Zhang *et al. Thrombosis Journal* (2025) 23:51
https://doi.org/10.1186/s12959-025-00739-3

Thrombosis Journal

RESEARCH Open Access

Liquiritin mitigates lower extremity deep vein thrombosis by inhibiting inflammation and oxidative stress via the NF-κB signaling pathway

Jiacheng Zhang[1], Nan Wang[1], Tianyou Xin[2], Xiaojun Zhu[3], Shengkun Lang[4*] and Xin Ge[1,5*]

Abstract

Background Lower extremity deep vein thrombosis (LEDVT) is a common vascular disease, with its pathogenesis mainly involving inflammatory responses and oxidative stress. Liquiritin (LIQ) is a flavonoid that exhibits pharmacological effects such as anti-inflammatory and antioxidant properties. This study aimed to investigate the role of LIQ in LEDVT and its potential mechanisms.

Methods We established an LEDVT model in mice by ligating the inferior vena cava (IVC) and performed in vitro experiments by stimulating human umbilical vein endothelial cells (HUVECs) with IL-1β (10 ng/mL) to simulate endothelial cell injury.

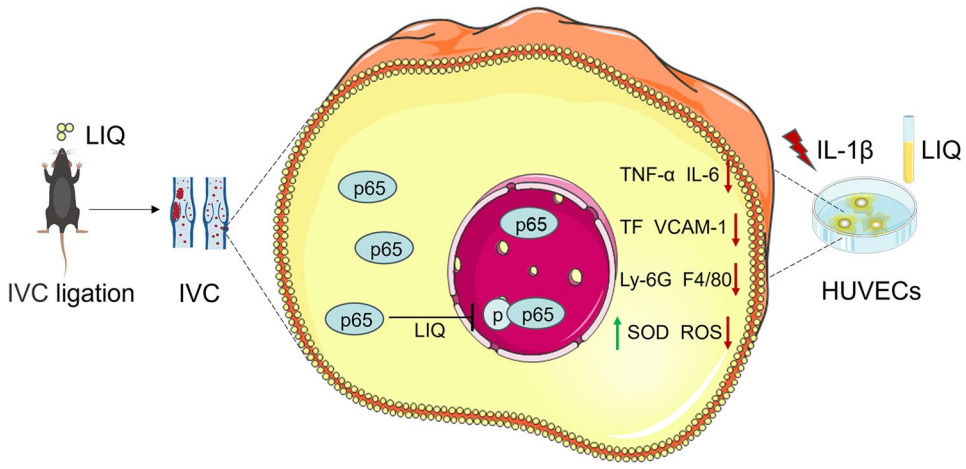
Results We found that LIQ significantly reduced the size and weight of thrombi and decreased the concentrations of inflammatory factors TNF-α and IL-6 in the IVC of LEDVT mice. Furthermore, LIQ inhibited the secretion of prothrombotic mediators such as tissue factor (TF) and vascular cell adhesion molecule-1 (VCAM-1). Administration of LIQ resulted in a notable reduction in immune inflammatory cells in the IVC of LEDVT mice. LIQ also demonstrated antioxidant properties, as the treatment of LIQ enhanced SOD activity and restored ROS levels to normal in the IVC. Similarly, LIQ reduced the formation of inflammatory factors and the secretion of prothrombotic mediators by HUVECs while inhibiting oxidative stress in HUVECs. Finally, LIQ effectively suppressed the levels of phosphorylated p65 in both the IVC and HUVECs.

Conclusions LIQ reduces inflammatory responses and oxidative stress in LEDVT by inhibiting the NF-κB signaling pathway. This finding provides new insights into the prevention and treatment of LEDVT.

*Correspondence:
Shengkun Lang
18600414110@163.com
Xin Ge
gexin2021@suda.edu.cn

Full list of author information is available at the end of the article

Graphical Abstract



Keywords Lower extremity deep vein thrombosis, Liquiritin, Inflammation, Oxidative stress,

Introduction

Deep vein thrombosis (DVT) poses a significant challenge to global public health, typically resulting from abnormal blood clotting in the deep veins, most commonly occurring in the lower extremities, known as lower extremity deep vein thrombosis (LEDVT). DVT leads to symptoms such as local pain, swelling, and fever; more importantly, if a thrombus dislodges and travels to the lungs, it may cause pulmonary embolism (PE) [1, 2]. Inflammatory responses play a crucial role in the pathogenesis of DVT. Inflammation can lead to endothelial dysfunction, increasing the risk of thrombosis [3]. Previous studies have shown that DVT occurs following the activation of inflammasomes and the secretion of IL-1β, marking a crossroads between inflammation and coagulation disorders [4]. Additionally, Liu et al. have indicated that oxidative stress also plays a critical role in DVT [5]. Therefore, researching anti-inflammatory and antioxidant strategies may be one of the fundamental approaches to addressing thrombosis.

Liquiritin (LIQ) is a flavonoid isolated from licorice, known for its various pharmacological effects, including cardiovascular protection, anti-tumor, and anti-inflammatory properties [6]. Zhai et al. demonstrated that LIQ alleviates rheumatoid arthritis by inhibiting inflammation, blocking MAPK signaling, and suppressing angiogenesis [7]. Mou et al. showed that LIQ reduces inflammation and oxidative stress, and inhibits apoptosis, thereby alleviating cardiac dysfunction in septic cardiomyopathy mice [8]. Some studies have reported that LIQ may be a potential drug for treating diabetic vascular complications [9]. Furthermore, traditional Chinese medicine formulas such as Banxia Baizhu Tianma Decoction have been noted for their protective effects on venous endothelial cells, with LIQ identified as one of the active components that exert antioxidant and anti-inflammatory effects [10]. Similarly, Wen Dan Decoction, which contains LIQ as an effective ingredient, has been found to alleviate palmitic acid-induced endothelial cell injury [11]. Therefore, we speculate that LIQ may become an effective drug for the treatment of LEDVT.

NF-κB is a key regulator of inflammatory responses, capable of activating the expression of various pro-inflammatory genes, such as TNF-α, IL-1β, and adhesion molecules. This activation promotes the accumulation of leukocytes at the site of damaged blood vessels, exacerbating the inflammatory response and facilitating the formation and development of thrombosis [12]. Some studies have reported that LIQ alleviates dysfunction in human umbilical vein endothelial cells (HUVECs) induced by advanced glycation end products through the RAGE/NF-κB pathway [9]. The team led by Tan found that a formula containing LIQ, known as Gegen Qinlian Decoction, reduces inflammatory thrombosis in mice by inhibiting the HMGB1/NF-κB/NLRP3 signaling pathway [13]. Furthermore, in the microenvironment of thrombus retention, excessive inflammatory responses may lead to oxidative stress [14]. The evidence suggests that LIQ may prevent endothelial cell damage by inhibiting inflammation and oxidative stress, ultimately reducing the likelihood of lower extremity deep vein thrombosis. This effect may involve the regulation of the NF-κB signaling pathway.

Therefore, this study aims to investigate the effects of LIQ on inflammatory factors, pro-thrombotic factors, and oxidative stress-related indicators in the inferior vena cava (IVC) of LEDVT mice by constructing an

animal model through IVC ligation. We will also explore the protective role of LIQ mediated by the NF-κB signaling pathway in LEDVT. Additionally, we will use IL-1β to induce endothelial cell injury in HUVECs for in vitro experiments. Our research provides new insights for future foundational studies on vascular diseases.

Materials and methods

Network pharmacology analysis

We initiated our study by retrieving the standard SMILES format for Liquiritin from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Thereafter, the obtained SMILES format was submitted to the SwissTargetPrediction database (http://www.swisstargetprediction.ch/) to identify potential targets of Liquiritin under the species setting of "Homo sapiens". To refine our search, we employed the GeneCards database (https://www.genecards.org/) to identify targets associated with lower extremity deep vein thrombosis. The overlapping targets were visualized using a Venn diagram generated by Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html). Subsequently, the shared targets were uploaded into the STRING database (https://string-db.org/) to construct a Protein–Protein Interaction (PPI) network. To further elucidate the functions and pathways of the target genes, we conducted a Gene Ontology (GO) enrichment analysis based on the predicted shared targets. Additionally, a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using Metascape (https://metascape.org/). These analyses colle ctively facilitated the exploration of Liquiritin's potential roles and mechanisms of action.

Animal model and treatment

In this experiment, healthy male C57BL/6 mice aged 8 weeks were randomly divided into four groups: (a) Sham; (b) LEDVT; (c) LEDVT + 40 mg/kg LIQ; (d) LEDVT + 80 mg/kg LIQ. The LEDVT mouse model was established using the IVC ligation method, with the following detailed steps: after anesthetizing the mice, a midline abdominal incision was made, and the IVC was separated from the aorta. A polypropylene suture was used to ligate the branches of the IVC. After ligating the IVC on the spacer, the spacer was gently removed, and the abdominal incision was sutured. The LIQ (CAS: 551-15-5, L886004, Macklin, China) groups received daily gavage of different doses of LIQ (40 mg/kg and 80 mg/kg) starting one week before modeling [15]. On the final day of gavage, the ligation surgery was performed, and two days later, IVC tissue was collected for subsequent experiments.

