Isoacteoside Attenuates Septic Acute Lung Injury by Inhibiting Inflammation, Oxidative Stress and Endothelial Hyperpermeability in Mice

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Research Article

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Abstract

The present study was aimed to explore the protective role of isoacteoside (ISO) in cecal ligation and puncture (CLP)-induced acute lung injury (ALI) in mice. Mice were divided into the following groups: sham control group, ALI group, and ALI+ISO group, in which mice received 10,50 or 100 mg/kg/day of ISO for 3 days before, 0h and 12h after CLP surgery. In the first experiment, all mice were maintained until 72 h after the CLP operation to calculate the survival rate. In the second experiment, mouse serum and lung and bronchoalveolar lavage fluid (BALF) were collected 24 h after model establishment for detection. The results revealed that ISO significantly improved the ALI associated survival rate, reduced the pathological injury, ALI score, infiltration of inflammatory cells, leakage of cells and proteins into BALF, systemic and local cytokine secretion, and pulmonary oxidative stress. Moreover, ISO significantly inhibited the expression levels of the pro-inflammatory proteins TLR4, MyD88, p-NF-κB p65, p-IKKαβ, and p-IκBα and increased the expression levels of the endothelial permeability related proteins ZO-1, claudin 5 and VE-cadherin. In conclusions, ISO mitigated acute lung injury in mice which was attributed to the capacity of ISO to inhibit inflammation, oxidative stress and endothelial hyperpermeability.

Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, and it is a worldwide health-care issue[1, 2]. Sepsis is a systemic disease involving the lung, kidney, gut, heart and central nervous system. Among these organs, the lung is the most highly affected organ because of its special vascular and alveolar architecture[3]. Data showed that nearly 50% of patients with septic shock developed acute lung injury (ALI), and approximately 40% of ALI was caused by sepsis[4]. ALI is characterized by hypoxemia and acute respiratory distress syndrome (ARDS) with approximately 30%mortality and infiltration, and unfortunately, there is no specific treatment[5]. Despite various efforts to explore treatments for ALI, none of them have achieved the expected therapeutic effect.

The pathophysiology of ALI in sepsis is complicated and less well understood. Increased alveolar capillary membrane permeability, massive inflammatory cell infiltration, and protein-rich fluid accumulation in alveolar cavities which jeopardizes gaseous exchange, are the classical pathological features[6]. On a deeper level, an uncontrolled pulmonary inflammatory response and excessive oxidative stress can contribute to the pathological changes[7]. Lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, can initiate of inflammation in sepsis and ALI. Toll-like receptor 4 (TLR4), a transmembrane receptor that can respond to LPS, activates myeloid differentiation factor 88 (MyD88) associated pathways to trigger the nuclear translocation of nuclear factor-κB (NF-κB), which can initiate the infiltration of inflammatory cells and the secretion of cytokines such as TNF-α, IL-6 and IL-1β during sepsis-induced ALI[8]. Moreover, the role of damaged of the integrity of endothelial barriers in ALI has been well established, which is manifested by vascular malformations, hemorrhage, and tissue edema[9]. Protecting endothelial cell junctions might be another effective therapy for ALI.

Isoacteoside, a phenylethanoid glycoside, has been identified and isolated from a number of plant species including *Monochasma savatieri* Franch. ex Maxim., *Bauhinia tarapotensis*, and *clerodendron trichotomum* leaves. [10–12]. Cognitive dysfunction, ear edema, endotoxic shock, acute kidney injury, acute liver injury and ovarian cancer cell growth could be inhibited by ISO treatment in vivo and in vitro. The protective effects are associated with the powerful antioxidant, anticancer, and anti-inflammatory effects of ISO ,as revealed by the above studies[10, 13–15]. However, the effect and relative mechanism of ISO in sepsis-induced ALI is still unknown. Thus, this study was launched to investigate the effect of ISO on cecal ligation and puncture treated mice in the ALI process to evaluate ISO as a potential drug therapy.

Materials And Methods

Animals and drugs

Wild-type male C57BL/6 mice (4–6weeks, 20–25g, 4–5 per cage) were procured for this experiment from Medical Experiment Center of Lanzhou University. The mice were housed under standard laboratory environment (23℃, 50% humidity, 12h light daily) for 7 days before formal experiment. All the operation was conducted according to the guidelines of China council on animal care and use. ISO was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cecal ligation and puncture (CLP) surgery

Cecal ligation and puncture (CLP) surgery was used to mimic septic acute lung injury in this study. The mice were anesthetized through intraperitoneal injection with 75 mg/kg ketamine and 10 mg/kg xylazine. And then they were fixed, shaved and made the 2-cm abdominal median incision to expose the cecum. The cecum was punctured with a 21-gauge needle after ligation below the ileocecal valve and squeezed a little feces. Next, we put the punctured cecum back and closed abdomen. Mice were resuscitated with saline and put into the incubator for recovery[16].

Study design

Mice were divided into the following groups: (1) sham control group, which contained mice that received normal saline during the sham operation; (2) ALI group, containing mice that received normal saline during CLP surgery; (3) ALI + ISO group, in which mice received 10,50 or 100 mg/kg/day of ISO for 3 days (daily) before and 0h and 12h after CLP surgery. ISO was dissolved in sterile phosphate-buffered saline, and the mice were treated through intraperitoneal injection. In the first experiment, mice in all groups (n = 20 per group) were maintained until 72 h after surgery to detect mortality. In the second experiment, mouse blood, lung samples and bronchoalveolar lavage fluid (BALF) (n = 6 per group) were collected 24 h after the operation.

Pulmonary edema analysis

The severity of pulmonary edema was determined by lung wet-to-dry weight (W/D) ratio. After sacrifice, the left lobe was separated immediately and weighed (wet weight). Then this lobe was put into incubator for dry and weighed (dry weight). Lung W/D ratio = wet weight/dry weight.

Histopathology examination

The collected lung samples were inflated with room air and fixed in formalin, embedded in paraffin, cut into 5-μm thickness of slice, stained with hematoxylin and eosin (H&E) and assessed by two experts blindly. A modified ALI score system (ranging from 0 to 12) was used to determine the histopathology injury[17].

Arterial blood gas analysis

Blood samples from all group mice were collected from the abdominal aorta and determined by using a pH/blood gas analyzer.

Enzyme-linked immunosorbent assays (ELISA)

Serum and BALF cytokines were detected by ELISA. For BALF collecting, mice were undergoing tracheotomy, ligated the left principal bronchus, lavaged by 1ml saline in the right lung for three times, and finally obtained about 0.8 ml BALF[18]. The serum and BALF TNF-α, IL-6, IL-1β, IL-2, CXCL1, CCL4 and CCL3 levels were measured by using relative ELISA kits (Beyotime Biotechnology, Beijing, China).

