Cite this as: Hanyu WANG, Yuanhao YANG, Xiao ZHANG, Yan WANG, Hui FAN, Jinfeng SHI, Xuelian TAN, Baoshi XU, Jingchao QIANG, Enzhuang PAN, Mingyi CHU, Zibo DONG, Jingquan DONG. Liensinine attenuates inflammation and oxidative stress in spleen tissue in an LPS-induced mouse sepsis model[J]. Journal of Zhejiang University Science B, 2023, 24(2): 185-190.
http://doi.org/10.1631/jzus.B2200340

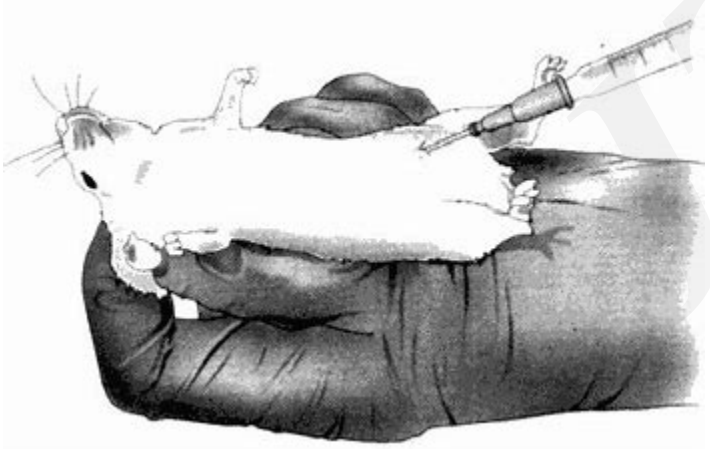
Liensinine attenuates inflammation and oxidative stress in spleen tissue in an LPS-induced mouse sepsis model

Key words: Sepsis; Inflammatory, Oxidative stress, Spleen, Liensinine

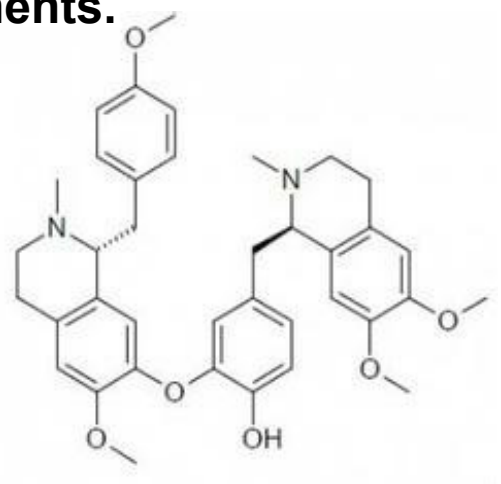
Methods and Results

1、Experimental design

The purpose of this study was to investigate the potential protective effect of liensinine (LIE) in spleen damage based on a model of sepsis induced by lipopolysaccharide (LPS). After the acclimation period (10 d), thirty healthy C57BL/6 mice (18-22 g, 6-8 weeks old) were divided randomly into five groups (n=6), including a control group, an LPS group (10 mg/kg), and LIE treatment groups with 3 doses (10, 20, 40 mg/kg) + LPS (10 mg/kg). Equal amounts of solvent or LIE were injected intraperitoneally each day for 5 d and LPS was administered after the last injection. Six hours later, the mice were cervically dislocated and executed for further experiments.



