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Original Article

# Levosimendan mitigates coagulopathy and organ dysfunction in rats with endotoxemia

Hsieh-Chou Huang [a], Hsin-Jung Tsai [b,c], Chao-Chun Wang [b,d], Cheng-Ming Tsao [e,f,*], Shuk-Man Ka [g], Wen-Jinn Liaw [f,h], Chin-Chen Wu [b,i]

[a] *Department of Anesthesiology, Cheng-Hsin General Hospital, Taipei, Taiwan, ROC*
[b] *Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan, ROC*
[c] *Department of Anesthesiology, Mackay Memorial Hospital, Taipei, Taiwan, ROC*
[d] *Department of Anesthesiology, Zhongxing Branch, Taipei City Hospital, Taipei, Taiwan, ROC*
[e] *Department of Anesthesiology, Taipei Veterans General Hospital and National Yang-Ming University, Taipei, Taiwan, ROC*
[f] *Department of Anesthesiology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC*
[g] *Graduate Institute of Aerospace and Undersea Medicine, National Defense Medical Center, Taipei, Taiwan, ROC*
[h] *Department of Anesthesiology, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan, ROC*
[i] *Department of Pharmacology, Taipei Medical University, Taipei, Taiwan, ROC*

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**Abstract**

*Background*: In patients with severe sepsis, pro-inflammatory cytokines and subsequent activation of tissue factors trigger a cascade of events that lead to coagulation dysfunction and multiple organ failure. It has been shown that levosimendan has protective effects against tissue injury caused by endotoxin. The purpose of this study was to evaluate the effects of levosimendan on consumptive coagulopathy and organ dysfunction in an endotoxemic animal model induced by lipopolysaccharide (LPS).

*Methods*: Forty-six male adult Wistar rats were randomly divided into four groups: 1) control group (n = 10), an intravenous infusion of 5% dextrose 1.2 mL/kg for 20 min and 0.03 mL/kg/min for 4 h; 2) the levosimendan-treated control group (n = 12), an intravenous levosimendan infusion (24 μg/kg for 20 min plus 0.6 μg/kg/min for 4 h); 3) the LPS group (n = 12), an intravenous LPS (4 mg/kg) infusion followed by dextrose administration; and 4) the levosimendan-treated LPS group (n = 12), an intravenous LPS infusion followed by levosimendan treatment. Various parameters of hemodynamics, biochemistry, hemostasis and inflammatory response were examined during the experimental period.

*Results*: The administration of levosimendan significantly attenuated (i) consumptive coagulopathy displayed by thromboelastography, (ii) the decreases of platelet count and plasma fibrinogen level, (iii) injury in the lung, liver and kidney, and (iv) the rise in plasma interleukin-6 in rats treated with LPS.

*Conclusion*: The treatment of LPS rats with levosimendan was found to reduce organ injury and coagulopathy. These protective effects may be attributed to the anti-inflammatory effects of this drug.

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*Keywords:* Coagulopathy; Endotoxemia; Levosimendan; Organ dysfunction; Thromboelastography

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Cheng-Ming Tsao, Department of Anesthesiology, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC.

*E-mail address:* cm.cmtsao@gmail.com (C.-M. Tsao).

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## 1. Introduction

Despite improvements in medical therapy and intensive care in the past decade, sepsis-related mortality rates have increased linearly according to the disease severity of sepsis ranging from 10−50%.[1] Sepsis is associated with initial activation of coagulation and fibrinolysis followed by late impairment of fibrinolytic and natural anticoagulant systems,[2] leading to thrombotic microangiopathy and disseminated intravascular coagulation. The outcomes of septic patients depend on the resolution of coagulopathy, which is related to multiple organ failures.[3]

In Gram-negative endotoxemia and sepsis, massive release of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were induced by endotoxins like lipopolysaccharide (LPS), leading to inflammation and coagulation cascades. These cytokines may trigger the expression of tissue factor on monocytes and endothelial cells,[4] and consequently activate coagulation and fibrin formation. In addition, cytokines impair fibrinolysis and further decrease fibrin removal, leading to microvascular thrombosis, tissue hypoperfusion, and even organ failure.[5] Therefore, the type of medical intervention used to alleviate the activation of inflammation and coagulation has been regarded as an important issue.

Levosimendan (LS) is a calcium sensitizer, which enhances cardiac contractility independent from the adrenergic system by binding to the troponin C within cardiomyocytes.[6,7] LS opens potassium channels in smooth muscle, leading to venous, arterial and coronary vasodilation.[8] Owing to its beneficial effects, LS may positively affect cardiac performance and tissue perfusion in patients with heart failure and/or septic shock.[9−11]

More importantly, both *in vitro* and *in vivo* studies demonstrate that LS has an anti-inflammatory property other than its cardiovascular effects.[12,13] In a rat model of severe sepsis evoked by cecal ligation and incision, LS was reported to alleviate plasma IL-1β and IL-6 releases, attenuate hypotension and improve survival.[14] LS is also known to be a potent phosphodiesterase-III (PDE-III) inhibitor leading to increased intracellular cAMP levels,[15] which may modulate platelet function. Kaptan et al. have demonstrated that levosimendan has a significant inhibition of platelet aggregation at clinically relevant concentrations in an *in vitro* study.[16] In contrast to the high dosage associated with *in vitro* findings, other *in vitro* and *in vivo* studies did not reveal a significant effect of LS at clinically relevant concentrations.[17−19] However, the effect of LS on platelet and coagulation function in sepsis remains unclear.

Therefore, the current study was designed to investigate the effect of LS on coagulopathy and organ dysfunction in rats with endotoxemia induced by LPS, and to explore possible pathophysiological mechanisms. Previous studies have demonstrated that thromboelastography (TEG) appears to be a reliable monitor to detect the early endotoxin-related activation of coagulation and the consecutive consumption of coagulation factors and platelets.[20,21] Therefore, we used TEG to measure the change of coagulation function induced by LPS in this study.

