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

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Sepsis is a complex syndrome caused by multiple pathogens and involves multiple organ failure, particularly spleen dysfunction. In 2017, the worldwide incidence was 48.9 million sepsis cases and 11 million sepsis-related deaths were reported (Rudd et al., 2020). Inflammation, oxidative stress, and apoptosis are the most common pathologies seen in sepsis. Liensinine (LIE) is a bisbenzylisoquinoline-type alkaloid extracted from the seed embryo of *Nelumbo nucifera*. Lotus seed hearts have high content of LIE which mainly has antihypertensive and antiarrhythmic pharmacological effects. It can exert anti-carcinogenic activity by regulating cell, inflammation, and apoptosis signaling pathways (Manogaran et al., 2019). However, its protective effect from sepsis-induced spleen damage is unknown. In this research, we established a mouse sepsis model induced by lipopolysaccharide (LPS) and investigated the protective effects of LIE on sepsis spleen injury in terms of inflammatory response, oxidative stress, and apoptosis.

Sepsis can cause tissue damage and is often accompanied by inflammation. The spleen facilitates phagocytosis and defends against infection in the normal body. People with a weakened spleen are more likely to suffer from fulminant sepsis, which is associated with a high mortality rate (Kanhutu et al., 2017). In sepsis damage to the spleen, an excessive inflammatory immune response can be triggered. In this situation, phagocytes release some pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-8, tumor necrosis factor-α (TNF-α), and IL-12 (Thompson et al., 1992). Along with the release of inflammatory factors, symptoms such as hyperthermia, disseminated coagulation, insufficient tissue perfusion, and oxidative damage occur in the unbalanced struggle between pro-inflammatory and anti-inflammatory organisms.

Aside from triggering a series of inflammatory responses, spleen injury caused by sepsis is often accompanied by oxidative stress and apoptosis. A hallmark of oxidative stress is an increase in malondialdehyde (MDA) levels, although several intracellular antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), can effectively protect cells and tissues from oxidative damage (Kumar et al., 2018). During the regulation of disease, it is not uncommon for immune cells in the spleen to undergo uncontrolled apoptosis and excessive release of pro-inflammatory mediators to the periphery, thus making the spleen more susceptible to stimulation and shrinking, and causing secondary infections (Kaur et al., 2022).

At present, there is no specific method for treating sepsis caused by bacterial or fungal infection. Meanwhile, medicines of natural origin have historically been used to alleviate many diseases, and have mainly been investigated as anticancer drugs. Studies have shown that LIE has potential for anti-oxidation and anti-inflammation. For example, a study on skin

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protection showed that rats fed lotus seed tea had reduced levels of the protein carbonyl, which is related to the degree of oxidation in skin tissue, indicating that LIE has a degree of antioxidant activity (Kim and Moon, 2015). The anti-inflammatory property of lotus seeds has been applied traditionally in the treatment of chronic diarrhea, enteritis, tissue inflammation, and other related disorders (Zhu et al., 2016). Because of its ability to treat inflammation and oxidative stress, LIE has the potential for the therapy of sepsis. Hence, we established an in vivo sepsis model of LPS-induced spleen injury in mice with the aim of revealing the influence of LIE on sepsis spleen injury.

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 three doses (10, 20, and 40 mg/kg)+LPS (10 mg/kg). Equal amount of solvent or LIE was 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.

To clearly observe the protective effect of LIE in pathological damage caused by sepsis, we carried out hematoxylin and eosin (H&E) staining. The results showed that the spleens in the control and LIE-treated groups were structurally normal, as evidenced by a clear red and white marrow demarcation (Figs. 1a and 1c). 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, probably due to the immune effect exerted by the macrophages in the spleen (Fig. 1b). These findings suggested that LIE can significantly alleviate the histopathological stress of spleen tissue damage in sepsis.

In the process of sepsis, many pro-inflammatory factors are produced. The inflammatory cytokine messenger RNA (mRNA) expression levels are provided in Fig. 2. Compared with the control group, the mRNA transcription levels of *TNF-α*, *IL-1β*, and *IL-6* were significantly upregulated, and *IL-10* was downregulated in the model group (Figs. 2a and 2c–2e). LIE pre-treatment (10, 20, and 40 mg/kg) 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. We found that LPS treatment significantly enhanced the level of *iNOS* expression compared to the control; however, LIE doses of 10, 20, and 40 mg/kg reversed the reduction in *iNOS* expression levels in a dose-dependent manner (Fig. 2b). In general, the 40 mg/kg dose of LIE greatly attenuated the inflammatory response and exhibited more anti-inflammatory capacity compared to the 10 and 20 mg/kg doses. Hence, it appeared that LIE had the potential to provide an anti-inflammatory response in sepsis.