2.8 Oxidative stress analysis

The lung tissue and BALF myeloperoxidase (MPO), malonaldehyde (MDA), 8-oxo-deoxyguanosine (8-OHdG), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GSH-px) levels were measured by using the commercial kits from Nanjing JianCheng Bioengineering Institute.

Calculation of the proteins and cells in BALF.

The cell pellets from BALF (centrifuged at 4°C, 3000 rpm, 10 min) were resuspended for cell counting using a hemocytometer. Macrophages and neutrophils were counted by Wright-Giemsa staining (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) under a microscope. Then, the BALF was centrifuged to collect the supernatant for ELISA detection of total protein and albumin levels (Beyotime Biotechnology, Beijing, China).

Quantitative reverse transcription–polymerase chain reaction (qRT–PCR) analysis.

RNAfast200 Kits (Fastagen Biotech, Shanghai, China) were used to isolate the pulmonary total RNA. PrimeScript RT reagent Kits (TaKaRa Biotechnology, Dalian, China) were used for reverse transcription. The target mRNA (TNF-α, IL-6, IL-1β,CCL3,CCL4 and CXCL1) expression was normalized to the 18S mRNA, which was calculated relative to the sham control group by using the Comparative-Ct Method ($2^{-\Delta\Delta Ct}$ method). The primers used in the study were listed in Table 1.

Table 1
Primers of the cytokines.

Gene	Sequence (5’-3’)
TNF-α	F: AAGCCTGTAGCCCACGTCGTA
	R: AGGTACAACCCATCGGCTGG
IL-1β	F: GGA GAC TTC ACA GAG GAT AC
	R: CCA GTT TGG TAG CAT CCA TC
IL-6	F: TCC ATC CAG TTG CCT TCT TG
	R: TTC CAC GAT TTC CCA GAG AAC
CCL4	F: CCCAATGAGTAGGCTGGAGA
	R: TCTGGACCCATTCCTTCTTG
CCL3	F: TTTTGAAACCAGCAGCCTTT
	R: CTGCCTCCAAGACTCTCAGG
CXCL1	F: GCACCCAAACCGAAGTCATA
	R: AGGTGCCATCAGAGCAGTCT
18S	F: AAACGGCTACCACATCCAAG
	R: CCTCCAATGGATCCTCGTTA

Western blotting

Western blotting was used to detect relative protein expression. RIPA lysis buffer was used to extract the total proteins in lung tissues. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the proteins. Isolated proteins were separated by SDS-PAGE electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membrane. The membranes were blocked with skimmed milk (8%) and then incubated with primary antibodies overnight at 4°C. The membranes were then incubated with secondary antibodies for 1 h at room temperature. The antibodies were purchased from San Ying Biotechnology, China, Abcam, USA and Boiss Biotechnology, China. The antibodies were used as follows: anti-TNF-α antibody (1:500), anti-IL-6 antibody (1:1000), anti-CCL3 antibody (1:200), anti-TLR4 antibody (1:1000), anti-MyD88 antibody (1:2000), anti- p-NF-κB p65 antibody (dilution 1:1500), anti- p-IκBα antibody (1:1000), anti- pIKKαβ antibody (1:10000), anti-VE-cadherin antibody (1:10000), anti-ZO-1 antibody (1:500), anti-claudin-5 antibody (1:2000), anti-β-actin antibody (1:5000), secondary antibody (1:5000). The relative density of protein expressions was quantitated by Image J software (n = 6). Protein levels were standardized by comparison with β-actin.

Statistical analysis

All the measurement data were expressed as mean ± SD. GraphPad Prism 6.0 is used for data statistics and mapping. One-way ANOVA followed by the Student-Newman-Keuls post hoc test is applied to analyze the difference between groups. Kaplan-Meier curve is used for survival analysis. $P < 0.05$ represents a significant difference.

Results

Isoacteoside improved the survival rate and histopathological damage in septic ALI in mice.

First, we performed the Kaplan-Meier survival analysis in all experimental groups, and the results showed that the higher dose of ISO (100 mg/kg/day, daily) could significantly improve the mouse 72 h survival rate under CLP surgery. Low doses (10 and 50 mg/kg/ day, daily) had no obvious treatment effect (Fig. 1A). Then, in the second experiment, the lung W/D ratio, which reflects pulmonary edema, was calculated in all groups. An increased lung W/D ratio was observed in the ALI group, which could be reduced by using a higher dose of ISO (Fig. 1B). The histopathological examination showed that hemorrhage, edema, necrosis, alveolar damage, increased thickness of the alveolar wall, infiltration of inflammatory cells and pulmonary consolidation were the classical features that were the easiest to observe in the ALI group. A higher dose of ISO could partially counter the development of these features, while some edema and inflammatory infiltration still exited (Fig. 1C). We also found that the ALI + ISO 100 group had lower scores than the ALI, ALI + ISO 10 and ALI + ISO 50 groups (Fig. 1D). Moreover, the alveolar wall thickness, inflammatory cell infiltration and pulmonary MPO were obviously increased in the ALI groups, which could also be reversed by high dose ISO treatment (Fig. 1D, 1E and 1F).

Isoacteoside reduced acid–base disturbance in septic ALI in mice.

Twenty-four hours after the experiment, arterial blood gas analysis was used to explore whether ISO could reduce the acid–base disturbance induced by ALI. A lower pH, decreased HCO_3-, PaO_2 and increased $PaCO_2$ were detected in the ALI group, while a higher dose of ISO obviously reduced acidosis and improved the pulmonary oxygenation (Fig. 2).

Isoacteoside reduced the systemic cytokine levels in septic ALI in mice.

On the basis of previous results, a higher dose of ISO (100 mg/kg/ day, daily) exhibited an evident protective effect. Therefore, we chose a dose of 100 mg/kg in the following experiment to reveal the potential mechanisms. CLP surgery resulted in severe local and systemic inflammatory reactions. Serum TNF-α, IL-6 and IL-1β levels in all groups were examined by ELISA to detect the severity of systemic inflammation. Significantly lower serum TNF-α, IL-6 and IL-1β levels were detected in the ALI + ISO100 group than in the ALI group (Fig. 3).

Isoacteoside reduced the BALF cytokine levels in septic ALI in mice.

In addition to the systemic inflammatory reaction, severe local inflammation could also be found in the lung, which was reflected by the BALF cytokine levels. BALF TNF-α, IL-6, IL-1β, IL-2, CXCL1, CCL4 and CCL3 levels were examined by ELISA to detect the severity of local inflammation. Significantly increased BALF TNF-α, IL-6, IL-1β, IL-2, CXCL1, CCL4 and CCL3 levels were also detected in the ALI group compared to those in the control group, which could be reduced by ISO treatment (Fig. 4).