## 2. Methods

### 2.1. Animals and experimental design

Forty-six healthy male adult Wistar rats, weighing 280−350 g, were purchased from the National Laboratory Animal Center of Taiwan, and maintained under a 12-h light/dark cycle at a controlled temperature (21 ± 2°C) with unrestricted access to food and tap water. The experimental protocol was approved by the appropriate review committee of the National Defense Medical Center, Taipei, Taiwan (Permit Number: IACUC-11-124), and provided assurance that all animals received humane care according to according to *Guide for the Care and Use of Laboratory Animals*, prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985).

The experiments were performed on pairs of rats, and the rats (n = 46) were all anesthetized with inhalational isoflurane (1%−2%) given via nosecone. Under anesthesia, two catheters were placed in the left carotid artery and right jugular vein for blood pressure measuring and drug administration, respectively. The catheters were cannulated, exteriorized and fixed to the back of the neck. Following catheter placement, the cannulated animals were allowed to recover for 4 h with tap water *ad libitum*.

After recovery, the catheter of the left carotid artery was connected to a transducer (P23ID, Statham Instruments, Oxnard, CA, USA) to measure phasic and mean arterial blood pressure (MAP) and heart rate (HR). Data were displayed on a multichannel recorder (XctionView Data Acquisition System, SINGA Technology Corporation, Taipei, Taiwan). After baseline hemodynamics were recorded and blood drawn, these rats were randomly divided into four groups as follows: (i) the control group, an intravenous infusion of 5% dextrose 1.2 mL/kg for 20 min and 0.03 mL/kg/min for 4 h; (ii) the LS group, an intravenous infusion of LS 24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h; (iii) the LPS group, an intravenous infusion of *Escherichia coli* LPS 4 mg/kg for 10 min followed by 5% dextrose 1.2 mL/kg for 20 min and 0.03 mL/kg/min for 4 h; and (iv) the LPS + LS group, an intravenous infusion of *E. coli* LPS (4 mg/kg for 10 min) followed by LS (24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h). Bacterial LPS (*E. coli* serotype 0127:B8, L3127) was obtained from Sigma Chemical (St. Louis, MO, USA). LS (*SIMDAX*®) was purchased from Abbott Labs., Orion Pharma, (Espoo, Finland), and dissolved in 5% dextrose solution (concentration: 0.01 mg/mL). Blood samples (total 3 mL) were collected at several time points: at time 0 (i.e., baseline, prior to LPS treatment), and at 1.5 and 4 h after LPS. Each volume of blood collected was replaced by the infusion of an equal volume of saline. The hemodynamic parameters were monitored for 4 h during this study.

All rats enrolled in the study were kept in the small in-house animal facility of our institute to enable optimal monitoring: their overall health status was checked every hour

for signs of distress. At the end of each experiment or upon signs of imminent death (i.e., unresponsive to external stimuli, inability to maintain upright position/tremor and prolonged/deep hypothermia and/or agonal breathing), rats were euthanized using an overdose of pentobarbital (100 mg/kg, i.v.). Then, the lung tissue samples were excised for histological examination.

### 2.2. Thromboelastography analysis of hemostatic changes

We used TEG analysis to detect the effects of LS on the characteristics of hemostasis as previously described in detail.[20,21] In brief, whole blood (0.36 mL) was withdrawn for TEG analysis which measured the global visco-elastic properties of whole blood clot formation with TEG 5000 Hemostasis System (Hemoscope Corporation, Niles, IL, USA). Neither anticoagulants nor coagulation activators were used in this study. Four main variables of TEG analysis were examined as shown in Fig. 1A.[22,23] *R*-time, related to coagulation factors, is the time of latency from beginning of analysis to initial fibrin formation. K-time is the time from the end of R-time until the amplitude of 20 mm, and α-angle is the slope between R- and K-time on TEG trace. K-time and α-angle are related to the speed of fibrin build-up and cross-linking, representing the kinetics of clot formation. Maximal amplitude (MA) represents the ultimate strength of the fibrin clot and is associated with the number and function of platelets and fibrinogen concentration.[24]

### 2.3. Assay of platelet count and plasma fibrinogen level

Whole blood was collected in an Eppendorf tube, to which 0.109 M sodium citrate had been added in a ratio of 1:9 sodium citrate/blood. The platelet count was measured by using an automated hematology analyzer (Sysmex KX-21N; SySmex America, Mundelein, IL, USA). Some citrated blood samples were centrifuged (15 min, 1,500 *g*) for the analysis of fibrinogen. We used rat fibrinogen ELISA kits (GenWay Biotech, San Diego, CA, USA) according to the manufacturer's instructions to obtain the level of plasma fibrinogen.

### 2.4. Quantification of organ dysfunction

Blood glucose was determined by a One-Touch II blood glucose monitoring system (Lifescan, Milpitas, CA, USA) with 10 μL of whole blood. Fuji DRI-CHEM 3030 Analyzer (Fuji Photo Film Co., Ltd., Tokyo, Japan) was used to evaluate the indexes of organ function. Liver function and renal function were determined by measuring plasma concentrations of alanine aminotransferase (ALT) and creatinine, respectively. In addition, lactate dehydrogenase (LDH) was surveyed to assess the severity of tissue injury.

