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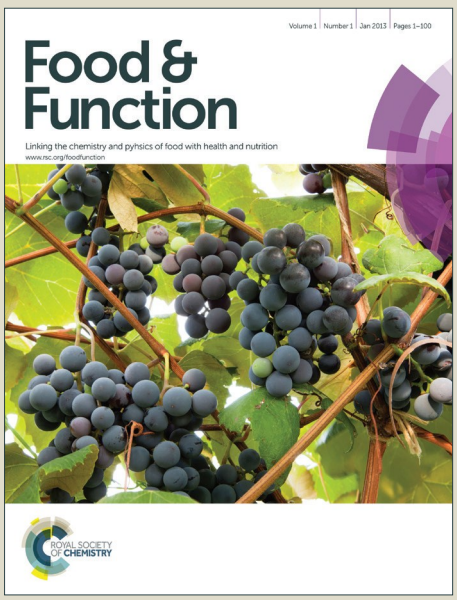


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# Protective effect of Ginsenoside Re on lipopolysaccharide-induced cardiac dysfunction in mice

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**Abstract:**

The impaired cardiac function caused by reduced myocardial contractility is a typical manifestation of sepsis/septic shock. Ginsenoside Re (GS-Re) is one of the most abundant ingredients of ginseng. This study was designed to investigate the protective effects of GS-Re on lipopolysaccharide (LPS)-induced septic cardiac dysfunction and inflammation response in mice. Mice were intragastrically administered with GS-Re (15 mg/kg) for 1 week before the LPS challenge (10 mg/kg, i.p.). Cardiac function was evaluated 6 h after LPS induction. GS-Re pretreatment significantly protected against LPS-induced cardiac dysfunction. GS-Re ameliorated the imbalance between iNOS and eNOS, prevented NF-κB activation and subsequent myocardial inflammatory responses in endotoxemic mice. The effects of GS-Re were closely associated with estrogen receptors (ERs), phosphatidlyinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling, and mitogen-activated protein kinase signaling pathway, as characterized by the GS-Re-induced preservation in ERα, ERβ, and phospho-Akt and inhibition in phospho-ERK1/2, phospho-JNK, phospho-P38. However, GS-Re had no effect on LPS-induced activation of TLR-4. All these results showed that GS-Re pretreatment significantly attenuated LPS-induced cardiac dysfunction and inflammatory response.

**KEYWORDS:** Ginsenoside Re, Cardiac dysfunction, Endotoxin, Estrogen receptor.

**Abbreviations:**

Akt, Protein kinase B; AST, Aspartate transaminase; CK, Creatine kinase; EF, Ejection fraction; ERK1/2, Extracellular signal-regulated kinase 1 and 2; ER, Estrogenic hormone; FS, Fractional shortening; GS-Re, Ginsenoside Re; IFN-γ, Interferon γ; I-kBα, Inhibitory-kBα; IL, Interleukin; iNOS, Inducible nitric oxide synthase; JNK, c-Jun N-terminal kinases; LDH, Lactate dehydrogenase; LPS, Lipopolysaccharide; LVIDs, Left ventricular internal diameter in systole; LVIDd, Left ventricular internal diameter in diastole; LVPWs, Left ventricular posterior wall thickness in systole; LVPWd, Left ventricular posterior wall thickness in diastole; LVVd, Left ventricular end-diastolic volume; LVVs, Left ventricular end-systolic volume; MAPK, Mitogen-activated protein kinase; MCP-1, membrane cofactor protein 1; NF-kB, Nuclear factor-kB; PI3K, Phosphatidlinositide 3-kinase; TLR, Toll-like receptor; TNF, Tumour necrosis factor.

## 1. Introduction

Severe sepsis and septic shock, also defined as a systemic inflammatory response to infection and progressive organ dysfunction, are among the most important causes of morbidity and mortality in hospitalized patients around the world [1]. Accumulating evidences suggest that the cardiovascular system is frequently affected by sepsis [2]. Cardiac dysfunction is a typical manifestation of sepsis/septic shock [3]. The prevention of cardiac dysfunction can significantly decrease the mortality of patients in sepsis/septic shock [1,2].

Ginseng (family Araliaceae) is a well-known medicinal plant that has been used in China for thousands of years to treat cancer, inflammation, stress, and diabetes [4]. Studies showed that the pharmacological and biological activities of ginseng are mainly attributed to ginsenosides, which are its most prominent and active components. As a major active ingredient, ginsenoside Re (GS-Re) possesses multifaceted pharmacological effects on the cardiovascular system [5]. GS-Re can alter cardiac electrophysiological properties, which may account for its antiarrhythmic effect [6]. Besides, GS-Re also exerts anti-ischemic effect and induces angiogenic regeneration [7].