Isoacteoside inhibited the mRNA level of cytokines in septic ALI in mice.

The mRNA level of pulmonary TNF-α, IL-6, IL-1β, IL-2, CXCL1, CCL4 and CCL3 was detected by RT-PCR. The results showed that ISO treatment could significantly reduce the transcriptional level of the aforementioned cytokines (Fig. 5A-5F).

Isoacteoside inhibited the TLR4/MyD88/NF-κB p65 signaling pathway in septic ALI in mice.

As the main classical inflammation-related signaling pathway, the TLR4/MyD88/NF-κB pathway was also examined in this study. TLR4, MyD88, p-NF-κB p65, p-IκBα and pIKKαβ expressions were markedly increased in the ALI group, and ISO treatment reversed the increase in protein expression levels (Fig. 6).

Isoacteoside reduced pulmonary oxidative stress in CLP-induced ALI in mice.

Excessive oxidative stress is another key mechanism involved in ALI. We detected the levels of MDA and 8-OHdG, which represented the pro-oxidative stress indexes, and the levels of CAT, SOD, GSH, and GSH-px, which represented the antioxidative stress indexes. The results showed that the MDA and 8-OHdG levels were significantly higher in the ALI group than in the ALI + ISO 100 group (Fig. 7A and 7B). In contrast, ISO treatment significantly increased the CAT, SOD, GSH and GSH-px levels after CLP surgery (Fig. 7C-7F). The results implied that ISO could inhibit pulmonary oxidative stress and improve antioxidative ability.

Isoacteoside inhibited pulmonary endothelial hyperpermeability in septic ALI in mice.

Excessive edema and increased endothelial permeability aggravated ALI. Not only proteins but also inflammatory cells can escape from the blood vessels to the alveoli because of severe vascular leakage. First, we detected the protein and albumin levels in BALF in all groups, and the results showed that the BALF protein and albumin levels were significantly increased after CLP surgery, and these increases could be reduced by ISO treatment (Fig. 8A and 8B). Then, we also tested the total cells, macrophages, neutrophils and MPO in the BALF, which can reflect the leakage of living cells. We found that the total cells, macrophages, neutrophils and MPO levels in BALF were significantly lower in the ALI + ISO 100 group than those in the ALI group (Fig. 8C-8F). Moreover, we tested endothelial junction proteins by western blotting for mechanistic research. The results showed that ISO treatment enhanced the expression levels of ZO-1, claudin-5 and VE-cadherin (Fig. 8G-8H).

Discussion

In the present study, we established a reliable and widely used mouse model of sepsis induced by CLP surgery to study the protective effect of ISO on sepsis-induced ALI. We found that a high dose of ISO (100 mg/kg/ day, daily) could: (1) improve the 72 h survival rate and reduce histopathological damage; (2) mitigate systemic and pulmonary inflammatory reactions through the TLR4/MyD88/NF-κB signaling pathway; (3) reduce pulmonary oxidative stress; and (4) inhibit pulmonary endothelial hyperpermeability in ALI. Although we performed ISO administration before and after CLP surgery, we mainly focused on the therapeutic effects rather than the preventive effect. A previous study using ultra-high performance liquid chromatography-tandem mass spectrometry found that isoacteoside could be rapidly absorbed, metabolized and eliminated in rats within 2 h after i.p administration ($MRT_{(0-\infty)}$ = 1.418 ± 0.077h, $T_{1/2}$= 1.002 ± 0.109 h, T_{max} =0.083 ± 0h). We speculated that ISO administration 3 days before CLP surgery may not lead to ISO accumulation but could be adapted for use in mice in a similar way. The therapeutic effects were mainly attributed to ISO administration 0 h and 12 h after CLP surgery.

Although improvements in advanced intensive care have been developed during the past decade, sepsis is still a global health issue causing unacceptable mortality and morbidity[19]. Sepsis most likely affects the respiratory system, leading to ALI and subsequent ARDS, both of which requrie advanced respiratory support and always indicate a poor prognosis in critically ill patients[20, 21]. Pulmonary edema, destruction of the normal architecture, and fluid accumulation induced by vascular leakage in the alveolus are the most common macroscopic changes. However, ISO treatment reduced the mortality, pulmonary water and wet/dry weight ratio in ALI mice. Our study is in accordance with the findings by Gao et al. that ISO treatment could improve the survival rate in endotoxic shock mice treated with LPS[10].

Overwhelming inflammation is the key process in sepsis-induced ALI. LPS produced by gram-negative bacteria can directly stimulate host inflammatory cell aggregation and then lead to the production of various cytokines after CLP [22]. Infiltration of neutrophils and macrophages in the lung tissues is the classical sign of ALI[23]. MPO is an indicator of neutrophil infiltration because it is mainly expressed on neutrophils. Our results showed that ISO could significantly reduce inflammatory cell infiltration and inhibit MPO activity. Moreover, proinflammatory cytokines ,such as TNF- α, IL-1β, and IL-6, and chemokines, such as CXCL1, CXCL2, and CCL1, are produced in abundance following sepsis, leading to the direct injury of alveolar epithelial cells and pulmonary vascular endothelial cells[24–26]. The current study showed that ISO treatment decreased systemic and local cytokine and chemokine levels, which was in agreement with previous studies[10]. Our study indicated that ISO may confer protection to mice with ALI through a reduction in inflammatory cell infiltration and the production of cytokines, which might be the underlying mechanisms. At the molecular level, TLR4/MyD88/NF-κB is the main classical signaling pathway that can initiate and aggravate inflammation during ALI[27]. TLR4 is a receptor that is expressed on the surface of immune cells and can be triggered directly by pathogen-associated molecular patterns (PAMPs), such as LPS or danger-associated molecular patterns (DAMPs). TLR4 triggers the MyD88-dependent pathway to translocate NF-κB from the cytoplasm into the nucleus, which finally controls immunity, oxidative stress, and inflammation[28]. When it is inactive, NF-κB is sequestered

in the cytoplasm, where it is bound to IκB. IκB is phosphorylated and degraded by IκB kinase upon MyD88 activation to release and translocate NF-κB to the nucleus, resulting in the transcription of numerous cytokine genes, such as TNF-α, IL-6 and IL-1β[29]. In this study, ISO inhibited the expression of TLR4, MyD88, p-NF-κBp65, p-IkBα, TNF-α and IL-6, which were upregulated by LPS, indicating that ISO might inhibit NF- κB activation by downregulating TLR4/MyD88 signaling. In agreement with our study, ISO has shown powerful anti-inflammatory effects in previous studies. Gao et al showed that ISO could suppress the TLR4/MyD88-TAK1-NF-κB/MAPK inflammatory signaling pathway to reduce TNF-α, IL-6 and IL-1β expression in LPS-treated RAW264.7 macrophages and bone marrow-derived macrophages, which implied that macrophages may be the direct target of ISO in ALI [10]. Sun-Young Nam et al. also found that ISO reduced the production and mRNA expression of TNF-α, IL-6, IL-8 and IL-1β in PMACI-stimulated HMC-1 cells via the caspase-1/NF-κB/MAPK pathway[30]. Thus, NF-κB might be the core target of ISO function, and future studies could focus on the detailed molecular interaction.

