D-galactosamine (D-GalN)-induced liver injury has been often used to investigate the pathogenesis of human liver injury and to find novel potential liver-protective agent [8–10].

Recently, a large amount of reports focused on the protective and therapeutic effects of natural herbal medicines for the treatment of inflammatory disease [11,12]. *Bupleurum falcatum*, a common Chinese herbal medicine, displays protective effects in the acute and chronic inflammation [13,14]. *Bupleurum falcatum* has been reported to have protective effects against liver injury. The extract of *Bupleurum falcatum* has been known to attenuate hepatic inflammation and fibrosis. Saikosaponin a (SSa), a triterpenoid saponin derived from *Bupleurum falcatum*, has a large number of pharmacological properties, including anti-cancer, anti-inflammation, and antioxidant effects [15–17]. Evidence suggested that SSa inhibited LPS-induced endometritis through regulating HO-1 and Nrf2 expression [12]. SSa protected agonist LPS-induced ALI through inhibiting NF-κB and NLRP3 inflammasome signaling pathways. In addition, SSa reduced TNF-α and IL-1β levels in septic mice induced by ligation and puncture. This protective effects mediated through inhibiting NOD2/NF-κB signaling pathway activation [18]. However, the effects of SSa on liver injury have not been reported. Thus, in the present study, we tested the protective effects and mechanisms of SSa on LPS/D-GalN-induced liver injury.

2. Materials and methods

2.1. Materials

Saikosaponin a (S8946), LPS (*Escherichia coli*, O55:B5), and D-GalN (G0500) were purchased from Sigma (St. Louis, MO, USA). ELISA kits for TNF-α and IL-1β were purchased from Biolegend (CA, USA). The MPO, MDA, AST, and ALT determination kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Antibodies specific for TLR4, NF-κB p65, p-NF-κB p65, IκBα, p-IκBα, and LXRα were purchased from Cell Signaling Technology Inc. (Beverly, MA). All other chemicals were of reagent grade.

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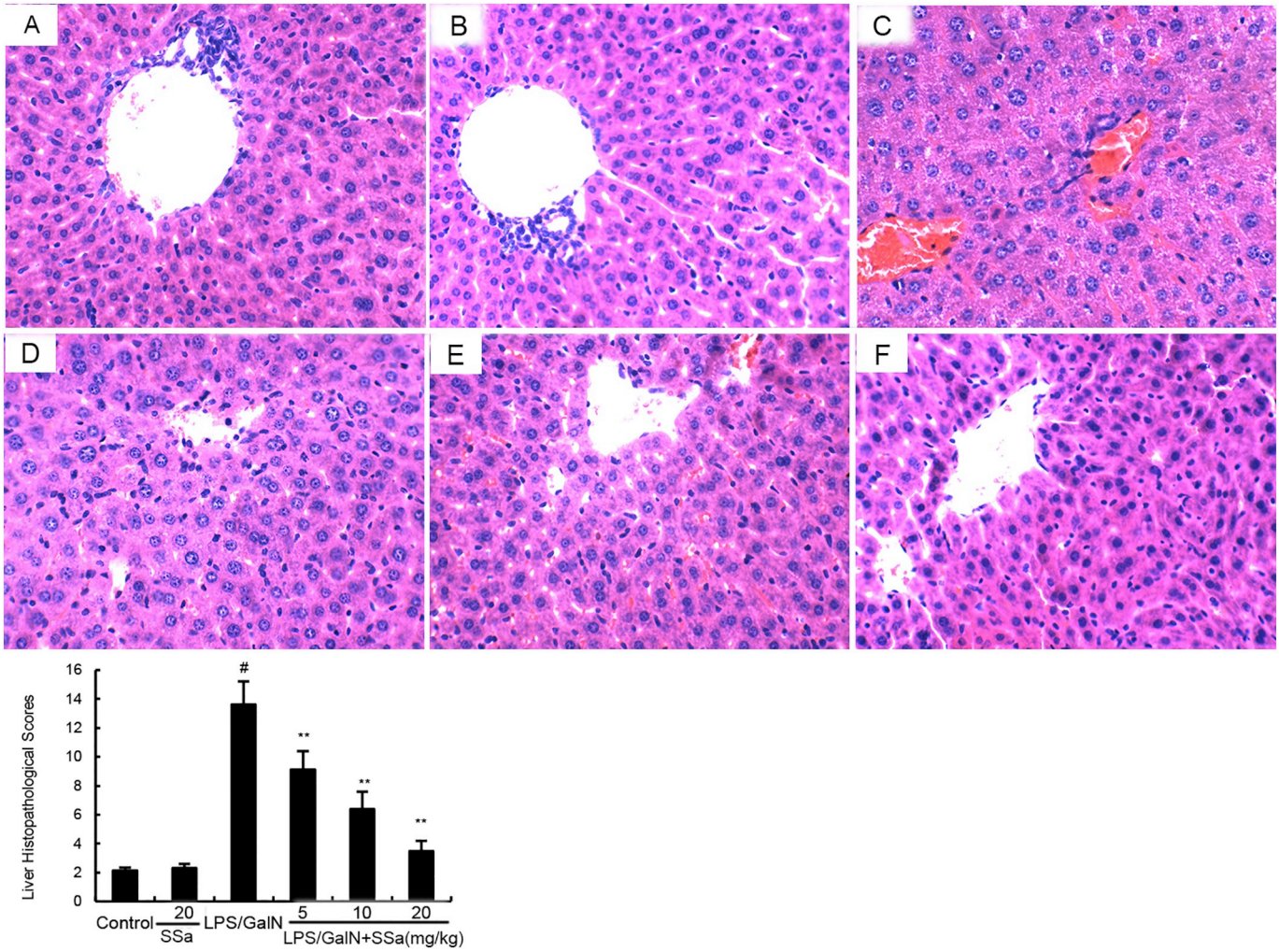