### 2.5. Assay of plasma IL-6

It has been shown that IL-6 plays a pivotal role in activation of coagulation.[25] Plasma IL-6 was used with ELISA kits

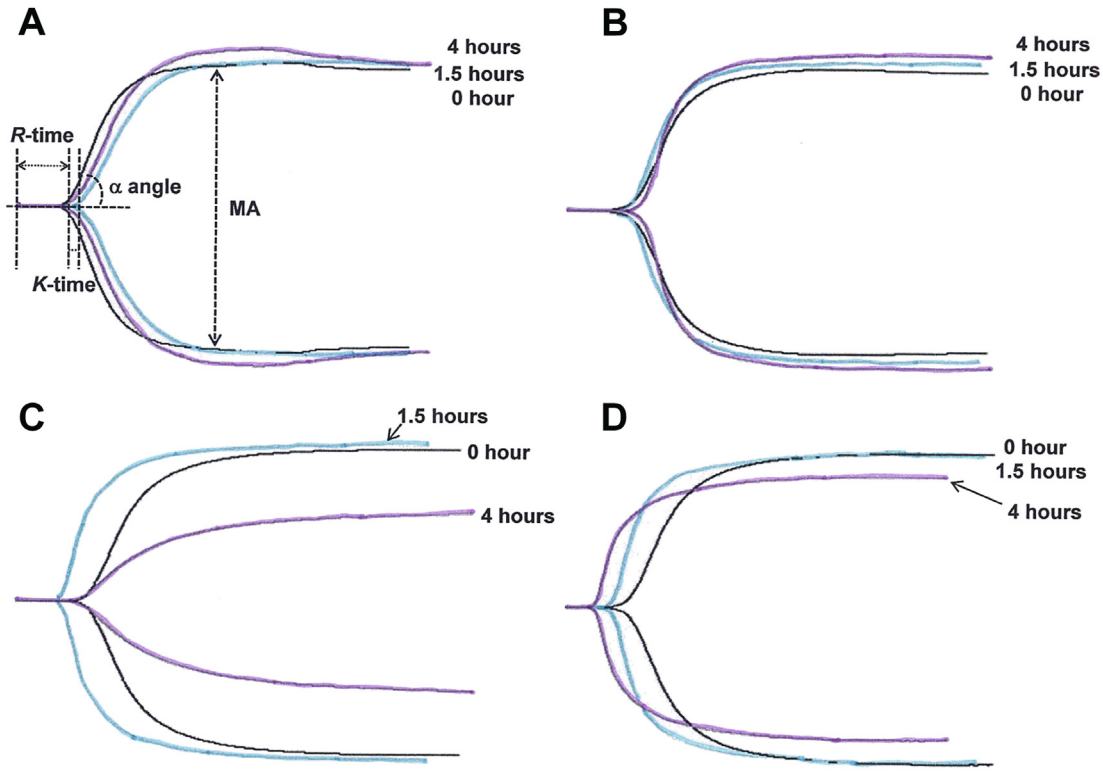


Fig. 1. The representative tracings of thromboelastography for (A) control group, showing primary parameters of thromboelastography, (B) LS group, (C) LPS group, and (D) LPS + LS group at the 0, 1.5 and 4 h. Control group: 5% dextrose infusion (same volume as levosimendan); LS group: levosimendan infusion 24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h; LPS group: *Escherichia coli* lipopolysaccharide (LPS) 4 mg/kg followed by 5% dextrose infusion; and LPS + LS group: *E. coli* LPS 4 mg/kg followed by levosimendan infusion. MA = maximal amplitude.

(R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's guidelines. All samples were performed in duplicate.

### 2.6. Histological examination

Lung tissues were excised and immediately fixed in 10% phosphate-buffered formaldehyde. The tissues were then embedded in paraffin, cut into slices of 3 μm, and placed on slides. The slides were stained with hematoxyline-eosin and examined by a pathologist who was blinded to the treatment group. The histological examination was analyzed at 400× magnification.

### 2.7. Statistical analysis

All data in the figures are described by the means ± standard deviation (SD) of n observations, where n represents the number of animals studied. Two-way analysis of variance with repeated measures was used to compare the difference between groups at distinct time points. A *P*-value of <0.05 was considered statistically significant.

## 3. Results

### 3.1. Cardiovascular changes

Baseline MAP and HR in all groups of animals were not significantly different between the groups (Fig. 2). In the control and LS group, there was no significant alteration in MAP and HR during the experimental period. In the LPS group, LPS infusion produced a rapid fall (48 ± 4 mmHg) in MAP from 0 to 1 h. Thereafter, MAP returned to the peak at 2 h after LPS administration and subsequently decreased at 3 and 4 h (Fig. 2A). Treatment of LPS rats with LS did not significantly affect the early hypotension or the delayed hypotension elicited by LPS. In addition, there was a significant increase in HR in endotoxemia after 1 h ($P < 0.05$, Fig. 2B), and treatment of LPS rats with LS had no significant effect on tachycardia.

### 3.2. Analysis of hemostatic changes by TEG

Fig. 1 showed that the representative tracings of TEG in four groups were at 0, 1.5 and 4 h during the period of study. We found no significant difference was observed in the basal values of TEG among all groups (Fig. 3). In the control or LS alone group, the four TEG parameters showed no significant changes during the experimental period. However, *R*-time and *K*-time significantly shortened at 1.5 h after LPS infusion ($P < 0.05$) and then reverted to the initial values at 4 h (Fig. 3A and B). Treatment of LPS increased the α-angle at the initial 1.5 h ($P < 0.05$) and then diminished progressively (Fig. 3C). The MA value decreased progressively and reached a significant level at 4 h in LPS group ($P < 0.05$, Fig. 3D).