The development of sepsis is accompanied by oxidative stress injury. Therefore, we investigated oxidative stress in the spleen tissue in the sepsis model. Mice were pre-treated with LIE at doses of 10, 20, and 40 mg/kg for 5 d, followed by intraperitoneal treatment with LPS (10 mg/kg). MDA content and CAT, SOD, and GSH-Px activity were determined by the kits purchased from the Nanjing Jiancheng Bioengineering Institute (China), following the instructions of the manufacturer. The relative index is provided in Fig. 3. Compared with the control group, LPS treatment disturbed the balance between oxidation and antioxidation. The MDA content was significantly increased (Fig. 3a), and the activity levels of CAT, SOD, and GSH-Px were all decreased (Figs. 3b–3d). Meanwhile in the LIE pre-treatment group, LIE decreased MDA content in a dose-dependent manner (Fig. 3a), and LIE significantly increased antioxidant capacity

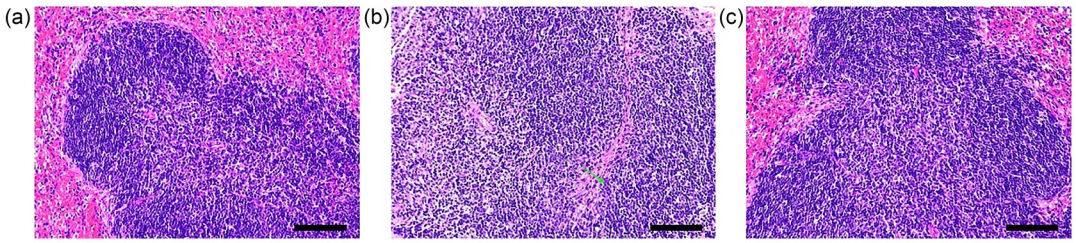

Fig. 1 Protective effect of liensinine (LIE) on spleen damage in lipopolysaccharide (LPS)-induced sepsis in mice. (a) Control group. (b) LPS-treated group (10 mg/kg). The green arrow indicates the white medullary area. (c) LIE (40 mg/kg)+ LPS-treated group. Scale bar=100 μm.