Cell culture and treatment

HUVECs were used for in vitro experiments and were purchased from iCell (iCell-h110, iCell, China). First, the cells were treated with different concentrations of LIQ (0, 5, 10, 20, 50, 100 μM) for 1 h. The inhibitory rates of LIQ on cells were measured. We selected the two highest concentrations (10 and 20 μM) that did not significantly affect cell viability for further experiments. Next, the cells were pre-treated with the determined concentrations of LIQ for 1 h, followed by treatment with IL-1β (10 ng/mL) for 6 h for subsequent analyses. The cell experiment groups were as follows: (a) Control; (b) IL-1β; (c) IL-1β + 10 μM LIQ; (d) IL-1β + 20 μM LIQ.

To further explore whether the effects of LIQ are mediated through the NF-κB pathway, we co-treated IL-1β-stimulated HUVECs with an NF-κB pathway activator (HY-134476, MCE, USA) and 20 μM LIQ.

HE staining

To observe the formation of thrombi in the IVC, we performed HE staining on the IVC tissue. The specific steps were as follows: after deparaffinization and rehydration of the sections, they were stained with hematoxylin for 5 min and eosin for 3 min. The slices were then permeabilized and mounted for subsequent observation.

ELISA assay

To determine the levels of inflammatory factors and SOD in the IVC tissue and HUVECs, samples were collected and centrifuged, following the instructions provided in the kits. The mouse TNF-α ELISA Kit (EK282, Lianke, China), mouse IL-6 ELISA Kit (EK206, Lianke, China), human TNF-α ELISA Kit (EK182, Lianke, China), human IL-6 ELISA Kit (EH0201, Wuhan Fine Biotech, China) and the SOD assay kit (A001, Nanjing Jiancheng, China) were purchased for the experiments. Specific procedures were conducted according to the manufacturers' instructions.

DHE staining

The DHE assay kit (S0063, Beyotime, China) was applied in this study. The DHE reagent was diluted at a ratio of 1:100 and sections were incubated at 37 °C in the dark for 30 min. After adding anti-fade reagent, the staining results were observed under a fluorescence microscope. The intensity of the red fluorescence indicated a significant accumulation of ROS in the mouse IVC.

Immunohistochemical (IHC) staining

Ly-6G and F4/80 are important markers for immune cells. We used IHC staining to determine the quantity of Ly-6G- or F4/80- positive immune cells in the IVC tissue. Antibody against Ly-6G (0809 – 11, HuaBio, China) and antibody against F4/80 (28463-1-AP, Proteintech, China) were obtained. The Anti-Ly-6G was diluted at 1:100 and the Anti-F4/80 was diluted at 1:4000. Then, the diluted antibodies were applied to completely cover the tissue,

and the samples were incubated overnight at 4 °C in a humidified chamber.

Immunofluorescence detection

To determine the levels of p-p65, p65, VCAM1, and tissue factor (TF) in the IVC tissue and HUVECs, we performed immunofluorescence detection. Antibody against p-p65 (AF2006, Affinity, China), antibody against p65 (A19653, Abclonal, China), antibody against VCAM1 (381014, Zenbio, China), and antibody against TF (R381361, Zenbio, China) were acquired. In brief, tissue sections were deparaffinized, subjected to antigen retrieval, and blocked with 1% BSA. The corresponding primary antibody was diluted and incubated overnight at 4 °C in a humid chamber. Under light-protected conditions, a fluorescent secondary antibody was added and incubated at room temperature for 60 min. Finally, DAPI was added for nuclear localization. Additionally, HUVEC staining required incubation with 0.1% Triton X-100 at room temperature for 30 min, without the need for antigen retrieval. Immunofluorescence intensity was quantified by ImageJ and results were normalized to the cell number.

Real-time PCR

After extracting RNA from the mouse IVC tissue and HUVECs, we measured the RNA concentration. The obtained RNA samples were reverse transcribed to obtain the corresponding cDNA. Fluorescent quantification was performed using the Exicycler™ 96 system, and the data were analyzed using the $2^{-\triangle\triangle CT}$ method. The relevant primer sequences are as follows: homo *TF* F: GAATGTGACCGTAGAAGATG R: TAACTGTTCGGGAGGGA; homo *VCAM1* F: GGGAATCTACAGCACCT R: CACAGCCCATGACACTA; mus *TF* F: GGAGGAGCCGCCATTTAC R: AGACTTGCCGCAGGGTGA; mus *VCAM1* F: TTGTGGAAATGTGCCCGAAAC R: TGTGCCTGGCGGATGGTGT.

Western blot

Western blotting was used to detect the specific expression levels of a molecule. The clear steps were as follows: lysis buffer supplemented with protease inhibitor buffer and PMSF was added to the IVC tissue or HUVECs to extract the protein. After quantifying the protein, an equal amount of protein was subjected to SDS-PAGE. Upon completion of SDS-PAGE, the proteins were transferred to a polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was then immersed in blocking solution for 60 min. The membranes were incubated overnight at 4 °C in a solution containing p65 antibody (A19653, ABclonal, China), p-p65 antibody (AP1294, ABclonal, China), and β-actin antibody (sc-47778, Santa Cruz, USA). Finally, the PVDF membranes were incubated at room temperature with the relevant secondary antibodies for 45 min, followed by ECL substrate chemiluminescence detection.

Data analysis

The data were presented as mean ± standard deviation (SD). GraphPad Prism 9 was used for data analysis. Shapiro–Wilk test was used to evaluate the normality distribution of the data. For the data with normal distribution, comparisons between groups were analyzed using one-way ANOVA followed by Tukey post-hoc test. For data with a non-normal distribution, Kruskal-Wallis test followed by post-hoc comparison was used. Additionally, Statistical significance was set at $p < 0.05$.

Results

Pharmacology prediction of liquiritin treatment of LEDVT

To investigate the therapeutic mechanism of Liquiritin on lower extremity deep vein thrombosis, we employed network pharmacology to elucidate the underlying effect targets. As illustrated in the Venn diagram, 46 overlapping genes were named candidate targets for Liquiritin in lower extremity deep vein thrombosis (Fig. 1A). Subsequently, we constructed a PPI network of these 46 targets (Fig. 1B). GO enrichment analysis was conducted to explore the probable biological process (BP), molecular function (MF), and cellular component (CC) terms associated with the candidate targets of Liquiritin-lower extremity deep vein thrombosis. The top 8 enriched terms in the GO were visualized, which included response to oxidative stress, regulation of inflammatory response, response to reactive oxygen species (Fig. 1C). In addition, we analyzed KEGG pathway enrichment for the 46 candidate targets (Fig. 1D-E). The results showed that the targets were associated with signaling pathways such as TNF-α and IL-17. The results indicated that the target is associated with signaling pathways such as TNF-α and IL-17. TNF-α and IL-17 are upstream of the NF-κB signaling pathway, suggesting that Liquiritin may regulate the disease progression of Lower extremity deep vein thrombosis through the NF-κB signaling pathway.

LIQ attenuates LEDVT in mice

We established the LEDVT model in mice using IVC ligation, with LIQ administered via oral gavage (Fig. 2A). First, thrombus formation in IVC was evaluated. The results indicated that large thrombi formed in IVC of LEDVT mice while treatment with LIQ significantly reduced the size of the thrombi (Fig. 2B). Additionally, we further confirmed the significant effect of administering 80 mg/kg of LIQ in reducing thrombus length and weight by measuring these parameters in IVC of the mice. However, compared to the LEDVT group, the 40 mg/kg dose of LIQ did not show a significant effect on