Oxidative stress is another important mechanism involved in the pathogenesis of ALI. Overproduction of ROS, such as superoxide, hydrogen peroxide (H2O2), and hydroxyl radicals, and reduced activity of antioxidases, including SOD, catalase, and GSH, which contributed to the oxidative stress[31]. Overproduction of ROS during ALI leads to the peroxidation of lipids, DNA, and proteins, which results in direct injury to cellular and mitochondrial membranes and eventual cell death [32]. MDA is an index of lipid peroxidation, and the 8-OHdG level represents DNA peroxidation; furthermore, SOD activity is a marker of the status of ROS scavenging[33]. In our study, a decrease in ROS production and an increase in antioxidantlevels were observed after ISO treatment in ALI. The antioxidant activity of ISO has been well documented in previous data. Sungwook Chae et al. found that ISO could protect cells exposed to H_2O_2 by scavenging intracellular ROS and 1,1-diphenyl-2-picrylhydrazyl radicals, inhibiting lipid peroxidation and increasing the activities of SOD and CAT[34]. Shi-Liang Ji et al found that acteoside/isoacteoside could protect PC12 cells against glutamate by decreasing intracellular ROS production, reducing MDA and increasing SOD and GSH-Px activities, and these findings were confirmed in an animal model[35]. Quanbo Xiong also found that ISO partially exerts hepatoprotective activity through its antioxidant effects[15]. Our research generated additional important data with regard to ISO antioxidant activity, which may extend the usage of ISO for treatment of different diseases.

Vascular endothelial injury is another mechanism in sepsis-induced ALI that results in the increased permeability of alveolar capillaries and promotes the infiltration of cell- and protein-rich substances and fluid into airspaces[36]. LPS could directly damage the integrity of microvascular endothelial cells during ALI. In our study, massive numbers of inflammatory cells and proteins were detected in the BALF, which means severe leakage occurred during ALI because of endothelial hyperpermeability. At the molecular level, this phenomenon was associated with the dysfunction of endothelial cell junctions, including tight junctions (TJs) and adherens junctions (AJs)[37]. ZO-1 belongs to TJs, which can form a complex to link the cytoplasmic domains of transmembrane proteins to the actin cytoskeleton. Previous studies showed that the deficiency of ZO-1 resulted in the instability of the TJ barrier. Claudin-5 is another TJ protein that contributes to the integrity of vessels. VE-cadherin is an important AJ protein that mediates the formation

of strong cell-to-cell adhesions, and the impairment of VE-cadherin increases endothelial permeability[38, 39]. We found that ISO treatment could upregulate ZO-1, claudin 5 and VE-cadherin levels in pulmonary tissue and then reduce the leakage of proteins and cells into BALF, which indicates that ISO could maintain the integrity of pulmonary capillary vessels. Although we did not perform the in vitro cell research, previous cell study showed that ISO might exert anti-inflammatory activity in vascular endothelium, which was a key mechanism of maintaining the integrity of endothelial permeability[40].

In conclusion, our data showed that ISO could alleviate septic acute lung injury, which might be associated with its capacity to inhibit inflammation through the TLR4/MyD88/NF-κB signaling pathway, alleviate oxidative stress and reduce endothelial hyperpermeability.

Declarations

AUTHORS' CONTRIBUTION

Yan-Nian Luo participated in the research design, mice modeling and writing the paper; Nan-nan He participated in the research design, WB and IHC performance. Juan Xu and Rui Wang participated in the WB performance and paper revising. Wen Cao and Dong-dong Chen provided substantial advice in designing the study and assisting in the division of labor.

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DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate. All the experimental protocols in this study were approved by the Institutional Animal Care and Use Committee of the Gansu Provincial Hospital. All authors consent to participate this research.

Consent for Publication. All authors consent to publish this article.

Competing Interests.: We declare that there is no conflict of interest regarding the publication of this article**.**