Fig. 1. Histopathologic sections of the livers (H&E, ×400). (A) Control group treated with PBS. (B) Group treated with LPS/D-gal. (C) Group pretreated with 5 mg/kg SSa 1 h before LPS/D-gal administration. (D) Group pretreated with 10 mg/kg SSa 1 h before LPS/D-gal administration. (E) Group pretreated with 20 mg/kg SSa 1 h before LPS/D-gal administration.

2.2. Animals

C57BL/6 mice, 20–25 g, were purchased from Experimental Animal Center of Zhejiang University. The mice were housed in microisolator cages under the controlled conditions (24 ± 1 °C, 12/12 h light/dark, and 40–80% humidity) and were given free access to food and water. The experiments were performed in accordance with the use and care of laboratory animal manual published by the US National Institutes of Health.

2.3. Animals treatment

Seventy-two mice were randomly divided into six groups (n = 12), including control group, SSa (20 mg/kg) group, LPS/D-GalN group, SSa (5, 10, and 20 mg/kg) + LPS/D-GalN groups. The mouse liver injury model was induced by injected intraperitoneally with LPS (60 mg/kg) and D-GalN (800 mg/kg) [19]. SSa (5, 10, and 20 mg/kg) were given intraperitoneally 1 h before LPS and D-GalN administration. The doses of SSa used in this study were based on previous study [12]. Eight hours later LPS/D-GalN challenge, the blood and liver tissues were collected for subsequent tests.

2.4. Histological analysis

H & E staining was used to test the pathological changes of liver tissues. The liver tissues were collected and fixed in 10% formalin for fixation. Then, the tissues were dehydrated, embedded, and cut into 4 μm sections. Finally, the sections were stained with hematoxylin and eosin (H & E) to evaluate the pathological changes of liver tissues under light microscope. Liver histological changes was scored as described previously [20]. 1–3: cases of minimal liver damage, 4–8: to the mild, 9–12: to moderate, 13–18: severe cases.

2.5. MPO, MDA, AST, and ALT levels analysis

The liver tissues were collected and homogenized with extraction buffer for MPO and MDA levels assess. The serum was collected used to assess the levels of AST and ALT. The levels of MPO, MDA, AST, and ALT were tested by specific determination kits (Jiancheng Bioengineering Institute of Nanjing) according to the manufacturer's instruction.