As shown in Fig. 3A, LS administration did not alleviate the trend of *R*-time induced by LPS. LPS-induced shortening of *K*-time was not affected after LS treatment at 1.5 h; however, LS prohibited *K*-time from reverting to the initial values at 4 h after LPS administration ($P < 0.05$; Fig. 3B). The administration of rats with LS significantly attenuated the decrease in α-angle at 4 h after LPS injection ($P < 0.05$, Fig. 3C), but not the increase in α-angle at the initial 1.5 h. Furthermore, we observed that the LPS-evoked decrease in the MA value was alleviated after LS administration ($P < 0.05$,

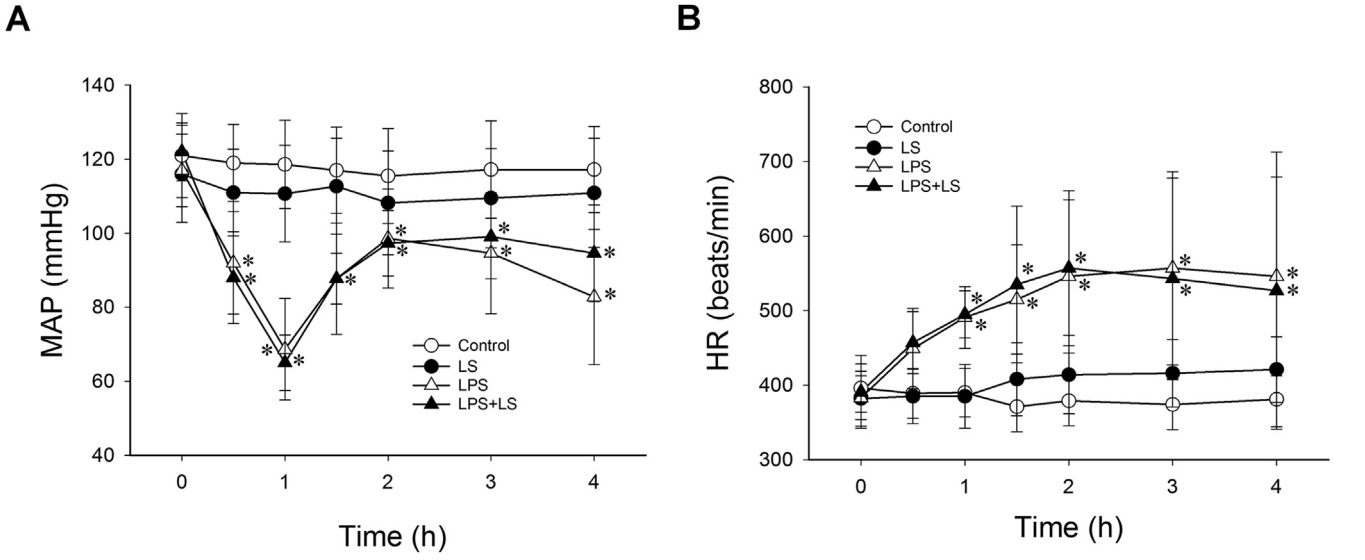


Fig. 2. Effects of levosimendan on changes of (A) mean arterial pressure and (B) heart rate in rats. Control group (n = 10): 5% dextrose infusion (same volume as levosimendan); LS group (n = 12): levosimendan infusion 24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h; LPS group (n = 12): *Escherichia coli* lipopolysaccharide (LPS) 4 mg/kg followed by 5% dextrose infusion; and LPS + LS group (n = 12): *E. coli* LPS 4 mg/kg followed by levosimendan infusion. Values are expressed as mean ± SD. *$P < 0.05$, all groups vs. Control. HR = heart rate; MAP = mean arterial pressure.