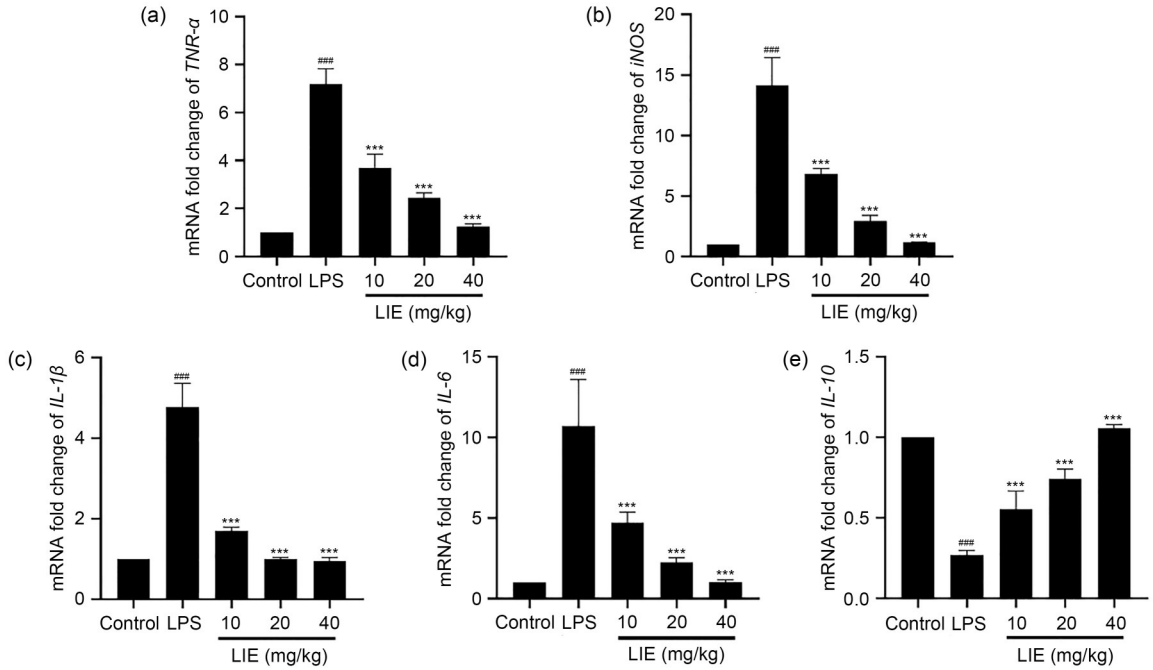

Fig. 2 Effects of liensinine (LIE) on spleen inflammatory response in lipopolysaccharide (LPS)-induced sepsis in mice. Pre-treatment of LIE at doses of 10, 20, and 40 mg/kg for 5 d was followed by intraperitoneal injection of LPS (10 mg/kg). The spleen tissues were collected for determination of the messenger RNA (mRNA) expression levels of inflammatory cytokines tumor necrosis factor-α (*TNF-α*) (a), inducible nitric oxide synthase (*iNOS*) (b), interleukin 1β (*IL-1β*) (c), *IL-6* (d), and *IL-10* (e) by quantitative real-time polymerase chain reaction (qPCR) analysis. Statistical significance was assessed by one-way analysis of variance (ANOVA). Data were represented as mean±standard deviation (SD), n=6. $^{###}$ P<0.001 vs. the control group; *** P<0.001 vs. the LPS group.

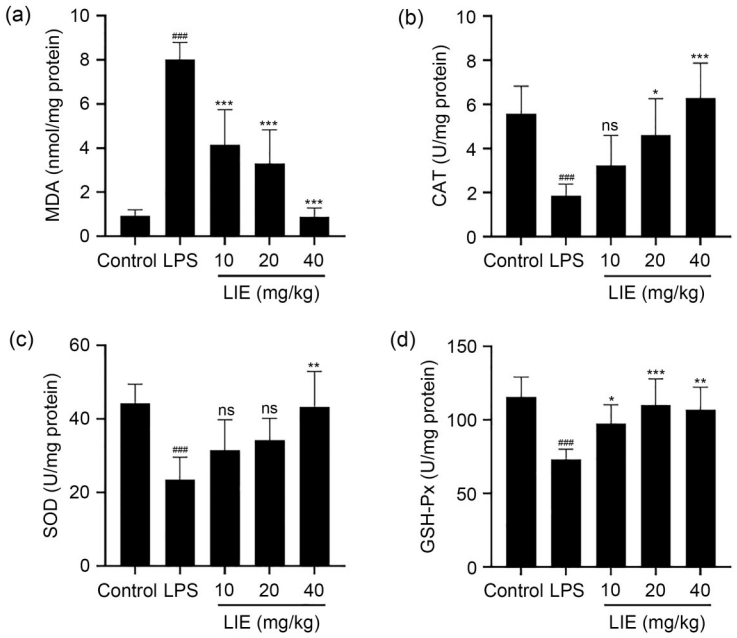

Fig. 3 Effects of liensinine (LIE) on spleen oxidative stress in lipopolysaccharide (LPS)-induced sepsis in mice. MDA content (a) and CAT (b), SOD (c), and GSH-Px (d) activity were determined. Statistical significance was assessed by one-way analysis of variance (ANOVA). Data were represented as mean±standard deviation (SD), n=6. $^{###}$ P<0.001 vs. the control group; * P<0.05, ** P<0.01, *** P<0.001, ^{ns} P>0.05 vs. the LPS group. MDA: malondialdehyde; CAT: catalase; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; ns: no significant difference.

at 20 and 40 mg/kg (Figs. 3b–3d). These results suggested that different doses of LIE can slow down sepsis-induced spleen damage by increasing the activity of antioxidant enzymes.

Patients with sepsis exhibit significant apoptosis in the spleen tissue, which is detrimental to the survival of the host, and prevention of this phenomenon may improve survival rates (Hotchkiss et al., 2001). LIE can significantly inhibit the apoptosis of spleen tissue induced by LPS. Spleen tissues were collected for terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) staining. The nucleus was stained blue by Hoechst 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 (Fig. 4). It was therefore clear that LIE could protect spleen tissue by reducing the degree of apoptosis caused by sepsis.