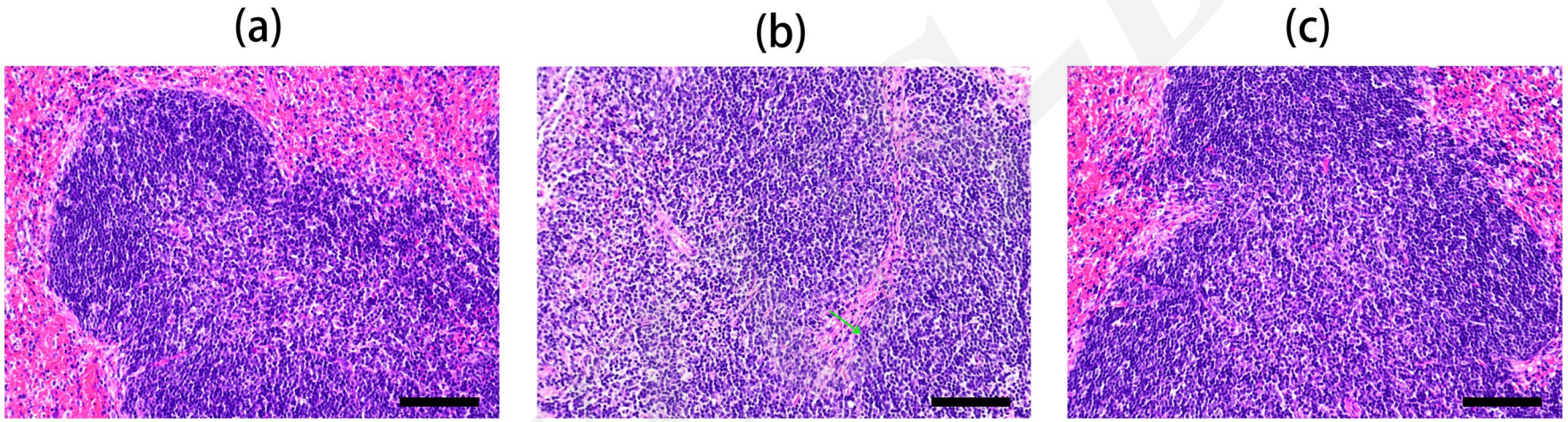
Intraperitoneal injection



Liensinine

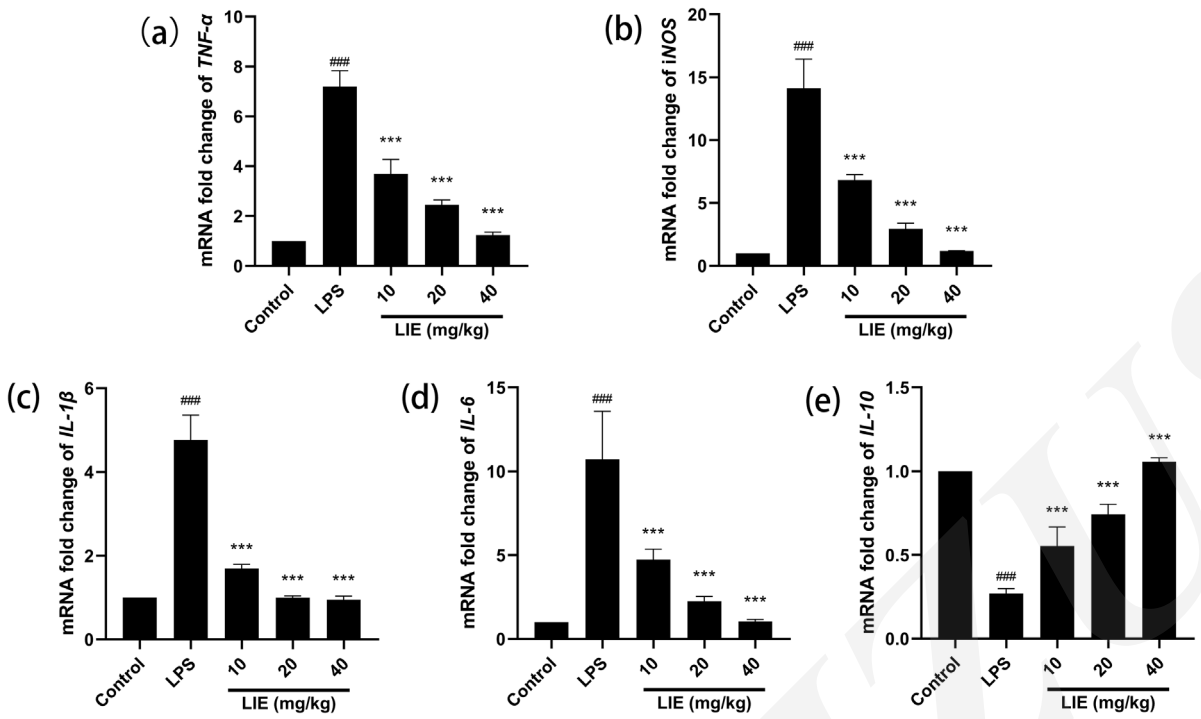
2、Histological analysis

Fresh spleen tissues from each group of mice were first fixed in 4% formaldehyde and then embedded well in paraffin. The tissues were cut into 4-μm-thick pieces and stained with hematoxylin-eosin (H&E), eventually observed and photographed with an optical microscope.