Lipopolysaccharide (LPS) is a component of the outer membrane of mainly Gram-negative bacteria and the most important pathogen leading to sepsis development. Studies have demonstrated that LPS induced myocardial inflammation and dysfunction by interacting with its ligand TLR-4, thus triggering the activation of multiple signaling pathways, such as MAPKs family [8], NAD(P)H oxidase [9], and GSK3β [10]. Besides, LPS could induce an imbalance between eNOS and iNOS in the myocardium. This imbalance may be triggered by LPS challenge and/or proinflamantory cytokine overproduction [11]. LPS can also increase intracellular $Ca^{2+}$ concentrations [12]. All these signaling pathways can induce inflammatory response in cardiomyocyte [13]. LPS-induced inflammatory response serves an important function in the progression of cardiac dysfunction [2]. NF-κB is an important signal integrator that can be triggered by TLR-4 activation. NF-κB controls the production of many pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, monocyte chemotactic protein-1 (MCP-1), and the cytokine-inducible nitric oxide synthase (iNOS) [14,15]. NF-κB signaling pathway has an important function in myocardial dysfunction during sepsis. Inhibition of NF-κB can prevent LPS-induced cardiac dysfunction [16]. The activity of NF-κB can be regulated by phosphatidylinositide 3-kinase (PI3K)/protein kinase B (Akt) and mitogen-activated protein kinase (MAPK) [17,18].

Studies have shown that estrogen receptors (ERs) play an important role in endotoxin-induced cardiac dysfunction [19,20]. Clinical studies revealed that women have lower mortality rate and TNF-α level than men during sepsis [21]. ERs are important in limiting inflammatory response during sepsis/septic shock. GS-Re is a phytoestrogen and can exert pharmacological effects via regulating the activities of ERs [22]. GS-Re reportedly exhibits anti-inflammatory effects [23,24]. Lee found that GS-Re could inhibit the binding of LPS to TLR4 on macrophages [25]. However, whether GS-Re protects the heart during sepsis/septic shock has not been investigated yet. The aim of the present study was to evaluate the effects of GS-Re on cardiac dysfunction during sepsis/septic shock in mice.

## 2. Materials and methods

### 2.1. Chemicals and materials

GS-Re with more than 98% purity was purchased from Shanghai Winherb Medical S & T Development Co., Ltd. (Shanghai, China). LPS (*Escherichia coli* O111:B4) was purchased from Sigma (St. Louis, MO, USA). The kit for determining lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate aminotransferase (AST) were obtained from the Jiancheng Bioengineering Institute (Nanjing, China). Primary antibodies against TNF-α, IL-6, IL-1β, eNOS, iNOS, I-κB, p-p65, p-ERK, ERK, p-JNK, JNK, p-P38, P38, TLR4, p-AKT, AKT, ERα, ERβ, β-actin were from Santa Cruz Biotechnology (CA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were from CWbiotech (Beijing, China). All other chemicals were purchased from Sigma (St. Louis, MO, USA). The purity of all chemical reagents was at least analytical grade, and all were commercially available.

**2.2 Experimental animals**

Male C57BL/6 mice (18 g to 20 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice were acclimatized for 1 week before experiments. The environment was controlled at 24 ℃ to 25 ℃ room temperature, 55% humidity, and 12:12 h light: dark cycle. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Peking Union Medical College (Permit Number: #IMPLAD2012112207). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

**2.3 Experimental design**

A total of 80 mice were randomly divided into four groups. Cont group: mice were dosed intragastrically with distilled water. Re group (distilled water dissolved): mice were dosed intragastrically with 15mg/kg GS-Re for 7 days. LPS group (normal saline dissolved): mice were treated with LPS intraperitoneal at a dose of 10 mg/kg. LPS + Re group: mice were treated with GS-Re for 7 days. At 1 h after the last administration, LPS were intraperitoneally injected. At 6 h after LPS administration, heart tissues were fixed in 4% buffered paraformaldehyde for histology and immunohistochemistry or were frozen at -80 °C for protein analyses. Another experiment was

conducted, and the survival rate of mice was monitored once every 2 h for up to 24 h. In a separate experiment, mice were pretreated with a non-selective ER antagonist, ICI 182780 (ICI; 2 mg/kg body weight) 1 h before LPS administration.

**2.4 Echocardiographic measurements**

M-mode echocardiography was performed using Vevo 770™ High Resolution Imaging System (VisualSonics Inc., Canada). After treatment, the mice were anesthetized and their chests were shaved. The mice were then placed in recumbent position. Left ventricular (LV) internal diameter in systole (LVIDs) and diastole (LVIDd), and LV posterior wall thickness in systole (LVPWs) and diastole (LVPWd) were measured using M-mode echocardiography. LV end-diastolic volume (LVVd), LV end-systolic volume (LVVs), fractional shortening (FS), and ejection fraction (EF) were automatically calculated using an ultrasound machine.

**2.5 Measurement the activity of LDH, CK and AST**

After the completion of the echocardiography, blood samples were obtained from the inner canthus using a capillary tube under chloral hydrate anesthesia. The samples were centrifuged at 3000×g for 15 min within 1 h after collection. The activities of lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate transaminase (AST) in the plasma were measured with the corresponding detection kit according to the manufacturers' instructions (Nanjing Jiancheng Bioengineering, China).