Since ancient times, traditional Chinese medicine has been extensively used to treat various diseases. Lotus seeds are used for both medicine and food, and are usually applied in folk medicine to treat tissue inflammation and cancer, or as an antiemetic. They mainly have antioxidative, anti-inflammatory, and antitumor biological activity (Arooj et al., 2021). Lotus seed extract exhibits high levels of antioxidant activity in vitro and in vivo, and the main active ingredients are alkaloids, saponins, and phenolics. LIE has been shown to inhibit heart disease, breast cancer, and

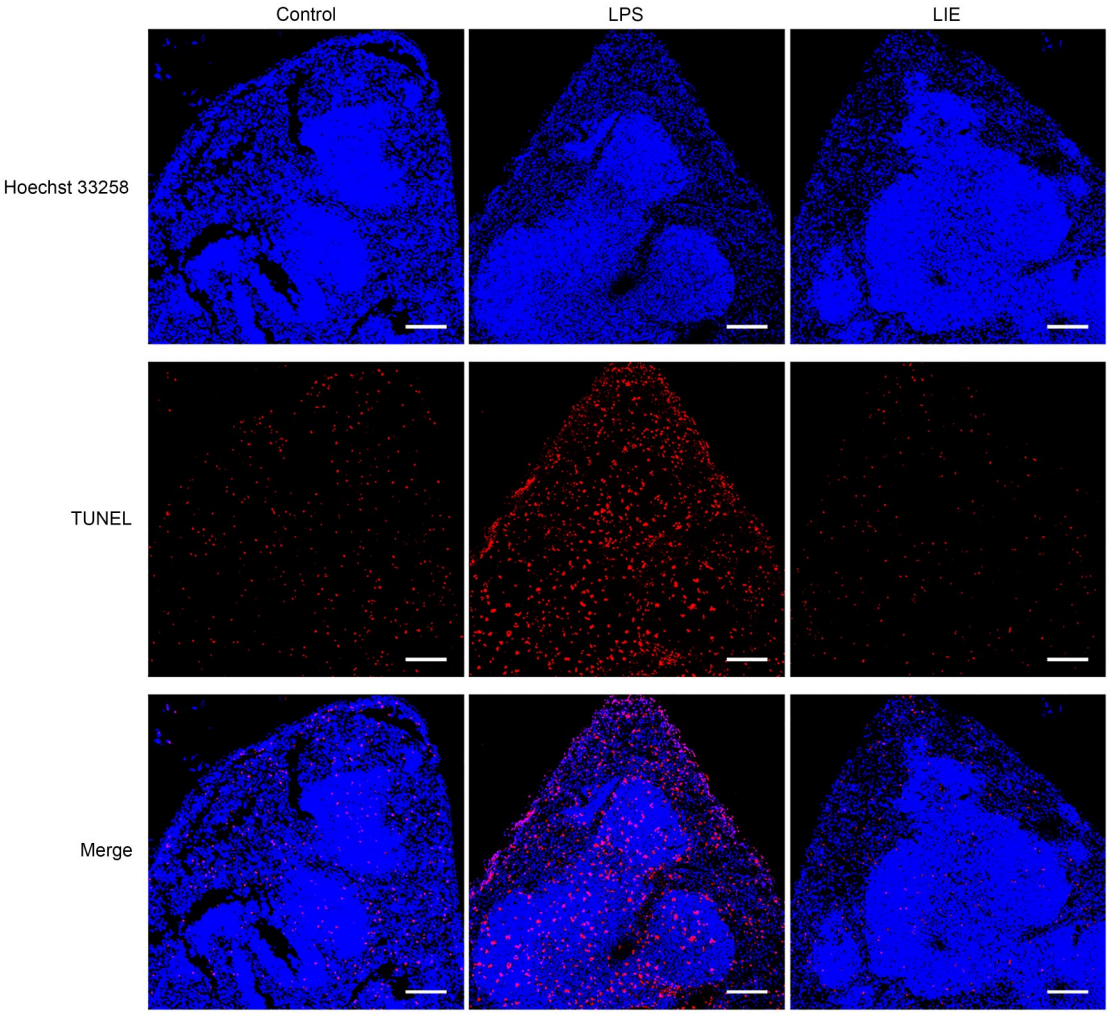

Fig. 4 Effects of pre-treatment with liensinine (LIE) on apoptosis in the spleen in lipopolysaccharide (LPS)-induced sepsis in mice. The nucleus was stained blue by Hoechst 33258 and apoptosis cells were stained red by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL). Scale bar=100 μm.

vascular inflammation by modulating the activity of various cells (Yu et al., 2016).

The pathogenesis of sepsis is due to over-activation of the innate immune system following infection by pathogenic microorganisms, which in turn triggers a systemic inflammatory syndrome. As a major component of the cytoderm of Gram-negative bacteria, LPS is a powerful inducer of pro-inflammatory immune responses and plays an important role in sepsis triggered by bacterial infections. Therefore, in our experiments, we used intraperitoneal injection of LPS to construct a sepsis model.