The results showed that the spleen in the control and LIE-treated groups was structurally normal, as evidenced by a clear red and white marrow demarcation. While in the LPS-stimulated model group, the red and white marrow demarcation of spleen tissue was not clear and the white marrow areas appeared enlarged and fused with each other. These findings suggested that LIE can significantly alleviate the histopathological stress of spleen tissue damage in sepsis.

3、Quantitative real-time PCR assay

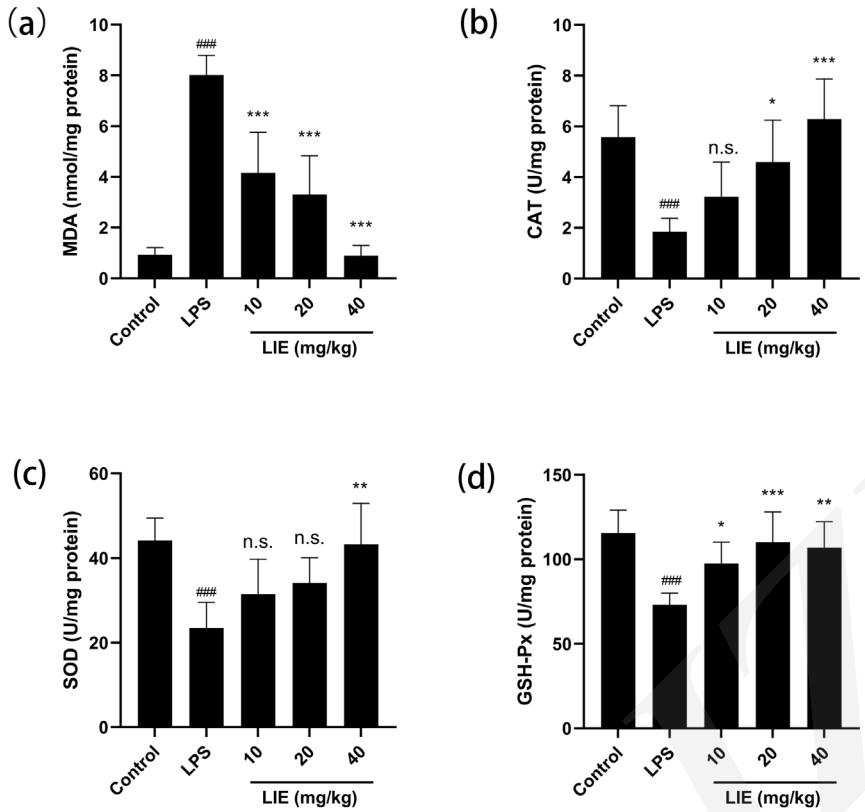


In the process of sepsis, many pro-inflammatory factors are produced including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL- 6), the release of which is significantly increased by the stimulation of lipopolysaccharide. LIE pre-treatment significantly reduced levels of TNF-α, IL-6, and IL-1β, while enhancing IL-10 levels in a dose-dependent manner.

Upregulation of inducible nitric oxide synthase (iNOS) expression is an important manifestation of inflammation in an organism. Compared to the control, LIE reversed the reduction in iNOS expression levels in a dose-dependent manner.

In general, the 40-mg/kg dose of LIE greatly attenuated the inflammatory response and exhibited more anti-inflammatory capacity compared to the 10 mg/kg and 20 mg/kg doses. Hence, it appeared that LIE had the potential to provide an anti-inflammatory response in sepsis.