**2.7 Histological and Immunohistochemical Analysis**

Heart tissues were fixed in 4% buffered paraformaldehyde, dehydrated in graded ethanol and then embedded in paraffin wax. The heart apex was sectioned, stained with hematoxylin and eosin (H&E), and then examined under a light microscope (CKX41, 170 Olympus, Tokyo, Japan).

For immunohistochemical analysis, slides were deparaffinized and hydrated. Endogenous peroxidases were blocked by hydrogen dioxide. Sections were incubated with goat anti-CD 68 monoclonal antibody and then stained using 3, 3'-diaminobenzidine kit. Finally, slides were

re-stained with hematoxylin and observed by light microscopy.

**2.8 Measurement the activity of TNF-α, IL-1β, IL-6, IFN-γ, MCP-1, and IL-10**

Heart tissues were mixed with saline at a ratio of 1:9 (mg/μL) to form a homogenate. After centrifugation at 7000 rpm for 5 min, the supernatant was used to measure the levels of TNF-α, IL-1β, IL-6, IFN-γ, MCP-1, and IL-10 by ELISA according to the manufacturer's instructions (R&D Systems, Wiesbaden, Germany).

**2.9 Western Blot Analysis**

Heart tissues were mixed with saline at a ratio of 1:9 (mg/μL) to form a homogenate. After centrifugation at 7000 rpm for 5 min, the precipitate was lysed on ice with tissue-protein extraction reagent containing 0.1 mM dithiothreitol and proteinase inhibitor cocktail. Protein concentration was determined using a BCA kit (Pierce Corporation, Rockford, USA). An equivalent amount of protein was added, and the mixture was loaded onto 12% SDS-polyacrylamide gels (Mini-PROTEAN II, Bio-Rad), separated, and transferred to nitrocellulose membranes. The membranes were blocked with 5% (w/v) non-fat milk powder in tris-buffer containing 0.05% (v/v) Tween-20 (TBST) for 2 h at room temperature. After overnight incubation with the appropriate primary antibodies at 4 °C, the membranes were washed thrice with TBST and incubated with secondary antibodies for 2 h at room temperature before re-washing thrice with TBST. The protein blots were developed using an enhanced chemiluminescence solution. Protein expression levels were visualized with Image Lab Software (Bio-Rad, USA).

**2.10 Statistical Analysis**

Results from at least three independent experiments were expressed as mean ± SE. Statistical comparisons between different groups were measured with Student's t-test or ANOVA using Prism 5.00 software. Statistical significance was considered at $p < 0.05$.

**3. Results**

### 3.1 Effects of GS-Re on survival rate of LPS-treated mice

We first evaluated the effects of GS-Re on sepsis-induced mortality. After LPS treatment, animal survival was monitored for up to 24 h. As shown in Figure 1B, mice began to die at 8 h after the LPS-treatment. However, mice in LPS+Re group exhibited significantly longer survival compared with the mice in LPS group. No mouse died within 6 h. Therefore, the mice treated with LPS for 6 h were used in the subsequent experiments.

### 3.2 Effects of GS-Re on heart function of LPS-treated mice

To investigate the effect of GS-Re on cardiac function of LPS-treated mice, M-mode echocardiography was used to measure cardiac parameters. Compared with control group, LPS administration significantly decreased ejection fraction (EF), fractional shortening (FS), left ventricular internal diameter at diastolic phase (LVDd), and left ventricular internal diameter at systolic phase (LVDs). GS-Re pretreatment attenuated LPS-induced cardiac dysfunction in mice (Figures 2A and 2B).

LDH, CK, and AST are three important indicators of cardiac injury. As shown in Figure 2C, LPS significantly increased the serum levels of LDH, CK, and AST, which was suppressed by pretreatment with GS-Re.

### 3.4 Effects of GS-Re on LPS-induced heart damage

H&E staining indicated that LPS administration significantly increased erythrocyte leakage and leukocyte infiltration into the cardiac interstitium (Figure 3A). Moreover, the number of CD 68-positive cells, representing monocyte/macrophage in an activated state, increased in the heart tissues after the LPS challenge (Figure 3B). GS-Re pretreatment obviously attenuated LPS-induced neutrophil/leukocyte infiltration.

### 3.5 Effects of GS-Re on inflammatory cytokine production in LPS-treated mice

The protein levels of TNF-α, IL-1β and IL-6 in cardiac tissues of mice significantly increased in the LPS group compared with the control group. This increase was significantly attenuated by

GS-Re pretreatment. No significant difference was found between saline- and GS-Re-treated mice (Figures 4A and 4B). ELISA was used to determine the levels of inflammatory cytokine in response to LPS stimulation. The levels of TNF-α, IL-1β, IL-6, IFN-γ, MCP-1, and IL-10 in cardiac tissues of LPS-treated mice increased significantly compared with those in the saline-treated controls. In contrast, GS-Re pretreatment significantly attenuated the increase of TNF-α, IL-1β, IL-6, INF-γ, and MCP-1 levels induced by LPS. However, the level of IL-10 increased further after GS-Re pretreatment (Figure 4C).