Firstly, in the H&E staining results, we found that LIE can effectively moderate the pathological changes in mouse spleen tissue caused by LPS stimulation and has a protective effect on the spleen. The inflammatory response is the key to organ damage in sepsis. iNOS was involved in the production of NO, an important pro-inflammatory mediator. In this experiment, we found that compared to the control group, LIE can significantly reduce the expression of pro-inflammatory cytokines in spleen tissue. It was evident that transcription levels of *TNF-α*, *IL-6*, *IL-1β*, and *iNOS* were dose-dependent and showed a clear downward trend, and the expression level of *IL-10* also showed a significant increase after administration, indicating that LIE can effectively reduce the inflammatory response in spleen tissue injury induced by LPS. In the in vitro experiment, emodin reduced IL-1, IL-6, and TNF-α expression in the sepsis model by affecting silent information regulator sirtuin 1 (SIRT1)-mediated phosphorylation of nuclear factor-κB (NF-κB) into the nucleus and inhibiting apoptosis, thereby alleviating lung injury (Liu et al., 2022). We speculated that the drug may inhibit production of TNF-α, IL-6, and IL-1β through the NF-κB signaling pathway; however, this hypothesis requires further research.

Neutrophils and phagocytes in an inflammatory state produce large amounts of reactive oxygen species (ROS), which enhance the triggering of oxidative stress and cause oxidative DNA damage. Excessive ROS also increases lipid peroxide MDA, which seriously damages cell structure and affects cell function. The main ROS responsible for oxidative damage include superoxide anion radicals ($\cdot O_2^-$), hydroxyl radicals (·OH), and non-radical oxidants such as hydrogen peroxide (H_2O_2) (Ali et al., 2020). SOD can effectively catalyze the $\cdot O_2^-$ to generate H_2O_2. CAT can degrade the oxidant H_2O_2 into water and oxygen that are harmless to the body. The presence of GSH-Px converts H_2O_2 to H_2O to form the antioxidant molecule glutathione. In non-alcoholic fatty liver disease, administration of LIE modulates metabolic disturbances in mice fed a high-fat diet, ameliorates high-fat diet-induced liver disease by mediating the nuclear factor-erythroid 2-related factor (Nrf2) and transforming growth factor-β (TGF-β)-activating kinase 1 (TAK1) signaling pathways, and mitigates the process of liver disease by improving the levels of oxidative stress and inflammatory response (Liang et al., 2022). The biochemical results from our experiment proved that LIE can significantly reduce oxidative stress by restoring the activity of antioxidant enzymes (SOD, CAT, and GSH-Px) in septic spleen tissue, while also reducing the content of lipid peroxidation marker MDA. Therefore, LIE does have the potential to alleviate the oxidative stress of splenic injury in sepsis.

Furthermore, uncontrolled apoptosis-induced changes in immune cells in sepsis are also considered to be a major cause of immunosuppression. Excessive apoptosis results in massive loss of immune cells, especially lymphocyte apoptosis, which is associated with a higher risk of secondary infection and poor outcomes (Cao et al., 2019). In this experiment, we found a significant reduction of apoptosis in the spleen tissue, indicating that LIE can reduce this type of damage caused by sepsis.

In general, sepsis-induced inflammation, oxidative stress, and apoptosis in spleen tissue can be alleviated by LIE treatment, so LIE has the potential to become a clinical drug for the treatment of sepsis. However, the specific mechanism still needs to be further explored.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Acknowledgments

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Author contributions

Hanyu WANG wrote and edited the manuscript; Yuanhao YANG managed and coordinated the planning and execution of research activities and provided research materials, reagents, and instruments; Xiao ZHANG, Yan WANG, and Hui FAN performed the experimental research and data analysis; Jinfeng SHI and Xuelian TAN performed the establishment of animal models; Baoshi XU and Jingchao QIANG contributed to the study design; Enzhuang PAN and Mingyi CHU performed data analysis; Zibo DONG was in charge of writing–review and editing; Jingquan DONG was in charge of validation and methodology. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Hanyu WANG, Yuanhao YANG, Xiao ZHANG, Yan WANG, Hui FAN, Jinfeng SHI, Xuelian TAN, Baoshi XU, Jingchao QIANG, Enzhuang PAN, Mingyi CHU, Zibo DONG, and Jingquan DONG declare that they have no conflict of interest.