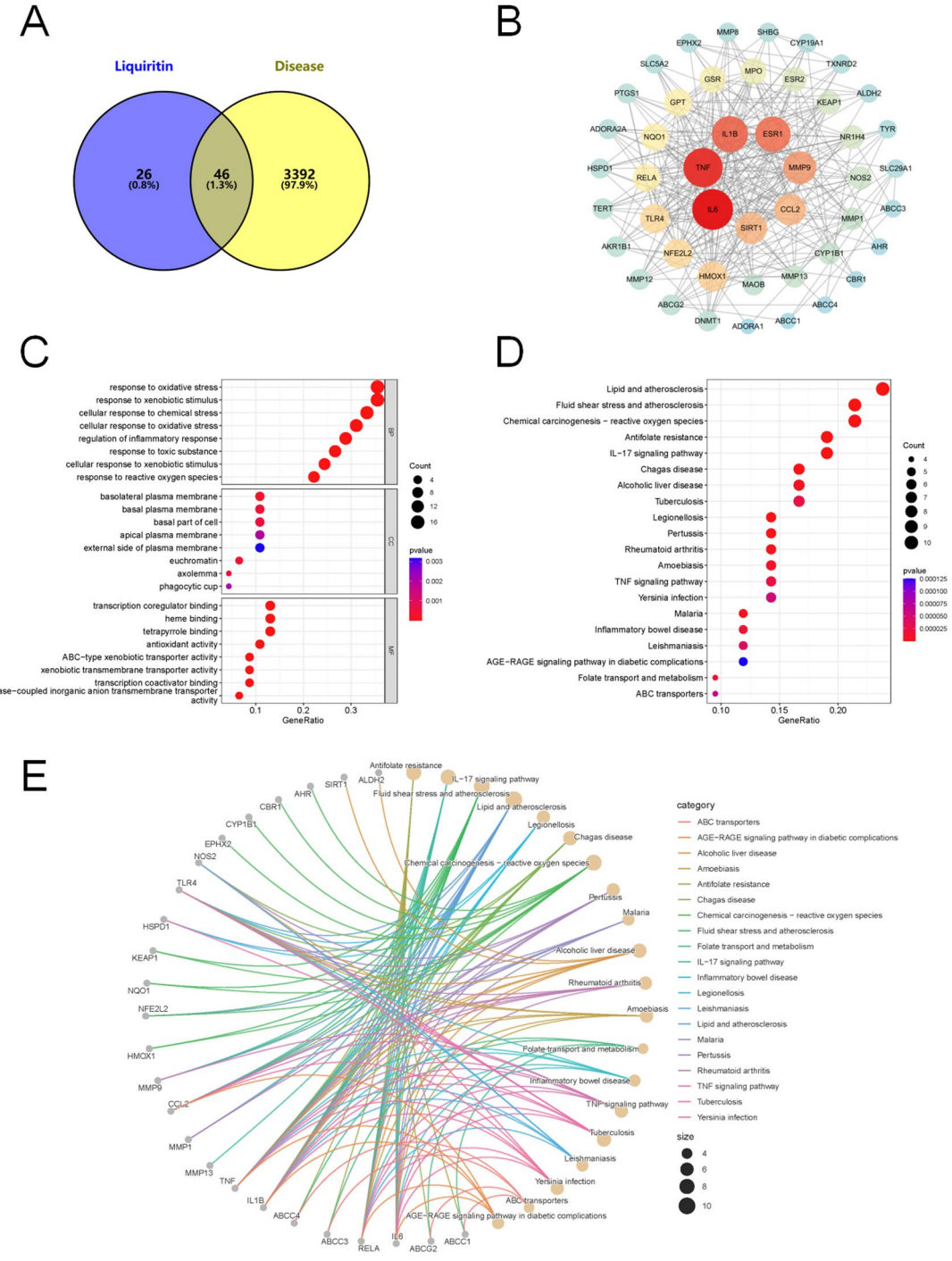


Fig. 1 Network pharmacology prediction of LIQ treatment of lower extremity deep vein thrombosis (LEDVT). (**A**) 46 common targets of LIQ and LEDVT; (**B**) PPI network of common targets; (**C**) The targets are studied for biological processes (BP), cellular components (CC), and molecular functions (MF). The top 8 GO categories were displayed; (**D**-**E**) KEGG enrichment analysis

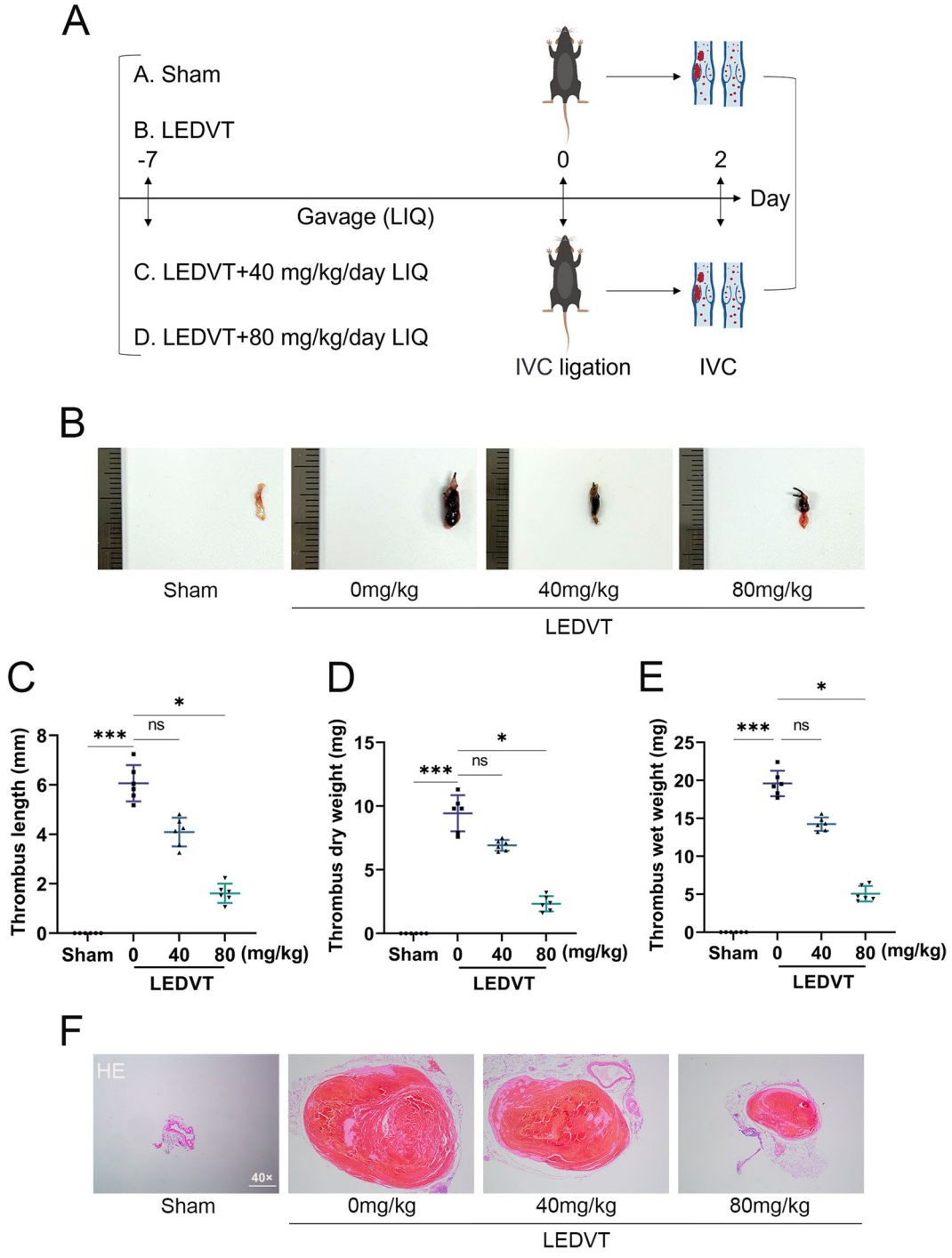


Fig. 2 LIQ attenuates LEDVT in mice. (**A**) Construction of an LEDVT mouse model using inferior vena cava (IVC) stenosis and the LIQ administration process; (**B**) Representative images of thrombus; (**C**) Thrombus length; (**D**) Thrombus dry weight; (**E**) Thrombus wet weight; (**F**) Thrombosis was examined in IVC by Hematoxylin–eosin (HE) staining (scale bar = 500 μm). Data were presented as means ± s.d

reducing thrombus length and weight (Fig. 2C-E). Finally, HE staining results showed that in the LEDVT group, a large amount of thrombus formed in IVC, with the vessel wall exhibiting a loose structure accompanied by edema and irregular intima. These pathological alterations associated with LEDVT were ameliorated by treatment with 80 mg/kg of LIQ (Fig. 2F).

LIQ inhibits inflammation and oxidative stress in the IVC tissue

Numerous studies have indicated that inflammation and oxidative stress induced by excessive inflammation are the main culprits of LEDVT. Although LIQ is an effective anti-inflammatory and antioxidant herbal medicine, its mechanisms of action in LEDVT remain unexplored. Thus, we aimed to investigate the mechanisms by which LIQ alleviates thrombus formation in a murine LEDVT model. First, we measured the concentrations of TNF-α in IVC of the mice. The results showed a dramatic increase in TNF-α levels in IVC of LEDVT mice, which significantly decreased following treatment with 80 mg/kg of LIQ (Fig. 3A). Additionally, IL-6 is a key pro-inflammatory cytokine that stimulates the synthesis of coagulation factors. We also measured the concentrations of IL-6 in the IVC, and the results indicated a significant upregulation of IL-6 in LEDVT mice. After treatment with 80 mg/kg of LIQ, IL-6 levels in the mice approached normal levels (Fig. 3B). To determine whether LIQ has antioxidant effects in IVC of LEDVT mice, we assessed the activity of SOD and the levels of ROS in IVC (Fig. 3C-E). The results showed that treatment with 80 mg/kg of LIQ significantly markedly reduced ROS levels and increased SOD activity in IVC of LEDVT mice. In summary, these results suggest that LIQ alleviates LEDVT in mice through its anti-inflammatory and antioxidant actions.