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Figures

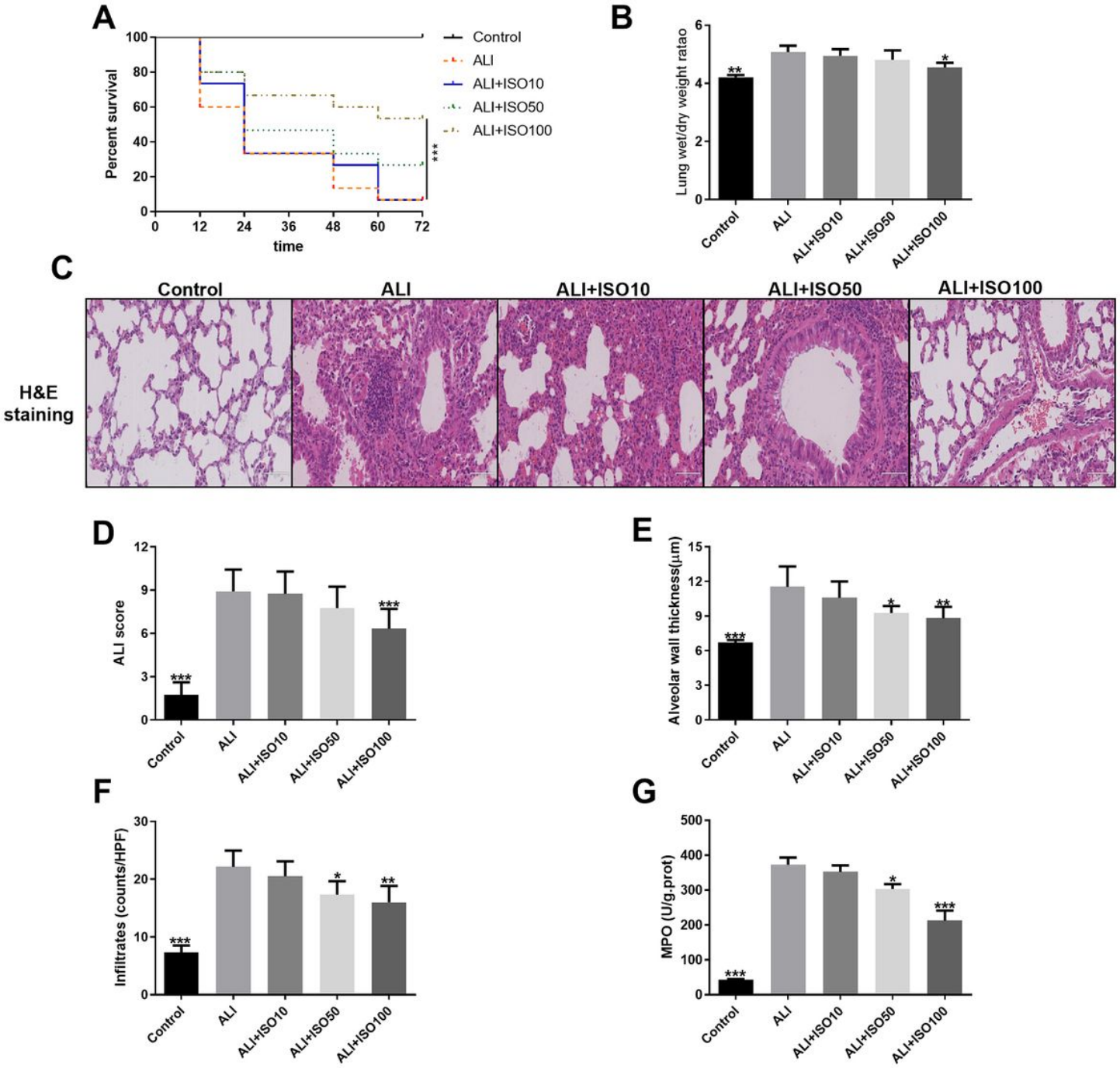

Figure 1

Isoacteoside improved the survival rate and histopathological damage in septic ALI in mice. (A): Kaplan-Meier survival curve (n=20); (B) lung wet-to-dry weight (W/D) ratio; (C) H&E staining of lung tissues (200×); (D) ALI score; (E) alveolar wall thickness ;(F) inflammatory cells infiltration and (G) MPO in lung tissues were detected. *p□0.05, **p□0.01, ***p□0.001vs ALI group.

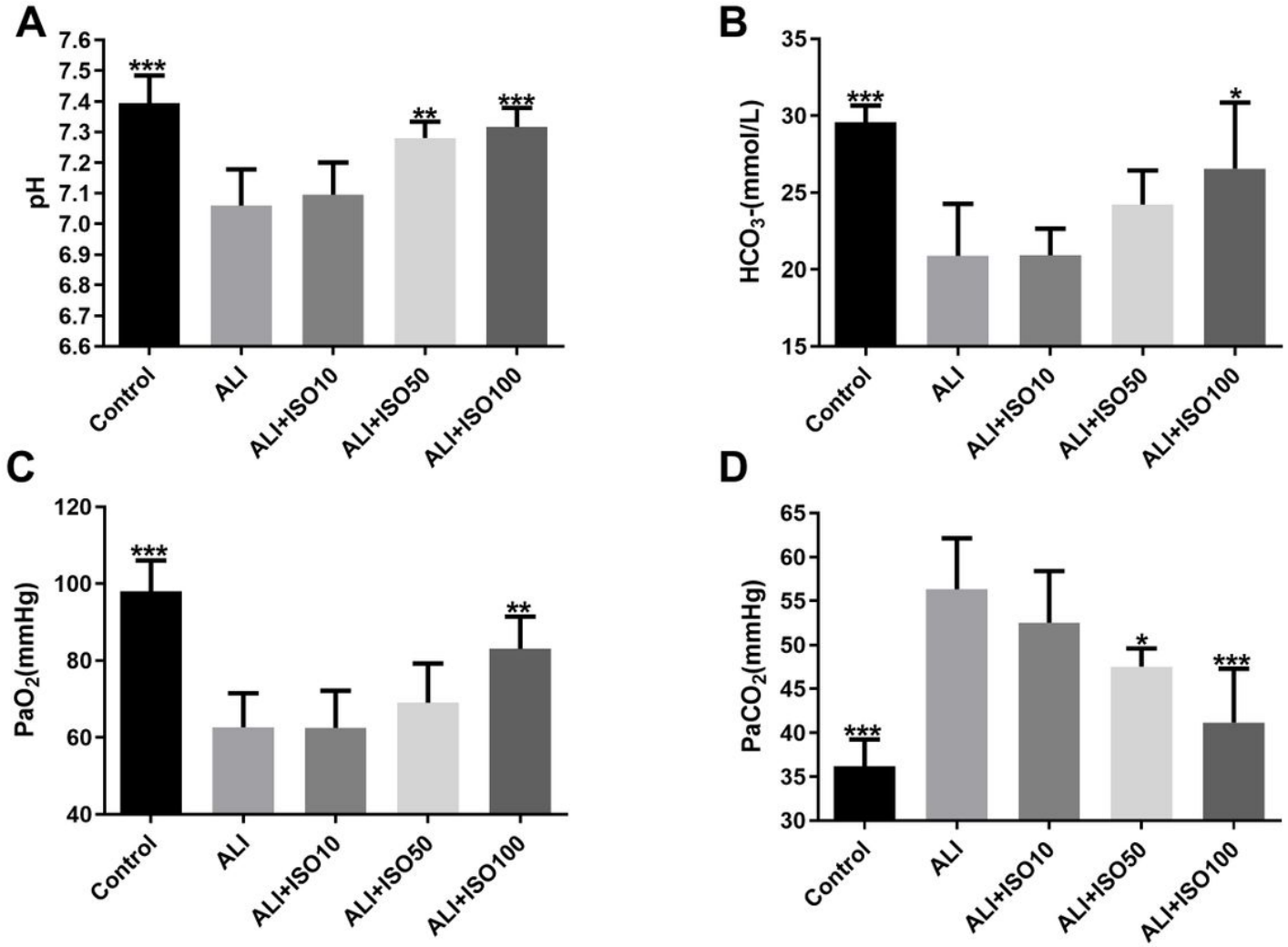

Figure 2

Isoacteoside improved acid–base disturbance in septic ALI in mice. (A) pH;(B) HCO3-;(C) PaO2 and (D) PaCO2 were calculated. *p□0.05, **p□0.01, ***p□0.001vs ALI group.