**3.6 Effects of GS-Re on LPS-induced imbalance between eNOS and iNOS in mice**

An imbalance between iNOS and eNOS serves an important function in myocardial dysfunction during sepsis. LPS administration significantly decreased the level of eNOS and increased the level of iNOS compared with the control group, which was significantly attenuated by GS-Re pretreatment (Figure 5A). No significant difference was found between the control group and the Re group.

**3.7 Effects of GS-Re on NF-κB signaling pathway**

NF-κB signaling pathway in myocardial tissue is activated after cardiac dysfunction during sepsis/septic shock. In our study, LPS administration significant increased IκB-α degradation and NF-κB p65 phosphorylation. By contrast, GS-Re pretreatment significantly attenuated IκB-α degradation and p65 phosphorylation as opposed to the LPS group (Figure 5B). No significant difference was found between the control group and Re group.

**3.8 Effects of GS-Re on MAPK signaling pathway**

MAPK signaling pathway has an important function in modulating the NF-κB signaling pathway. As shown in Figure 8, LPS stimulation significantly increased phosphorylation of JNK, ERK, and p38 MAPK in comparison to the control group. GS-Re pretreatment dramatically inhibited the phosphorylation of JNK, ERK, and p38 MAPK (Figure 6). These results demonstrated that GS-Re inhibited the activity of MAPK pathways in LPS-induced endotoxemia.

### 3.9 Effects of GS-Re on TLR4, ERs, and phospho-Akt

As shown in Figure 7, GS-Re significantly increased ERα and ERβ expression in the LPS+Re and the Re groups. The PI3K/AKT is a downstream signaling pathway of ERs that plays a key role in cardiac dysfunction during sepsis/septic shock. As shown in Figure 7, LPS administration significantly reduced the level of phospho-Akt compared with control group, which was preserved by GS-Re pretreatment. These results suggested that GS-Re may attenuate LPS-induced NF-κB activation and inflammation response partially through ERs and PI3K signaling. However, GS-Re had no obvious effect on the LPS-induced up-regulation of TLR-4 (Figure 7).

### 3.10 ERs inhibition abolished the protective effect of GS-Re on LPS-induced cardiac dysfunction

To assess further whether ERs were associated with the cardioprotective properties of GS-Re, we evaluated cardiac function in mice by echocardiography upon stimulation with LPS, GS-Re, or pharmacological inhibitor. Figures 8A show that non-selective (ICI 182780) ERs antagonist decreased EF, FS, LVDd, and LVDs in GS-Re and LPS co-treated mice. Importantly, this pharmacological inhibitor abolished the surpression of GS-Re on the production of proinflammatory cytokines in LPS-induced sepsis (Figures 8B).

## 4. Discussion

In the present study, GS-Re was proved for the first time to reduce LPS-induced cardiac dysfunction in mouse. We observed that GS-Re significantly decreased the serum levels of CK, LDH, and AST in LPS-treated mice and inhibited LPS-induced neutrophil/leukocyte infiltration into the myocardium. GS-Re ameliorated the imbalance between iNOS and eNOS, prevented NF-κB activation and the subsequent myocardial inflammatory. The mechanism underlying the cardioprotective effect of GS-Re may have depended on the inhibition of MAPK signaling pathway and activation of ERs and PI3K/Akt.

Cardiac dysfunction during sepsis/septic shock is always accompanied by neutrophil/leukocyte infiltration [1]. Our study showed that LPS administration significantly increased

neutrophil/leukocyte infiltration in the myocardium. By contrast, this pathological change was significantly inhibited by GS-Re pretreatment. In LPS-treated mice, we can see an elevation of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IFN-γ, and MCP-1 and anti-inflammatory cytokine IL-10. Pre-treatment with GS-Re decreased the level of TNF-α, IL-1β, IL-6, IFN-γ, and MCP-1, surprisingly, increased the level of IL-10. These pro-inflammatory cytokines are major triggers of cardiac dysfunction in endotoxin [1]. As a potent anti-inflammatory cytokine, IL-10 controls the degree and duration of the inflammatory response. An enhancement of IL-10 production is effective for the treatment of septic shock [26].

In our study, LPS significantly increased iNOS expression and reduced eNOS expression. Studies indicated that constitutively expressed eNOS has beneficial effects on myocardial function during sepsis. However, iNOS may induce deterioration of cardiac cells [27]. Interestingly, GS-Re pretreatment significantly inhibited the induction of iNOS and maintained normal eNOS levels following the LPS challenge. Our data suggested that a balance between eNOS and iNOS was important in the cardioprotective effect of GS-Re during endotoxemia.