LIQ downregulates the secretion of prothrombotic mediators

TF is a key initiator of the extrinsic coagulation pathway. When blood vessels are damaged, TF is exposed to the bloodstream and binds to coagulation factors, thereby activating the coagulation cascade and promoting thrombus formation. Vascular Cell Adhesion Molecule-1 (VCAM-1) is an adhesion molecule located on the surface of endothelial cells, primarily involved in the rolling, adhesion, and migration of leukocytes. Therefore, we measured the levels of TF and VCAM-1 in IVC of the mice. Immunofluorescence results showed a significant increase in TF and VCAM-1 protein levels in IVC of LEDVT mice (Fig. 4A-D). Similarly, PCR results indicated a marked elevation of TF and VCAM-1 RNA levels in IVC of LEDVT mice (Fig. 4E-F). However, after treatment with 80 mg/kg of LIQ, the levels of TF and VCAM-1 in IVC of LEDVT mice approached normal levels (Fig. 4A-F).

LIQ reduces excessive accumulation of neutrophils and macrophages in the IVC of LEDVT mice

Next, we sequentially assessed the accumulation of neutrophils marked by Ly-6G and macrophages marked by F4/80 in the IVC of the mice. Immunohistochemical results showed elevated levels of Ly-6G and F4/80 in the IVC tissue of LEDVT mice, indicating a significant accumulation of neutrophils and macrophages. However, after gavage treatment with 80 mg/kg of LIQ, the number of inflammatory cells was significantly reduced (Fig. 5A-B). In summary, our results suggest that the upregulation of TF and VCAM-1 in LEDVT promotes the adhesion of leukocytes, ultimately leading to the infiltration of neutrophils and macrophages. Treatment with 80 mg/kg of LIQ alleviated these pathological changes.

LIQ alleviates IL-1β-induced damage in HUVECs

We used HUVECs for in vitro experiments to validate our findings. First, the results showed that treatment with 50 and 100 μM of LIQ for 1 h significantly impaired HUVEC viability (Fig. 6A). Furthermore, we evaluated the cytotoxicity of 20 μM LIQ on HUVECs. The results showed that pretreatment with LIQ did not significantly alter the levels of IL-6 and TF in HUVECs. Additionally, 20 μM LIQ did not affect the SOD activity in HUVECs. Moreover, Western blot analysis revealed that the protein levels of p65 and p-p65 remained unchanged with 20 μM LIQ treatment (Supplementary Fig. 1A-E).

Based on the above results, we selected non-cytotoxic concentrations of 10 and 20 μM LIQ for subsequent experiments. ELISA analysis demonstrated that IL-1β stimulation significantly elevated TNF-α and IL-6 levels in HUVECs. While 10 μM LIQ pretreatment only modestly reduced IL-6 without affecting TNF-α, 20 μM LIQ pretreatment significantly attenuated both inflammatory cytokines compared to the IL-1β-treated control group (Fig. 6B-C). At the transcriptional level, PCR results showed that IL-1β stimulation markedly upregulated TF and VCAM-1 mRNA expression, which was normalized by 20 μM LIQ pretreatment (Fig. 6D-E). These findings were further confirmed at the protein level by immunofluorescence, demonstrating that 20 μM LIQ significantly reduced IL-1β-induced TF and VCAM-1 expression in HUVECs (Fig. 6F-G). Furthermore, oxidative stress analysis revealed that 20 μM LIQ effectively mitigated IL-1β-induced ROS accumulation (Fig. 5H-I) while significantly enhancing SOD activity (Fig. 6J).

LIQ mitigates LEDVT by inhibiting the NF-κB pathway

In DVT, NF-κB is a classical pro-inflammatory pathway; however, there are no published reports

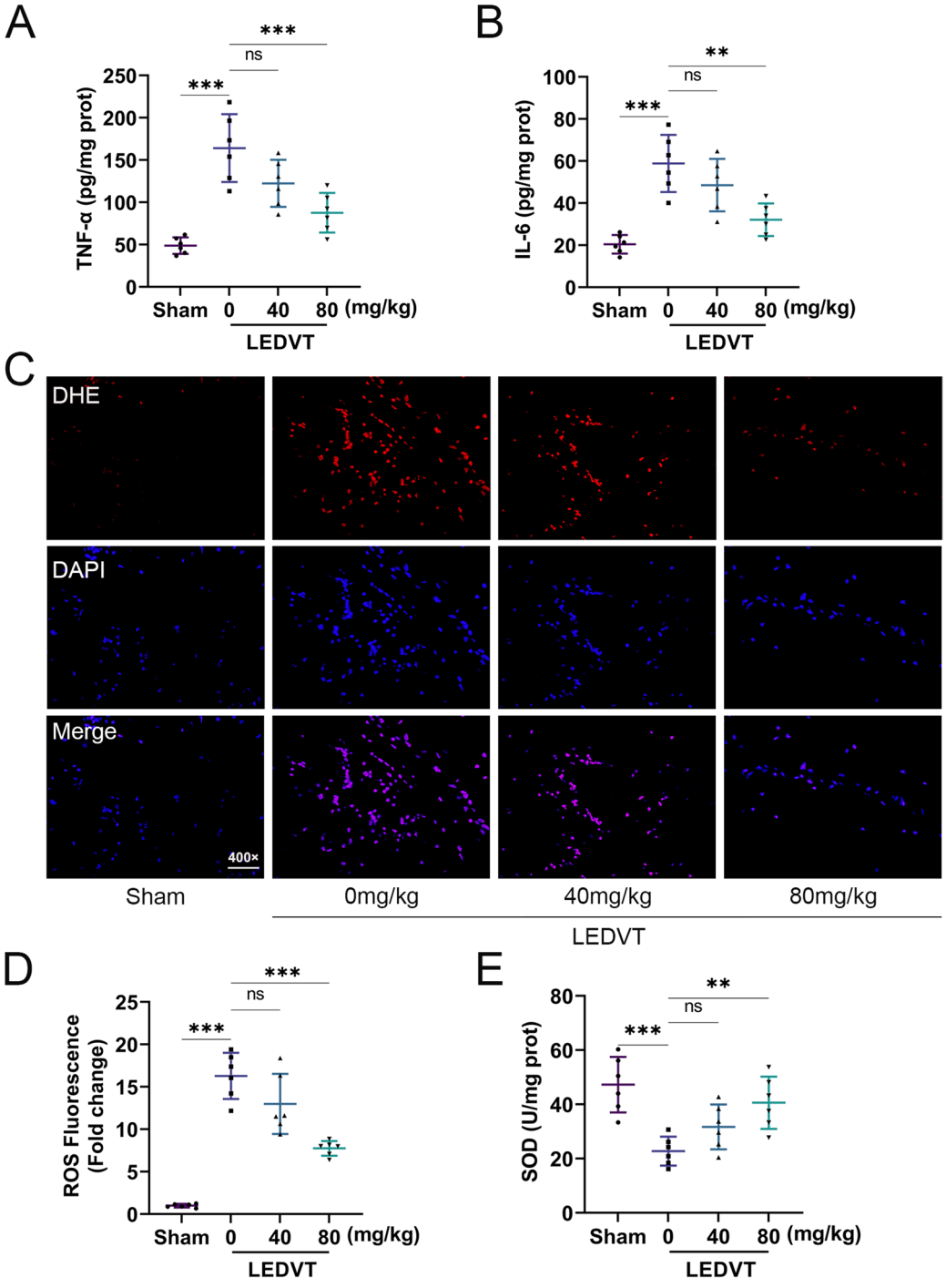


Fig. 3 LIQ inhibits inflammation and oxidative stress in the IVC tissue. (**A**) TNF-α concentrations in IVC were examined using ELISA; (**B**) IL-6 concentrations in IVC were examined using ELISA; (**C**) Dihydroethidium (DHE) staining in IVC (scale bar = 50 μm); (**D**) Quantitative analysis of fluorescence level; (**E**) SOD activity in IVC was examined using ELISA. Data were presented as means ± s.d

indicating that LIQ alleviates LEDVT through NF-κB. Immunofluorescence and Western blot results showed that pre-treatment with 20 μM LIQ significantly attenuated IL-1β-induced phosphorylation of p65 (p-p65) in HUVECs (Fig. 7A-D). Consistent with cellular observations, administration of 80 mg/kg of LIQ effectively suppressed p-p65 upregulation in IVC of LEDVT mice (Fig. 7E-J). Notably, total p65 levels remained unaltered in both models. To further investigate whether the effects of LIQ are mediated through the NF-κB pathway, we co-incubated IL-1β-stimulated HUVECs with an NF-κB pathway agonist alongside 20 μM LIQ. The