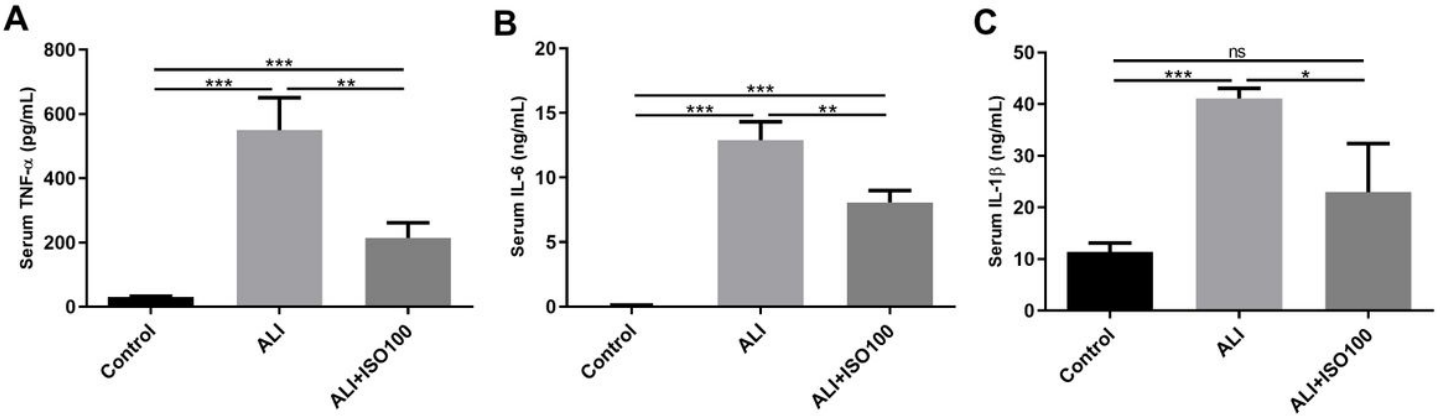

Figure 3

Isoacteoside reduced the systemic cytokine levels in septic ALI in mice. Serum (A) TNF-α; (B)IL-6; and (C) IL-1β levels were detected by ELISA. *p<0.05, **p<0.01, ***p<0.001vs ALI group.

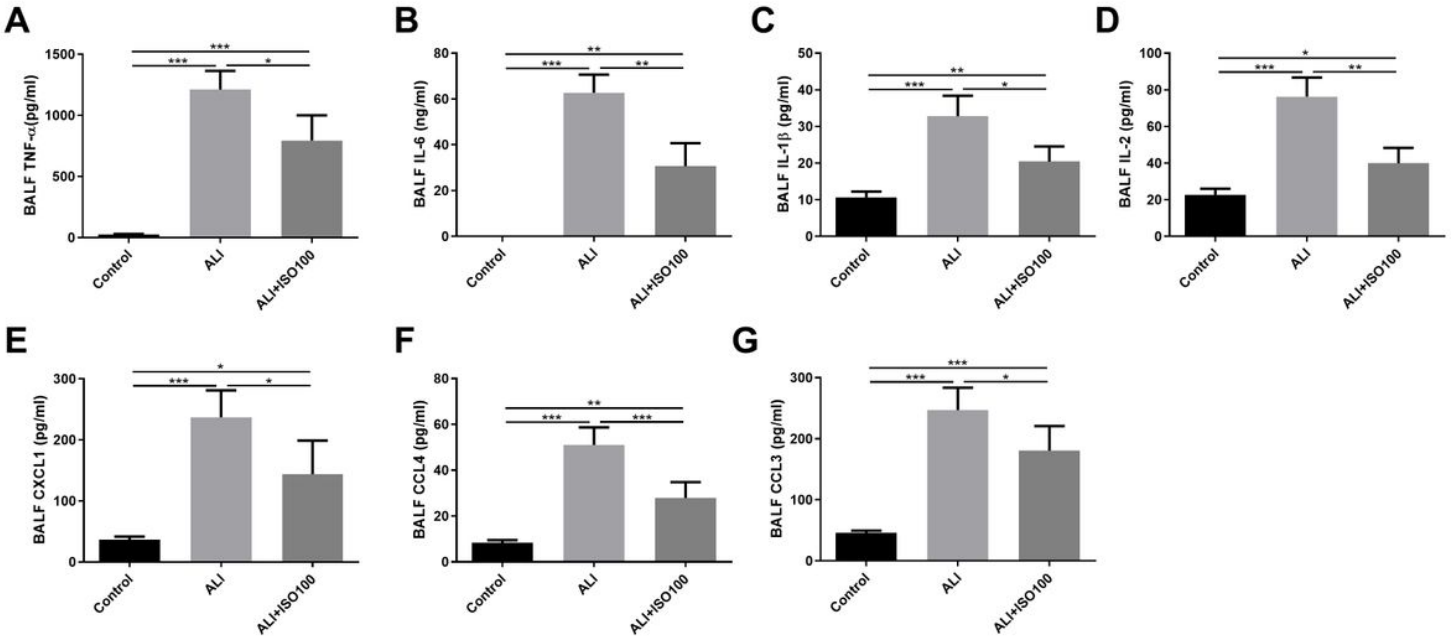

Figure 4

Isoacteoside reduced the BALF cytokine levels in septic ALI in mice. BALF (A) TNF-α; (B)IL-6; (C) IL-1β;(D) IL-2; (E) CXCL1; (F)CCL4 and (G) CCL3 levels were detected by ELISA. *p<0.05, **p<0.01, ***p<0.001vs ALI group.