NF-κB prominently regulates most inflammatory genes and controls the production of many pro-inflammatory cytokines [15]. Under normal conditions, NF-κB is sequestered in the cytoplasm by IκB-α. Following phosphorylation by IκB kinases, I-κBα was subsequently ubiquitinated and degraded. The degradation of I-κBα releases the NF-κB from IκB-α. NF-κB enters nuclear and binds to NF-κB promoter elements, thereby resulting in the activation of target genes expression [28]. Substantial evidence indicates that LPS induced myocardial inflammation and dysfunction by interacting with its ligand TLR4 on cardiomyocytes, thereby activating NF-κB signaling pathway [15]. LPS induced a significant degradation of I-κBα and activation of NF-κB in the heart, which was blocked by pretreatment with GS-Re. However, GS-Re pretreatment could not attenuate the protein level of TLR-4 after the LPS challenge. Hence, GS-Re-induced NF-κB inactivation and less inflammatory cytokine release may not be directly mediated by TLR-4.

MAPK family serves an important function in LPS-induced inflammatory response, thereby contributing to the development of septic cardiac dysfunction [29]. MAPK family can modulate the activity of NF-κB and the production of inflammatory factor. A previous study suggested that

the activation of ERK1/2, JNK, and p38 is required for LPS-induced TNFα expression [18]. Therefore, GS-Re may have inhibited inflammatory mediators by inactivating the MAPK pathway. Here, we demonstrated that GS-Re indeed decreased the levels of p-ERK1/2, p-JNK, and p-p38 in LPS-treated mice.

ERs are implicated in many pathophysiological processes and serve an important function in cellular survival by regulating the PI3K/Akt pathway [30]. The nuclear localization and activity of phospho-Akt in the myocardium is higher in females than in age-matched men [31]. ERα can inhibit cardiomyocyte apoptosis through the activation of Akt [32]. GS-Re could increase the expression of ERα and ERβ and the level of phospho-Akt in the myocardium, which may have negatively regulated the LPS-induced, NF-κB-dependent inflammatory responses. Importantly, these findings were supported by the pharmacologic inhibition of ERs (by ICI) resulting in the abolished protection of GS-Re against LPS-induced cardiac dysfunction as well as abrogated GS-Re-inhibited the level of proinflammatory cytokines following LPS challenge. The data suggests that GS-Re, as a novel phytoestrogen, promotes ERs expression via an unknown mechanism. MAPK signaling pathway can also be regulated by ER activation. 17β-Estradiol prevents smooth muscle cell proliferation and migration by inhibiting p38 MAPK activation, whereas it promotes these events in endothelial cells [33]. However, the specific contributions of ERα and ERβ on these events remain require further study.

Although GS-Re pretreatment significantly inhibited the LPS-induced cardiac dysfunction and inflammatory reactions in mice, the results cannot be translated to a septic patient admitted to a hospital primarily because the current study is that of prevention. In addition, the duration of the experiment was only 6 h, and GS-Re did not completely prevent the adverse events associated with endotoxemia. Thus, the therapeutic functions of GS-Re on sepsis-related mortality have yet to be determined in future studies. Besides, six metabolites of GS-Re are detected in rat feces after oral administration by HPLC-ESI-MS/MS analysis. Their structures are identified as 20(S)-ginsenoside Rg2, 20(S)-ginsenoside Rh1, 20(R)-ginsenoside Rh1, ginsenoside F1, 3-oxo-ginsenoside Rh1 and protopanaxatriol [34]. So, it is interesting to study the pharmacological action of metabolites of GS-Re.

## Conclusion

In summary, pretreatment with GS-Re attenuated LPS-induced myocardial inflammatory cytokines production, the imbalance between iNOS and eNOS, and NFκB activation, as well as improved myocardial dysfunction and reduced myocardial injury during endotoxemia. The mechanisms by which GS-Re attenuated cardiac dysfunction involve the inhibition of MAPK and preserved activation of ERs and the PI3K/Akt signaling pathway. However, more advanced research is necessary to further explore the mechanisms underlying GS-Re's protective effects against LPS-induced myocardial dysfunction.

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## Figure legends

**Figure 1** (A) Molecular structure of GS-Re. (B) Effects of GS-Re on mice survival rate.

**Figure 2** Effects of GS-Re on left ventricular functions in mice. (A) Representative images of M-mode echocardiogram. (B) Echocardiography values are expressed as mean ± SD. EF, ejection fraction; FS, fractional shortening; LVVd, left ventricular end-diastolic volume; LVVs, left ventricular end-systolic volume. (C) Effects of GS-Re on myocardial enzyme activities in LPS-treated mice. The results were expressed as the mean ± SD of three independent experiments. $^{*}$ indicates significant differences from the control ($P$ <0.05). $^{\#}$ indicates significant differences from treatment with LPS alone ($P$ <0.05).

**Figure 3** Effects of GS-Re on neutrophil/leukocyte infiltration. After LPS treatment, hearts were harvested and sectioned for HE counterstaining (A) or immunohistochemistry (B). Infiltrated leukocytes or CD68-positive cells were examined under a microscope. The results were expressed as the mean ± SD of three independent experiments. * indicates significant differences from the control ($P$ <0.05). # indicates significant differences from treatment with LPS alone ($P$ <0.05).