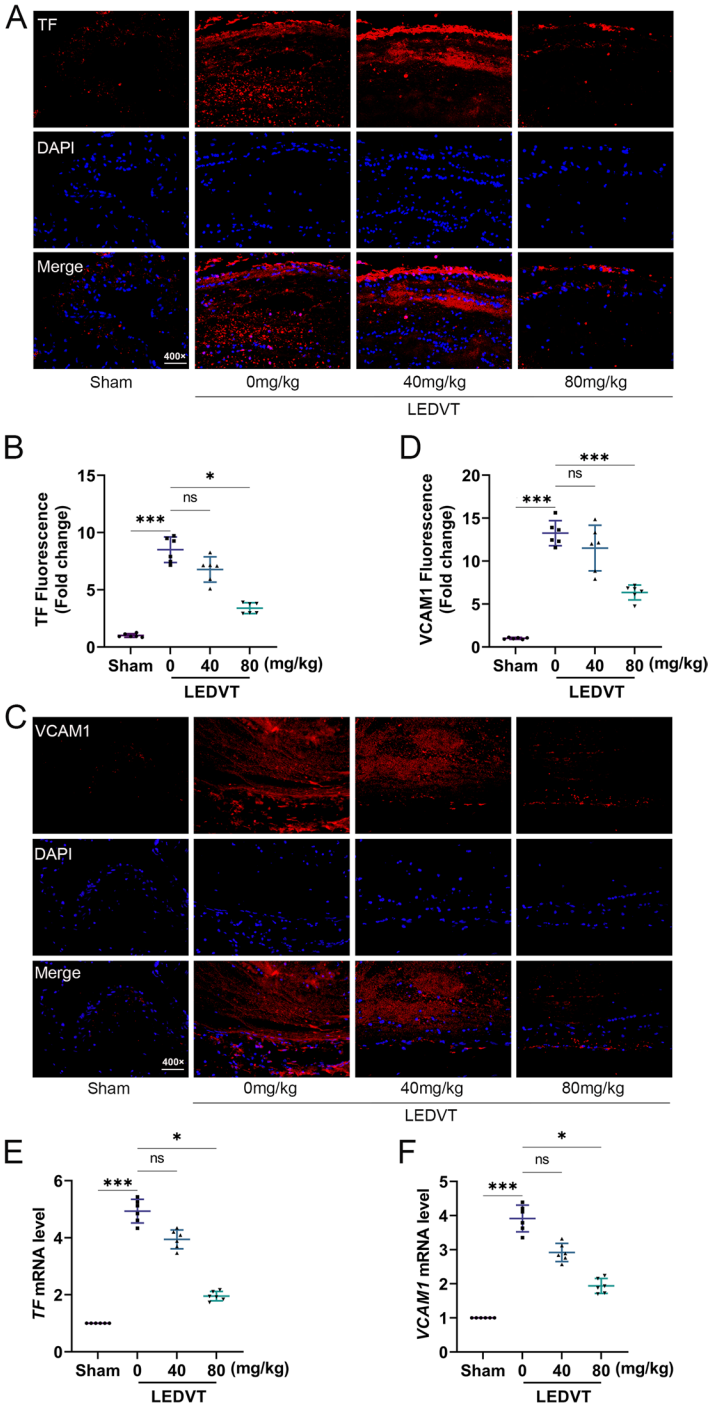


Fig. 4 LIQ downregulates the secretion of prothrombotic mediators. (**A**) The expression of TF in IVC was examined by immunofluorescence (scale bar = 50 μm); (**B**) Quantitative analysis of TF level; (**C**) The expression of VCAM1 in IVC was examined by immunofluorescence (scale bar = 50 μm); (**D**) Quantitative analysis of VCAM1 level; (**E**) *TF* mRNA levels in IVC; (**F**) *VCAM1* mRNA levels in IVC. Data were presented as means ± s.d

results demonstrated that the protective effect of LIQ on HUVECs was negated by the agonist, leading to significant upregulation of IL-6 and TF levels, while intracellular SOD activity decreased (Supplementary Fig. 2A-C). In summary, our data indicate that LIQ inhibits p-p65 in the NF-κB pathway, thereby dampening NF-κB-driven inflammation and thrombosis.

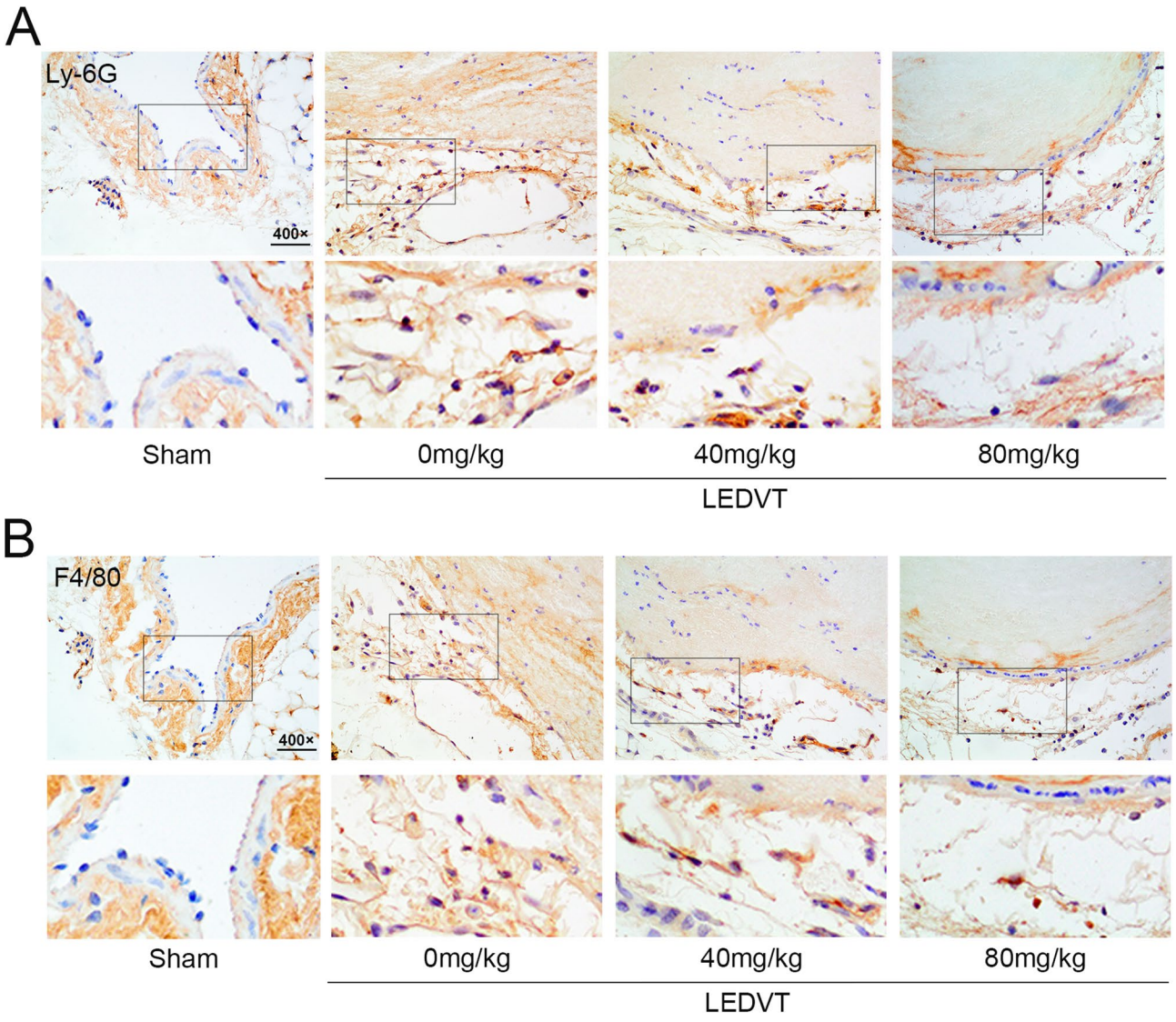


Fig. 5 LIQ reduces excessive accumulation of neutrophils and macrophages in IVC of LEDVT mice. (**A**) The positive expression of Ly-6G in the IVC was examined by immunohistochemistry (scale bar = 50 μm); (**B**) The positive expression of F4/80 in the IVC tissue was examined by immunohistochemistry (scale bar = 50 μm)

Discussion

Inflammation is considered a key factor in the occurrence of DVT. Yadav et al. found that neutralizing antibodies against IL-1β significantly reduced the risk of thrombosis in mice [16]. An article in *BLOOD* indicates that DVT occurs at the intersection of dysregulated inflammation and coagulation upon activation of the inflammasome and IL-1β under conditions of restricted venous flow [4]. Additionally, research has shown that IL-1β and TNF-α can further activate transcription factors, leading to the production of IL-6 [17]. Therefore, we chose to stimulate HUVECs with IL-1β to simulate endothelial cell injury. LIQ, a flavonoid compound extracted from licorice, possesses various pharmacological activities, including anti-inflammatory and immunomodulatory effects [18]. In DVT, elevated concentrations of TNF-α are believed to be associated with thrombosis. TNF-α activates endothelial cells, increases vascular permeability, and promotes the expression of pro-inflammatory factors, thereby exacerbating local inflammation. It may also enhance platelet aggregation, increasing the tendency for thrombosis [19]. Further, IL-6 is a critical pro-inflammatory cytokine that stimulates the synthesis of coagulation factors, increasing blood coagulability and promoting the formation of DVT [20]. Our findings clearly indicate that LIQ inhibits TNF-α and IL-6 levels in the IVC of LEDVT mice.