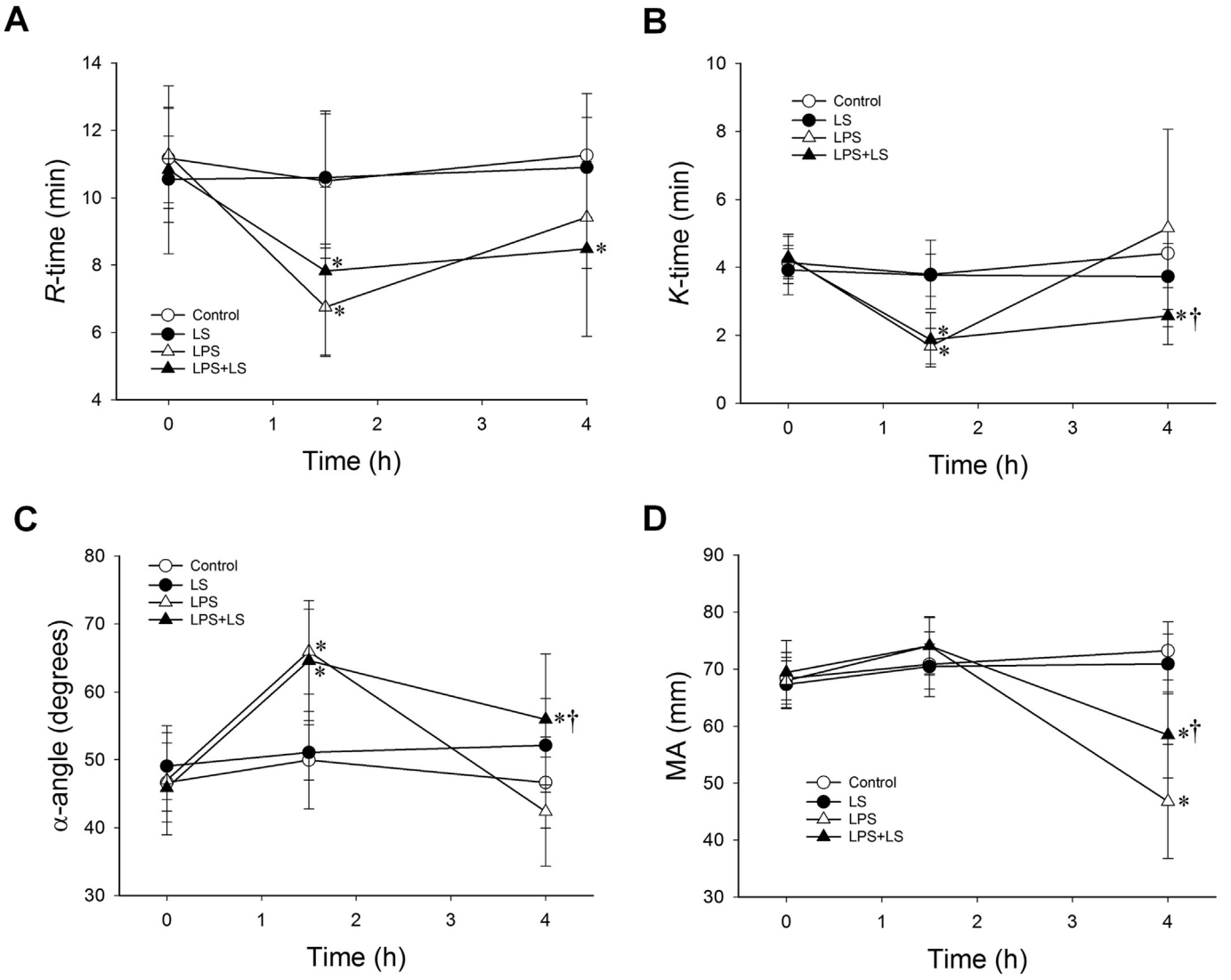


Fig. 3. Changes of thromboelastography parameters in rats. These parameters include (A) *R*-time, (B) *K*-time, (C) α-angle, and (D) maximal amplitude. Control group (n = 10): 5% dextrose infusion (same volume as levosimendan); LS group (n = 12): levosimendan infusion 24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h; LPS group (n = 12): *Escherichia coli* lipopolysaccharide (LPS) 4 mg/kg followed by 5% dextrose infusion; and LPS + LS group (n = 12): *E. coli* LPS 4 mg/kg followed by levosimendan infusion. Values are expressed as mean ± SD. *$P < 0.05$, all groups vs. Control. †$P < 0.05$, LPS + LS vs. LPS. MA = maximal amplitude.

Fig. 3D). Thus, these results indicate that LS administration attenuates the LPS-induced consumptive coagulopathy in rats.

### 3.3. Platelet count and plasma fibrinogen levels

At the beginning of the experiment, all groups had similar levels of platelet count and plasma fibrinogen (Fig. 4A and B). In the control or LS alone group, there was no significant change in platelet counts and plasma fibrinogen levels during the experimental period. After LPS administration, no significant alterations in platelet counts and plasma fibrinogen levels were found at the initial 1.5 h. However, significant decreases in these parameters were observed at 4 h ($P < 0.05$, Fig. 4A and B). Interestingly, we found that endotoxemic rats treated with LS significantly attenuated the decrease in platelet counts and plasma fibrinogen levels at 4 h ($P < 0.05$).

### 3.4. Blood glucose and plasma indexes of organ injury

There were no significant difference in blood glucose and plasma ALT, creatinine, and LDH at baseline among all groups (Fig. 5). As shown in Fig. 5A, the administration of rats with LPS caused a biphasic change in blood glucose: dramatic elevation at 1.5 h and then a significant drop at 4 h ($P < 0.05$). In addition, significant increases in plasma ALT, creatinine, and LDH at 4 h were found in the LPS group, which indicates that LPS induces tissue injury and impairs organ function ($P < 0.05$, Fig. 5B–D). Compared with the LPS-treated group,