**Figure 4** Effects of GS-Re on the levels of inflammatory cytokines in LPS-treated mice. (A) Myocardial TNFα, IL-1β, and IL-6 expressions were assayed by Western blot analysis. (B) Quantification of protein expression. (C) The levels of TNF-α, IL-1β, IL-6, MCP-1, IFN-γ, and IL-10 in heart tissues of mice were measured by ELISA. The results were expressed as the mean ± SD of three independent experiments. * indicates significant differences from the control ($P$ <0.05). # indicates significant differences from treatment with LPS alone ($P$ <0.05).

**Figure 5** Effects of GS-Re on the levels of eNOS, iNOS and NF-κB activation. (A) Protein levels of eNOS and iNOS in the myocardium, as examined by Western blot analysis (B) Protein levels of IκB and p-P65 in the myocardium, as examined by Western blot analysis. The results were expressed as the mean ± SD of three independent experiments. * indicates significant differences from the control ($P$ <0.05). # indicates significant differences from treatment with LPS alone ($P$ <0.05).

**Figure 6** Effects of GS-Re on the expression of phosphorylation of ERK1/2, JNK, and P38. The Protein levels of phospho-ERK, phospho-JNK, and phospho-P38 in the myocardium were examined by Western blot analysis. The results were expressed as the mean ± SD of three independent experiments. * indicates significant differences from the control ($P$ <0.05). # indicates significant differences from treatment with LPS alone ($P$ <0.05).

**Figure 7** Effects of GS-Re on the expression of TLR-4, ERα, ERβ and phosphorylation of AKT. The protein levels of TLR-4, ERα, ERβ, and the phosphorylation of AKT in the myocardium were examined by Western blot analysis. The results were expressed as the mean ± SD of three independent experiments. * indicates significant differences from the control ($P$ <0.05). # indicates significant differences from treatment with LPS alone ($P$ <0.05).

**Figure 8** Effect of ERs inhibition on the protective effects of GS-Re against LPS-induced cardiac dysfunction. (A) EF, ejection fraction; FS, fractional shortening; LVVd, left ventricular end-diastolic volume; LVVs, left ventricular end-systolic volume were automatically calculated by the ultrasound machine. (B) The levels of TNF-α, IL-1β, IL-6, MCP-1, IFN-γ, and IL-10 in heart tissues of mice were measured by ELISA. The results were expressed as

the mean ± SD of three independent experiments. * indicates significant differences from the control ($P$ <0.05). # indicates significant differences from treatment with LPS alone ($P$ <0.05). $ indicates significant differences from GS-Re and LPS co-treatment ($P$ <0.05).

**Figure 1**

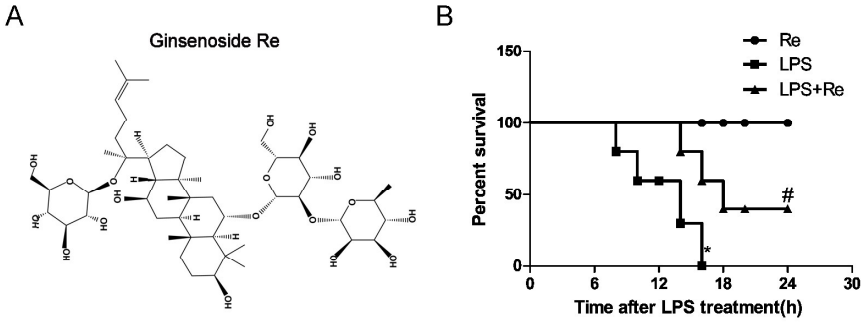
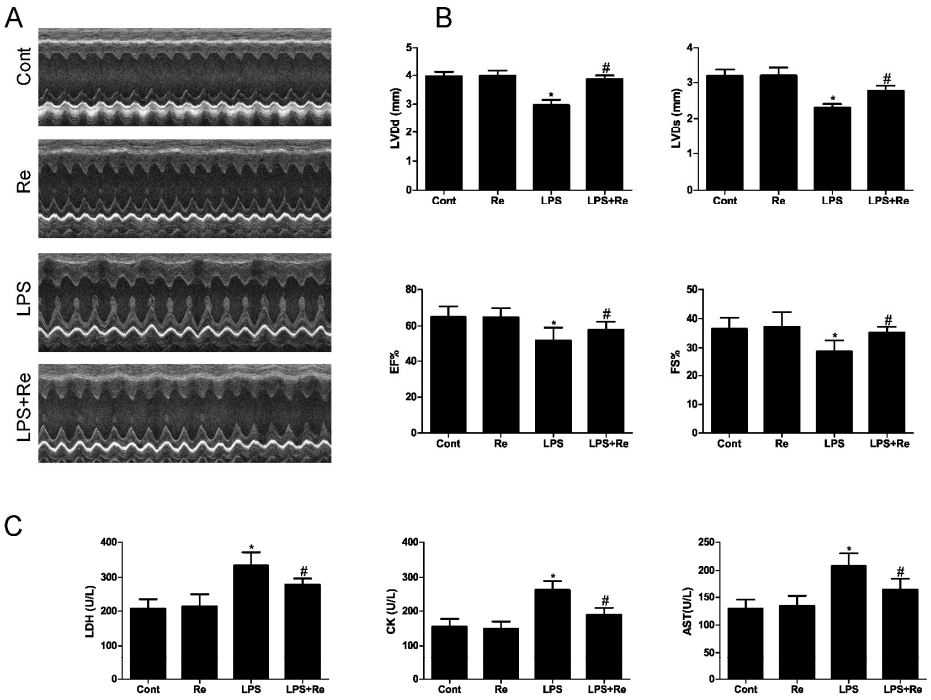