2.6. Pro-inflammatory cytokines analysis

TNF-α, and IL-1β levels in liver tissues and serum were tested by ELISA. The liver tissues were collected and homogenized with PBS at a ratio of 1:9 (w/v). The homogenates were centrifuged at 2000g for 40 min. The supernatants were collected and assessed the levels of TNF-α and IL-1β according the manufacturer's instructions. The gene expression of TNF-α and IL-1β were measured by qRT-PCR. Total RNA

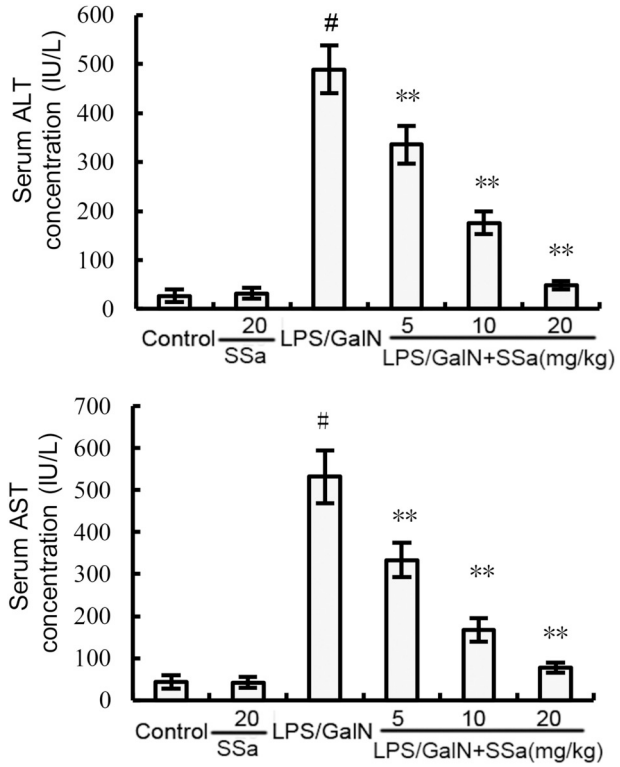

Fig. 2. Effects of SSa on MPO activity and MDA content in LPS/D-gal induced mice. Mice were given an intraperitoneal injection of SSa 1 h before LPS/D-gal administration. The values presented are the mean ± SD. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. LPS/D-gal group.

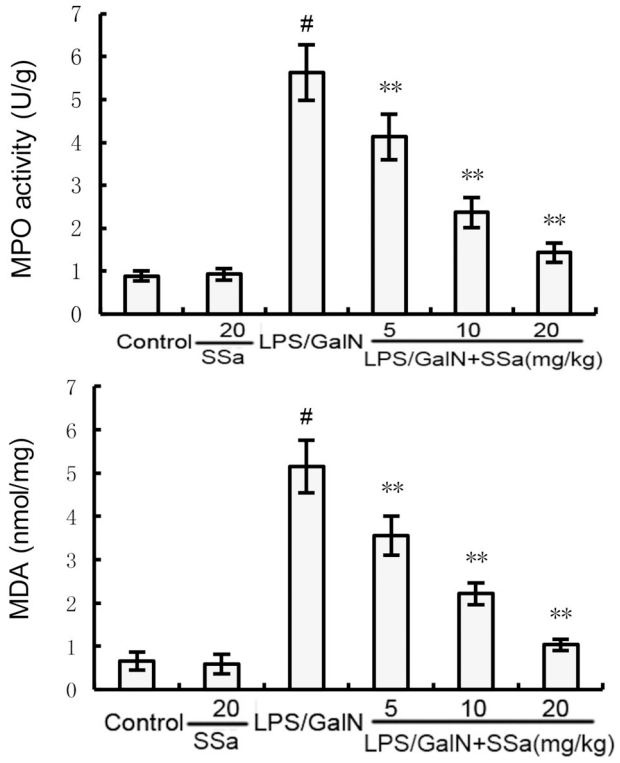

Fig. 3. Effects of SSa on ALT and AST activities in mice after LPS/D-gal treatment. The values presented are the mean ± SD. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. LPS/D-gal group.