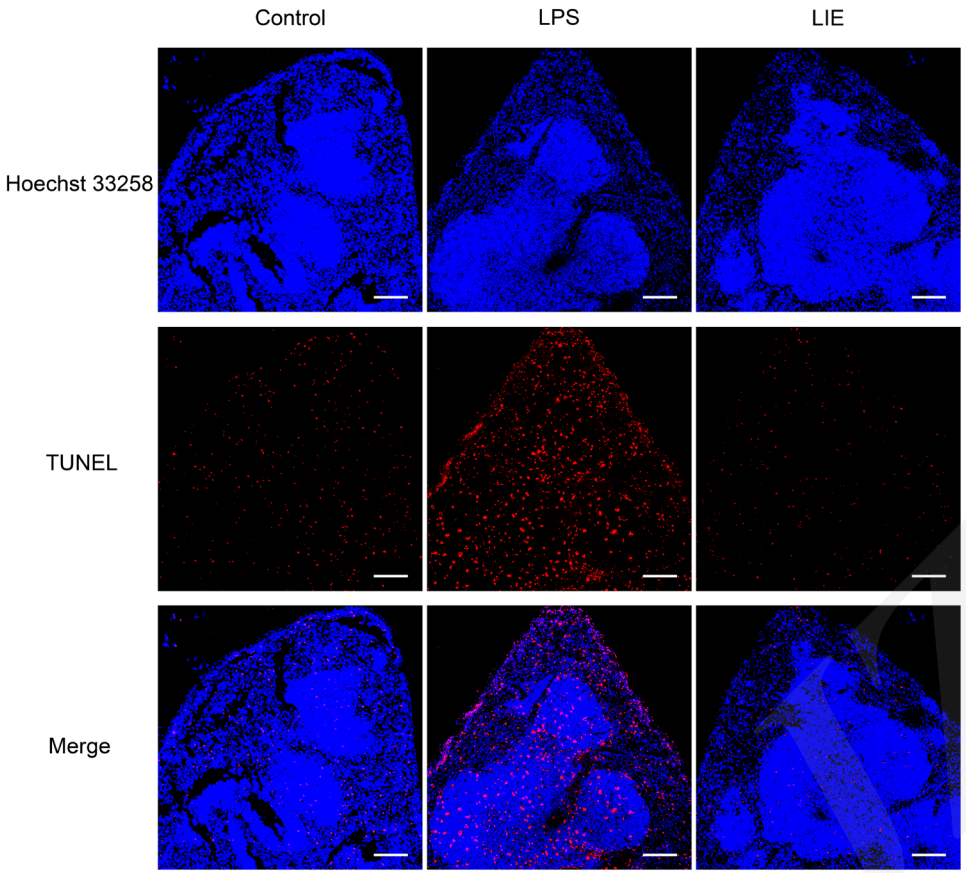
4、Biochemical indicators measurement



Compared with the control group, LPS treatment disturbed the balance of oxidation and anti-oxidation. The malondialdehyde (MDA) content was significantly increased, and the activity of CAT, SOD, and GSH-Px were all decreased. Meanwhile in the LIE pre-treatment group, LIE decreased MDA content in a dose-dependent manner, and LIE significantly increased antioxidant capacity at 20 mg/kg and 40 mg/kg.

These results suggested that different doses of LIE can slow down sepsis-induced spleen damage by increasing the activity of antioxidant enzymes.

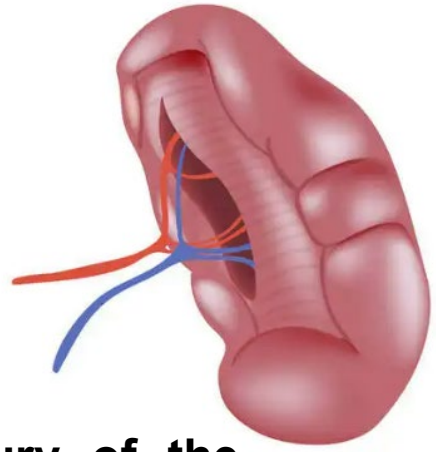
5、TUNEL assay



Spleen tissues were collected for TUNEL staining. The nucleus was stained blue by Hochest 33258 and apoptosis cells were stained red by TUNEL. Compared with the control group, we observed a significant increase in the number of apoptotic cells in the LPS group, and the number of these cells was significantly downregulated after pre-treatment with LIE, compared with the LPS group. LIE can significantly inhibit the apoptosis of spleen tissue induced by LPS.

It was therefore clear that LIE could protect spleen tissue by reducing the degree of apoptosis caused by sepsis.

Conclusion



The results suggested that LIE relieved histopathological injury of the spleen and inhibited apoptosis. LIE significantly reduced transcript levels of the pro-inflammatory factors TNF-α, IL-6, IL-1β, and iNOS, and increased expression of the anti-inflammatory factor IL-10. In addition, LIE pretreatment decreased indicators of lipid peroxidation of MDA and enhanced the antioxidant activities of CAT, SOD, and GSH-Px. In conclusion, our study showed that LIE has potential antioxidant and anti-inflammatory capacity to protect the spleen from sepsis damage.

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Innovation points

1、This study was the first to use LIE in a mouse model of sepsis induced by lipopolysaccharide to investigate the effects of LIE on sepsis splenic injury in three important pathways: inflammation, oxidative stress and apoptosis.

2、The results suggest that Liensinine has potential as a therapeutic agent for the treatment of sepsis.

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