**Figure 2**

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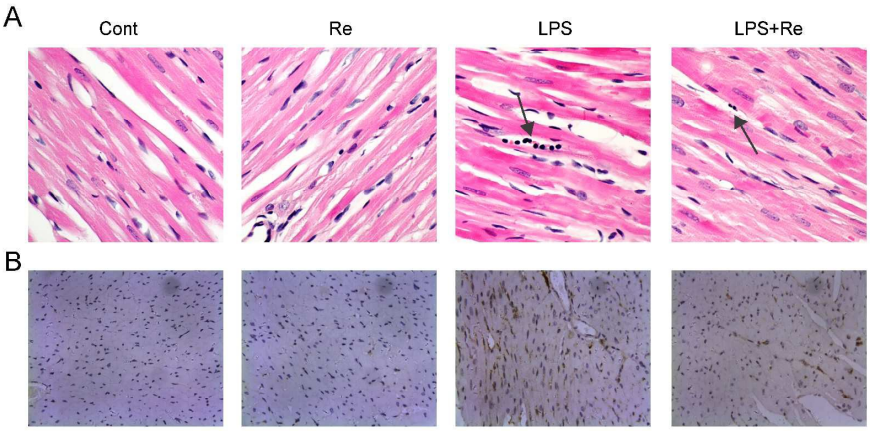