from liver tissues was isolated using TRIzol (Sangong Biotech, Shanghai, China). Real time PCR was performed in a 7500 Fast Real-Time PCR System (Applied Biosystems). The primers used were the following: TNF-α: forward primer, CCTCCCTCTCATCAGTTCTA, reverse primer GGC AGC CTT GTC CCT TGA C, IL-1β: forward primer, TTC GAG GCA CAA GGC ACA ACA, reverse primer, AGG TCC TGG AAG GAG CAC TTC A; β-actin: forward primer, TAA AAC GCA GCT CAG TAA CAG TCG G, reverse primer TGC AAT CCT GTG GCA TCC ATG AAA C.

2.7. Western blot analysis

The liver tissues were collected and total proteins from liver were extracted with T-PER Protein Extraction Reagent (Thermo, USA). The protein content was assessed by the BCA assay kit. Equal amounts of protein (40 μg) were separated on 10% SDS-PAGE and electro-transferred to PVDF membranes. The membranes were blocked in 5% skimmed milk for 2 h and incubated with specific primary antibodies at 4 °C overnight. The membranes were washed with TBST for three times and incubated with secondary antibody at room temperature for 1 h. The membranes were washed for three times and were detected by chemiluminescence western blotting detection kit (Thermo, USA).

2.8. In vitro experiment

HepG2 cells were purchased from ATCC (Manassas, VA, USA) and cultured in DMEM-F12 containing 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2. The cells were pretreated with SSa (12 μM) 1 h before LPS treatment. 24 h later, the levels of TNF-α and IL-1β were measured by ELISA. For LXRα inhibition assay, the cells were pretreated with or without 20 μM GGPP for 2 h. Then, the cells were pretreated with SSa 1 h before LPS treatment.

2.9. Statistical analysis

All the data were analyzed by SPSS 21.0 software (IBM Corp, Armonk, NY, USA). Measurement data were expressed as the mean ± SD. Statistical significance was analyzed using one-way analysis of variance (ANOVA). p < .05 was considered statistically significantly.

3. Results

3.1. SSa inhibited LPS/D-GalN-induced liver pathological changes

To assess the protective effects of SSa on LPS/D-GalN-induced liver injury, the liver tissues were detected by H & E staining. As shown in Fig. 1, the liver tissues from the control group and SSa alone group did not show any pathological injury. However, the liver tissues from LPS/D-GalN group showed severe damages, including numbers of hepatocyte necrosis and a large number of inflammation cells infiltration. Whereas, these changes in LPS/D-GalN + SSa groups were less severe than in LPS/D-GalN group. These results represented that SSa was able to alleviate liver pathological injuries induced by LPS/D-GalN.

3.2. SSa inhibited LPS/D-GalN-induced liver MPO and MDA levels

MPO activity is an important index of neutrophil infiltration. MDA level reflects the levels of oxidative stress in the liver. The results showed that SSa alone did not affect the levels of MPO and MDA. MPO and MDA levels were significantly increased in the LPS/D-GalN group compared with the control group. However, treatment with SSa markedly inhibited MPO and MDA levels induced by LPS/D-GalN (Fig. 2).