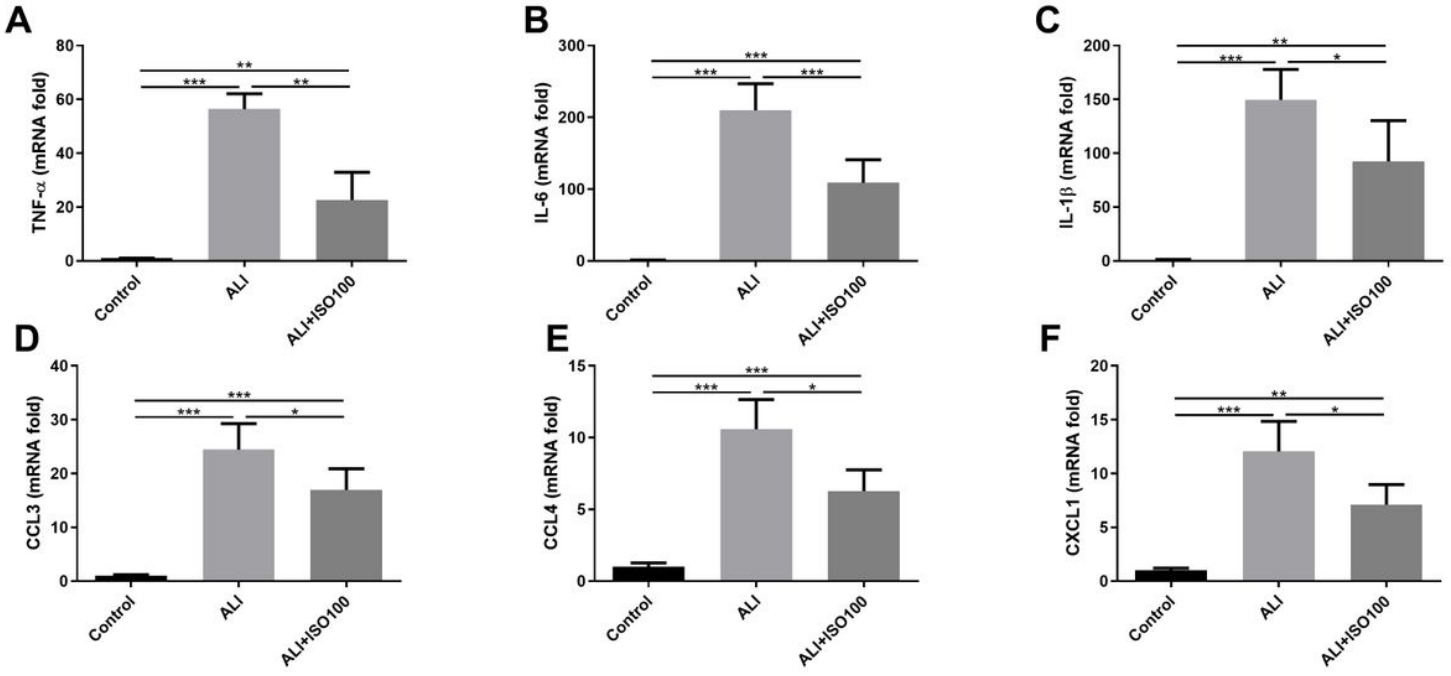

Figure 5

Isoacteoside inhibited the transcription and translation of cytokines in septic ALI in mice. mRNA of (A) TNF-α; (B)IL-6; (C) IL-1β;(D) CCL3; (E) CCL4; and (F)CXCL1 were detected by qRT-PCR. *p<0.05, **p<0.01, ***p<0.001vs ALI group.

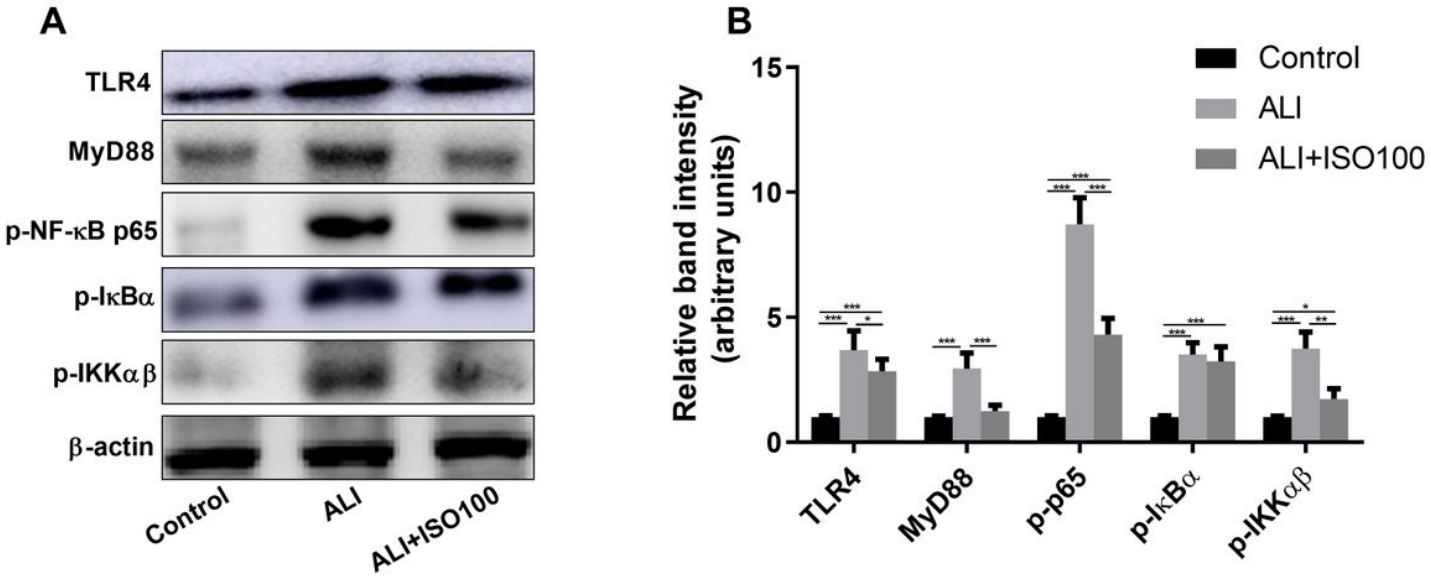

Figure 6

Isoacteoside inhibited the TLR4/MyD88/NF-κB p65 signaling pathway in septic ALI in mice. Protein expression of (A) TLR4, MyD88, p-NF-κB p65, p-IκBα and pIKKαβ were detected by western blotting, (B) Semi-quantitative results. *p<0.05, **p<0.01, ***p<0.001vs ALI group.