**Figure 4**

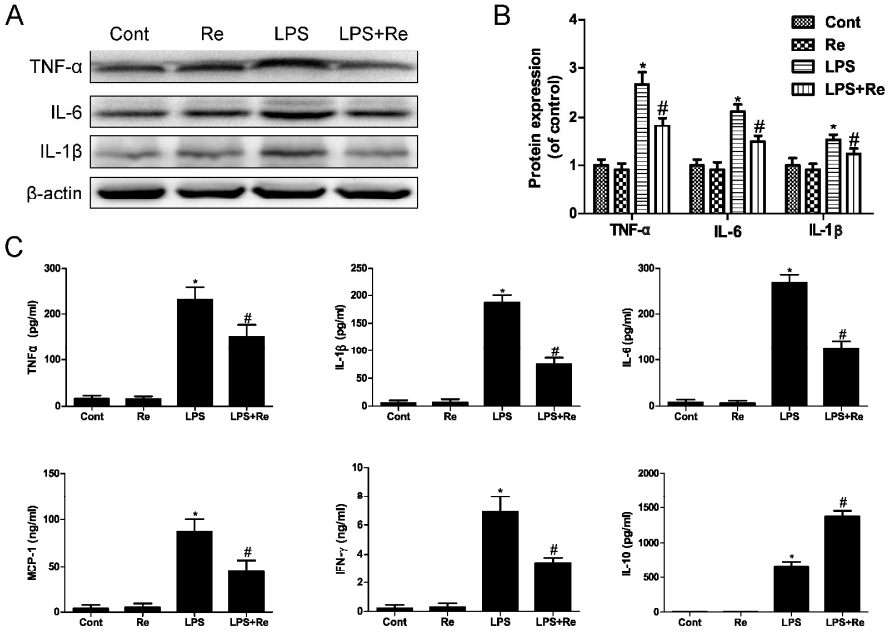



**Figure 5**

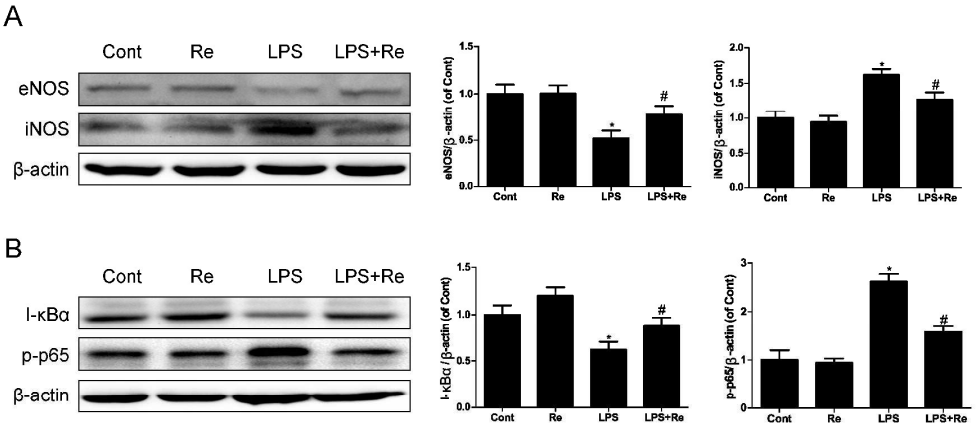


**Figure 6**

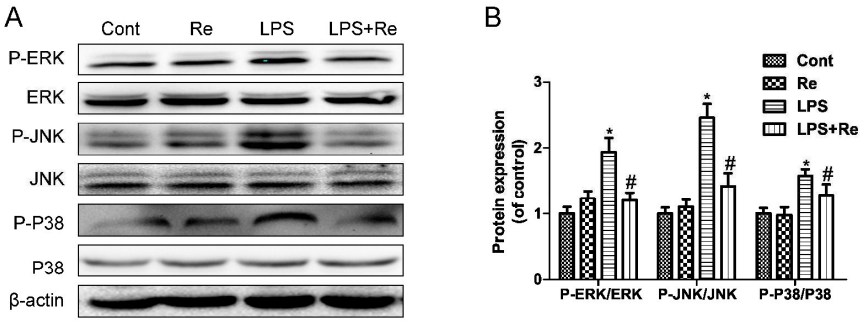



**Figure 7**

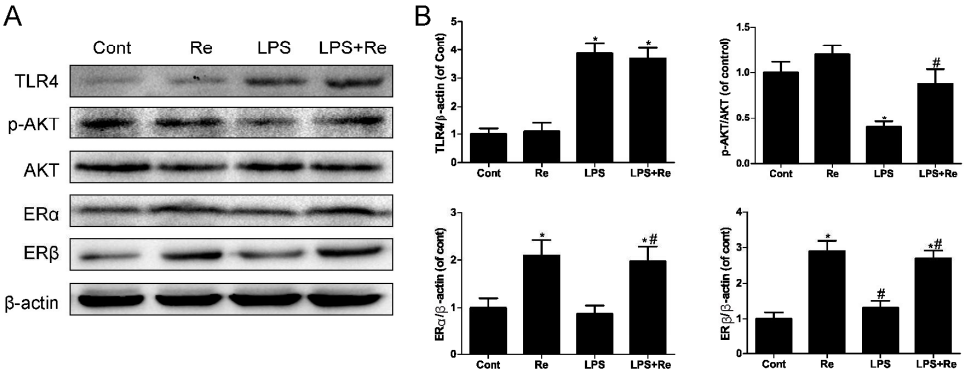
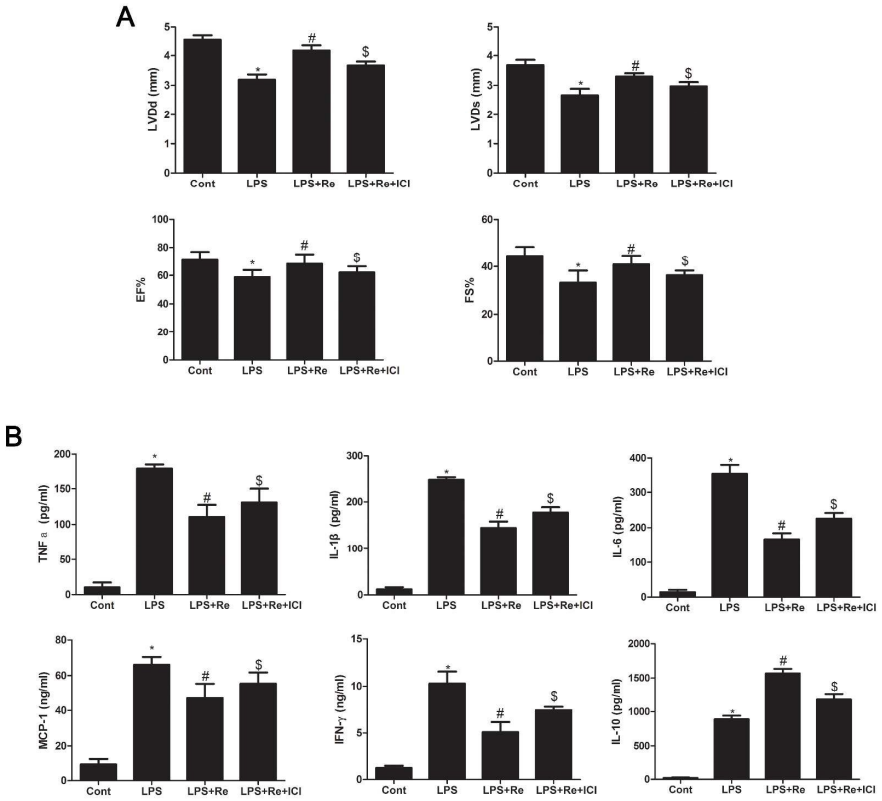



**Figure 8**

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**Ginsenoside Re protected against lipopolysaccharide-induced cardiac dysfunction in mice via ERs and PI3K/AKT mediated NFκB inhibition**