TF is an important coagulation factor that is exposed to the bloodstream when blood vessels are damaged or inflammation occurs, facilitating the generation of thrombin and accelerating thrombus formation. TF also

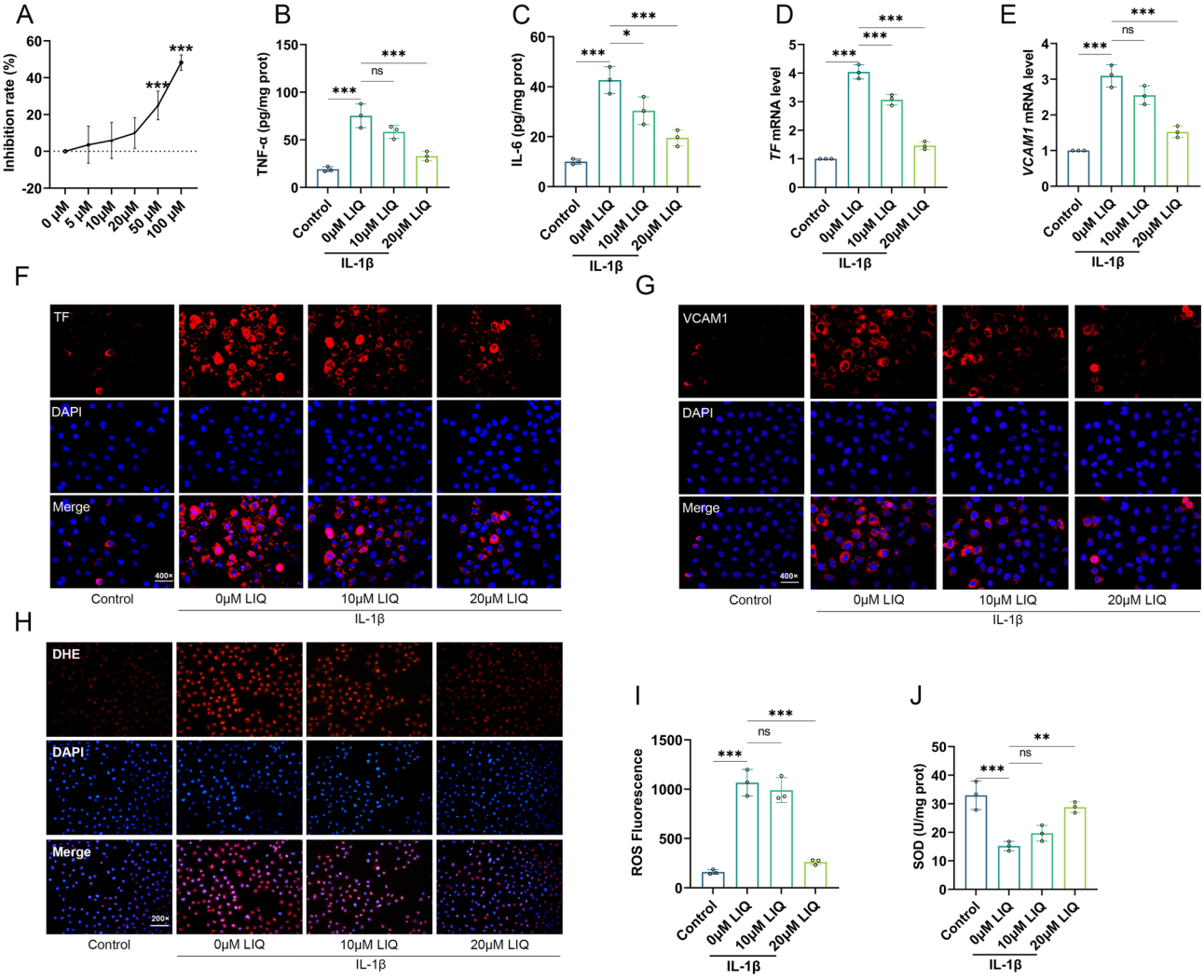


Fig. 6 LIQ alleviates IL-1β-induced damage in HUVECs. (**A**) Toxicity assessment of LIQ on HUVECs; (**B**) TNF-α concentrations in HUVECs were examined using ELISA; (**C**) IL-6 concentrations in HUVECs were examined using ELISA; (**D**) *TF* mRNA levels in HUVECs; (**E**) *VCAM1* mRNA levels in HUVECs; (**F**) The expression of TF in HUVECs was examined by immunofluorescence (scale bar = 50 μm); (**G**) The expression of VCAM1 in HUVECs was examined by immunofluorescence (scale bar = 50 μm); (**H**) DHE staining in HUVECs (scale bar = 100 μm); (**I**) Quantitative analysis of fluorescence level; (**J**) SOD activity in HUVECs was examined using ELISA. Data were presented as means ± s.d

plays a role in regulating inflammatory responses by activating neutrophils and macrophages, further exacerbating DVT [21]. Liang et al. demonstrated that increased expression of TF in vascular endothelial cells leads to endothelial dysfunction, promoting thrombosis [22]. VCAM-1 is primarily expressed in endothelial cells and promotes the binding of leukocytes to the vascular endothelium, enhancing the inflammatory response. Lu et al. indicated that lowering the levels of ICAM-1 and VCAM-1 proteins in venous endothelium is beneficial for alleviating thrombosis [23]. Fu et al. found that LIQ regulates vascular processes by blocking the MK/α6 signaling pathway [24]. Research indicates that LIQ inhibits oxidative stress and endoplasmic reticulum stress, promoting cellular angiogenesis and maintaining the integrity of the blood-brain barrier [25]. These findings suggest that LIQ plays a significant role in maintaining vascular integrity. However, its effects on the coagulation system in the treatment of venous thrombosis remain inconclusive. Surprisingly, our results demonstrate that LIQ effectively reduces the formation of thrombi in the IVC tissue, aligning with our hypothesis. In summary, during the pathogenesis of LEDVT, TNF-α and IL-6 act as early pro-inflammatory factors that activate the coagulation factor TF and promote leukocyte adhesion, ultimately leading to the infiltration of neutrophils and macrophages. The interactions among these factors form a complex network of inflammation and coagulation, resulting in endothelial damage and collectively promoting the occurrence of LEDVT. LIQ effectively alleviates the symptoms associated with this process. However, the