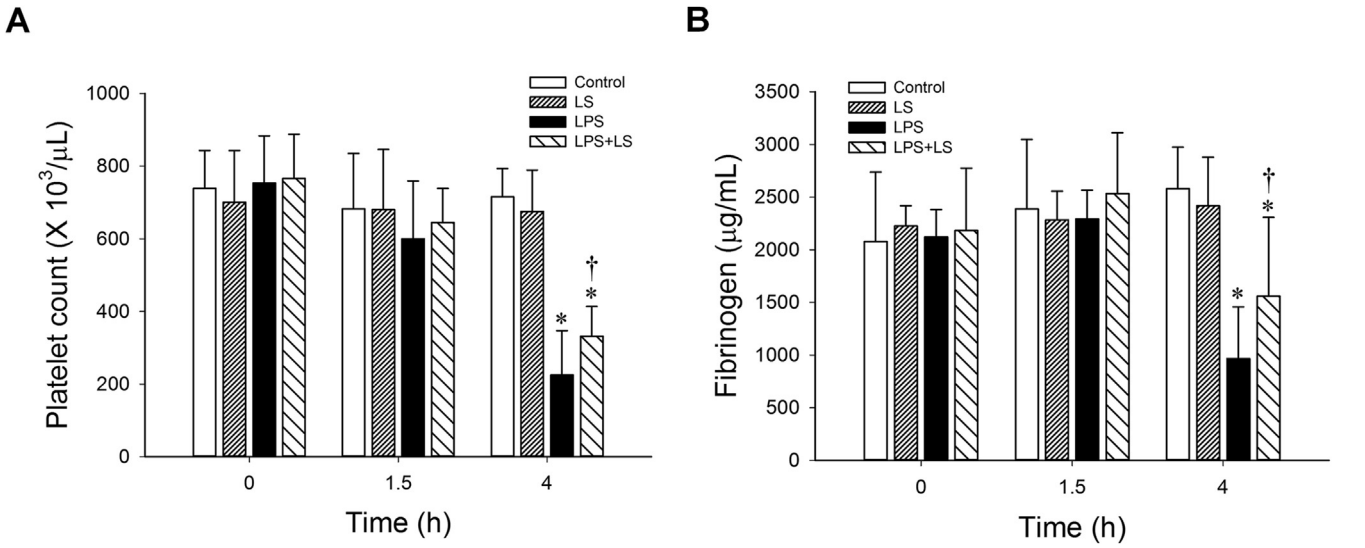


Fig. 4. Effects of levosimendan on changes of (A) platelet count and (B) plasma fibrinogen levels in rats. Control group (n = 10): 5% dextrose infusion (same volume as levosimendan); LS group (n = 12): levosimendan infusion 24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h; LPS group (n = 12): *Escherichia coli* lipopolysaccharide (LPS) 4 mg/kg followed by 5% dextrose infusion; and LPS + LS group (n = 12): *E. coli* LPS 4 mg/kg followed by levosimendan infusion. Values are expressed as mean ± SD. *P < 0.05, all groups vs. Control. †P < 0.05, LPS + LS vs. LPS.

blood glucose, plasma ALT, creatinine, and LDH level were obviously attenuated in the LPS + LS group ($P < 0.05$).

### 3.5. Plasma IL-6 level

The plasma IL-6 concentration was detected only at 1.5 and 4 h in the LPS and LPS + LS group (Fig. 6). LPS treatment increased plasma IL-6 levels at 1.5 and 4 h ($P < 0.05$, Fig. 6), however, the increase at 4 h was alleviated after administration of LS ($P < 0.05$).

### 3.6. Histology

Decreased alveolar airspaces, markedly diffused congestion and neutrophil infiltrations were observed in the lung at 4 h after LPS administration (Fig. 7C), but not in the control (Fig. 7A) or LS alone group (Fig. 7B). However, treatment of LPS rats with LS seemed to reduce the changes in lung morphology, with less edema, inflammatory infiltrates and septal widening present (Fig. 7D).

## 4. Discussion

It had been reported that inflammation-related coagulopathy, such as microvascular thrombosis, participated in the pathogenesis of multiple organ dysfunction syndrome.[26] The medical strategies aimed at mitigating systemic coagulopathy may improve organ injury in sepsis.[27,28] Our study demonstrated that LS administration partially attenuated hemostasis derangement and mitigated multiple organ dysfunctions in the *in vivo* rat model of endotoxemia. The protective effects of LS in acute endotoxemic rats may be associated with the inhibition of plasma IL-6 production.

Comparable to our previous study,[20] the analysis of TEG parameters revealed that the shortened clotting time (*R*-time) and the increased kinetics of clot formation (*K*-time and α-angle) were found in the initial period of LPS infusion in this experiment. Thereafter, the rate of clot formation was diminished, and eventually followed by the attenuation in the strength of the blood clot (the MA value). This indicates the reduction in the number and function of platelets and fibrinogen,[24] which was indeed confirmed in our study (shown in Figs. 4 and 5). These results are comparable to alterations in clinical status when severe sepsis patients usually developed coagulation dysfunction to hypocoagulable profiles (defined as the value of MA < 51 mm).[29]

In addition to calcium sensitizer and potassium channel opener, LS is a potent PDE-III inhibitor which increases intracellular cAMP levels and leads to platelet inhibition. Therefore, an *in vitro* study showed that LS decreased the blood aggregation response in a dose-dependent correlation.[16] However, in our *in vivo* study, the LS application in a clinical relevant concentration did not significantly change coagulation and platelet function in rats (Fig. 3). This is consistent with *in vivo* studies either in human[17] or in rats,[18] showing that LS has no significant effect on blood coagulation in a normal condition.

Intriguingly, in this study, the changes of TEG parameters and decreases of platelet count and plasma fibrinogen levels after acute LPS administration in rats were attenuated by LS, suggesting that the therapeutic effect of LS is most likely associated with alleviating the coagulation activation and minimizing the degree of consumptive coagulopathy. This is contrary to Bent and Plaschke's data,[18] showing that LS had no significant effects on the function and numbers of platelets in endotoxemic rats. The difference between our study and theirs