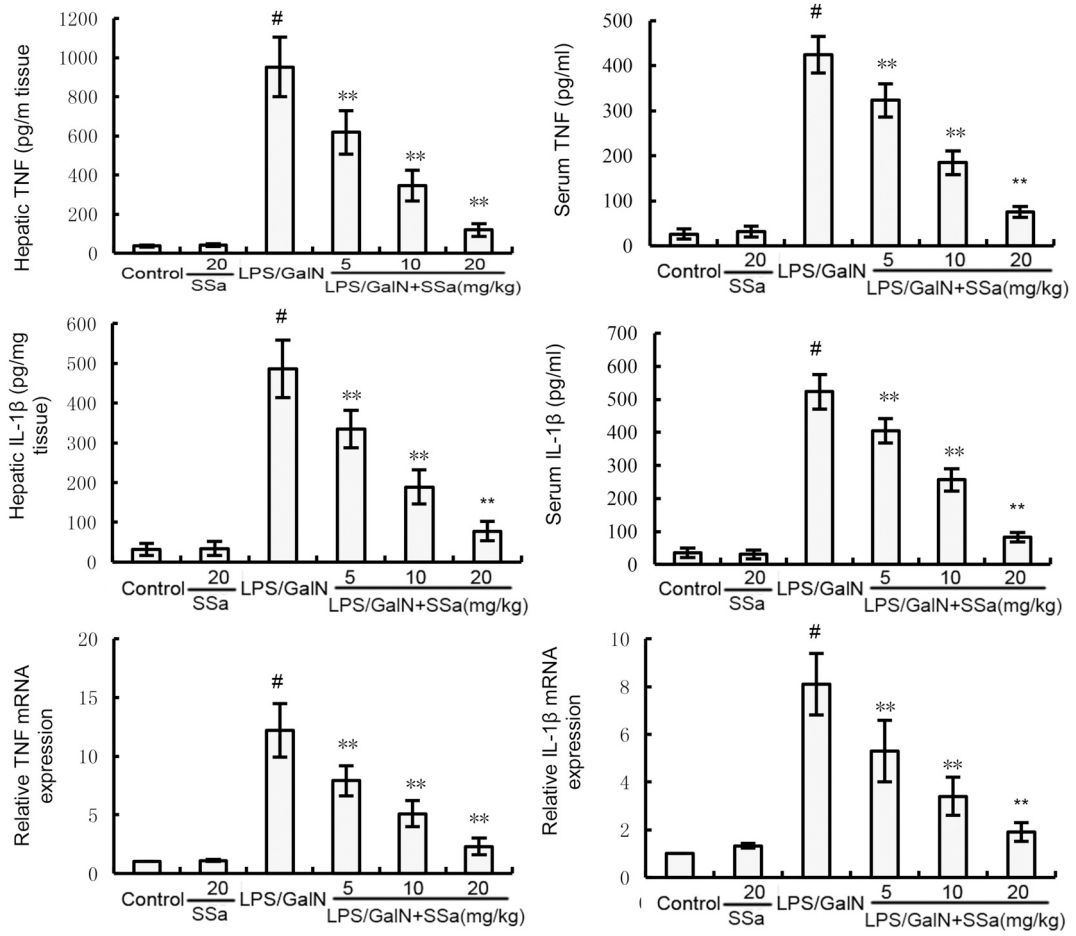

Fig. 4. Effects of SSa on hepatic TNF-α and IL-1β levels in LPS/D-gal induced mice. The values presented are the mean ± SD. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. LPS/D-gal group.

3.3. SSa inhibited LPS/D-GalN-induced serum AST and ALT levels

The serum AST and ALT levels were tested to assess the effects of SSa on liver injury. The results showed that SSa did not affect the levels of serum AST and ALT. Serum AST and ALT levels were significantly increased during liver injury induced by LPS/-D-GalN. However, the levels of AST and ALT were reduced by SSa in a dose-dependent manner (Fig. 3).

3.4. SSa inhibited LPS/D-GalN-induced TNF-α and IL-1β levels

Pro-inflammatory cytokines TNF-α, and IL-1β levels in the liver tissues and serum were tested in the present study. As shown in Fig. 4, the mRNA and protein levels of TNF-α, and IL-1β in the liver tissues and serum from the LPS/D-GalN group were higher than that from the control group. However, treatment of SSa inhibited LPS/D-GalN-induced TNF-α and IL-1β production, and in a dose dependent manner.

3.5. SSa inhibited LPS/D-GalN-induced TLR4 expression and NF-κB signaling pathway activation

Pro-inflammatory cytokines TNF-α, and IL-1β production are required for the activation of NF-κB signaling pathway. Thus, the effect of SSa on LPS/D-GalN induced NF-κB signaling pathway proteins expression were assessed in the present study. The results showed that the expression of phosphorylation of NF-κB p65 and phosphorylation of IκBα significantly increased after LPS/D-GalN treatment. However, treatment of SSa inhibited LPS/D-GalN-induced phosphorylation of NF-κB p65 and phosphorylation of IκBα (Fig.5). Meanwhile, the expression of TLR4 increased significantly after LPS/D-GalN treatment. However, treatment of SSa did not affect the expression of TLR4 induced by LPS/D-GalN treatment (Fig. 5).