All experiments were conducted according to the "Principles of Laboratory Animal Care" (National Institutes of Health (NIH) publication No. 85-23, revised 1985) and National Institute for Pharmaceutical Research and Development (NIPRD)'s standard operating procedures (No. NIPRD/05.03.05-1). All institutional and national guidelines for the care and use of laboratory animals were followed. The study was approved by Jiangsu Ocean University Animal Ethics Committee (Permission No. 2020220650), China.

References

Ali SS, Ahsan H, Zia MK, et al., 2020. Understanding oxidants and antioxidants: classical team with new players. *J Food Biochem*, 44(3):e13145. https://doi.org/10.1111/jfbc.13145

Arooj M, Imran S, Inam-ur-Raheem M, et al., 2021. Lotus seeds (*Nelumbinis semen*) as an emerging therapeutic seed: a comprehensive review. *Food Sci Nutr*, 9(7):3971-3987. https://doi.org/10.1002/fsn3.2313

Cao C, Yu MM, Chai YF, 2019. Pathological alteration and therapeutic implications of sepsis-induced immune cell apoptosis. *Cell Death Dis*, 10(10):782. https://doi.org/10.1038/s41419-019-2015-1

Hotchkiss RS, Tinsley KW, Swanson PE, et al., 2001. Sepsis-induced apoptosis causes progressive profound depletion of B and $CD4^+$ T lymphocytes in humans. *J Immunol*, 166(11):6952-6963. https://doi.org/10.4049/jimmunol.166.11.6952

Kanhutu K, Jones P, Cheng AC, et al., 2017. Spleen Australia guidelines for the prevention of sepsis in patients with asplenia and hyposplenism in Australia and New Zealand. *Intern Med J*, 47(8):848-855. https://doi.org/10.1111/imj.13348

Kaur S, Allan SM, Al-Ahmady ZS, 2022. Re-directing nanomedicines to the spleen: a potential technology for peripheral immunomodulation. *J Control Release*, 350:60-79. https://doi.org/10.1016/j.jconrel.2022.04.005

Kim SY, Moon GS, 2015. Photoprotective effect of lotus (*Nelumbo nucifera* Gaertn.) seed tea against UVB irradiation. *Prev Nutr Food Sci*, 20(3):162-168. https://doi.org/10.3746/pnf.2015.20.3.162

Kumar S, Gupta E, Kaushik S, et al., 2018. Evaluation of oxidative stress and antioxidant status: correlation with the severity of sepsis. *Scand J Immunol*, 87(4):e12653. https://doi.org/10.1111/sji.12653

Liang LP, Ye SW, Jiang RL, et al., 2022. Liensinine alleviates high fat diet (HFD)-induced non-alcoholic fatty liver disease (NAFLD) through suppressing oxidative stress and inflammation via regulating TAK1/AMPK signaling. *Int Immunopharmacol*, 104:108306. https://doi.org/10.1016/j.intimp.2021.108306

Liu FJ, Gu TJ, Wei DY, 2022. Emodin alleviates sepsis-mediated lung injury via inhibition and reduction of NF-κB and HMGB1 pathways mediated by SIRT1. *Kaohsiung J Med Sci*, 38(3):253-260. https://doi.org/10.1002/kjm2.12476

Manogaran P, Beeraka NM, Padma VV, 2019. The cytoprotective and anti-cancer potential of bisbenzylisoquinoline alkaloids from *Nelumbo nucifera*. *Curr Top Med Chem*, 19(32):2940-2957. https://doi.org/10.2174/1568026619666191116160908

Rudd KE, Johnson SC, Agesa KM, et al., 2020. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *Lancet*, 395(10219):200-211. https://doi.org/10.1016/s0140-6736(19)32989-7

Thompson PN, Cho E, Blumenstock FA, et al., 1992. Liver and spleen phagocytic depression after peripheral ischemia and reperfusion. *Am J Surg*, 164(3):248-253. https://doi.org/10.1016/s0002-9610(05)81080-3

Yu YY, Sun SN, Wang SF, et al., 2016. Liensinine- and neferine-induced cardiotoxicity in primary neonatal rat cardiomyocytes and human-induced pluripotent stem cell-derived cardiomyocytes. *Int J Mol Sci*, 17(2):186. https://doi.org/10.3390/ijms17020186

Zhu MZ, Liu T, Guo MQ, 2016. Current advances in the metabolomics study on lotus seeds. *Front Plant Sci*, 7:891. https://doi.org/10.3389/fpls.2016.00891

Supplementary information

Materials and methods