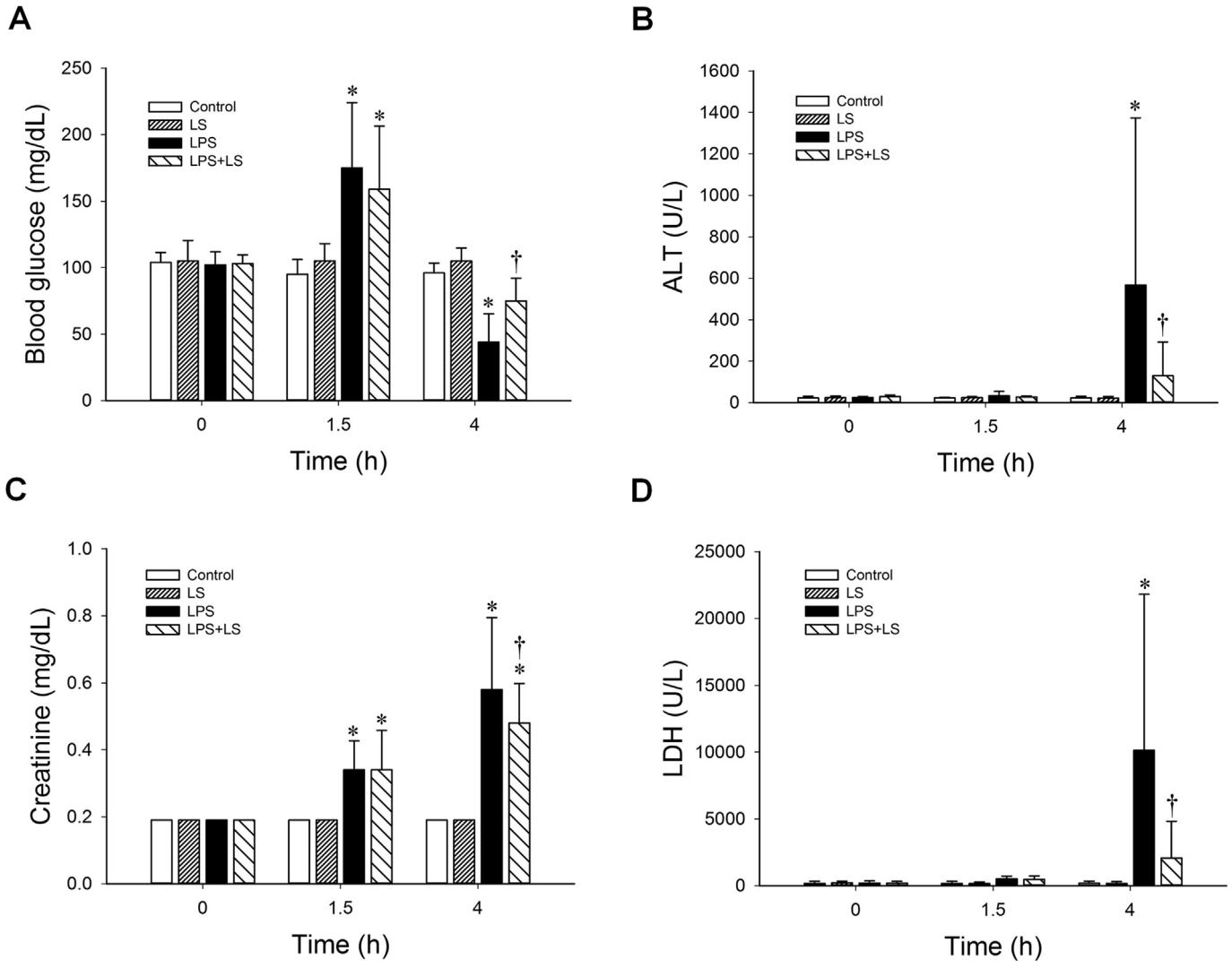


Fig. 5. Effects of levosimendan on changes of (A) blood glucose, (B) plasma alanine aminotransferase, (C) plasma creatinine, and (D) plasma lactate dehydrogenase in rats. Control group (n = 10): 5% dextrose infusion (same volume as levosimendan); LS group (n = 12): levosimendan infusion 24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h; LPS group (n = 12): *Escherichia coli* LPS 4 mg/kg followed by 5% dextrose infusion; and LPS + LS group (n = 12): *E. coli* lipopolysaccharide (LPS) 4 mg/kg followed by levosimendan infusion. Values are expressed as mean ± SD. *P < 0.05, all groups vs. Control. †P < 0.05, LPS + LS vs. LPS. ALT = alanine aminotransferase; LDH = lactate dehydrogenase.

could be due to: (i) LPS dosage, 4 mg/kg vs. 8 mg/kg; (ii) initial infusion time of LS after LPS administration, co-treatment vs. 24 h post-treatment; (iii) duration of LS infusion, 4 h vs. 1 h; and (iv) analysis time after LPS infusion, 4 h vs. 1 and 6 days.

However, our results demonstrated that LS alleviated the LPS-induced rise of biochemical parameters of the liver and the kidney and pathological deteriorations of the lung. Thus, LS may attenuate organ injury and dysfunction induced by endotoxemia, at least in part, through its anticoagulation effect. In addition to coagulation, inflammation plays an important role in the pathogenesis of sepsis.[25] Several studies have showed that LS offers potential effects on inhibition of the inflammatory response in patients with decompensated heart failure.[30–32] Furthermore, in rats with endotoxemia, LS can inhibit the production of IL-1β and IL-6,[14] but not TNF-α.[33] Indeed, our study showed that the increase in serum IL-6 levels was significantly attenuated after LS treatment in endotoxemic rats. Van der Poll et al. demonstrated that anti-IL-6 markedly attenuates endotoxin-induced activation of coagulation, monitored by the plasma levels of the prothrombin fragment F1+2 and thrombin-antithrombin III complexes in mild endotoxemia in chimpanzees.[34] Thus, the protective effects of LS on coagulation in endotoxemic rats may be mediated partly by the inhibition of plasma IL-6 production.

Bacterial LPS stimulates monocytes and tissue macrophages to release TNF-α and IL-1, which then stimulate secondary response cytokines, including IL-6.[35] In addition to hematopoietic cells, IL-6 is synthesized by many different types of nonhematopoietic cells, including endothelium, lasting for an extended period in response to various stimuli.[35,36] Activation of the inflammatory cascade impacts the coagulation pathway, and vice versa, and affect the endothelium.[37] Widespread

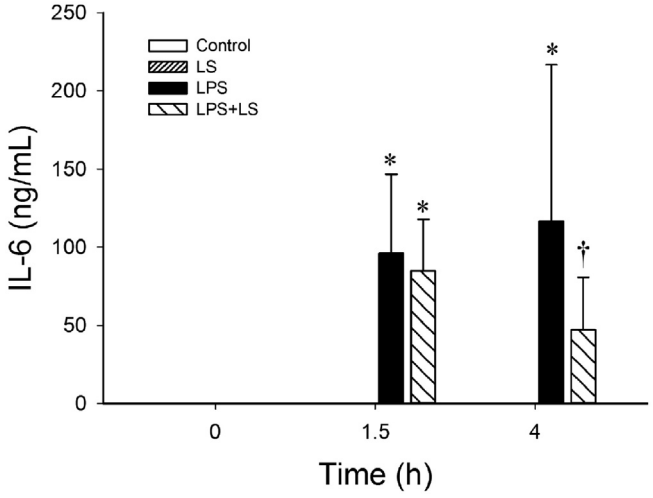


Fig. 6. Effects of levosimendan on changes of plasma interleukin-6 levels in rats. Control group (n = 4): 5% dextrose infusion (same volume as levosimendan); LS group (n = 4): levosimendan infusion 24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h; LPS group (n = 8): *Escherichia coli* lipopolysaccharide (LPS) 4 mg/kg followed by 5% dextrose infusion; and LPS + LS group (n = 8): *E. coli* LPS 4 mg/kg followed by levosimendan infusion. Values are expressed as mean ± SD. *$P < 0.05$, all groups vs. Control. †$P < 0.05$, LPS + LS vs. LPS. IL-6 = interleukin-6.