3.6. SSa protected against liver injury by activating LXRα

LXRα is a member of the nuclear hormone receptor superfamily that participated in the regulation of metabolism of lipids and inflammatory response. In order to test the anti-inflammatory mechanism of SSa, the expression of LXRα in liver was detected in the present study. As shown in Fig. 6A, treatment of SSa dose-dependently increased the expression of LXRα in the liver. In vitro, our results showed SSa significantly inhibited LPS-induced NF-κB activation, TNF-α and IL-1β production in HepG2 cells. And the inhibitory effects were reversed by LXRα inhibitor GGPP. These results suggested SSa inhibited LPS-induced inflammation through activating LXRα (Fig. 6B).

4. Discussion

Liver injury is a life-threatening disease associated with a high rate of mortality [21,22]. LPS/D-GalN-induced liver injury mouse model was wildly used to explore the efficacy of liver-protective medicine [23,24]. Saikosaponin a (SSa), isolated from *Bupleurum falcatum*, has

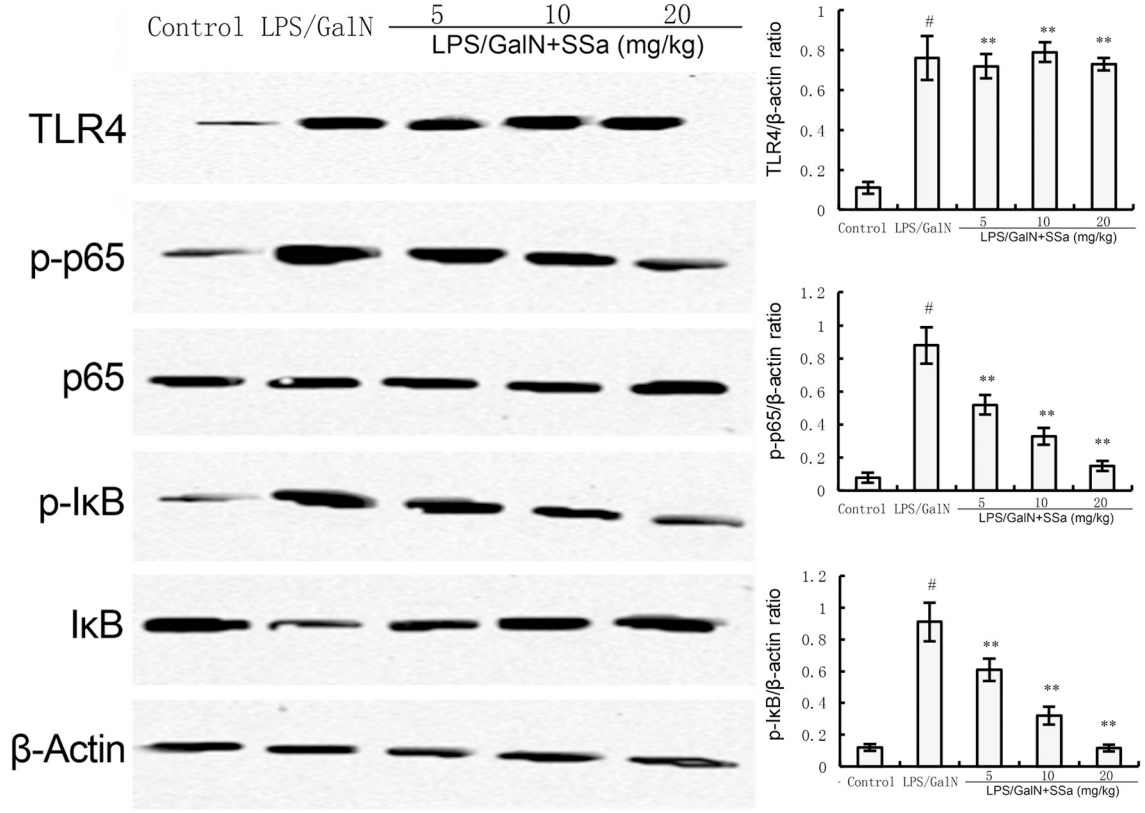

Fig. 5. Effects of SSa on LPS/D-gal induced NF-κB activation in mice. β-Actin was used as a control. The values presented are the mean ± SD. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. LPS/D-gal group.