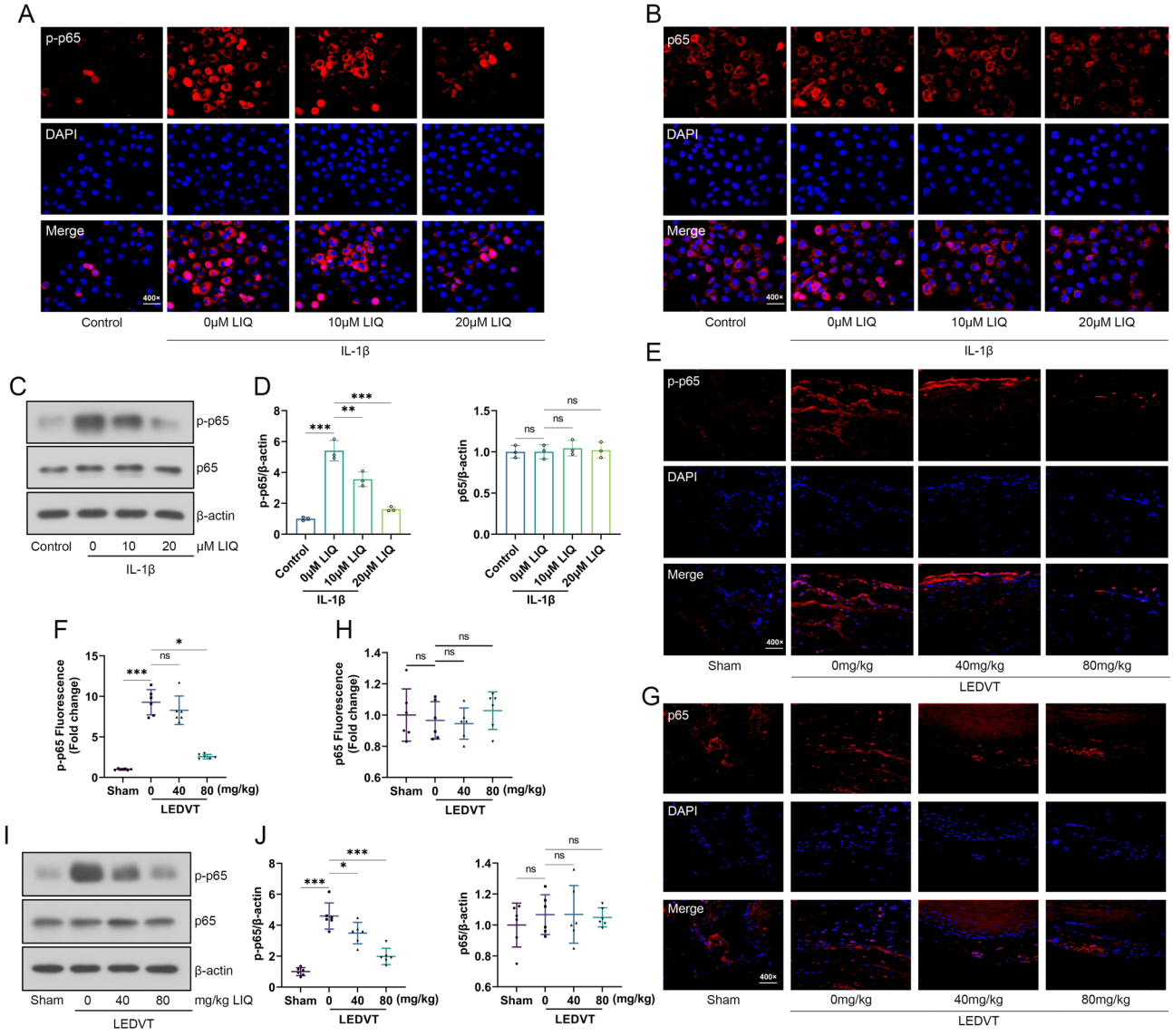


Fig. 7 LIQ mitigates LEDVT by inhibiting the NF-κB pathway. (**A**) Immunofluorescence staining of p-p65 in HUVECs (scale bar = 50 μm); (**B**) Immunofluorescence staining of p65 in HUVECs (scale bar = 50 μm); (**C**) Western blot was used to test p-p65 and p65 proteins in HUVECs; (**D**) Quantitative analysis of p-p65 and p65 expression in HUVECs; (**E**) Immunofluorescence staining of p-p65 in IVC (scale bar = 50 μm); (**F**) Quantitative analysis of p-p65 level; (**G**) Immunofluorescence staining of p65 in IVC (scale bar = 50 μm); (**H**) Quantitative analysis of p65 level; (**I**) Western blot was used to test p-p65 and p65 proteins in IVC; (**J**) Quantitative analysis of p-p65 and p65 expression in IVC. Data were presented as means ± s.d

study did not specifically explore the inhibitory effects of LIQ on platelets. Our research team will address this gap in future studies.

The occurrence of DVT is closely related to oxidative stress [26]. Previous studies have shown that the formation of DVT is influenced by ROS, which regulates coagulation and modulates effector cells such as platelets, endothelial cells, and neutrophils [27]. Effective management of oxidative stress can prevent abnormal platelet activation and the formation of new thrombi [28]. Research indicates that LIQ possesses antioxidant properties and attenuates lipopolysaccharide-induced cardiomyocyte injury by regulating oxidative stress [8]. Zhang et al. found that pre-treatment with LIQ reduces the generation of reactive oxygen species in HUVECs induced by advanced glycation end products (AGEs) [9]. Remarkably, our experimental results demonstrate that the antioxidant properties of LIQ can scavenge ROS in vivo, alleviating oxidative damage and thereby helping to maintain normal vascular function and blood fluidity. SOD is an important antioxidant enzyme that catalyzes the conversion of superoxide anions into hydrogen peroxide and oxygen, reducing intracellular oxidative stress. Meng et al. indicated that lowering SOD levels in DVT mice inhibits thrombus formation. Enhanced SOD activity protects vascular endothelial cells from oxidative

damage, thereby reducing the risk of DVT [29]. Our experiment is the first to demonstrate the role of LIQ in combating oxidative stress in LEDVT, suggesting that reducing oxidative stress may be an important strategy for the future treatment of LEDVT.

The NF-κB signaling pathway plays a critical role in inflammatory responses. Activation of this pathway leads to the expression of various pro-inflammatory factors, which promote thrombus formation. Specifically, inhibition of NF-κB lowers the levels of inflammatory factors such as IL-1β and TNF-α, thereby mitigating endothelial damage and platelet activation [12]. Huang et al. demonstrated that inhibiting the SIRT1/NF-κB pathway alleviates endothelial cell injury and neutrophil activation, thereby reducing DVT [30]. Cheng et al. found that activation of the TLR-4/NF-κB inflammatory signaling pathway exacerbates thrombus and inflammation in DVT rats [31]. LIQ reduces the release of pro-inflammatory factors by inhibiting NF-κB activation, which may be one of its important mechanisms for alleviating DVT. Interestingly, our experimental results indicate that a certain dose of LIQ significantly inhibits the upregulation of phosphorylated p65 protein expression. This aligns with previous findings that blocking NF-κB can partially obstruct the secretion of inflammatory factors, thereby inhibiting thrombus formation. Therefore, exploring and utilizing the NF-κB signaling pathway may offer new solutions for the prevention and treatment of thrombosis. Additionally, ROS also interacts with NF-κB signaling transduction and can interfere with the NF-κB pathway in various ways, affecting downstream targets [32]. However, the specific interactions between ROS and the NF-κB pathway were not explored in this experiment, which requires further investigations in future research.

Although our current experimental results indicate that LIQ has a protective effect on LEDVT mice under laboratory conditions, more randomized controlled trials are needed to verify its efficacy and safety in clinical applications. Moreover, it is necessary to investigate the specific mechanisms of LIQ in the NF-κB signaling pathway. In addition, exploring the combined effects of LIQ with other anticoagulant drugs or therapeutic approaches to enhance the treatment outcomes for DVT. If LIQ is validated in clinical studies, it may have significant potential as an adjunctive therapy for the prevention and treatment of DVT.

Conclusions

LIQ inhibits the activation of the NF-κB signaling pathway, reduces inflammation and oxidative stress in IVC, and thereby suppresses thrombus formation in IVC. This research provides an important theoretical basis for the application of natural medicines in thromboembolic diseases and is crucial for subsequent clinical studies.

Abbreviations

LEDVT	Lower extremity deep vein thrombosis
LIQ	Liquiritin
IVC	Inferior vena cava
HUVECs	Human umbilical vein endothelial cells
TF	Tissue factor
VCAM-1	Vascular cell adhesion molecule-1
DVT	Deep vein thrombosis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12959-025-00739-3.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

JC.Z.: Investigation, Formal analysis, Writing – original draft N.W.: Data curation, Formal analysis, Writing – original draft T.Y.X.: Validation, Project administration X.J.Z.: Visualization, Data curation. SK.L.: Conceptualization, Resources, Writing – review & editing X.G.: Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Funding

This research was supported by the Top Talent Support Program for young and middle-aged people of Wuxi Health Committee (grant No. BJ2023108), Major Project of Wuxi Specialized Program for the Development of Traditional Chinese Medicine Science and Technology(grant No.ZYZD202401) and the Binhu Advanced Medical Expert Team Program (grant No. BH202401).

Data availability

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

Declarations

Ethical approval

The animal experiment protocol received approval from the Ethics Committee of Wuxi 9th People's Hospital Affiliated to Soochow University (Approval number KS2024016). All animal experiments were performed following the Guide for the Care and Use of Laboratory Animals (8th edition).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Emergency and Critical Care Medicine, Wuxi 9th People's Hospital Affiliated to Soochow University, Liangxi Road 999, Wuxi, Jiangsu 214000, People's Republic of China
[2]Department of Ultrasound, Wuxi 9th People's Hospital Affiliated to Soochow University, Wuxi, Jiangsu, People's Republic of China
[3]Department of Vascular Surgery, Wuxi 9th People's Hospital Affiliated to Soochow University, Wuxi, Jiangsu, People's Republic of China
[4]Department of Neurosurgery, The First Medical Center of Chinese PLA General Hospital, Fuxing Road 28, Beijing, People's Republic of China
[5]Orthopedic Institution of Wuxi City, Liangxi Road 999, Wuxi, Jiangsu 214000, People's Republic of China