involvement of endothelium and monocytes/tissue macrophages, together with the more generalized activation of inflammation and coagulation, may lead to multiple organ dysfunction.[37] According to our results, it is speculated that LS attenuates the late response induced by LPS and further mitigated multiple organ dysfunctions at 4 h after LPS injection, while early response protein TNF-α is not suppressed by LS.[33]

According to the dosage of LS used in the previous study done by Fries et al.,[38] we applied the preliminary dosage of LS (12 μg/kg for 10 min and 0.3 μg/kg/min for 4 h), which is compatible with the dosage used in patients with heart failure.[39] However, the study showed disappointing results in the treatment of sepsis-induced coagulopathy (data not shown). In the current study, therefore, we used a twofold dosage (24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h), which did not lead to a significant decrease in blood pressure in both the control and LPS rats.

However, the current study has some limitations which need to be addressed. First, we used the experimental rat model which does not imitate the clinical manifestation of sepsis. However, Patel et al. reported that polymicrobial sepsis induced by cecal ligation/perforation and endotoxemia promote microvascular thrombosis under different mechanisms.[40] More importantly, endotoxemia, but not cecal ligation/perforation, causes significant thrombocytopenia and coagulation derangement, comparable to hemostatic features in patients with sepsis.[41] Second, it has been shown that an initial 3-h management bundle remains very important regarding early detection of the onset of severe sepsis and septic shock.[42] In this study, LS was given at 10 min after LPS administration, namely co-administered with LPS. Therefore, it may be a potential adjuvant of early therapy for sepsis.

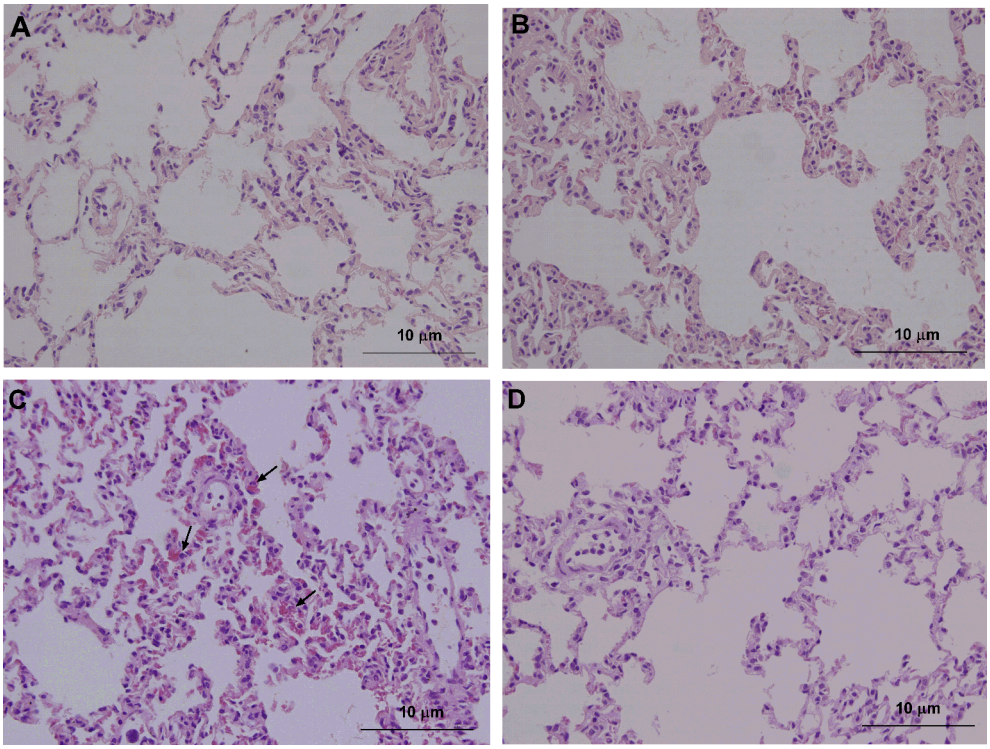


Fig. 7. Hematoxyline and eosin staining of lung tissues in rats. Representative lung histology pictures in three animals from each group. (A) control group: 5% dextrose infusion (same volume as levosimendan); (B) LS group: levosimendan infusion 24 μg/kg for 20 min and 0.6 μg/kg/min for 4 h; (C) LPS group: *Escherichia coli* lipopolysaccharide (LPS) 4 mg/kg followed by 5% dextrose infusion; and (D) LPS + LS group: *E. coli* LPS 4 mg/kg followed by levosimendan infusion. Arrows indicate the infiltration of neutrophils. Magnification ×400.

In conclusion, our present study demonstrated that LS treatment attenuated coagulopathy and improved organ dysfunction in endotoxemic rats. The protective effects of LS may be due to its inhibition of coagulation and inflammation. However, these beneficial effects of LS need to be further confirmed, particularly by clinical trials in the early treatment of patients, in particular, with Gram-negative sepsis.

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