been wildly used to treat inflammatory disease. In the present study, the protective effects and mechanisms of SSa on LPS/D-GalN-induced liver injury were assessed. The results showed that SSa relieved LPS/D-GalN-induced liver injury through activating LXRα.

MPO activity is an indicator of the amount of neutrophil. The increased MPO activity could lead to severe oxidative stress and oxidative tissue damages [9]. MDA is a marker of oxidative stress [25]. In this study, we found that SSa inhibited MPO and MDA levels induced by LPS/D-GalN. These data suggested that SSa protected against LPS/D-GalN-induced liver injury by inhibiting inflammatory cells infiltration and oxidative response. Serum ALT and AST are important biochemical indicators of liver injury [26,27]. In the present study, we tested the effects of SSa on the serum ALT and AST levels induced by LPS/D-GalN. The results showed that SSa reduced AST and ALT levels in serum induced by LPS/D-GalN. In addition, liver tissues pathological evaluation also proved that SSa had protective effects on LPS/D-GalN-induced liver injury.

The levels of pro-inflammatory cytokines are closely associated with the development of liver injury [28]. Over-production of TNF-α could lead to hepatocyte apoptosis and necrosis [29]. In addition, TNF-α can induce the inflammatory cascade and then to induce other cytokine production [30]. IL-1β is another pro-inflammatory cytokine which could stimulate the generation of several secondary cytokines. Pro-inflammatory cytokines production is regulated by NF-κB [31]. NF-κB is a nuclear transcription factor that plays an important role in the development of inflammatory disease [32]. Normally, NF-κB was located in the cytoplasm and bound to its inhibitory proteins IκBα. Once stimulated by agonists, IκBα was degradated and NF-κB p65 enter the nucleus and activated the expression of pro-inflammatory cytokines [33,34]. In the present study, we assessed the expression of NF-κB signaling pathway in liver tissues. The results showed that SSa reduced NF-κB signaling pathway activation on LPS/D-GalN-induced liver injury in a dose-dependent manner.

LXRα is a member of the nuclear hormone receptor superfamily that has been reported to regulate the inflammatory response [35,36]. T0901317, an LXRα agonist, treatment inhibited inflammatory response through suppressing NF-κB and MAPKs signaling pathways in ALI [37,38]. T0901317 protected against inflammation through activating LXRα. Because of activation of LXRα competed with IRF3 for GRIP binding, and inhibited NF-κB transcriptional activity in LPS-induced Kupffer cells [39]. GW3965, the other one LXR agonists, reduced IL-8 production correlates to the inhibition of NF-κB signaling pathway in LPC-stimulated endothelial cells [40]. In the present study, we found that SSa dose-dependently increased the expression of LXRα in LPS/D-GalN-induced liver injury. Furthermore, our results showed that the anti-inflammatory effects of SSa can be reversed by LXRα inhibitor GGPP. These data suggested that SSa inhibited LPS/D-GalN-induced inflammatory response in liver by activating LXRα.

In conclusion, we found that SSa inhibited LPS/D-GalN-induced liver injury through activating LXRα, which subsequently inhibited NF-κB signaling pathway and inflammatory response. These results indicated that SSa may provide a beneficial effect for preventing liver injury.

Author's contribution

Y. Z. and X. C. design the experiment; Y. Z., X. R., C.Z., and X. P. did the experiment; X.C. and C.Z. analyzed the data; Y.Z. writes the article.

Conflict of interest

All authors declare that they have no conflict of interest.