Received: 26 January 2025 / Accepted: 9 May 2025
Published online: 20 May 2025

References

1. Chu C, Wang B, Zhang Z, Liu W, Sun S, Liang G, Zhang X, An H, Wei R, Zhu X, et al. miR-513c-5p suppression aggravates pyroptosis of endothelial cell in deep venous thrombosis by promoting Caspase-1. Front Cell Dev Biol. 2022;10:838785. https://doi.org/10.3389/fcell.2022.838785.
2. Zhao Z, Li X, Wang Y, Liu C, Ling G, Zhang P. Biomimetic platelet-camouflaged drug-loaded polypyrrole for the precise targeted antithrombotic therapy. J Nanobiotechnol. 2023;21:439. https://doi.org/10.1186/s12951-023-02197-3.
3. Navarrete S, Solar C, Tapia R, Pereira J, Fuentes E, Palomo I. Pathophysiology of deep vein thrombosis. Clin Exp Med. 2023;23:645–54. https://doi.org/10.1007/s10238-022-00829-w.
4. Kondreddy V, Keshava S, Das K, Magisetty J, Rao LVM, Pendurthi UR. The Gab2-MALT1 axis regulates thromboinflammation and deep vein thrombosis. Blood. 2022;140:1549–64. https://doi.org/10.1182/blood.2022016424.
5. Liu H, Lu Q. Fisetin alleviates inflammation and oxidative stress in deep vein thrombosis via MAPK and NRF2 signaling pathway. Int J Mol Sci. 2024;25. https://doi.org/10.3390/ijms25073724.
6. Qin J, Chen J, Peng F, Sun C, Lei Y, Chen G, Li G, Yin Y, Lin Z, Wu L, et al. Pharmacological activities and pharmacokinetics of liquiritin: A review. J Ethnopharmacol. 2022;293:115257. https://doi.org/10.1016/j.jep.2022.115257.
7. Zhai KF, Duan H, Cui CY, Cao YY, Si JL, Yang HJ, Wang YC, Cao WG, Gao GZ, Wei ZJ. Liquiritin from Glycyrrhiza uralensis attenuating rheumatoid arthritis via reducing inflammation, suppressing angiogenesis, and inhibiting MAPK signaling pathway. J Agric Food Chem. 2019;67:2856–64. https://doi.org/10.1021/acs.jafc.9b00185.
8. Mou SQ, Zhou ZY, Feng H, Zhang N, Lin Z, Aiyasiding X, Li WJ, Ding W, Liao HH, Bian ZY, Tang QZ. Liquiritin attenuates Lipopolysaccharides-Induced cardiomyocyte injury via an AMP-Activated protein Kinase-Dependent signaling pathway. Front Pharmacol. 2021;12:648688. https://doi.org/10.3389/fphar.2021.648688.
9. Zhang X, Song Y, Han X, Feng L, Wang R, Zhang M, Zhu M, Jia X, Hu S. Liquiritin attenuates advanced glycation end products-induced endothelial dysfunction via RAGE/NF-κB pathway in human umbilical vein endothelial cells. Mol Cell Biochem. 2013;374:191–201. https://doi.org/10.1007/s11010-012-1519-0.
10. Xu N, Li M, Wang P, Wang S, Shi H. Spectrum-Effect relationship between antioxidant and anti-inflammatory effects of Banxia Baizhu Tianma Decoction: an identification method of active substances with endothelial cell protective effect. Front Pharmacol. 2022;13:823341. https://doi.org/10.3389/fphar.2022.823341.
11. Xu N, Ijaz M, Shi H, Shahbaz M, Cai M, Wang P, Guo X, Ma L. Screening of active ingredients from Wendan Decoction in alleviating palmitic Acid-Induced endothelial cell injury. Molecules. 2023;28. https://doi.org/10.3390/molecules28031328.
12. Wang Z, Fang C, Yao M, Wu D, Chen M, Guo T, Mo J. Research progress of NF-κB signaling pathway and thrombosis. Front Immunol. 2023;14:1257988. https://doi.org/10.3389/fimmu.2023.1257988.
13. Wei X, Zhang B, Wei F, Ding M, Luo Z, Han X, Tan X. Gegen Qinlian pills alleviate carrageenan-induced thrombosis in mice model by regulating the HMGB1/NF-κB/NLRP3 signaling. Phytomedicine. 2022;100:154083. https://doi.org/10.1016/j.phymed.2022.154083.
14. Cao Z, Jiang X, He Y, Zheng X. Metabolic landscape in venous thrombosis: insights into molecular biology and therapeutic implications. Ann Med. 2024;56:2401112. https://doi.org/10.1080/07853890.2024.2401112.
15. Chen M, Zhang C, Zhang J, Kai G, Lu B, Huang Z, Ji L. The involvement of DAMPs-mediated inflammation in cyclophosphamide-induced liver injury and the protection of Liquiritigenin and liquiritin. Eur J Pharmacol. 2019;856:172421. https://doi.org/10.1016/j.ejphar.2019.172421.
16. Yadav V, Chi L, Zhao R, Tourdot BE, Yalavarthi S, Jacobs BN, Banka A, Liao H, Koonse S, Anyanwu AC, et al. Ectonucleotidase tri(di)phosphohydrolase-1 (ENTPD-1) disrupts inflammasome/interleukin 1β-driven venous thrombosis. J Clin Invest. 2019;129:2872–7. https://doi.org/10.1172/jci124804.
17. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6:a016295. https://doi.org/10.1101/cshperspect.a016295.
18. He Y, Ci X, Xie Y, Yi X, Zeng Y, Li Y, Liu C. Potential detoxification effect of active ingredients in liquorice by upregulating efflux transporter. Phytomedicine. 2019;56:175–82. https://doi.org/10.1016/j.phymed.2018.10.033.
19. Li Y, Chen J, Yang Y, Wang Y, Zhang Y, Zhou Y, Bao Y, Zhang Z, Lu Y. Necroptosis plays a crucial role in vascular injury during DVT and is enhanced by IL-17B. J Immunol Res. 2022;2022(6909764). https://doi.org/10.1155/2022/6909764.
20. Salemi R, Gattuso G, Tomasello B, Lavoro A, Gaudio A, Libra M, Signorelli SS, Candido S. Co-Occurrence of Interleukin-6 Receptor Asp358Ala Variant and High Plasma Levels of IL-6: An Evidence of IL-6 Trans-Signaling Activation in Deep Vein Thrombosis (DVT) Patients. Biomolecules. 2022; 12.https://doi.org/10.3390/biom12050681
21. von Brühl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, Khandoga A, Tirniceriu A, Coletti R, Köllnberger M, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. J Exp Med. 2012;209:819–35. https://doi.org/10.1084/jem.20112322.
22. Liang W, Lu H, Sun J, Zhao G, Wang H, Guo Y, Eitzman D, Chen YE, Fan Y, Zhang J. KLF11 protects against venous thrombosis via suppressing tissue factor expression. Thromb Haemost. 2022;122:777–88. https://doi.org/10.1055/s-0041-1735191.
23. Lu Z, Ye Y, Liu Y, Yang X, Ding Q, Wang Y, Wu Z, Zhan Y, Gui S, Lin B, Lin B. Aqueous extract of paeoniae Radix rubra prevents deep vein thrombosis by ameliorating inflammation through inhibiting GSK3β activity. Phytomedicine. 2021;92:153767. https://doi.org/10.1016/j.phymed.2021.153767.
24. Fu C, Zhang Y, Xi WJ, Xu K, Meng F, Ma T, Li W, Wu L, Chen Z. Dahuang Zhechong pill attenuates hepatic sinusoidal capillarization in liver cirrhosis and hepatocellular carcinoma rat model via the MK/integrin signaling pathway. J Ethnopharmacol. 2023;308:116191. https://doi.org/10.1016/j.jep.2023.116191.
25. Li M, Ke J, Deng Y, Chen C, Huang Y, Bian Y, Guo S, Wu Y, Zhang H, Liu M, Han Y. The protective effect of liquiritin in Hypoxia/Reoxygenation-Induced disruption on blood brain barrier. Front Pharmacol. 2021;12:671783. https://doi.org/10.3389/fphar.2021.671783.
26. Ekim M, Sekeroglu MR, Balahoroglu R, Ozkol H, Ekim H. Roles of the oxidative stress and ADMA in the development of deep venous thrombosis. Biochem Res Int. 2014;2014:703128. https://doi.org/10.1155/2014/703128.
27. Gutmann C, Siow R, Gwozdz AM, Saha P, Smith A. Reactive oxygen species in venous thrombosis. Int J Mol Sci. 2020;21. https://doi.org/10.3390/ijms21061918.
28. Xiao S, Sun X, Wang C, Wu J, Zhang K, Guo M, Liu B. Nanomicrosphere sustained-release urokinase systems with antioxidant properties for deep vein thrombosis therapy. RSC Adv. 2024;14:7195–205. https://doi.org/10.1039/d3ra07221e.
29. Meng Y, Yin Q, Ma Q, Qin H, Zhang J, Zhang B, Pang H, Tian H. FXII regulates the formation of deep vein thrombosis via the PI3K/AKT signaling pathway in mice. Int J Mol Med. 2021;47. https://doi.org/10.3892/ijmm.2021.4920.
30. Huang B, Tang P, Liu Y, Liu F, Zheng Y, Yang X, Zhang X, Xie H, Lin L, Lin B, Lin B. Xuefu Zhuyu Decoction alleviates deep vein thrombosis through inhibiting the activation of platelets and neutrophils via Sirtuin 1/nuclear factor kappa-B pathway. J Ethnopharmacol. 2024;333:118485. https://doi.org/10.1016/j.jep.2024.118485.
31. Cheng Z, Jia W, Tian X, Jiang P, Zhang Y, Li J, Tian C, Liu J. Cotinine inhibits TLR4/NF-κB signaling pathway and improves deep vein thrombosis in rats. Biosci Rep. 2020;40. https://doi.org/10.1042/bsr20201293.
32. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res. 2011;21:103–15. https://doi.org/10.1038/cr.2010.178.

Publisher's note