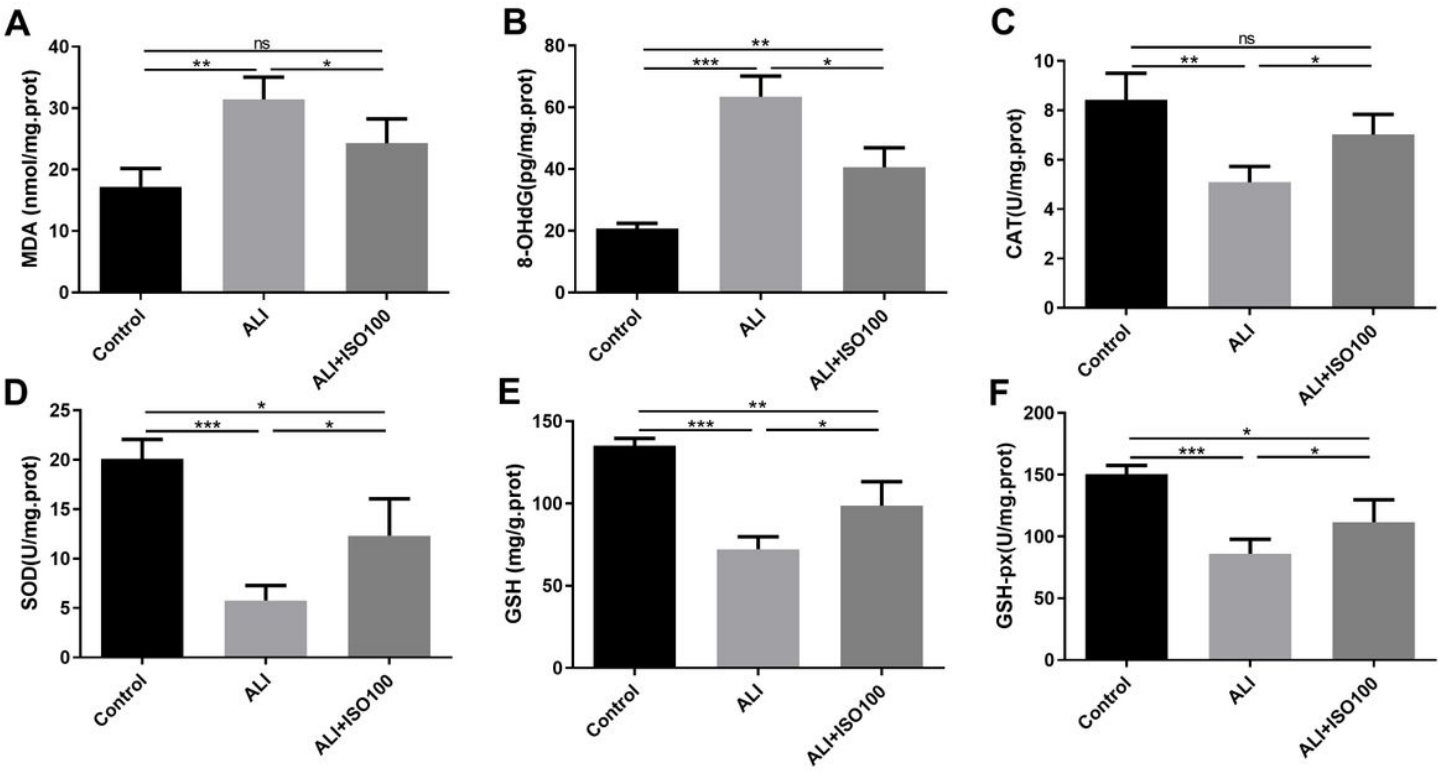

Figure 7

Isoacteoside reduced pulmonary oxidative stress in septic ALI in mice. The level of (A) MDA;(B) 8-OHdG; (C) CAT; (D)SOD;(E) GSH;(F) GSH-px was detected by activity assay kits. *p□0.05, **p□0.01, ***p□0.001vs ALI group.

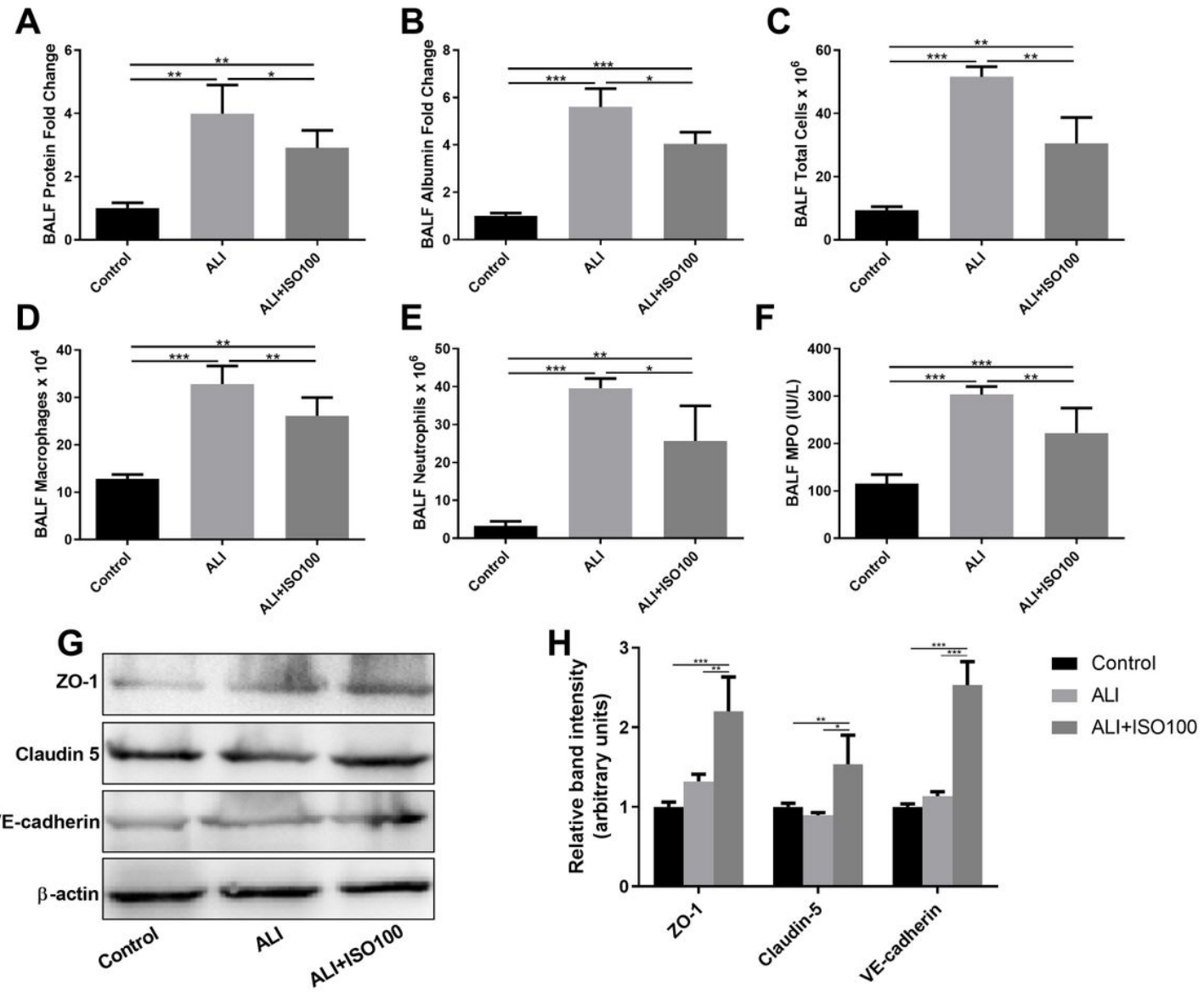

Figure 8

Isoacteoside inhibited pulmonary endothelial hyperpermeability in septic ALI in mice. BALF (A) protein;(B) albumin;(C) total cells;(D) macrophages, (E)neutrophils and (F)MPO were calculated. Protein expression of (A) ZO-1, claudin 5 and VE-cadherin were detected by western blotting, (B) Semi-quantitative results. *p □0.05, **p□0.01, ***p□0.001vs ALI group.