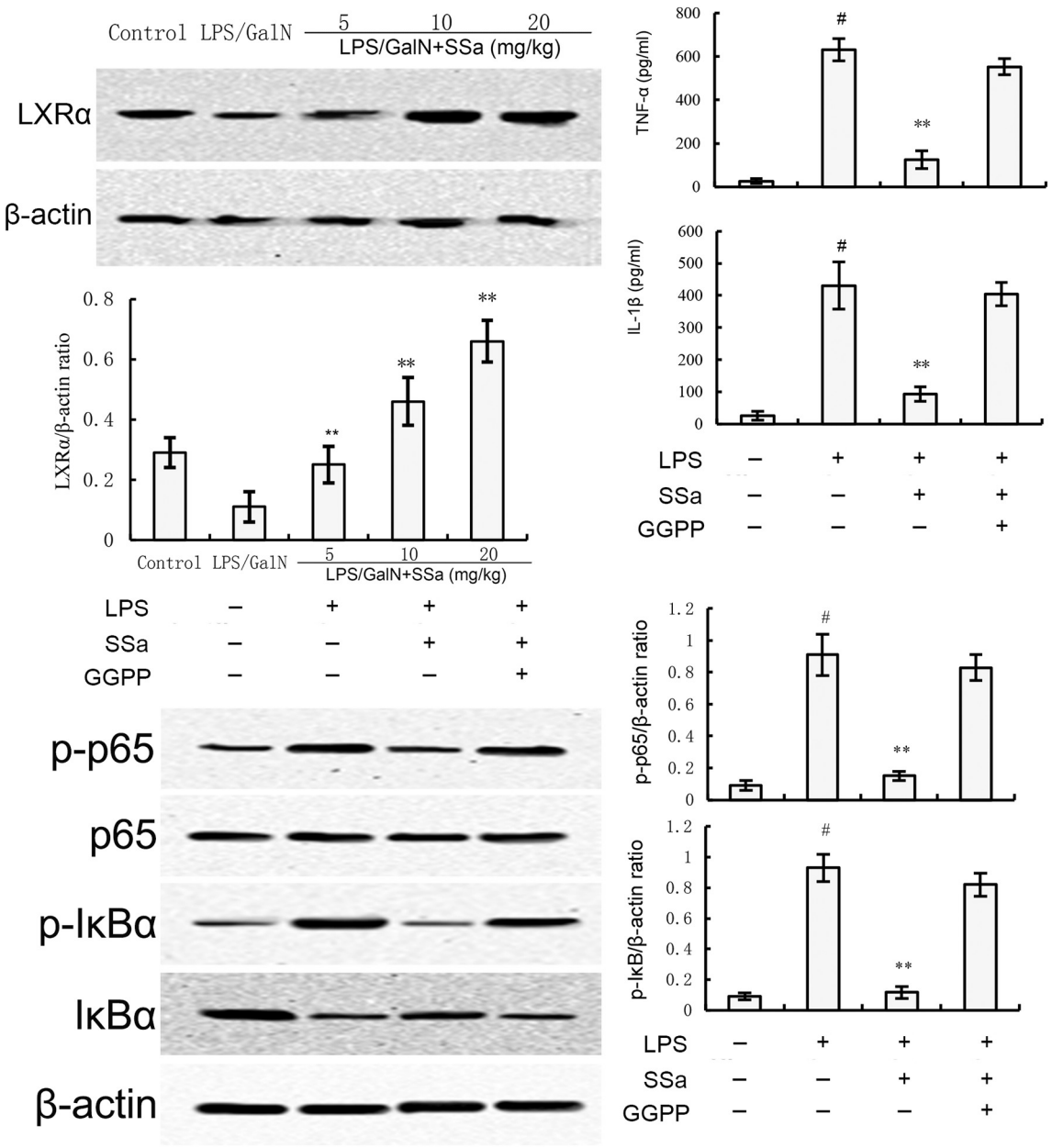

Fig. 6. (A) Effects of SSa on LXRα expression in mice. β-Actin was used as a control. The values presented are the mean ± SD. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. LPS/D-gal group. (B) Anti-inflammatory effects of SSa was through activating LXRα. The values presented are the mean ± SD. p# < 0.01 vs. control group, p* < 0.05, p** < 0.01 vs. LPS group.

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